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COLORIMETRIC IMAGE ANALYSIS AS A FACTOR IN ASSESSING THE QUALITY OF PORK HAM SLICES DURING STORAGE

ANÁLISIS COLORIMÉTRICO DE IMÁGENES COMO FACTOR DE EVALUACIÓN DE LA CALIDAD DE REBANADAS DE JAMÓN DE CERDO DURANTE ALMACENAMIENTO

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Abstract

This study analysed the colour changes of stored pork ham slices at two temperatures (4 and 8°C) to compare two imaging methodologies for estimating colour changes over time in CIELAB colour spaces through a DigiEye® and stereoscope with digital image analysis. Colour space changes were analysed using a computer vision system for image segmentation analysis. It was determined that from the ninth day, changes could be perceived in the representative colour of ham slices using DigiEye®. Finally, colour prediction equations with R^2 >0.85 were determined as a tool for electronic monitoring to assessing the quality of pork ham slices during storage.

Keywords: colorimetric analysis, ham, ΔE^*_{ab}, storage conditions.

Resumen

En este estudio se analizaron los cambios de color de rodajas de jamón de cerdo almacenadas a dos temperaturas (4 y 8°C) con el fin de comparar dos metodologías de captura de imagen para la estimación de los cambios de color en el tiempo en los espacios de color CIELAB a través de un DigiEye®, y estereoscopio con análisis digital de imágenes. Los cambios de espacio de color se analizaron utilizando un sistema de visión por computador para el análisis de segmentación de las imágenes. Se determinó que a partir del noveno día, los cambios de color representativos podrían ser percibidos en las rebanadas de jamón utilizando DigiEye®. Por último, se lograron obtener ecuaciones de predicción de color con R^2 > 0.85 como una herramienta para el monitoreo electrónico para la evaluación de la calidad de las rodajas de jamón de cerdo durante el almacenamiento.

Palabras clave: análisis colorimétrico, jamón, ΔE^*_{ab}, condiciones de almacenamiento.

1 Introduction

The colour of food products is considered a fundamental physical property directly correlated with perceived quality (Iqbal *et al*. 2010); for this reason, colour is a parameter that affects acceptance by the consumer, who has the power to decide on the purchase. Colour determination enables detection of certain potential anomalies or defects in food (León *et al*. 2006). Eyiler & Oztan (2011) have recognised the importance of these attributes in meat products, such as sausages, emphasising the need to adopt different

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methodologies for measuring the colour of products in the meat industry. For this purpose, several authors, such as Wu and Sun (2013), have mentioned that it remains important to develop inspection and colour measuring systems, especially during the processing and storage of food products. Thus, in recent years, the perception of colour in foods has been consolidated within instrumental parameters through predictive, comparative and validated models (Quevedo *et al*. 2008; Blasco *et al*. 2009; Jackman *et al*. 2012).

It is currently difficult to compare results for the same products due to differences in instrumentation for measuring colour. For this reason, it is necessary to standardise colour analysis and improve the traceability and transferability of measurements (Pathare *et al*. 2013). For colour, as for other quality characteristics of food products, reproducibility and low variability become so important that data analysis requires computer vision systems. These systems have been considered by several authors as a promising method for both predicting colour changes in meat and detecting colour changes during processing (Mancini & Hunt, 2005).

Moreover, image analysis has been used to objectively measure the colour of different foods; this technique uses colour spaces and numeric values to create, represent and display colours in two and three spatial dimensions (Trusell *et al*. 2005). It also provides certain advantages over other conventional colorimetric techniques, such as the possibility of analysing each pixel on the entire surface of the food and quantifying its characteristics and defects (Brosnan *et al*. 2004). With a digital camera, the colour of any pixel in the image of the object can be recorded using three colour sensors per pixel (León *et al*. 2006). The colour model used most often is RGB, in which each sensor captures the light intensity of the spectrum in red (R), green (G) or blue (B). However, because the RGB colour space is not continuous, each channel can only take integer values between 0 and 255. To calculate the colorimetric coordinates recommended by the International Commission on Illumination (CIE, for its initials in French) for the evaluation of foods, it is necessary to transform RGB to CIELAB colour spaces. This transformation requires calibration and depends on the lighting when taking pictures (León *et al*. 2006).

