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Application of biospeckle laser technique for determining biological phenomena related to beef aging

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ABSTRACT

Among the features of beef quality, color is a major factor that influences marketing since it is the only characteristic consumers can see at the time of purchase, although tenderness is the organoleptic trait that most affects consumer acceptance of beef. Thus, an effective technology to predict meat quality is highly desirable for the meat industry. Among many emerging technologies, optical methods have the greatest potential since they are fast, nondestructive, and generally low cost. This study evaluated the potential application of laser biospeckle technique and its methods of image processing in order to assess and quantify biological phenomena related to beef aging. Samples of muscle *Longissimus thoracis* were aged for 21 days and underwent biospeckle analysis, objective color and Warner–Bratzler Shear Force (WBSF). According to the results, biospeckle laser parameters may, possibly, assess biological activity resulting from action of endogenous enzymes (calpains and cathepsins) responsible for the aging process through its correlation ($R = 0.6146$) with analysis of WBSF and of the correlation (-0.7973) with aging time. High correlation of biospeckle analysis, related to traditional out puts and to new proposals, were obtained to color parameters, especially hue angle (h^*) whose R value was 0.7953, redness intensity ($R = 0.8120$) and percentage of metmyoglobin (MMb) showed that R value of 0.9119, demonstrates the potential of this technique for evaluating quality of meat color.

1. Introduction

Color is a major factor influencing meat sale, as it is the only characteristic a consumer sees (Veraverbeke et al., 2006; Carpenter et al., 2001). In addition, taste quality of product is highly influenced by sensory properties such as juiciness, flavor, and especially tenderness. However, consumers are willing to pay a higher price for products with additional quality assurance (Koohmaraie and Geesink, 2006).

A technology capable of effectively grouping cuts or carcasses based on the expected sensitivity is highly desirable in meat industry (Ranasinghesagara et al., 2010). Among various emergent technologies, the methods of image analysis assist human perceptions of the visual phenomena arising from the optical creation of measurement systems, also provides greatest potential for application since they are fast, nondestructive and generally low cost (Hernández et al., 2008). Much research, concerned with the non-destructive techniques, has been using computer vision, near infrared, thermography or imaging spectroscopy (Xia et al., 2007; Fito et al., 2004). All these techniques rely on propagation of light within the meat, which is strongly modulated by optical absorption and scattering properties. Thus, currently there is increasing interest in optical scattering measurements especially to characterize meat tenderness (Rabal and Braga, 2008).

Dynamic laser speckle or biospeckle laser technique allows for optical monitoring of activities related to various physicochemical phenomena in biological tissues (Rabal and Braga, 2008; Braga et al., 2005, 2009). Speckle results from generation of interference patterns formed on a surface illuminated by a coherent light such as laser source (Janusz et al., 2002), when in the case of the dispersed particles are in motion they cause a temporal variation of speckle pattern in each image pixel, which is called dynamic speckle or biospeckle (Li et al., 2011).

As research trends in developing non-destructive methods for assessing meat quality, we studied variations in biological phenomena in beef, considering temporal variations of dynamic speckle when subjected to aging process and compared to routine methods related to color and tenderness.

2. Biospeckle image processing technique

Numerical analysis of speckle patterns in time mostly uses Temporal History of Speckle Pattern (THSP), which is a matrix

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formed by a collection of pixels in time (Oulomara et al., 1989; Xu et al., 1995; Arizaga et al., 1999).

According to Cardoso and Braga (2011), the single numerical value known as the inertia moment is obtained from THSP when it is transformed into matrix of occurrence (MOC) of successive intensity levels, and then into Inertia Moment (IM) as shown in equation:

$$\mathrm{IM} = \sum_{x,y} \frac{\mathrm{MOC}}{\mathrm{NORM}}(x-y)^2$$

where MOC is the occurrence matrix with lines (x) and columns (y) varying from 1 to 256 representing gray levels (8 bits). MOC or dispersion of intensity matrix is formed by occurrence of intensity of pixel x (1–256) followed by intensity y (1–256) that represents the next pixel in THSP matrix. The variable NORM represents the normalization type, which may be the sum of each line equal to one (Arizaga et al., 1999) or the sum of occurrences in the whole matrix equal to one (Cardoso and Braga, 2011).

According to Braga et al. (2011) the alternative to this activity index (IM) is the Absolute Values of the Differences (AVD) expressed in equation:

$$\mathrm{IM} = \sum_{x,y} \frac{\mathrm{MOC}}{\mathrm{NORM}}|x-y|$$

3. Materials and methods

3.1. Experimental design

We used completely randomized design (CRD) with six replicates. Results were analyzed by ANOVA considering 5% significance and interpreted by correlation of parameters with coefficients assessed by Student's *t*-test. All analyses were performed with Statistica 5.0 software (Statsoft).

