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Color stability of *Bos indicus* bull steaks in modified atmosphere packaging (MAP)

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Abstract

Evaluations of meat quality, including color, influence purchasing decisions and can be affected by type of fresh meat the packaging system. In this study, fresh steaks from *Bos indicus* bull were packaged in the vacuum (vacuum), 75% O_2/25% CO_2 (HiOx-MAP) and 40% CO_2/0.4% CO/59.6% N_2 (CO-MAP). Emphasis is placed on the color and lipid oxidation of bull beef steaks. Results reveal that the steaks stored in CO-MAP and HiOx-MAP exhibited similar or brighter red color than fresh steaks (exposed only to oxygen) or vacuum. The red color of the LD bull beef steaks packaged in CO-MAP was more intense than the color of meat stored in HiOx-MAP after the 14th day of storage. Vacuum packing dramatically impaired the color of the LD bull steaks, which were severely discolored (brown) after all storage times. *Bos indicus* steaks of all treatments showed extremely low TBARS values in all storage times. The results suggested that HiOx-MAP or CO-MAP may be utilized to stabilize or improve the red color of fresh steaks from bull of so appreciated by the consumer.

Keywords: MAP; beef color; carboxymyoglobin; non-castrated males; shelf-life.

1. Introduction

More than three-fourths of the Brazilian beef cattle are *Bos indicus* animals, of which Nellore is a major breed. *Bos indicus* or *Bos indicus* crossbreed with European breeds are commonly raised for beef in the tropical areas of Brazil, with the common husbandry practice of growing bulls until late castration at approximately 18 to 24 months of age to capture the growth advantages of bull animals, and then slaughter at 30 to 36 months of age (Silva *et al.*, 2003). The meat obtained from bulls is characterized by the susceptibility to pre-slaughter stress resulting in a varying quality aspects, such as color, tenderness and oxidative stability (dos Santos *et al.*, 2015).

Modified atmosphere packaging with a high oxygen concentration (commonly composed of 70–80% O_2 and 20–30% CO_2) is widely used to preserve fresh beef (Zakrys-Waliwander *et al.*, 2012; Seyfert *et al.*, 2005). However, this system can promote lipid and pigment oxidation (Eilert, 2005).

CO-MAP was approved (FDA, 2004) for use with meats and has become increasingly relevant to case-ready beef merchandising in the United States to

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reduce oxidation reactions caused by high oxygen concentration (Cornforth and Hunt, 2008; Eilert, 2005). CO binds strongly to the meat pigment myoglobin to form stable carboxymyoglobin, which displays a cherry red color similar to oxymyoglobin (Jeong and Claus, 2011) widely accepted by consumers. Although several studies examined the effects of modified atmosphere on raw surface color stability and lipid oxidation in fresh beef (McMillin, 2008, Hur *et al*., 2013, Owczarek-Fendor *et al*., 2014), the influence of HiOx-MAP or CO-MAP on color of fresh beef steaks from *Bos indicus* bulls was not investigated. Therefore, the objective of the present study was to examine the effects of vacuum or modified atmosphere on color stability and lipid oxidation of beef steaks (*M. longissimus dorsi*) from *Bos indicus* bulls.

2. Materials and methods

Materials

The following chemicals were used in this study: 1,1,3,3-tetraethoxypropane (TEP; approximately 97%) and 2-thiobarbituric acid (TBA; minimum 98%) (Sigma-Aldrich, St. Louis, Missouri, USA), propyl-3,4,5-trihydroxybenzoate (PB) (Merck, Hohenbrunn, Baviera, Germany), trichloroacetic acid (TCA), ethylenedinitrilo-tetraacetic acid, and disodium salt dihydrate (Titriplex III) (Merck, Darmstadt, Hessen, Germany).