Image analysis has been used as a technique to evaluate quality parameters for characterize pork or turkey slices (Vestergaard *et al*. 2005; Iqbal *et al*. 2013) or other food products (Camelo-Méndez *et al*. 2013). One of the most important steps in image analysis is segmentation. This process is based on dividing an image into multiple segments (array of pixels). The goal of segmentation is to simplify and/or change the representation of an image into something more meaningful and easier to analyse (Zheng *et al*. 2008). However, there are no known studies comparing different image capturing systems for colour evaluations based on response surface methodologies. Thus, the objective of this investigation was to analyse the colour changes of stored pork ham slices at two temperatures (4 and 8°C) to compare two imaging methodologies for estimating colour changes over time in CIELAB colour spaces through a DigiEye® and stereoscope with digital image analysis.

2 Materials and methods

378 slices of cooked pork ham were produced in the Zenú S.A.S. Food Industry (Medellín, Colombia) Nutresa Group from standard formulas based on established preparation protocols. The product was chopped and vacuum packaged by a Tiromat Compac 320® compact packer in rooms with controlled temperature, relative humidity and air speed using a packaging film consisting of two layers: the upper laminate with a high gas barrier and an oxygen permeability at 23°C and 0% relative humidity of 8 gm^2/day and the lower one with a 90 μm thickness, coextruded, a high gas barrier, an oxygen permeability at 23°C and 0% relative humidity of 6 gm^2/day and a water vapour permeability at 38°C and 90% relative humidity of 8 gm^2/day. Ham was stored at 4°C and 8°C in Thermo Scientific Model 815® temperature controlled chambers with a sensitivity of ± 1°C for 41 days. These temperatures were selected as refrigeration and abuse temperatures respectively. The slices, the muscle section and the ham brine section were analysed separately to differentiate the heterogeneity of the samples.

2.1 Image acquisition

2.1.1 Image capture by stereoscope

Images of 126 cooked pork ham slices were acquired over a white background using a stereomicroscope (transmitted light) with a 4.0x Nikon SMZ 800 objective and a Nikon DS-Vi1 digital camera connected through a USB interface adapted to a capture system. Images were sent to a Hewlett Packard laptop, Intel (R) Core (TM) i5-3230M processor CPU

@ 2.60 GHz, installed memory (RAM) of 8.00 GB (7.90 GB useable) and a 64 bit operating system. Capture was performed with a constant focal length and standardised lighting conditions. The captured images had a resolution of 1280 x 960 pixels and were stored in *.BMP colour format (24 bits). For each slice, 5 images were captured for muscle and 5 images for ham brine sections and finally were analysed with Image J software (V2.31, NIH USA) in RGB coordinates. In addition, CIELAB values were obtained using the "Colour Space Converter" plugin (León *et al*. 2006).

2.1.2 Image capture by DigiEye®

Image capture was performed using the DigiEye® Version 2.62 (Rodríguez-Pulido *et al*., 2013) imaging system (reflected light), which consists of a Nikon® D90 10.2-megapixel digital camera connected to a controlled lighting cabin (VeriVide, Leicester, United Kingdom) and a computer provided with appropriate software (DigiPix®) through USB. For these measurements, the samples were illuminated by an illuminant D65. The camera was calibrated with a colour chart (DigiTizer) certified and provided by VeriVide Ltd. (Leicester, United Kingdom) to characterise the camera response, relating the RGB signals to the CIE specifications under fixed lighting conditions in the chamber. For colour measurements, ham slices (252) were placed on a white background surface.

2.2 Image segmentation

Images of cooked pork ham slices were segmented into two sections (muscle and ham brine) according to a* values using the "Colour Histogram" tool as can be observed in Figure 1. The selected intervals for muscle were 123 to 136. Of the segmented images of muscle and ham brine, 5 measurements were performed per image with segmentations of 50 × 50 pixels and 200 × 200 pixels, respectively.

2.3 Experimental design

Three experimental designs were applied via Response Surface Models of Optimisation (RSMO) to identify the maximum values of colour change ΔE^*_{ab} from Equation 1. To this end, different factors that influence colour changes were varied, such as storage time, evaluated in days (A); temperature, evaluated at 4°C and 8°C (B); and section of the slice (C). Two designs were used to evaluate the colour change ΔE^*_{ab} on images acquired using a DigiEye®, and the third was used to evaluate the colour change by stereoscope. The commercial statistical package Design Expert Version 8.0 (Statease Inc., Minneapolis, USA) was used.

