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

Isis Celena Amaral, Roberto Alves Braga Jr.*, Eduardo Mendes Ramos, Alcinéia Lemos Souza Ramos, Estevam Abreu Rezende Roxael

Departamento de Engenharia (DEG), Universidade Federal de Lavras (UFLA), P.O. Box 3037, 37200-000 Lavras, Minas Gerais, Brazil

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 ($R = 0.7973$) with aging time. High correlation of biospeckle analysis, related to traditional outputs 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 speckle pattern in each image pixel, which is called dynamic speckle or biospeckle (Li et al., 2011).

2. Biospeckle image processing technique

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

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

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:

$$IM = \sum_{xy} \frac{MOC(x, y)}{NORM} |x - y|$$

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 lactic acid solution 1%. Thirty steaks about 2 cm thick were cut, individually vacuum packed in high resistance polyethylene and lactic acid solution 1%. Thirty steaks about 2 cm thick were cut, individually vacuum packed in high resistance polyethylene and lactic acid solution 1%. Thirty steaks about 2 cm thick were cut, individually vacuum packed in high resistance polyethylene and lactic acid solution 1%. Thirty steaks about 2 cm thick were cut, individually vacuum packed in high resistance polyethylene and lactic acid solution 1%. Thirty steaks about 2 cm thick were cut, individually vacuum packed in high resistance polyethylene and lactic acid solution 1%

3.3. Warner–Bratzler shear force (WBSF)

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

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 splitter, lens, camera, mirror and table.](image-url)
(2011) was identified in the methods using the tag IM and AVD, and the traditional normalization was identified as IM_ARI and AVD_ARI. The term ARI was used as a reference to Ricardo Arizaga, the first author of the IM method (Arizaga et al., 1999).

4. Results and discussion

4.1. Warner–Bratzler shear force

Descriptive statistics of results for WBSF and biospeckle is shown in Table 1, while Table 2 presents Pearson correlation coefficients with the same parameters under study.

Results show the significant correlation of the biospeckle laser with the changes in meat WBSF. In turn, the outputs of the WBSF analysis correlated with meat tenderness. Since during aging the alteration in the meat tenderness along time are likely coming from the activity of the endogenous enzymes (cathepsins and calpains), it is possible, that the biospeckle laser analysis have measured the cumulative action of these enzymes. Nevertheless, as tenderness is a quality parameter influenced by many factors besides aging such as breed, management, muscles, among others, we conclude that some additional studies shall be done to isolate the sources of the tenderness and their relation with the biospeckle outputs.

For data analysis of shear force, the inertia moment with normalized co-occurrence matrix AVD_ARI showed a better fit of data related to packed meat (Fig. 2) and along time (Fig. 3). However, IM and AVD achieved the best correlations for analysis of unpacked meat exposed to air for 30 min.

Since the normalizations in IM and AVD are conducted using the whole matrix, instead at each line as in AVD_ARI, the expected inhomogeneity present in the unpacked samples were better observed by that alternative normalization.
Table 3
Simple descriptive analysis of results found in the experiment (n=3).

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>F test (time)</th>
<th>Average</th>
<th>Standard deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>L'</td>
<td>30</td>
<td>0.0902</td>
<td>42.62</td>
<td>1.58</td>
<td>40.10</td>
<td>46.9</td>
</tr>
<tr>
<td>a'</td>
<td>30</td>
<td>&lt;0.0001</td>
<td>15.51</td>
<td>4.08</td>
<td>6.8</td>
<td>22.86</td>
</tr>
<tr>
<td>b'</td>
<td>30</td>
<td>&lt;0.0001</td>
<td>17.55</td>
<td>14.16</td>
<td>6.64</td>
<td>48.26</td>
</tr>
<tr>
<td>C'</td>
<td>30</td>
<td>&lt;0.0001</td>
<td>24.91</td>
<td>11.94</td>
<td>9.50</td>
<td>49.97</td>
</tr>
<tr>
<td>h'</td>
<td>30</td>
<td>&lt;0.0001</td>
<td>42.31</td>
<td>16.95</td>
<td>25.85</td>
<td>76.03</td>
</tr>
<tr>
<td>MMb (%)</td>
<td>28</td>
<td>&lt;0.0001</td>
<td>37.22</td>
<td>12.71</td>
<td>21.94</td>
<td>56.12</td>
</tr>
<tr>
<td>Mb⁺ (%)</td>
<td>28</td>
<td>&lt;0.0001</td>
<td>12.98</td>
<td>7.25</td>
<td>2.60</td>
<td>30.49</td>
</tr>
<tr>
<td>O₂Mb (%)</td>
<td>28</td>
<td>&lt;0.0001</td>
<td>49.80</td>
<td>12.57</td>
<td>32.39</td>
<td>68.83</td>
</tr>
</tbody>
</table>

