

Effect of Muscle pH and Display Conditions on Surface Color in *Hanwoo* (Korean Native Cattle) Beef**

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ABSTRACT : The effects of light exposure and light intensity on surface color of *Hanwoo* (Korean native cattle) beef and color stability of fresh normal and DFD (dark, firm, dry) muscles during 7 days at $4 \pm 1^\circ\text{C}$ under three display conditions (Dark, Light1000 and Light3000) were investigated. The L^* , a^* , b^* , C^* values and R630-R580 were significantly ($p < 0.05$) higher in normal beef than in DFD beef. The a^* , b^* , C^* values and R630-R580 of normal beef increased during the first day of display except Light3000 group, then gradually decreased over time. The surfaces of *Hanwoo* beef accumulated more metmyoglobin in the light than in the dark. Also, the rate of decrease in redness during refrigerated storage was enhanced by light exposure and increase in light intensity. Discolorations were more rapid in DFD beef than normal beef. Increasing light intensity promoted not only discoloration but also lipid oxidation. Therefore storage in the dark is effective in retarding the formation of a brown color in *Hanwoo* beef. (*Asian-Aust. J. Anim. Sci.* 2001. Vol. 14, No. 3 : 365-371)

Key Words : DFD, Light Intensity, Surface Color, Metmyoglobin, *Hanwoo* Beef

INTRODUCTION

The color of fresh meat is an important quality attribute which determines whether consumers will purchase the product (Faustman and Cassens, 1990). Fresh meat color is largely dependent on the chemical state of myoglobin, a heme-containing protein. In the reduced form, deoxymyoglobin (Mb) is purplish. In the oxygenated form, oxymyoglobin (MbO₂) is a bright cherry red preferred by consumers, and in the oxidized form, metmyoglobin (MetMb) is brown (Govindarajan, 1973). Visual color of muscle is largely due to the relative proportions of oxymyoglobin, metmyoglobin, and reduced myoglobin. Accumulation of the undesirable brown color of fresh meat is due to oxidation of oxymyoglobin to metmyoglobin (Renerre, 1987; Renerre, 1990). When brown metmyoglobin reaches 30-40% of total pigments on the surface of fresh retail beef, consumers make a no-purchase decision (Greene et al., 1971).

The rate of metmyoglobin accumulation is muscle dependent (Ledward, 1971). Light, pH, temperature, bacterial contamination, lipid oxidation, pO₂ (partial pressure of O₂) and metmyoglobin reducing activity are known to influence the oxidation of myoglobin

(Cornforth, 1994). In general, low pH favors oxidation of myoglobin, due in part to destabilization of the heme-protein linkage (Livingston and Brown, 1981). Dark color may be observed at meat pH values above 6.0, and especially so at pH values above 6.2 (Hedrick, 1981). High pH DFD meat spoils more rapidly, and the unusually dark meat is generally not marketable (Newton and Gill, 1981). Also, retail display lighting influences meat discoloration during storage (Kropf, 1980). Marriott et al. (1967) demonstrated increased color deterioration of fresh meat at -1°C under direct illumination, and concluded that illumination caused an increase in meat surface temperature, enhancing bacterial growth. The relatively short shelf-life of fresh meats is the single greatest concern to retailers (Cornforth, 1994). Delaying oxidation of oxymyoglobin to metmyoglobin is one way to increase shelf-life and the stability of fresh meats (Demos et al., 1996). Display conditions should accordingly be optimized to improve color and color stability, but little is known about the effect of light intensity during display on the color stability of beef.

The objective of this study was to determine color stability of fresh normal and DFD (dark, firm, dry) muscles during refrigerated display, and to investigate the effects of light exposure and light intensity on surface color and lipid oxidation of *Hanwoo* (Korean native cattle) beef.

MATERIALS AND METHODS

Samples and display conditions

The *M. semimembranosus* from *Hanwoo* carcasses used in this study were obtained from a local slaughter house. According to the pH at 24 h

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postmortem, the 6 carcasses were grouped into normal (pH 5.4-5.7, n=3) and DFD (pH 6.0-6.1, n=3) muscles. Muscles were sliced (1.5 cm thickness), then overwrapped in polyethylene wrap film (oxygen transmission rate 35,273 cc/m²/24hr/atm, thickness 0.01 mm; 3M Co., Seoul, Korea). The samples allocated to the 'Dark' group were stored in a refrigerated dark room to block the light. Samples allocated to 'Light' groups were placed at a distance of either 50 cm or 30 cm from the light source (natural white fluorescent bulbs, 35W). The light intensities at the surface of these samples were 1,000 lux (Light1000) and 3,000 lux (Light3000), respectively. Samples were stored at 4 ± 1°C for 7 days under the three display conditions. Day 0 (before storage) corresponded to analysis at 24 h postmortem. There were three replications per carcass.

pH measurement

pH was determined by homogenizing a 10 g sample of muscle with 100 mL distilled water for 1 min using a homogenizer at the 8,000 rpm (AM-7, Nihonseiki kaisha Ltd., Japan). The pH of the resultant suspension was measured with a pH meter (F-12, Horiba, Japan) equipped with a combination pH electrode calibrated to pH 4.0 and 7.0. The pH of the distilled water was 5.86.