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad (1)$$

2.3.1 DigiEye® experimental design (time and temperature)

The coding of the independent factors used for the first experimental design is shown in Table 1. The experiments were planned according to the "D-optimal" pattern, as shown in Table 1, in independent assays. The order of experiments was completely randomised. Data were analysed by multiple least squares regressions. A cubic model was used to express the response variable ΔE^*_{ab} in whole slices as a function of independent factors, where A and B are values coded by time (numerical factor) and temperature (categorical factor), respectively. These factors were fixed to consider that both extrinsic factors (time and temperature) and structural composition of the ham have influence over colour changes in complete surface of the slices (Garrido, García-Jalón and Vitas, 2010).

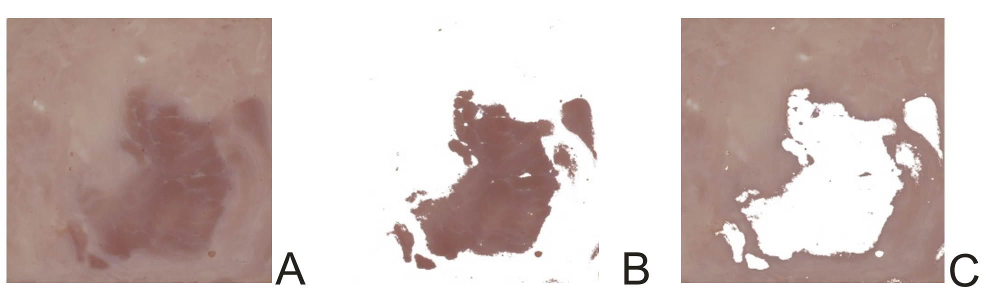

Fig. 1. Example of segmentation of the slices cooked pork ham. Slice (A), muscle (B) and ham brine (C).

Table 1. Experimental designs in images acquired by DigiEye® and stereoscope, modifying the evaluated factors (time, temperature and section).

	Design 1: DigiEye® (2 Factors)			Design 2: DigiEye® (3 Factors)				Design 3: Stereoscope (3 Factors)			
Std	Factor 1 : A - Time (days)	Factor 2: B - Temperature (°C)	ΔE^*_{ab}	Factor 1: A - Time (Days)	Factor 2: B - Temperature (°C)	Factor 3: C - Section	ΔE^*_{ab}	Factor 1 : A - Time (days)	Factor 2: B - Temperature (°C)	Factor 3: C - Section	ΔE^*_{ab}
1	0.00	4	0.00	0.00	8	Slice	0.00	41.00	4	Muscle	11.68
2	10.00	4	8.52	10.00	4	Slice	13.46	0.00	4	Muscle	0.00
3	10.00	8	7.21	41.00	8	Muscle	8.04	15.00	8	Muscle	2.77
4	0.00	8	0.00	10.00	8	Muscle	11.29	7.00	4	Muscle	9.59
5	20.00	8	4.98	41.00	8	Slice	0.94	34.00	4	Brine	12.93
6	40.00	8	3.69	20.50	4	Brine	5.5	7.00	4	Muscle	9.59
7	0.00	4	0.00	0.00	4	Muscle	0	0.00	8	Muscle	0
8	27.00	8	4.16	10.00	4	Slice	13.46	34.00	4	Brine	12.93
9	27.00	4	1.46	37.92	8	Brine	15.16	7.00	8	Brine	11.41
10	20.00	4	8.78	10.00	8	Muscle	11.29	7.00	8	Brine	11.41
11	20.00	4	8.78	0.00	4	Brine	0.00	41.00	4	Muscle	11.68
12	40.00	8	3.69	20.50	4	Brine	5.5	24.00	8	Brine	7.81
13	0.00	8	0.00	31.00	4	Muscle	11.08	0.00	4	Brine	0.00
14	40.00	4	1.74	31.00	4	Muscle	11.08	41.00	8	Brine	10.89
15	40.00	4	1.74	31.00	8	Slice	1.69	15.00	8	Muscle	2.77
16	-	-	-	3.07	8	Brine	1.35	41.00	8	Muscle	2.16
17	-	-	-	0.00	8	Muscle	0.00	15.00	4	Brine	16.87
18	-	-	-	31.00	8	Slice	1.69	34.00	8	Muscle	4.75
19	-	-	-	41.00	4	Brine	2.74	-	-	-	-
20	-	-	-	20.50	8	Brine	7.66	-	-	-	-
21	-	-	-	41.00	4	Slice	2.91	-	-	-	-

