REPEATABILITY AND REPRODUCIBILITY OF TWO INSTRUMENTS TO MEASURE MEAT COLOUR

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Abstract – The aim of this study was to evaluate the repeatability and reproducibility of colour measurements obtained by different operators using two instruments. Twenty samples of *Longissimus thoracis* muscle of Piemontese young bulls were utilized. Three operators measured each sample twice, with a colorimeter and a spectrophotometer. L*, a* and b* parameters were recorded. The correlation coefficients between CIEL*a*b* values obtained by the two instruments and the colour difference between the two measurements obtained by each operator and between the three operators were calculated. Repeatability and reproducibility of each instrument was analyzed by ANOVA.
The L*a*b* absolute values depend on the instrument used. L*, a* and b* correlations between the two instruments were highly significant. The spectrophotometer was more precise than colorimeter in repeated colour measurements. In general, data on repeatability and reproducibility showed that the measurements carried out with the spectrophotometer are more precise than the colorimeter. In fact, L* and b* values obtained with the spectrophotometer showed the best repeatability and reproducibility. The colorimeter was more precise for a* values. This could depend on the fact that colorimeter utilizes larger measuring area than spectrophotometer. The larger aperture leads to more averaged measuring results and, thus, to smaller measurements uncertainty.

Key Words – colorimeter, spectrophotometer, Piemontese young bulls.

I. INTRODUCTION

Meat color is the major quality trait based on which consumers make their purchase decisions. It depends on myoglobin (muscle pigment) and, to a lesser extent, on haemoglobin (blood pigment) concentration, their chemical state, and the light scattering properties of meat [1; 2]. Colour may be assessed subjectively, using a standard colour chart, or objectively, using a reflectometry based instrument [3]. Subjective assessment of colour is open to bias, hence, colour measuring devices, such as tristimulus colorimeter and spectrocolorimeter, are frequently used. These instruments differ in the way they measure the reflected light. The tristimulus method uses a light source that illuminates the sample and is then reflected through red, green, and blue filters onto photo-detectors. The microprocessor can convert the reflected values to CIEL*a*b* values. The spectrophotometer illuminates the sample and the reflected waves are either scanned or read simultaneously by a photo diode array. These values are sent to a microprocessor and can be presented as the reflected spectra or converted to CIEL*a*b* values [4].
When different instruments are used to measure colour, different data are generated, even when measuring the same tissue, and even under the same environmental and instrument (colour space, standard illuminant) condition.
Therefore, the aim of this study was to evaluate the repeatability and reproducibility [5] of colour measurements obtained by different operators using two instruments.

II. MATERIALS AND METHODS

In this study two instruments commonly used in meat colour evaluation have been analysed.
The Minolta Chroma Meter model CR-331C is a compact portable colorimeter which uses a circumferential ring optical fiber illuminant source at 45° angle of incidence and 0° viewing angle for measuring glossy surface. With this illuminant/sensor arrangement the gloss component is excluded from the measurements. The colorimeter averages the readings over a 25 mm diameter measuring area to provide a more uniform response. It uses a pulsed xenon arc lamp and the 1932 CIE 2° Standard Observer [4].

The Minolta CM-600d is a compact, portable spectrophotometer with an aperture of 8 mm. It uses a silicon photodiode array (dual 36-element) detector and a light source of pulsed xenon lamp with UV cut filter. The illumination/viewing system is arranged with 8-degree viewing angle (diffused illumination) for the detection of specular component included (SCI) or excluded (SCE). The instrument uses the CIE 10° Standard Observer, which was developed in 1964 [4].

Before conducting the experiment, usual warm up and calibration procedures were followed. Each instrument was calibrated on its own white reference tile supplied by the manufacturer and set with the illuminant D65, which simulates average north sky daylight with a color temperature of 6500 K. For the spectrophotometer, the SCE mode was chosen because it "mimicks" the specular excluded 45°/0° geometry of the colorimeter. The SCE mode provides measurements that correspond to the visual changes in appearance of the sample due to both changes in pigment colour and surface gloss or texture.

Twenty meat samples of *Longissimus thoracis* muscle of Piemontese young bulls were utilized. Three operators measured each sample twice with each instrument.

The colour measurements were obtained on a freshly cut surface after 1 hour of blooming. The procedure for sample preparation was strictly adhered to in order to reduce other sources of variability as much as possible.

L*, a* and b* parameters were recorded. L* is the lightness and extends from 0 (black) to 100 (white). The other two coordinates, a* and b*, represent redness-greenness and yellowness-blueness respectively.

In order to determine the level of agreement between the CIEL*a*b* values obtained by the two instruments, correlation coefficients were calculated.