Raw material, preparation and packaging

Twelve *Bos indicus* bulls were slaughtered on the same day using standard procedures (MAPA, 2007) at a commercial slaughter house. All animals (aged 30 - 36 months) came from one farm and were administered the same feeding regime, *ad libitum* access to pasture. The carcasses were electrically stimulated (low voltage, 30 s) after exsanguination and randomly selected for the experiment. The carcasses were hung by the Achilles tendon and stored at 2 °C overnight until the deboning 24 hours *post-mortem*. During commercial deboning, the M. *longissimus* dorsi (M. *longissimus thoracis* et *lumborum*) muscles were separated from the left side of the half-carcasses and transported under vacuum to the laboratory at 2 °C. All muscles were cut into 1.50 cm thick steaks 48 hours *post-mortem*. Two steaks were packed under vacuum in each *barrier bags* (shrinkable ethylene-vinyl acetate) with O_2TR < 25 cm^3 m^{-2} for 24 h or on polyester/polyethylene trays (23.6 x 16.4 x 4.5 cm; Bemis Company-Dixie Toga, São Paulo, São Paulo, Brazil) with O_2 TR< 1.0 cm^3 m^{-2} for 24 h at 23 °C, 0% RH. The trays were evacuated, filled with gas mixture (HiOx-MAP: 75% O_2/25% CO_2 and CO-MAP: 40% CO_2/0.4% CO/59.6% N_2) and sealed with a laminated polyethylene-based barrier film with a thickness of 102 µm and an O_2 TR = 2.5 cm^3 m^{-2} for 24 h at 23 °C, 0% RH (Bemis Company-Dixie Toga, São Paulo, São Paulo, Brazil) using a Multivac semi-automatic tray sealer machine (Model T200; Multivac, Campinas, São Paulo, Brazil). Liquid-absorbing pads (Dri-loc, Cryovac-Sealedair, São Paulo, São Paulo, Brazil) with 100 ml of absorbing capacity were placed in the MAP trays. The effects of packaging on pH, instrumental color and lipid oxidation (TBARS) were assayed on the same day that the steaks were processed (day = 0) and after 7, 14, 21 and 28 days of storage at 2 ºC.

Headspace analysis

Oxygen and carbon dioxide concentrations in MAP packages were determined immediately after packaging (extra packages) and during days the storage display using a CheckPoint® gas analyser (PBI Dansensor A/S, Ringsted, Denmark) and expressed as % O_2 and % CO_2.

pH

After each storage period, the pH of the samples was determined directly using a potentiometer (Oakton pH 300 series 35618, Vernon Hills, Illinois, USA) with automatic temperature compensation and a glass penetration electrode (Digimed, Presidente Prudente, São Paulo, Brazil).

Thiobarbituric acid reactive substances (TBARS)

The extent of lipid oxidation was measured via thiobarbituric acid reactive substances (TBARS) using the extraction method described by Vyncke (1975) and Sørensen and Jørgensen (1996) with modifications. For extraction, 5 g of meat was homogenized in an Ultra Turrax (Ika T18 basic, Wilmington, North Carolina, USA) at 10,000 rpm for 30 s with 15 ml of solution (7.5% TCA, 0.1% PB and 0.1% EDTA). After filtration with qualitative filter paper (12.5 mm), 5 ml of the filtrate was mixed with 5 ml of an aqueous solution (0.02 M TBA) in capped test tubes. The samples were incubated in a water bath at 100 °C for 40 min and cooled in cold water. The absorbance was measured at 532 nm and 600 nm by a spectrophotometer (Shimadzu, UV–Vis mini 1240, Chiyoda-ku, Tokyo, Japan) against a blank containing 5 ml of the same TCA, PB and EDTA solution and 5 mL of TBA solution. The differences (A532 nm–A600 nm) were expressed as absorbance values corrected for turbidity. The results were calculated from the standard TEP curve and expressed as mg of malonaldehyde (MDA) per kg of meat. The TBARS value determination was performed after processing (0 day) and after seven, 14, 21 and 28 days of ageing.

Analysis of surface instrumental color

The surface of steaks were evaluated for instrumental color using a HunterLab MiniScan XE Plus spectrophotometer (HunterLab Associates, Reston, Virginia, USA) with an optical geometry of 45/0, a 2.54 cm diameter aperture, illuminant D65, and 10° standard observer. Six trays (or bags) containing totally 12 steaks were used to perform the analyses. The reflectance spectra (from 400 to 700 nm) and the CIE L*, a*, b* values were measured at six random locations on each steak. The 630 nm/580 nm reflectance ratio was used to evaluate the color oxidation (AMSA, 2012).