2.3.2 DigiEye® experimental design (time, temperature and section of the slice)

The coding of independent factors used for the second design is shown in Table 1. The experiments were planned according to the "D-optimal" design, as shown in Table 1, in independent assays. A quadratic model was used to express the response variable as a function of the three independent variables A, B and C, which are the coded values of time, temperature and section of the slice (whole slice, ham brine or muscle), respectively.

2.3.3 Stereoscope experimental design (time, temperature and section of the slice)

The coding of independent factors used for the third experimental design is also shown in Table 1. Experiments were planned according to the "D-optimal" design, as shown in Table 1, in independent assays. A cubic model was used to express the response variable as a function of three independent factors, where A, B and C are the coded values of time, temperature and section of the slice (ham brine or muscle), respectively, for images acquired by stereoscope. For the three designs, a significance test was used on the total error criteria with a 95% confidence level (CI). Significant terms in the model were found by analysis of variance (ANOVA). The model fit was evaluated by R^2 and adjusted R^2 values. A graphical and numerical optimisation technique from Design Expert Software was used to optimise the response.

3 Results and discussion

3.1 DigiEye® experimental design (time and temperature)

Table 1 shows the colour change (ΔE^*_{ab}) results for each of the evaluated designs. This parameter represents the magnitude of the difference in colour change between slices of stored ham and the control taken as Day 0 (Maskan, 2001; Saricoban *et al*. 2010; Pathare *et al*. 2013). Values of ΔE^*_{ab} were calculated in slices of pork ham during 41 days of storage at 4 ºC and 8ºC.

Table 2 shows a summary of the ANOVA results with the significance of the regression model coefficients for the experimental data of the design in which the colour of slices of cooked ham was evaluated through a DigiEye®. For any term of the model, a high regression coefficient and low probability value indicate a significant effect in the corresponding response variable. The coefficient of determination (R^2) is the proportion of variation in the response attributed to the model instead of to random error; for a well-adjusted model, the value of R^2 should be greater than 80% (Little *et al*. 1978; Rodríguez-Bernal *et al*. 2014). The ANOVA results for this design suggest that the models used in this study were adequate for identifying colour change (ΔE^*_{ab}), as $R^2 > 0.80$. The colour changes ΔE^*_{ab} in sliced and stored pork ham can be attributed to the oxidation of heme pigments (Fernández *et al*., 2000). This was explained by Penfield *et al*. (1990), when the

Table 2. ANOVA for a cubic surface response model of the experiment design on images acquired by the three experimental designs, 1: DigiEye® (time and temperature). 2: DigiEye® (time, temperature and section of the slice) and 3: Stereoscope (time, temperature and section of the slice).

