Effects of ultimate pH and high-oxygen modified atmosphere package on color stability of *Longissimus lumborum* from Nellore bulls

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Abstract – Red bright color is an important quality attribute that influences beef purchasing and is affected by beef pH. The aim of this study was to determine how three pH_u ranges affect color stability during cold storage of LL steak packaged in HiOx atmosphere. Fifteen LL muscles from Nellore bulls were grouped into 3 pH_u ranges: normal (n = 5; 5.40 < pH_u <5.79), intermediate (n = 5; 5.80 < pH_u <6.19) and high (n = 5; pH_u > 6.20). All muscles were cut into 2.5 cm steaks, which were packaged in 80% O_2/ 20% CO_2 (v/v) and stored at 2 ± 1 ºC under dark conditions until day 5 and under fluorescent light onwards. On days 0, 5, 8, 11, and 14 of retail display, pH, gas composition and instrumental color were determined. Instrumental color was determined with HunterLab MiniScan XE Plus spectrophotometer. High pH_u steaks were darker (L*), redder (a*/b*), and with greater tone (Hue) and color intensity (Chroma) than normal and intermediate groups (p < 0.05). High pH_u shows great importance for meat color stability and should be more investigated by meat researchers and producers.

Key Words – CIE L*a*b*, beef shelf-life, high ultimate pH.

I. INTRODUCTION

Brazil is one of the world's largest beef producer and exporter. Nellore is the predominant breed in Brazilian cattle herd, with great importance for internal consumption and for beef export.

Meat color is the initial attribute that influences purchasing by consumers and loss of ideal beef color – bright-cherry red – reduces consumer interest in beef [1]. This attribute results from beef myoglobin content, the redox state of myoglobin's heme iron and the type of the ligand of heme iron [2]. In fresh beef, myoglobin can be present as: deoxymyoglobin (DeoxyMb), with water as ligand of Fe^{+2} (purplish color); oxymyoglobin (OxyMb), with molecular oxygen as ligand of Fe^{+2} (bright red color); and metmyoglobin (MetMb), with water as ligand of Fe^{+3} (brownish color) [2].

High pH_u can occur as a consequence of pre-slaughter stress. In higher pH_u muscles (>6.2) is reported dark muscles. Dark cutting beef is a quality defect frequently attributable to meats with high pH_u [3]. Dark color can range from slight to severe dark and darker steaks contained less superficial OxyMb than beef with low pH [4].

Some strategies can be employed in order to increase color stability. One of them is the packaging of beef steaks in high-oxygen atmosphere (HiOx MAP), such as 80% O_2/ 20% CO_2. The high amount of oxygen in trays headspace increases the proportion of OxyMb on beef surface, extending meat cherry-red color [1].

Therefore, this research aims to study how three different pH_u ranges affect color stability of *Longissimus lumborum* (LL) steaks from Nellore bulls and packaged in HiOx MAP, during 14 day of retail time.

II.MATERIALS AND METHODS

Raw material and processing: Fifteen Nellore bulls, raised in a pasture fattening regime, with 30-36 months were slaughtered in a conventional slaughterhouse. After 48h post-mortem, *Longissimus lumborum*, deboned from 1st to 6th lumbar vertebra, were grouped into 3 pH_u ranges: normal (n = 5; 5.40 < pH_u <5.79), intermediate (n = 5; 5.80 < pH_u <6.19) and high (n = 5; pH_u > 6.20). The superficial

fat was removed and then, all muscles were cut into 2.5 cm steaks for posterior packaging.

Packaging: Individual steaks were placed in modified atmosphere trays (model 13D65, 24 x 16 x 65 cm, white polypropylene with ethylene vinyl alcohol (EVOH) barrier, O_2 permeability < 0.5 $cm^3/m^2/24h$ at 50% relative humidity, Sealed Air Corp., US). Air was removed from each package and a high oxygen (HiOx) atmosphere (80% O_2/ 20% CO_2; Air Liquide Ltd., Brazil) was flushed, with subsequent sealing with a high barrier sealing film (4532-G lid, with nominal thickness of 70 µm, O_2 permeability < 5 $cm^3/m^2/24$ h at 23ºC, 66% of relative humidity and water vapor permeability < 5 $g/m^2/24$ h at 38ºC e 90% relative humidity, Bemis Company - Dixie Toga, Brazil) by using a Multivac machine (model T200, Multivac' Ltd., Germany).

Display conditions: All the trays were stored at 2.0 ± 1.4 ºC at a cold room under dark conditions from day 0 until day 5 and under fluorescent light (1,980 ± 150 lux) from day 6 until day 14. The cold room was defrosted at 4-h intervals.

Analyses: All the attributes studied were evaluated on days 0 (without MAP), 5 (dark condition), 8 (beginning of bright condition), 11 and 14 of steak storage.