Experimental design and statistical analysis

A factorial design with two factors (storage time and packaging system) was used, with five levels of storage time (0, 7, 14, 21 and 28 days) and three packaging system (vacuum, HiOx-MAP and CO-MAP). Six bags or trays were performed for each combination treatment x time for a total of 90 samples. The effects of storage time and system on the color and lipid oxidation of fresh *Bos indicus* beef were studied by analysis of variance (ANOVA) using Statistica™ (Statsoft Inc., Tulsa, OK, USA). Significant differences between means were determined by the Tukey´s test. The significance level used for all statistical analyses was 5%.

3. Results and discussion

pH

Immediately before vacuum or modified atmosphere packaging (day = 0), the pH of the LD bull steaks ranged from normal to high (5.6 - 6.4; Table 1). The observed high pH in steaks from bulls could be due to possible depletion of muscle glycogen.

Table 1

pH from LD bulls steaks in vacuum and modified atmosphere packaging for 28 days at 2 °C

Storage time (days, S)	Treatment: Vacuum	Treatment: HiOx-MAP	Treatment: CO-MAP	Overall mean
0	6.0 ± 0.4 abA	5.8 ± 0.1 bA	6.0 ± 0.3 aA	5.9 ± 0.3
7	5.9 ± 0.3 aA	5.8 ± 0.3 aA	5.9 ± 0.4 aA	5.9 ± 0.3
14	5.7 ± 0.2 aA	5.7 ± 0.2 aA	5.7 ± 0.3 aA	5.7 ± 0.2
21	5.8 ± 0.2 aA	5.8 ± 0.2 aA	5.8 ± 0.2 aA	5.8 ± 0.2
28	5.8 ± 0.2 aA	5.8 ± 0.2 aA	5.9 ± 0.2 aA	5.8 ± 0.2
Overall mean	5.8 ± 0.2	5.8 ± 0.2	5.9 ± 0.3	5.8 ± 0.2

a-e: Averages in the same row with different letters are significantly different ($p < 0.05$).
A-E: Averages in the same column with different letters are significantly different ($p < 0.05$).

Bulls, due to their temperament, are more susceptible to stress than steers, resulting in a low drop in pH in post-mortem muscles (dos Santos *et al*., 2015).

Understanding further the role of glycogen in modulating the frequency of dark-firm-dry (DFD) beef may prove useful in developing strategies to reduce its occurrence, however it is possible that some unknown biological mechanisms or management practices modify beef color independent of pH decline or initial glycogen deposition. Metabolic intermediates of glycolysis and the tricarboxylic cycle can stabilize beef color through improved metmyoglobin-reducing activity (Purohit *et al*., 2014). In the in vitro model, mitochondria influence the maintenance of ATP, and inhibition of mitochondria enzyme activity contributes to accelerated metabolism and pH decline (Scheffler *et al*., 2015).

Thiobarbituric acid reactive substances (TBARS)

High O_2 atmosphere can significantly affect the onset, the rate and extent of lipid oxidation. But in the present study, levels of lipid oxidation in steaks beef remained extremely low in all packaging treatments throughout the storage period and below the sensory threshold of 3 mg MDA/kg beef (Popova *et al*., 2009) up to 28 days of storage (Table 2). One possible explanation for the low TBARS values found in samples stored in HiOx atmosphere can be the formation of MDA-protein complex, which leads to an underestimation of TBARS values. The other probable reason for low TBARS values is the low intramuscular fat (0.9 %) observed in the fresh steaks of *Bos indicus* bulls. Similar values were observed with the values found by Oliveira *et al*. (2013) for the oxidation of fresh meat from zebu steers.

Instrumental color

The mean values for the effects of the interactions between the treatment and storage time on the instrumental color are displayed in Table 3. The initial storage time (day=0) was used to represent the color of fresh meat that was exposed to atmospheric oxygen and was 48 hours post-mortem but immediately before placement in modified atmosphere and vacuum packaging. The color saturation (C*) of the *Longissimus dorsi* steaks from bulls stored in carbon monoxide modified atmosphere packaging (CO-MAP) increased significantly over time and was greater than that of fresh meat throughout all storage periods. The intensity of the red color (a*) of the meat stored in carbon monoxide (CO-MAP) was similar to the meat color stored in an atmosphere containing high oxygen levels (HiOx-MAP) up to the 7th day of storage and higher in the subsequent periods (14, 21 and 28 days). The bright red carboxymyoglobin that forms due to the addition of CO may be slightly more stable (less likely to discolor via metmyoglobin) during storage than the oxymyoglobin that is formed in traditional packaging (Hunt *et al*., 2004). Interestingly, steaks stored in CO-MAP for 28 days were redder (higher values of a* and C*; p < 0.05) than the steaks stored for only 7 days.