	Sum of Squares	**Degrees of Freedom**	**Least Squares**	**F value**	**p-value**
Model 1	144.45	6	24.08	16.07	0.0005*
A – Time	34.38	1	34.38	22.95	0.0014*
B – Temperature	3.90	1	3.90	2.60	0.1452
AB	3.43	1	3.43	2.29	0.1689
A^2	84.99	1	84.99	56.73	0.0001*
A^2B	5.86	1	5.86	3.91	0.0832
A^3	42.46	1	42.46	28.34	0.0007*
Residuals	11.99	8	1.50	-	-
Lack of Fit	11.99	3	4.00	-	-
Pure Error	0.000	5	0.000	-	-
Total Error	156.44	14	-	-	-
Model 2	476.60	10	47.66	5.81	0.0051*
A - Time	39.62	1	39.62	4.83	0.0527
B - Temperature	0.56	1	0.56	0.068	0.7992
C - Section	46.70	2	23.35	2.84	0.1052
AB	22.37	1	22.37	2.72	0.1298
AC	87.24	2	43.62	5.31	0.0268*
BC	145.77	2	72.88	8.88	0.0061*
A^2	109.18	1	109.18	13.30	0.0045*
Residuals	82.10	10	8.21	-	-
Lack of Fit	82.10	5	16.42	-	-
Pure Error	0.000	5	0.000	-	-
Total Error	558.70	20	-	-	-
Model 3	469.53	11	42.68	21.98	0.0006*
A - Time	1.61	1	1.61	0.83	0.3971
B - Temperature	84.53	1	84.53	43.52	0.0006*
C - Section	0.32	1	0.32	0.16	0.7000
AB	36.75	1	36.75	18.92	0.0048*
AC	9.27	1	9.27	4.77	0.0716
BC	39.69	1	39.69	20.43	0.0040*
A^2	54.35	1	54.35	27.98	0.0018*
ABC	0.30	1	0.30	0.16	0.7067
A^2B	44.12	1	44.12	22.72	0.0031*
A^2C	3.07	1	3.07	1.58	0.2552
A^3	9.286 E-003	1	9.286 E-003	4.781E-003	0.9471
Residuals	11.65	6	1.94		
Lack of Fit	11.65	1	11.65		
Pure Error	0.000	5	0.000		
Total Error	481.19	17			

*Significant difference ($p<0.05$)

meat is heated, as it often is during the curing process, the more stable pink pigment, nitrosyl hemochrome, is formed. This pigment is responsible for the colour of ham, bacon, and corned beef as well as that of many table-ready processed meats. Its colour is stable during cooking, but becomes brown when exposed to light and air because the iron of the pigment is oxidized from the ferrous to the ferric state, thus changing the pigment to denatured nitrosyl hemichrome. In this way less nitrosyl hemochrome should relate to a less pink product (Claus, Sawyer and Vogel, 2010).

Table 3. Conditions of experimental design optimisation for the three experimental designs.

Design	Factors	Goal	Lower limit	Upper limit	Minimum weight	Maximum weight	Importance
Design 1: DigiEye® (2 Factors)	A: Time		0	40	1	1	3
	B: Temperature		4	8	1	1	3
	R1 : ΔE^*_{ab}	Maximise	0	8.78	1	1	3
Design 2: DigiEye® (3 Factors)	A: Time		0	40	1	1	3
	B: Temperature		4	8	1	1	3
	C: Section		Within the range				
	R1 : ΔE^*_{ab}	Maximise	0	15.16	1	1	3
Design 3: Stereoscope (3 Factors)	A: Time		0	40	1	1	3
	B: Temperature		4	8	1	1	3
	C: Section		Within the range				
	R1 : ΔE^*_{ab}	Maximise	0	16.87	1	1	3

Table 4. Solutions for factor combination for optimisation of experimental designs.

Design	Solution No.	Time	Temperature	Section	R1 : ΔE^*_{ab}	Desirability
Design 1: DigiEye® (2 Factors)	1	12.00	4	-	9.4552	1.000
	2	15.00	4	-	9.2110	1.000
	3	10.00	4	-	9.1638	1.000
	4	10.37	4	-	9.2488	1.000
	5	14.27	4	-	9.3364	1.000
	6	15.47	4	-	9.1088	1.000
	7	9.33	4	-	8.9733	1.000
	8	15.83	4	-	9.0228	1.000
	9	12.53	4	-	9.4674	1.000
	10	9.75	4	-	9.0973	1.000
Design 2: DigiEye® (3 Factors)	1	28.57	8	Muscle	13.5277	0.892
	2	14.38	4	Slice	13.4661	0.888
	3	30.66	8	Brine	12.3767	0.816
	4	23.57	4	Muscle	10.5264	0.694
	5	25.65	4	Brine	6.7818	0.447
	6	19.37	8	Slice	5.0166	0.331
Design 3: Stereoscope (3 Factors)	1	24.60	4	Brine	17.2186	1.000
	2	15.20	4	Muscle	19.121	1.000
	3	32.80	4	Muscle	19.6128	1.000
	4	23.20	4	Brine	17.3348	1.000
	5	25.80	4	Brine	17.0199	1.000
	6	28.70	4	Muscle	21.687	1.000
	7	25.18	4	Muscle	22.4135	1.000
	8	26.50	4	Muscle	22.256	1.000
	9	14.39	4	Muscle	18.5047	1.000
	10	24.22	4	Muscle	22.4419	1.000

The analysis of the response surface data showed that the relationship between (ΔE^*_{ab}) and the independent variables fit a cubic model with 0.8234 and 0.8659 for R^2 and adjusted R^2, respectively. The model and factors A, A^2 and A^3 were significant (p <0.05); however, the interactions of the other factors were not considered significant (Table 2). These results indicate that only time is a significant variable in the design evaluated.