3.2. Preparation of samples

The ribeye roll (*Longissimus thoracis*) cuts were obtained at a commercial packing plant, with Federal Inspection, in state of Minas Gerais, Brazil. The cuts were previously cleaned and sanitized in lactic acid solution 1%. Thirty steaks about 2 cm thick were cut, individually vacuum packed in high resistance polyethylene and aged for 0, 5, 10, 15, 21 days in a BOD incubator (model EL202, EletroLab) at controlled temperature (1 °C).

After each aging period the steaks were removed and analyzed by biospeckle laser and by objective color. Subsequently they were frozen and stored at −18 °C until analysis for cooking loss (CL) and Warner–Bratzler Shear Force (WBSF) were performed.

3.3. Instrumental color

Instrumental color was analyzed with spectrophotometric colorimeter CM-5 (Kônica Minolta) aperture 30 mm, illuminant A, 10° standard observer, specular component excluded (SCE) and CIELAB color system. Steaks were unpacked and exposed to air for 30 min, when color values L^*, a^* and b^* were obtained by the average value of 10 readings performed on the exposed surface. Chroma (C^*) was calculated with the following equation: $C^* = (a^{*2} + b^{*2})1/2$. Hue angle ($h^*$) was calculated as $\tan^{-1}$ (b^*/a^*) (Hunt et al., 1991).

Simultaneously with the objective color readings we evaluated changes in the chemical state of myoglobin during the aging period. The parameters for spectrophotometric colorimeter CM-5 (Konica Minolta) measurement were the same ones used for objective color, except for using specular component included (SCI) instead of specular component excluded (SCE).

Relative contents of pigments oxymyoglobin (O_2Mb), reduced myoglobin (Mb^+) and metmyoglobin (MMb) in meat were assessed with the mathematical method by Krzywicki (1979) described by Ramos and Gomide (2007), using reflectance spectra obtained between 400 and 730 nm at 10 nm intervals. Intermediate values (473, 525 and 572 nm) were calculated by linear interpolation.

3.4. Warner–Bratzler shear force (WBSF)

Samples were subjected to cooking loss measured according to the methodology described by Ramos and Gomide (2007) for further analysis of Warner–Bratzler Shear force (WBSF). WBSF was assessed according to the American Meat Science Association (AMSA) protocol for evaluating objective tenderness in muscle *Longissimus thoracis* with adaptations described by Rodrigues (2007). Samples from the cooking loss (CL) assessment were cut with a knife in parallel to the direction of muscle fibers in 1×1 cm^2 samples, avoiding meat with aponeurosis or fats. We used at least five rectangles of each sample.

WBSF was measured with a TAXT2 texturometer attached to a Warner Bratzler blade. Samples were sheared transversely to the direction of muscle fibers at 5.0 mm s^{-1} speed in pre-and post-test, and 3.3 mm s^{-1} in test speed.

The shear force WBSF was obtained and related to sample tenderness with results expressed in kilogram force (kgf).

3.5. Biospeckle laser

Meat samples were illuminated with coherent light, in an homogeneous way, and the interference patterns formed on them were captured by a CCD camera 640×486 pixels and shutter speed 1/60 s. Coherent light was a HeNe laser, wavelength 632 nm, 10 mW power, enlarged by a lens assembly sufficient to cover the entire sample (Fig. 1). In each illumination session we stored a set of 128 images in gray levels (8 bits) related to dynamic speckle at 0.08 s intervals. Analysis of images from laser illumination of the samples was performed by constructing the Temporal History of Speckle Pattern (THSP) and finding the Inertia Moment (Arizaga et al., 1999) and its variation, the Absolute Values of the Differences (AVD) (Braga et al., 2011).

We performed five illumination sessions in different points of each steak and found the average to ensure a biological activity that should be representative of the entire sample. We used packed and unpacked samples, which were unpacked after exposure to air for 30 min for blooming before the illumination.

During processing of each set of 128 frames in MATLAB software we found values for Inertia Moment evaluating different normalizations. The normalization proposed by Cardoso and Braga

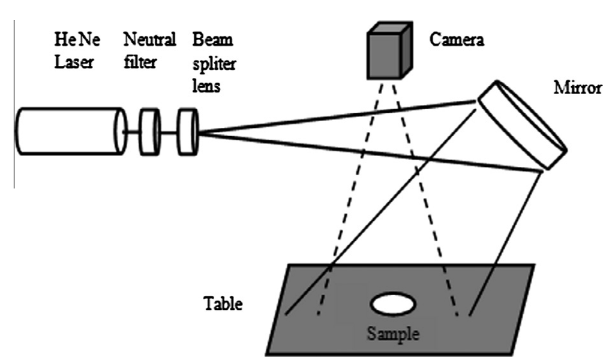

Fig. 1. Experimental setup to illuminate the sample using HeNe laser, neutral filter, beam splite rens, camera, mirror and table.