Table 2

TBARS (mg kg^{-1}) from LD bull steaks in vacuum or modified atmosphere packaging for 28 days at 2 °C

Storage time (days, S)	Treatment		
	Vacuum	75%O_2 / 25%CO_2	0,4%CO/40%CO_2/59,4% N_2
0	0.11 ± 0.03 aAB	0.14 ± 0.04 aA	0.15 ± 0.04 aB
7	0.15 ± 0.05 bA	0.17 ± 0.05 abA	0.22 ± 0.05 aAB
14	0.09 ± 0.02 bB	0.21 ± 0.11 aA	0.22 ± 0.04 aAB
21	0,15 ± 0.02 bA	0.23 ± 0.07 abA	0.28 ± 0.07 aA
28	0.09 ± 0.01 bB	0.21 ± 0.08 aA	0.13 ± 0.06 abB
Overall mean	0.12 ± 0.03	0.19 ± 0.07	0.20 ± 0.05

a-e: Averages in the same row with different letters are significantly different (p < 0.05).
A-E: Averages in the same column with different letters are significantly different (p < 0.05).

Table 3
Instrumental color of *Longissimus dorsi* steaks from *Bos indicus* bull stored under vacuum or in a modified atmosphere for 28 days at 2°C

	Storage time (days, S)	Treatment: Vacuum	Treatment: 75%O_2 / 25%CO_2	Treatment: 0,4%CO / 40%CO_2 / 59,4% N_2	Overall mean
*L**	0	39.5 ± 3.4 aA	39.4 ± 2.9 aA	38.7 ± 2.8 aA	39.2 ± 3.0
	7	32.8 ± 2.8 aB	44.2 ± 2.2 bB	43.5 ± 3.1 bB	40.2 ± 2.7
	14	33.6 ± 3.2 aB	44.4 ± 1.1 bB	44.7 ± 1.8 bB	40.9 ± 2.0
	21	33.5 ± 4.1 aB	44.6 ± 1.9 bB	44.7 ± 3.5 bB	40.9 ± 3.2
	28	33.0 ± 3.3 aB	44.1 ± 2.2 bB	44.2 ± 2.6 bB	40.4 ± 2.7
	Overall mean	34.5 ± 3.4	43.3 ± 2.1	43.2 ± 2.7	40.3 ± 2.7
*a**	0	14.5 ± 1.9 aC	15.2 ± 1.7 aA	14.3 ± 1.5 aA	14.7 ± 1.7
	7	10.4 ± 1.6 aA	19.3 ± 1.1 bC	20.8 ± 2.1 bB	16.8 ± 1.6
	14	10.6 ± 0.8 aAB	17.2 ± 0.9 bB	21.9 ± 2.9 cBC	16.5 ± 1.5
	21	13.4 ± 4.8 aBC	17.3 ± 1.5 bB	23.6 ± 2.2 cCD	18.1 ± 2.8
	28	10.8 ± 0.8 aAB	15.6 ± 1.9 bAB	24.8 ± 1.4 cD	17.1 ± 1.3
	Overall mean	11.9 ± 2.0	16.9 ± 1.4	21.1 ± 2.0	17.6 ± 1.8
*b**	0	12.4 ± 2.0 aC	12.9 ± 1.8 aA	12.2 ± 1.4 aA	12.5 ± 1.7
	7	7.2 ± 1.6 aA	13.8 ± 0.9 bA	11.3 ± 2.2 cA	10.8 ± 1.5
	14	8.2 ± 1.3 aAB	12.7 ± 1.1 bA	11.8 ± 2.1 bA	10.9 ± 1.5
	21	9.2 ± 1.7 aB	13.2 ± 1.3 bA	12.6 ± 2.0 bA	11.6 ± 1.7
	28	8.4 ± 1.3 aAB	12.8 ± 1.0 bA	13.2 ± 1.3 bA	11.5 ± 1.2
	Overall mean	9.1 ± 1.6	13.1 ± 1.2	12.2 ± 1.8	11.5 ± 1.5
C*	0	19.1 ± 2.8 aA	19.9 ± 2.4 aB	18.8 ± 2.0 aD	19.3 ± 2.4
	7	12.7 ± 2.2 bC	23.7 ± 1.2 aA	23.7 ± 2.9 aC	20.0 ± 2.1
	14	13.4 ± 1.3 cBC	21.4 ± 1.1 bB	24.9 ± 3.5 aBC	19.9 ± 2.0