L' = luminosity; a' = redness value; b' = yellowness value; C' = chroma; h' = hue angle; MMb = metmyoglobin; Mb⁺ = reduced myoglobin; O₂Mb = oxymyoglobin.

Table 4
Pearson correlation coefficients of color values (L', a', b', C', h') in relation to WBSF and biospeckle readings (n=3).

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>L'</th>
<th>a'</th>
<th>b'</th>
<th>C'</th>
<th>h'</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBSF</td>
<td>30</td>
<td>-0.3489</td>
<td>0.059</td>
<td>-0.3978</td>
<td>0.029</td>
<td>-0.3524</td>
</tr>
<tr>
<td>IM</td>
<td>30</td>
<td>-0.4012</td>
<td>0.028</td>
<td>0.7102</td>
<td>0.000</td>
<td>-0.4664</td>
</tr>
<tr>
<td>AVD</td>
<td>30</td>
<td>-0.3720</td>
<td>0.043</td>
<td>0.7724</td>
<td>0.000</td>
<td>-0.5043</td>
</tr>
<tr>
<td>IM_ARI</td>
<td>30</td>
<td>-0.3658</td>
<td>0.047</td>
<td>0.7257</td>
<td>0.000</td>
<td>-0.3722</td>
</tr>
<tr>
<td>AVD_ARI</td>
<td>30</td>
<td>-0.2358</td>
<td>0.210</td>
<td>0.7107</td>
<td>0.000</td>
<td>-0.4575</td>
</tr>
<tr>
<td>IM (30 m)</td>
<td>29</td>
<td>-0.3857</td>
<td>0.039</td>
<td>0.8041</td>
<td>0.000</td>
<td>-0.5218</td>
</tr>
<tr>
<td>AVD (30 m)</td>
<td>29</td>
<td>-0.3832</td>
<td>0.040</td>
<td>0.8120</td>
<td>0.000</td>
<td>-0.5445</td>
</tr>
<tr>
<td>IM_ARI (30 m)</td>
<td>29</td>
<td>-0.2586</td>
<td>0.176</td>
<td>0.6843</td>
<td>0.000</td>
<td>-0.4810</td>
</tr>
<tr>
<td>AVD_ARI (30 m)</td>
<td>29</td>
<td>-0.2862</td>
<td>0.132</td>
<td>0.7074</td>
<td>0.000</td>
<td>-0.4085</td>
</tr>
</tbody>
</table>

L' = luminosity; a' = redness value; b' = yellowness value; C' = chroma; h' = hue angle; IM = inertia moment of packed meat with new normalization; AVD = inertia moment of packed meat with new normalization; IM_ARI = inertia moment of packed meat with traditional normalization; AVD_ARI = inertia moment of packed meat with traditional normalization; IM (30 m) = inertia moment of unpacked meat exposed to air for 30 min with new normalization; AVD (30 m) = inertia moment of unpacked meat exposed to air for 30 min with new normalization; IM_ARI (30 m) = inertia moment of unpacked meat exposed to air for 30 min with traditional normalization; AVD_ARI (30 m) = inertia moment of unpacked meat exposed to air for 30 min with traditional normalization.

Table 5
Pearson correlation coefficients of pigments (MMb, Mb⁺, O₂Mb) in relation to WBSF and biospeckle readings (n=3).