Gas composition: MAP gas composition of each package was measured by using a headspace oxygen/carbon dioxide analyzer (CheckPoint®, PBI Dansensor A/S, Denmark) during the shelf life time. This analysis was performed immediately after sealing the tray on day 0 and in the other evaluation periods before opening the trays.

pH: pH was determined with a calibrated (pH 4.0 to 7.0) combination pH electrode attached to a pH-meter (Hanna Instruments, USA). Steaks were measured at three random locations and values were averaged for statistical analyses.

Instrumental color: In the surface of each steak, CIE lightness (L*), redness (a*), yellowness (b*) were read with HunterLab MiniScan XE Plus spectrophotometer (Hunter Associates Laboratory, USA), integrated to an Easy Match QC system. Readings were performed immediately after removal of steaks from MAP and at five random locations on the light-exposed steak surfaces of each steak, with 2.54 cm diameter aperture, 10º standard observer and illuminant A. The spectrophotometer was calibrated using black and white reference standards provided by the manufacturer. With a* and b* from readings, ratio of a*/b*, hue (arctan (b/a)) and Chroma ($\sqrt{a^2+b^2}$) were calculated.

Statistical analysis: Data were analyzed using the Univariate Procedure of SAS (SAS, Inst. Inc., Cary, NC, US), using MIXED Procedure of SAS, considering time (storage days) as repeated measure on the model. All the effects from the model were assumed as fixed (treatments, pH_u ranges, time and its interactions). After ANOVA, least square means were estimated as well as their standard errors (SE). Tukey test was used to compare the average differences between treatments, pH_u ranges, time and its interactions ($p < 0.05$).

III. RESULTS AND DISCUSSION

pH ranged between 5.55 and 6.94 (48h post mortem). pH values of each group were stable during all storage time: normal: 5.56 ± 0.09, intermediate: 5.82 ± 0.02, and high: 6.29 ± 0.03. There was O_2 concentration reduction in tray headspace as CO_2 proportion increased for all pH_u ranges and treatments ($p < 0.05$).

Regarding color stability, there was alteration in all the color attributes during storage time and among pH_u ranges ($p<0.05$). The high pH_u group showed difference compared with other both pH_u ranges. For L*, high pH_u was lower than normal and intermediate pH_u ranges on each storage day ($p<0.05$). The darker beef for high pH_u is in agreement with reported by Abril et al. [3] and McKeith et al. [4]. Some factors can cause this darkening, such as higher water-holding capacity of miofibrilar proteins, with decreasing in light scattering inside the structure of fiber [5] and greater oxidative metabolism with low efficient mitochondria [4]. In agreement with Knock et al. [6] found in the not enhanced (control) group, L* in normal pH_u was stable during all retail time.

Redness can be measured by a* and the ratio a*/b*, whose increasing indicates less discoloration during steak storage [7]. For a*, there was a decrease for normal pH_u after exposition to fluorescent light. Intermediate and high pH_u steaks, on the other hand, showed an increasing after HiOx packaging. It can indicate that the higher proportion of oxygen in trays improved the blooming. Since oxygen penetration, to form OxyMb, is

hindered in higher pH_u [7], a massive proportion of oxygen (80%, compared with 21% in air) could be enough to bloom MAP steaks, which was not necessary in normal pH_u. There was a falling on a* during time in normal pH_u, as was observed by Knock et al. [6] for control group.

The values of b* showed an increasing between days 0 and 5, stabilizing afterwards, for intermediate or high pH_u ranges. For normal pH_u, on the other hand, b* showed stable until day 11, with significant growth on day 14 ($p < 0.05$).

Higher ratio indicates less discoloration during retail time, which was observed for high pH_u [8]. This pH_u range presented greater ratio of a*/b* than normal and intermediate ($p < 0.05$). The ratio gradually decreased along time, being more stable in high pH_u range, whose ratio decrease after MAP packaging and stabilized onwards ($p < 0.05$). Hue data are in agreement with the ratio results. High pH_u showed lower hue values than other both pH_u ranges ($p < 0.05$). The more stability in these attributes can be related to the less oxidative susceptibility presented by myoglobin in higher pH values [9]. Therefore, lower hue and greater ratio of a*/b* values indicate lower discoloration, possibly due to less MMb formation.

M High pH_u passed from the lowest value of Chroma on day 0 to the highest on day 11, with stabilization onwards, in comparison with normal and intermediate pH_u ranges ($p < 0.05$). Along storage time for high pH_u, there was a continuing growth on Chroma, indicating saturation of the red color and correlating to hue results. Chroma for normal pH_u storage is in concordance Knock et al. [6]. The authors found lower Chroma after 14 days in HiOx MAP for control group.