In addition, colour differences (ΔE*) between the two measurements obtained by each operator and between the three operators were calculated by the following equation: $\Delta E^*=(\Delta L^{*2}+\Delta a^{*2}+\Delta b^{*2})^{1/2}$. The colour CIEL*a*b* difference (ΔE*) represents the distance from the measured values L*, a*, and b* to the three-dimensional space of 2 colours.

Repeatability and reproducibility of each instrument were analyzed by ANOVA as described by Youden and Steiner [5].

The statistical model included three operators, 20 samples and two measurements. The variance components relative to the operator effect, its interaction with the sample effect, and error variance were used to calculate the standard deviation of repeatability and reproducibility according to Youden and Steiner [5]. Finally, relating the standard deviation of repeatability and reproducibility to the corresponding general means of the tested parameters, the percent variability coefficients were obtained, named relative repeatability and relative reproducibility.

III. RESULTS AND DISCUSSION

The mean values obtained by the three operators with the two instruments are reported in table 1.

Table 1. Mean values and standard deviation (s.d.) of L*, a* and b*

	Operators				
	1	2	3	Mean	s.d.
331					
L*	39.27	39.15	38.78	39.06	3.28
a*	28.61	28.63	29.04	28.76	1.93
b*	9.52	9.21	9.80	9.51	1.94
600					
L*	40.24	39.83	39.45	39.84	3.09
a*	18.41	19.00	18.67	18.70	2.13
b*	15.88	15.91	15.65	15.81	2.12

331: Minolta colorimeter CR-331C
600: Minolta spectrophotometer CM-600d

CIEL*a*b* absolute values were affected by the instrument used. Compared with the spectrophotometer, the colorimeter gave much higher values of a* and lower values of b* while the L* values were more similar.

The observed differences can be easily explained on the basis of the different optical geometry of the two instruments. The term "geometry" refers to the placement of a sample relative to the light source and measuring lens. In fact, Minolta CR-331C has 45°/0° geometry while Minolta CM-600d has a diffuse 8° geometry.

Different optical geometries of different instruments are important source of variation in this regard [6].

Moreover the two instruments have different viewing aperture area and illuminating light

spot which can greatly affect both the direction and amount of light returned from translucent materials. Using a LabScan spectrophotometer with different aperture size in pork, Yancey and Kropf [7] demonstrated that L*, a* and b* values decrease with decreasing aperture size, when using illuminant D65.

Honikel [8] recommended that aperture (viewing port) size should be as large as the instrument will allow. The larger aperture leads to more averaged measuring results and, thus, to smaller measurements uncertainty.

Therefore the L*a*b* absolute values depend on the instrument used.

Although a direct comparison of the results obtained by the two instruments was not possible, the correlations between L*, a* and b* parameters of the two instruments were r=0.883, r=0.689, r=0.790, respectively, and highly significant (P<0.01).

Table 2 show the ΔE* between the two measurements obtained by each operator and between the operators.

Table 2. Colour differences (ΔE*) between the two measurements obtained by each operator and between operators

	Within Operator			
	1	2	3	P-value
331				
ΔE*	2.3	2.5	2.0	0.596
600				
ΔE*	1.7	1.7	1.3	0.245
	Between Operators			
	1 *vs* 2	1 *vs* 3	2 *vs* 3	P-value
331				
ΔE*	2.0	2.1	2.0	0.498
600				
ΔE*	2.0	2.2	2.1	0.720

331: Minolta colorimeter CR-331C
600: Minolta spectrophotometer CM-600d

It can be noticed that the spectrophotometer was more precise than colorimeter in repeated colour measurements. In fact, CM-600d showed ΔE* values lower in comparison with CR-331C and always below 2. On the contrary, the ΔE* values between operators were very similar for both the instruments.

The repeatability defines the variation between readings of the same sample repeated with the same instrument and operator over a certain period.

The reproducibility defines the variation between readings of the same sample from two or more operators.

An adequate level of precision of the instrument is critical as it determines the capacity to detect differences between the samples. It is therefore necessary to quantify the detection capability of the measurement process which is achieved by quantifying the variability therein. The total variation can be decomposed into variation due to the operator, its interaction with sample and variation due to the sample.

The main objectives in performing a repeatability and reproducibility study are to identify and quantify the absolute and relative contribution of each source of variation, to decide if the measurement process is adequate or not and, if not, to correct the errors by recalibrating the instrument, training the operators, including mathematical corrections, and so on.

Both repeatability and reproducibility were expressed in terms of standard deviation or, relating to general mean of the corresponding determination, as variability coefficient, which allow the comparison of determination with different means.