	21	16.3 ± 4.9 cAB	21.7 ± 1.5 bB	26.8 ± 2.8 aAB	21.6 ± 3.1
	28	13.7 ± 1.3 cBC	20.2 ± 1.8 bB	28.1 ± 1.7 aA	20.7 ± 1.6
	Overall mean	15.0 ± 2.5	21.4 ± 1.6	24.4 ± 2.6	20.3 ± 2.2
h*	0	40.4 ± 1.3 aA	40.4 ± 1.5 aA	40.4 ± 1.1 aA	40.4 ± 1.3
	7	34.6 ± 2.4 aB	35.7 ± 1.9 aC	28.3 ± 2.3 bB	32.9 ± 2.2
	14	37.6 ± 3.4 aAB	36.5 ± 2.3 aBC	28.2 ± 1.4 bB	34.1 ± 2.4
	21	35.4 ± 4.6 aB	37.3 ± 3.5 aBC	27.9 ± 2.1 bB	33.5 ± 3.4
	28	37.8 ± 3.3 aAB	39.3 ± 3.3 aAB	28.0 ± 1.3 bB	35.1 ± 2.6
	Overall mean	37.2 ± 3.0	37.8 ± 2.5	30.6 ± 1.6	35.2 ± 2.4
630/580 nm	0	3.68 ± 0.25 Aa	3.68 ± 0.25 Ab	3.68 ± 0.25 Ac	3.68 ± 0.25
	7	2.94 ± 0.03 Ba	3.35 ± 0.09 Bb	3.49 ± 0.03 Bc	3.26 ± 0.05
	14	3.22 ± 0.14 Ca	3.07 ± 0.04 Cb	3.70 ± 0.01 Cc	3.33 ± 0.06
	21	3.75 ± 0.07 Da	3.13 ± 0.18 Db	3.97 ± 0.07 Dc	3.62 ± 0.11
	28	3.34 ± 0.34 Ea	2.74 ± 0.19 Eb	4.46 ± 0.26 Ec	3.51 ± 0.26
	Overall mean	3.39 ± 0.17	3.19 ± 0.15	3.86 ± 0.12	3.48 ± 0.15

The significant increase ($p < 0.05$) in a* and C* values during CO storage was accompanied by a reduction in the h* value because of the ability of CO to reverse metmyoglobin to carboxy-myoglobin (Jeong and Claus, 2010), which is the most stable redox (reduction-oxidation) form of myoglobin in anoxic conditions (Raines and Hunt, 2010). Steaks stored in CO-MAP presented significantly smaller hue angles (h*) when compared to steaks stored under vacuum or in HiOx-MAP ($p < 0.05$). The luminosity (L*) values of steaks stored in CO-MAP were similar to those of the steaks stored in HiOx-MAP throughout the storage period. The increased red color intensity in the samples stored in an atmosphere with high oxygen content was only observed up to the 7th day of storage ($p < 0.05$). The C* and a* values during this period were higher than those of fresh meat, whereas the h* values were significantly lower. In subsequent periods, the C* and h* values for LD steaks stored in HiOx-MAP were similar to those of fresh meat ($p > 0.05$). Storing beef steaks in HiOx-MAP increases the depth of oxygen penetration, forming a deeper layer of oxymyoglobin inside the steaks and thus increasing the resistance to the formation of metmyoglobin when compared to vacuum-stored steaks (Hunt *et al.*, 2004).

