EFFECTS OF IRON CONTENT AND LIPID OXIDATION ON COLOUR OF THE KNUCKLE IN BEEF

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Abstract – The effects of iron content and lipid oxidation on meat colour have been assessed throughout display under MAP in pre-aged knuckles for 15 or 22 days under vacuum conditions, using 48 beef crossbred 12 mo young bulls raised on concentrates. Samples were divided in three groups according the level of iron (Fe <0.89 mg/100g muscle; 0.9-1.1 mg/100g muscle; Fe >1.10 mg/100g muscle) and to the level of lipid oxidation after 9 days of display in knuckles pre-aged for 15 days (low, TBA<1; medium, TBA 1-2; high, TBA>2). Iron content highly influenced the colour of no oxygenated meat, with increased redness and Chroma when the content of iron was higher, whereas lipid oxidation had a negative effect on colour throughout display, especially on the knuckle that was pre-aged for longer time.

Keywords – Young bulls, meat colour, Fe, TBA

I. INTRODUCTION

Beef aging is widely used by the meat industry to improve tenderness, resulting in a more homogeneous and acceptable product for consumers [1, 2]. Vacuum packaging (or wet aging) is the most commonly used ageing system. When the air is removed, its oxidizing effect is also eliminated, which allows longer ageing times delaying meat discoloration and lipid oxidation [3].

After ageing, packaging in modified atmospheres (MAP) using an enriched oxygen concentration is a common practice in red meats. Under this format, meats and, particularly, beef cuts, are directly sold to the consumers in display cases. The objective is to maintain a bright cherry red colour, keeping myoglobin in the oxygenated form [4]. But the colour stability of meat during commercial display in MAP can be affected by the previous ageing conditions of meat [5].

Iron is the most abundant trace metal in muscle food and is necessary for several biochemical functions. Myoglobin, a predominant skeletal muscle hemeprotein, provides red color appearance to fresh beef when, in an aerobic environment, the deoxygenated redox form of myoglobin with heme-iron present in the ferrous state binds with oxygen to form oxymyoglobin. Oxidation of oxymyoglobin or deoxymyoglobin results in the formation of metmyoglobin and meat discoloration, which influences consumer purchasing decisions at retail [6-8]. However, the concentration of iron forms has not been fully related with colour development and quality changes in beef muscles [9]. The aim of this work was to assess the influence of iron content in the muscle and lipid oxidation on the colour of the long-term pre-aged knuckle throughout display.

II. MATERIALS AND METHODS

Samples and display

The knuckle (including *vastus lateralis* and *rectus femoris* of the *cuadriceps femoris*) from 48 beef crossbred, 12 mo young bulls, raised with concentrates and cereal straw, were aged intact at 3 ± 1º C for 15 d (left side) or 22 d (right side) under vacuum conditions. Then, pH was measured by a penetration probe (Crison 507) and two-cm thick steaks were placed individually in polyethylene and polyamide laminate trays flushed with O_2 (80 %) and CO_2 (20%). Samples were displayed with cool white fluorescent illumination 16 h daily on, at 4 ºC for 5 or 9 days.

Colour

On the moment of sampling (0 days of blooming), and after 5 or 9 days of display, colour was measured (CIE L*a*b*) with a spectrophotometer MINOLTA CM2002 using a D65 Illuminant and a 10º Observer. Chroma* ($\sqrt{(a^2+b^2)}$) and Hue* (arctangent (b*/a*) * 57.29) were calculated. The average of three measures was obtained per sample.

Fe content

Minerals were extracted with acid digestion by nitric acid [10] and detection and quantification by ICP-OES (Thermo Elemental IRIS Intrepid). Based on the content in the muscle, samples were divided into three groups: A, with less than 0.89 mg Fe/100 g muscle; B, between 0.9 and 1.1 mg Fe/100 g muscle and C, with more than 1.1 mg Fe/100 g muscle.

Lipid oxidation

Thiobarbituric Acid Reacting Substances (TBARS) values were expressed as mg malonaldehyde (MDA) per kg of meat [11]. Based on the results obtained at day 9 of display in samples previously aged for 15 days in vacuum conditions, samples were grouped according to the extent of lipid oxidation in low oxidative, when less than 1 mg MDA/kg was reached on day 9 of display, medium oxidative, when TBARS ranged between 1 and 2 mg MDA/kg, and high oxidative, when TBARS exceeded 2 mg MDA/kg. The highest value over 2 coincides with an established value for sensory perception of rancidity over beef flavour [12].