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>MMb</th>
<th>Mb⁺</th>
<th>O₂Mb</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBSF</td>
<td>28</td>
<td>-0.5866</td>
<td>0.001</td>
<td>0.6148</td>
</tr>
<tr>
<td>IM</td>
<td>28</td>
<td>-0.8312</td>
<td>0.000</td>
<td>0.4957</td>
</tr>
<tr>
<td>AVD</td>
<td>28</td>
<td>-0.8834</td>
<td>0.000</td>
<td>0.4317</td>
</tr>
<tr>
<td>IM_ARI</td>
<td>28</td>
<td>-0.7923</td>
<td>0.000</td>
<td>0.3446</td>
</tr>
<tr>
<td>AVD_ARI</td>
<td>28</td>
<td>-0.7961</td>
<td>0.000</td>
<td>0.3067</td>
</tr>
<tr>
<td>IM (30 m)</td>
<td>27</td>
<td>-0.9580</td>
<td>0.000</td>
<td>0.3716</td>
</tr>
<tr>
<td>AVD (30 m)</td>
<td>27</td>
<td>-0.9119</td>
<td>0.000</td>
<td>0.3457</td>
</tr>
<tr>
<td>IM_ARI (30 m)</td>
<td>27</td>
<td>-0.7822</td>
<td>0.000</td>
<td>0.4168</td>
</tr>
<tr>
<td>AVD_ARI (30 m)</td>
<td>27</td>
<td>-0.7706</td>
<td>0.000</td>
<td>0.2820</td>
</tr>
</tbody>
</table>

MMb = metmyoglobin; Mb⁺ = reduced myoglobin; O₂Mb = oxymyoglobin; IM = inertia moment of packed meat with new normalization; AVD = inertia moment of packed meat with new normalization; IM_ARI = inertia moment of packed meat with traditional normalization; AVD_ARI = inertia moment of packed meat with traditional normalization; IM (30 m) = inertia moment of unpacked meat exposed to air for 30 min with new normalization; AVD (30 m) = inertia moment of unpacked meat exposed to air for 30 min with new normalization; IM_ARI (30 m) = inertia moment of unpacked meat exposed to air for 30 min with traditional normalization.

The values of "N" are variable due to the missing points during the experimental execution.

The study shows that optical properties of laser light scattering have great potential to provide valuable information on physico-chemical characteristics responsible for meat tenderness. The ability to provide high correlation with the packed meat gives to the biospeckle a robust way to evaluate that complex phenomenon contactless.

4.2. Instrumental color

The descriptive statistics of results obtained for color, chemical forms of myoglobin, and biospeckle are presented in Table 3. While the Table 4 shows Pearson correlation coefficients of biospeckle laser with changes in color parameters (L'; a'; b'; C'; h') in the aging process.

We also found correlations concerning yellowness intensity (b') and especially redness intensity (a'), which indicates that the tool is not only able to detect and quantify color changes as a whole through hue angle (h'), but is also capable of quantifying color changes related to intensity of redness and yellowness.

Table 5 shows results of biospeckle correlations through values of moments of inertia with pigments metmyoglobin (MMb), reduced myoglobin (Mb⁺) and oxymyoglobin (O₂Mb).

Fresh meat color is associated with proportion and relative distribution of three chemical forms of myoglobin: purple red reduced myoglobin or deoxymyoglobin (Mb⁺), bright red
oxymyoglobin (O₂Mb), and brown metmyoglobin (MMb). Such chemical forms are rarely found alone on meat surface, which usually shows two or more forms. However, the resulting color is always that of the predominant chemical form (Mancini and Hunt, 2005). Biospeckle analysis was performed on the samples packed and unpacked and exposed at room temperature for 30 min. While, although colorimeter analysis was performed only with unpacked samples exposed to room temperature for 30 min (blooming), biospeckle readings showed high correlations in both packed and unpacked samples. This suggests a strong potential of the technique to predict information on hue and metmyoglobin content in meat, without having to remove product from packaging and expose it to air.

Normalizations associated with IM and especially with AVD (Fig. 4) showed high correlation with several physicochemical parameters of meat. AVD with the alternative normalization showed a better fit of data and consequently better correlations. Thus, it was deemed ideal for analysis of animal tissue. Therefore, the correlations of the biospeckle laser with aging time, WBSF and the color parameters are relevant which make of the biospeckle laser a potential technique to predict contactlessly the meat quality, in especially, color and tenderness.

5. Conclusions

Biospeckle technique combined with analysis of inertia moment offers an efficient tool for monitoring and quantifying biological activity of meat during aging process, which demonstrates the technique potential for evaluating and predicting beef quality. However, further study is needed to elucidate biospeckle contributions and confirm these results.

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