The F-value (16.07) suggests that the model is significant and that there is only a 0.05% probability that this value is due to experimental error p-values>F less than 0.05 indicate that the model terms are significant; values greater than 0.1 indicate that the model terms are not statistically significant. Equation 2 describes the relationship between independent variables (factors A and B) and ΔE^*_{ab}.This equation can predict colour change with respect to the time and temperature tested.

$$\Delta E^*_{ab} = 6.64 - 8.58A - 0.85B + 0.63AB - 5.33A^2 + 1.40A^2B + 9.95A^3 \quad (2)$$

Saricoban *et al*. (2010) reported the influence of composition on the prediction equations of colorimetric parameters in meatballs. Although the object of study and evaluated factors are not the same, it is interesting to note that this type of methodology can be valid for predicting colour changes in food systems.

The program in which experimental design was evaluated allowed for transforming surface response values to desirability values, where 0 and 1 correspond to the minimum and maximum values, respectively. ΔE^*_{ab} was considered optimal if maximum values were reached. Table 3 shows established conditions for optimising the cubic model, in which 22 possible solutions were obtained; Table 4 shows 10 of these solutions. These solutions correspond to optimal storage conditions that make it possible to assess or identify the maximum colour change.

It is important to note that from the ninth day, maximum colour changes are identified. This result could suggest that the microbiological and texture evaluations associated with shelf life tests could begin on the ninth day, when colour changes are maximal. Although temperature did not significantly influence the model, all optimisation responses suggest evaluating colour change at 4°C.

3.2 DigiEye® experimental design (time, temperature and section of the slice)

Table 2 shows a summary of the ANOVA results with the significance of the regression model coefficients for the experimental data of the design in which the colour of cooked ham slices was evaluated using the DigiEye®. The ANOVA results suggest that the models used in this study were adequate for identifying colour change ΔE^*_{ab}, with $R^2 > 0.80$.

For the proposed experimental design, analysis of the response surface data showed that the relationship between ΔE^*_{ab} and the independent variables fit a quadratic model with 0.8531 and 0.7061 for R^2 and adjusted R^2, respectively. The model and interaction between the factors AC, BC and A^2 were significant ($p < 0.05$). However, the interactions of the other factors were not significant.

An F-value of the model of 5.81 means it is significant. p-values>F lower than 0.05 indicate that the model terms are significant. Equation 3 describes the relationship between the independent variables (factors A, B and C) and ΔE^*_{ab}. This equation can predict colour change with respect to the time and temperature evaluated.

$$\Delta E^*_{ab} = 9.71 + 1.86A - 0.20B - 0.74C[1] - 1.05C[2] + 1.45AB - 3.96AC[1] + 2.58AC[2] - 3.77BC[1] + 2.46BC[2] - 5.94A^2 \quad (3)$$

Saricoban *et al*. (2010) also obtained second order colour change prediction equations. It is important to note that in this type of model, the interaction of the evaluated factors always shows synergistic effects over the evaluated response variables, results that coincide in this study with the interaction of time and section in which ΔE^*_{ab} was evaluated.

The program that evaluated the experimental design allowed for transformation of the surface response values to desirability values, where 0 and 1 correspond to minimum and maximum values, respectively. ΔE^*_{ab} was considered optimal if maximum values were achieved. Table 3 shows established conditions for optimising the quadratic model, in which 6 possible solutions were obtained (Table 4).

It can be seen that maximum colour changes are perceived from day 14 at a temperature of 4°C in the whole slice. It is worth noting that changes in ham brine or muscle could be determined from day 23. However, such analysis would involve image segmentation and could overlook phenomena

Table 5. Summary of the three experimental designs

Model	Minimum Time (day)	Temperature (°C)	Section	Maximum ΔE^*_{ab}	R^2
DigiEye®	9*	4	--------	8.79	0.82
DigiEye®	14*	4	Slice	13	0.85
Stereoscope	14	4*	Muscle	18	0.97

* Significant factor in the model

implicated in the storage of ham that begin during earlier stages of senescence.