IV. CONCLUSION

Ultimate pH had significant effect on color stability. Beef with high pH_u packaged in HiOx MAP can be interesting for purchase for longer period of storage time.

Packaging in HiOx was beneficial to steaks with high pH_u. The highest saturation of color (more red) at the end of storage makes HiOx MAP can be a strategy to improve redness in beef with high pH in retail.

Due to this effect on meat color, different pH_u ranges should be more studied by meat color researchers and taken into account by meat producers and retailers.

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Table 1. Effect of pH_u ranges on color parameters during beef steaks storage at 2 ± 1 ºC in HiOx MAP

	pH_u	Display-time (day)				
		0	5	8	11	14
L*	Normal	49.33 ± 0.69^{ay}	48.44 ± 0.79^{ay}	47.51 ± 0.69^{ay}	49.32 ± 0.69^{ay}	47.58 ± 0.69^{ay}
	Intermediate	47.53 ± 0.69^{ay}	48.87 ± 0.79^{ay}	47.89 ± 0.69^{ay}	47.67 ± 0.69^{axy}	47.09 ± 0.69^{axy}
	High	41.59 ± 0.69^{ax}	43.93 ± 0.79^{ax}	43.02 ± 0.69^{ax}	44.71 ± 0.73^{bx}	43.94 ± 0.69^{bx}
a*	Normal	24.97 ± 0.80^{by}	25.71 ± 0.86^{bx}	22.30 ± 0.80^{ax}	21.90 ± 0.80^{ax}	24.25 ± 0.80^{bx}
	Intermediate	22.93 ± 0.80^{ay}	27.23 ± 0.80^{bx}	25.63 ± 0.80^{bxy}	25.86 ± 0.80^{by}	26.15 ± 0.80^{bx}
	High	17.55 ± 0.80^{ax}	27.67 ± 0.86^{bx}	26.60 ± 0.80^{by}	26.07 ± 0.82^{by}	27.23 ± 0.80^{bx}
b*	Normal	16.88 ± 0.60^{ay}	18.10 ± 0.66^{abx}	16.35 ± 0.60^{ax}	16.41 ± 0.60^{ax}	18.65 ± 0.60^{bx}
	Intermediate	15.35 ± 0.60^{ay}	19.42 ± 0.66^{bx}	18.38 ± 0.60^{bx}	18.49 ± 0.60^{bx}	18.99 ± 0.60^{bx}
	High	9.72 ± 0.60^{ax}	18.27 ± 0.66^{bx}	17.64 ± 0.60^{bx}	17.84 ± 0.62^{bx}	17.73 ± 0.60^{bx}
Redness (a*/b*)	Normal	1.49 ± 0.02^{bx}	1.48 ± 0.02^{bx}	1.46 ± 0.02^{bx}	1.34 ± 0.02^{ax}	1.30 ± 0.02^{ax}
	Intermediate	1.50 ± 0.02^{bcx}	1.52 ± 0.02^{cx}	1.44 ± 0.02^{abx}	1.40 ± 0.02^{ax}	1.38 ± 0.02^{ax}
	High	1.82 ± 0.02^{by}	1.57 ± 0.02^{ax}	1.54 ± 0.02^{ay}	1.53 ± 0.02^{ay}	1.54 ± 0.02^{ay}
Hue	Normal	33.94 ± 0.31^{ay}	34.15 ± 0.37^{ax}	34.55 ± 0.31^{ay}	36.82 ± 0.31^{by}	37.64 ± 0.31^{bz}
	Intermediate	33.71 ± 0.31^{ay}	33.46 ± 0.37^{ax}	34.88 ± 0.31^{by}	35.55 ± 0.31^{by}	36.02 ± 0.31^{by}
	High	28.81 ± 0.31^{ax}	32.57 ± 0.37^{bx}	32.95 ± 0.31^{bx}	33.26 ± 0.33^{bx}	33.01 ± 0.31^{bx}
Chroma	Normal	30.14 ± 0.83^{cy}	28.14 ± 0.94^{bcx}	24.76 ± 0.83^{ax}	27.37 ± 0.83^{abx}	30.60 ± 0.83^{cx}
	Intermediate	27.60 ± 0.83^{ay}	28.77 ± 0.94^{abx}	28.06 ± 0.83^{ax}	31.80 ± 0.83^{bcx}	32.33 ± 0.83^{cx}
	High	20.07 ± 0.83^{ax}	29.71 ± 0.94^{bcx}	28.25 ± 0.83^{bx}	32.13 ± 0.87^{cy}	32.50 ± 0.83^{cx}

^{abc} Means with different letters in the same row are different ($P < 0.05$) for each attribute. ^{xyz} Means with different letters in the same column are different ($P < 0.05$) for each attribute. Mean ± Standard Error