As regard the results obtained with the colorimeter, the ANOVA (table 3) showed the significant influence of the operator for a* and b*.

Table 3. Results of analysis of variance for L*, a* and b*

	Variances				
	Between Operators d.f. = 2		Operator x Sample d.f. = 38		Error of Variance d.f. = 60
331					
L*	2.88	ns	0.56	ns	1.64
a*	4.39	*	0.53	ns	1.01
b*	7.57	*	0.45	ns	1.01
600					
L*	6.14	*	1.83	*	0.56
a*	2.16	ns	2.48	*	0.64
b*	0.87	ns	1.81	*	0.52

331: Minolta colorimeter CR-331C
600: Minolta spectrophotometer CM-600d
d.f. = degrees of freedom
ns = not significant
*= P<0.05

In fact, operator 3 obtained higher values than the other two operators (table 1). No significant interaction operator x sample

was found and the variance ratio for all the three parameters was lower than 1.
When the spectrophotometer was utilized, significant differences between operators for L* and significant interactions operator x sample for all the CIEL*a*b* values were found.
Operator 1 obtained higher value of L* in comparison with the other two operators (table 1).
Because the interaction term is significant, the measurements will show greater variation when carried out by different operators than within one operator.
Repeatability and reproducibility values are reported in table 4.

Table 4. Mean values of L*, a* and b*, standard deviation of repeatability and reproducibility (s.d.) and relative repeatability and reproducibility

		Repeatability		Reproducibility	
	Mean	s.d.	relative	s.d.	relative
331					
L*	39.06	1.28	3.28	1.15	2.95
a*	28.76	1.00	3.48	0.90	3.13
b*	9.51	1.00	10.52	0.90	9.46
600					
L*	39.84	0.75	1.88	0.96	2.41
a*	18.70	0.80	4.28	1.08	5.78
b*	15.81	0.72	4.55	0.86	5.44

331: Minolta colorimeter CR-331C
600: Minolta spectrophotometer CM-600d

The more repeatable and reproducible the method was, the lower the values were.
Colorimeter had lower values of relative reproducibility compared with the values of relative repeatability. The use of a circumferential "ring" in the illumination resolves problems of poor reproducibility of the bi-directional 45°/0° geometry colorimeters.
On the contrary, the spectrophotometer had lower values of relative repeatability compared with the values of relative reproducibility. The values of relative repeatability and relative reproducibility of spectrophotometer varied less in comparison with that of the colorimeter. The values of relative repeatability and relative reproducibility obtained with spectrophotometer in comparison with that of colorimeter were lower for all the parameters, except for a* parameter.

IV. CONCLUSION

This study showed that there were differences between the CIEL*a*b* values measured with the two instruments. Therefore, researchers working on meat colour should use the same instrument to compare their results and be aware that some factors like illuminant, aperture size and observer angle can affect instrumental meat colour measurements.
The overall results showed that the spectrophotometer was slightly more precise than the colorimeter.

ACKNOWLEDGEMENTS

The research project "QualiPiem – Metodi innovativi per la selezione della qualità nella razza Piemontese" was financially supported by Fondazione Cassa di Risparmio di Cuneo. This study was conducted as part of a research project supported by the Italian Ministry for Education, University and Research (MIUR) ex60% - BRUA_RILO_16_01.

REFERENCES

1. Lawrie, R. A. 2002. The Eating Quality of Meat. In Meat Science, 5th Ed., pp. 173-176, 184-188, New York, Pergamon Press.
2. MacDougall, D.B. (1982) Changes in the colour and opacity of meat. Food Chemistry, 9, 75-88.
3. Kerry, J., Kerry, J. & Ledward, D. (2002). Meat Processing, Improving Quality. Woodhead Publishing Limited, Cambridge.
4. A.M.S.A. (2012). Meat color measurement guidelines. American Meat Science Association 201 West Springfield Avenue, Suite 1202 Champaign, Illinois USA 61820 800-517-2672.
5. Youden, W.J. & Steiner, E.H., (1975). Statistical manual of the AOAC. Association of official analytical chemists, Arlington, VA, USA.
6. MacDougall, D.B. (1994). Colour of meat in: Quality attributes and their measurement in meat, poultry and fish products. Advances in Meat Research Series. Volume 9, Ed. By Pearson A.M. & Dutson T.R., Chapman & Hall, 79-83.
7. Yancey, J.W.S. & Kropf, D.H. (2008). Instrumental reflectance values of fresh pork are dependent on aperture size. Meat Science, 79, 734-739.
8. Honikel, K.O. (1988). References methods for the assessment of physical characteristics of meat. Meat Science, 49, 447-457.