3.3 Stereoscope experimental design (time, temperature and section of the slice)

Table 2 shows a summary of the ANOVA results with the significance of the regression model coefficients for the experimental data of the design in which the colour of cooked ham slices was evaluated by stereoscope. The ANOVA results suggest that the models used in the study were adequate for identifying colour change (ΔE^*_{ab}), with an R^2 >0.80.

For the proposed experimental design, analysis of the surface response data showed that the relationship between ΔE^*_{ab} and the independent variables fit a cubic model with 0.97584 and 0.9314 for R^2 and adjusted R^2, respectively.

An F-value of 21.98 implies that the model is significant. p-values>F less than 0.05 indicate that the model terms are significant. Therefore, in this case, B, AB, BC, A^2, A^2B are significant model terms. For this experimental design, different equations were obtained given that the categorical factor temperature was significant. Equation 4 describes the relationship between independent variables at 4°C in muscle. Equation 5 describes the same relationship at 8°C in muscle. Equations 6 and 7 describe the relationship for ham brine at 4°C and 8°C, respectively.

$$\Delta E^*_{ab} = 1.3670 + 1.9717Time - 0.0414Time^2 + 2.4327x10^{-0.005}Time^3 \quad (4)$$

$$\Delta E^*_{ab} = 0.3565 + 0.2159Time - 0.45024x10^{-0.003}Time^2 + 2.4327x10^{-0.005}Time^3 \quad (5)$$

$$\Delta E^*_{ab} = 0.7693 + 1.4796Time - 0.0336Time^2 + 2.4327x10^{-0.005}Time^3 \quad (6)$$

$$\Delta E^*_{ab} = 12.5432 - 0.2350Time + 3.3467x10^{-0.003}Time^2 + 2.4327x10^{-0.005}Time^3 \quad (7)$$

The program that evaluated the experimental design allowed for transformation of the response surface values to desirability values, where 0 and 1 correspond to minimum and maximum values, respectively. ΔE^*_{ab} was considered optimal if maximum values were attained. Table 3 shows established conditions for optimising the cubic model in which 49 possible solutions were obtained; Table 4 shows 10 of these solutions.

For this model, maximum colour changes can be perceived from day 14 at 4°C in the muscle section of the slice, values that match the experimental design evaluated in the images acquired from the DigiEye®. Finally, Table 5 summarises the optimisation conditions of each of the evaluated designs in which maximum ΔE^*_{ab} was obtained. Although the largest adjustments are obtained with RSMO of images acquired by stereoscope, colour changes can be perceived in the whole slice from images acquired in the DigiEye®. The maximum values of (ΔE^*_{ab}) obtained from the experimental designs were greater than 3 and can be perceived by the human eye (Escudero-Gilete *et al.* 2010; Fernández-Vázquez *et al.* 2013). Therefore, these differences may be directly related to quality characteristics and their effect on consumer acceptance.

Conclusions

Two instrumental methods (DigiEye® and a stereomicroscope) were used that differed in their optical design and approach to capturing images, with the goal of identifying colour changes (ΔE^*_{ab}) in slices of pork ham stored at different conditions. The results indicate that regardless of the instrument used, models were generated with acceptable correlation coefficients (R^2 >0.80), determining the day in which the greatest changes occurred in terms of colour, storage temperature and section analysed. However, DigiEye® is still a much more accurate colour capture machine than a stereoscope in assessing segmented slices of ham. Together, these observations suggest that both techniques prove useful for assessing colour changes in slices of cooked pork ham, a factor that is directly related to quality characteristics and their effect on consumer acceptance. Temperature had a significant effect in ΔE^*_{ab} predictions. Furthermore, when predicting ΔE^*_{ab} values, the effect of temperature is more pronounced in the sections (muscle or ham brine) of ham slices samples. For these reasons, when implementing these methodologies in industry, predictive models would have to be constructed at the storage temperature and specific models to predict ΔE^*_{ab}.

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Nomenclature

ΔE^*_{ab}	color change
A	time (days)
B	temperature (°C)
C	section (dimensionless)

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