Data analysis

A General Lineal Model (SPSS, 22.0) was applied with Fe content group, TBA group as fixed effects and their interaction, within ageing and display. A Duncan test was performed to see differences between Fe content or TBA groups.

III. RESULTS AND DISCUSSION

No significant differences were found in pH between the different groups (data not shown) without abnormal values (DFD or PSE) that might have affected the assessed values. The content of Fe had an important effect on colour parameters when meat had not been displayed in MAP and the knuckle had been aged for 15 days (Table 1). The highest lightness and yellowness corresponded to those samples with an iron content between 0.9 and 1.1 mg/100g muscle. However, it was very clear that the lowest the content in Fe, the less redness, Chroma and Hue values. Once the muscle was exposed to oxygen and displayed, these differences completely disappeared. Nevertheless, after 9 days of display, it seems that the content in iron higher than 1.1 mg/100g muscle provokes darker meat (P=0.052).

All these differences disappeared when the knuckle was aged for 22 days under vacuum conditions before being displayed (Table 2), although it maintained the tendency (P<0.1) to obtain darker meat and higher Hue values in those samples with higher content in iron, even after 9 days of display.

The highest value found in the knuckle was 1.60 mg Fe/100g muscle, which is characteristic of young animals such as those used in the study. Also the type of fibre is related to the mineral content, since a reduction in glycolytic fibres has been associated with an increase in iron in the M. *Longissimus thoracis* [13]. Then, muscle becomes more oxidative with the reduction of glycolytic myofibres and increases the proportion of oxidative myofibres with age and, therefore, the meat looks redder [13].

Lipid oxidation had a different pattern of influence. In knuckles aged for 15 days, it affected colour only after 9 days of display, when clearly those samples from the high oxidative group showed the lightest meat with the lowest redness, Chroma and Hue values (Table 1). This effect was also showed in the muscle aged for 22 days under vacuum conditions (Table 2). Besides, in this case the effect was also observed after 5 days of display. Since the knuckle had been pre-aged without being sliced under vacuum conditions, no oxidation had affected the muscle previously to the oxygenation. But it seems that MAP affects more rapidly and negatively those samples aged for longer, reducing the shelf life, which could imply that 22 days it is too long for the M. *Cuadriceps femoris* when it comes from Frisian young bulls raised on concentrates. No effect has been found of a short-term pre-ageing period (7 days in vacuum conditions) in colour

parameters [5, 14]. But in the M. *Longissimus dorsi* muscle, pre-ageing times longer than 14 days affect negatively colour and lipid stability, resulting in decreasing shelf life [15].

A level of TBARS over 2 has been suggested as the limiting level for beef acceptability after consumption [12] due to rancid flavour in relation to beef flavour. After 9 days of display, none of the samples of the high oxidative group (with TBA >2) would be accepted if consumed, but probably they would neither be visually accepted with a Hue value of 22.27 (Table 1) or 20.93 (Table 2).

IV. CONCLUSIONS

Iron content in the muscle influences colour parameters when meat is not oxygenated. In long-term pre-aged knuckles, lipid oxidation negatively affects colour parameters, especially at long displays. Pre-ageing under vacuum conditions for 22 days seems excessive for the knuckle.

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Table 1. Effect of iron content (FE) and oxidation group after 9 days of display (OX) on colour parameters in the knuckle aged for 15 days under vacuum conditions and displayed under MAP packaging for 0, 5 or 9 days.