Vacuum packaging dramatically affected the color of LD beef steaks. The decreases in a* and C* ($p < 0.05$) observed from the 7th day of storage indicate that the loss of red color (discoloration) due to the oxidation of deoxymyoglobin to metmyoglobin was caused by the depletion of the reducing activity of metmyoglobin (Limbo *et al.*, 2013). Concomitantly, LD beef steaks displayed relatively lower L* values and higher h* values ($p < 0.05$) in vacuum packaging than steaks packaged in modified atmosphere (HiOx-MAP or CO-MAP) throughout the storage period, suggesting a greater formation of metmyoglobin on the surface of these products, because complete removal of oxygen is extremely difficult (Karpińska-Tymoszczyk, 2013). Rapid discoloration may occur on the surface of beef steaks stored in anoxic systems if the residual oxygen concentration is high enough (> 0.05%) (Venturini *et al*., 2006; Venturini *et al.*, 2014). The CO-MAP caused an expressive increase in reflectance (Figure 1) in the red region (620 – 700 nm) of the spectra. The overall shape of the reflectance curves was similar for all steaks in CO-MAP, displaying a maximum between 600 and 630 nm. During storage in CO-MAP, the formation of carboxymyoglobin resulted in reflectance curves that were superior to the initial scans. The addition of low concentrations of CO can eliminate the problem of discoloration in meat stored in an oxygen-free atmosphere for prolonged periods, restoring the red color through the formation of a strong partially ionic bond with the ferrous ion of myoglobin (Møller and Skibsted, 2006). This reaction is limited to a thin superficial layer that corresponds to the depth of CO penetration (Hunt *et al*., 2004). The overall shape of the reflectance curve was unaffected by the vacuum storage time. However, vacuum storage decreased the reflectance between 600 and 630 nm compared with the initial reflectance, indicating that deoxymyoglobin was the dominant pigment in the LD steaks and thus yielded a purplish deoxygenated color. A shoulder at 610 nm and a plateau at 630 nm in HiOx-MAP atmosphere, which were not present in fresh beef (0 d), is indicative of pigment oxidation (Figure 1). This decreased reflectance at 630 nm and the increased reflectance between 540 and 580 nm are typical of the conversion of oxy to metmyoglobin (Mancini *et al.*, 2005). Carboxymyoglobin is much more resistant to oxidation than oxymyoglobin in the presence of CO_2 (Luño *et al.*, 1998; Sørheim *et al*., 1999).

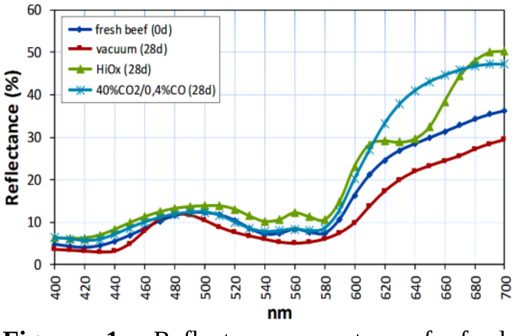

Figure 1. Reflectance spectra of fresh *Longissimus dorsi* beef steaks and 28 days after packaging in vacuum, HiOx-MAP and CO-MAP.

The Brazilian meat market is adapting and investing new production patterns and processing to achieve the increasingly high levels of quality demanded by consumers. Among the technologies developed and applied by the meat industry in other countries, modified atmosphere packaging (MAP) aims to maintain the quality and appearance of fresh meat during distribution and marketing. Details are still limited regarding the applicability of MAP and its effect on the quality parameters of beef from *Bos indicus* (Nellore). There is not report available in the scientific literature on the influence of HiOx_MAP or CO-MAP on fresh meat steaks of *Bos indicus* bull. In this kind of meat, the red color is usually much darker than in castrated animal. So, it is important to obtain reliable, practical, technical and scientific information concerning the effects of different commercially used packaging system on *Bos indicus* bull.

4. Conclusions

The red color of the LD beef steaks fresh steaks from *Bos indicus* bull packaged in a modified atmosphere of 0.4% CO-MAP or HiOx-MAP was higher than initial color of fresh beef and remained stable to oxidation for 28 days of refrigerated storage. Vacuum packing dramatically impaired the color of the LD bull beef steaks, which were severely discolored (brown) after 7, 14, 21 and 28 days of storage at 2 °C. CO-MAP or HiOx-MAP provides a means of improving meat quality of intact male from *Bos indicus* cattle for meat industry.

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