		FE[1]			OX[2]			RMSE	FE	OX	FExOX
		A	B	C	low	med	high				
n	display	14	19	15	16	15	17				
L*	0	40.53b	44.21a	39.15b	41.87	41.52	41.29	4.29	0.011	0.936	0.593
a*	0	6.93c	7.91b	8.65a	8.18	7.72	7.67	1.02	0.003	0.790	0.368
b*	0	10.39b	12.37a	11.20b	11.91	11.46	10.95	1.53	0.037	0.729	0.951
Ch*	0	8.31b	9.35a	9.87a	9.54	9.09	8.99	0.94	0.004	0.803	0.426
Hue*	0	33.73b	32.73b	37.69a	34.59	33.99	35.07	4.15	0.005	0.653	0.471
L*	5	38.30	40.31	38.21	39.76	39.39	38.14	2.89	0.167	0.351	0.744
a*	5	13.72	13.56	14.79	14.86	13.72	13.41	1.70	0.278	0.380	0.924
b*	5	14.71	15.08	15.00	15.39	14.82	14.64	0.85	0.803	0.280	0.456
Ch*	5	14.76	14.64	15.78	15.88	14.77	14.47	1.63	0.300	0.366	0.928
Hue*	5	42.91	41.94	44.29	43.70	42.75	42.43	2.74	0.092	0.781	0.543
L*	9	41.56	41.48	39.22	39.10b	39.88b	43.21a	2.79	0.052	0.006	0.376
a*	9	7.27	10.22	10.48	12.93a	10.05b	5.62c	2.35	0.945	<0.001	0.687
b*	9	13.37	14.04	14.05	14.69	13.44	13.42	1.23	0.971	0.057	0.876
Ch*	9	9.14	11.64	11.89	14.03a	11.34b	7.82c	1.97	0.960	<0.001	0.649
Hue*	9	27.12	35.27	35.46	41.13a	36.35b	22.27c	6.68	0.912	<0.001	0.671

L*: Lightness; a*: redness; b*: yellowness; Ch*: Chroma; RMSE: Root mean standard error. [1]A: Fe <0.89 mg/100g muscle; B: Fe = 0.9-1.1 mg/100g muscle; C. Fe >1.10 mg/100g muscle; [2]low, TBA <1; medium TBA = 1-2; high, TBA >2.
a-c: different letters in the same effect within row indicate significant differences ($P \leq 0.05$).

Table 2. Effect of iron content (FE) and oxidation group after 9 days of display (OX) on colour parameters in the knuckle aged for 22 days under vacuum conditions and displayed under MAP packaging for 0, 5 or 9 days.

		FE[1]			OX[2]			RMSE	FE	OX	FExOX
		A	B	C	low	med	high				
n	display	14	19	15	16	15	17				
L*	0	43.09	42.38	39.07	41.07	40.48	42.96	4.22	0.079	0.847	0.235
a*	0	8.07	7.79	8.88	8.07	8.81	7.81	1.09	0.241	0.424	0.911
b*	0	11.45	11.60	11.21	11.65	11.37	11.29	1.67	0.665	0.763	0.658
Ch*	0	9.39	9.17	10.09	9.42	10.04	9.15	1.05	0.364	0.527	0.971
Hue*	0	35.31	33.97	38.12	34.75	37.75	34.66	3.59	0.075	0.278	0.248
L*	5	37.50	40.22	38.87	39.41	37.67	39.61	2.60	0.139	0.061	0.019
a*	5	13.63	13.97	13.67	14.86a	14.10a	12.59b	1.65	0.880	0.038	0.047
b*	5	14.71	15.30	14.76	15.43	14.78	14.68	1.18	0.850	0.665	0.359
Ch*	5	14.68	15.04	14.73	15.87a	15.11a	13.72b	1.59	0.891	0.047	0.061
Hue*	5	42.66	42.22	42.51	43.79a	43.58a	40.37b	2.52	0.691	0.006	0.001
L*	9	42.06	41.55	40.24	40.67b	39.59b	43.24a	2.80	0.093	0.005	0.455
a*	9	5.43	9.19	9.72	11.53a	8.32b	5.10c	3.03	0.089	0.018	0.806
b*	9	13.39	13.31	13.27	13.62	13.12	13.23	1.33	0.843	0.807	0.509
Ch*	9	7.71	10.75	11.24	12.81a	9.97b	7.39c	2.52	0.124	0.018	0.842
Hue*	9	21.43b	32.84a	34.28a	38.62a	30.88b	20.93c	8.98	0.048	0.063	0.725

L*: Lightness; a*: redness; b*: yellowness; Ch*: Chroma; RMSE: Root mean standard error. [1]A: Fe <0.89 mg/100g muscle; B: Fe = 0.9-1.1 mg/100g muscle; C. Fe >1.10 mg/100g muscle. [2]low, TBA <1; medium TBA = 1-2; high, TBA >2.
a-c: different letters in the same effect within row indicate significant differences ($P \leq 0.05$).