Surface color measurement

CIE (Commission Internationale de l'Éclairage) L^* (lightness), a^* (redness), and b^* (yellowness) values for Illuminant C were measured by a color difference meter (CR-310, Minolta Co., Tokyo, Japan). Also, chroma (C^*) value and hue-angle (h^*) were calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$, and $h^* = \tan^{-1}(b^*/a^*)$, respectively (Little, 1975). The samples of day 0 were measured 30 min after cutting a fresh surface.

Surface metmyoglobin percent measurement

The relative content of metmyoglobin at the meat surface was measured by the method of Kryzwicki (1979) using reflectance at 473, 525, 572, and 730 nm. Reflectance readings were converted to absorbance [$2 - \log(\% \text{reflectance})$] and used in the following equation (Demos et al., 1996).

$$\text{Metmyoglobin}(\%) = 1.395 - [(A_{572} - A_{730}) / (A_{525} - A_{730})] \times 100$$

Percent reflectance differences at 630 nm minus 580 nm (R630-R580) were used to indicate differences in redness (Strange et al., 1974). Reflectance at selected wavelengths was measured by a dual beam spectrophotometer (UV-2401PC, Shimadzu, Kyoto, Japan) provided with a diffuse reflectance attachment adjusted to 100% reflectance with a BaSO₄ block.

Assessment of lipid oxidation

The level of thiobarbituric acid reactive substances (TBARS) was used as an index of lipid oxidation in meat. TBARS was measured according to the modified method of Sinnhuber and Yu (1977). To 0.4 g portions of mince weighed into test-tubes were added three drops of antioxidant solution, 3 mL of TBA solution and 17 mL of trichloroacetic acid-HCl reagent. The tubes were flushed with N₂, the screw caps were tightly closed, and the tubes were placed in a boiling water bath for 30 min. And then cooled to room temperature in tap water. About 5 mL of the color solution was transferred into a centrifuge tube, 2 mL of chloroform was added, and the tubes were centrifuged for 10 min at 3,000 rpm. The absorbance of the aqueous clear color solution was analyzed spectrophotometrically at 532 nm, and the TBARS were expressed as milligrams of malonaldehyde per kilogram of meat with the following equation.

$$\text{TBARS}(\text{mg malonaldehyde/kg sample}) = [(\text{absorbance of sample} - \text{absorbance of blank}) \times 46] / [\text{sample}(\text{g}) \times 5]$$

Statistical analysis

Data were analyzed as a 2 (muscle pH conditions) by 8 (storage times) by 3 (display conditions) factorial design using the General Linear Model (GLM). Least square means were used, and when F-values were significant, least square mean differences were compared by using PDIFF at $p < 0.05$. No interactions were detected. The relationships between the measured variables were assessed by Pearson correlation coefficients (SAS Institute, Inc., 1993).

RESULTS AND DISCUSSION

Effects of muscle pH and display conditions

Effects of muscle pH conditions on quality characteristics in Hanwoo (Korean native cattle) beef during display were compared (table 1). The L^* , a^* , b^* , C^* , R630-R580 and TBARS values of normal beef were significantly ($p < 0.05$) higher than those of DFD beef. In contrast, hue angle and metmyoglobin were higher in DFD beef.

Effects of display conditions on quality characteristics were compared (table 2). The a^* , C^* and R630-R580 were different ($p < 0.05$) in order of: Dark > Light1000 > Light3000, indicating that Dark group was redder. Hue angle and metmyoglobin were higher in Light3000 group than in the other two. TBARS of Dark group was significantly ($p < 0.05$) lower than light-exposed groups (Light1000 and Light3000). In general, lipid oxidation in beef was influenced mostly by the light exposure regardless of whether the intensity was 1,000 lux or 3,000 lux.

Table 1. Effects of muscle pH conditions on quality characteristics in *Hanwoo* (Korean native cattle) beef

Muscle pH condition	pH	L^*	a^*	b^*	Chroma	Hue angle	MetMb ^c	R630-R580	TBARS
Normal	5.35 ^b	38.17 ^a	15.57 ^a	6.12 ^a	16.74 ^a	21.64 ^b	32.02 ^b	10.48 ^a	1.06 ^a
DFD	5.91 ^a	36.56 ^b	13.10 ^b	5.43 ^b	14.20 ^b	22.96 ^a	34.58 ^a	8.70 ^b	0.56 ^b
SEM ^d	0.05	0.08	0.11	0.06	0.11	0.17	0.18	0.12	0.08

^{a,b} Least square mean values within the same column with different superscripts are significantly different ($p < 0.05$).

^c Metmyoglobin.

^d Standard error of the least square mean.

Table 2. Effects of display conditions on quality characteristics in *Hanwoo* (Korean native cattle) beef

Display condition	pH	L^*	a^*	b^*	Chroma	Hue angle	MetMb ^d	R630-R580	TBARS
Dark	5.62 ^b	37.33 ^b	15.26 ^a	5.95 ^a	16.39 ^a	21.44 ^c	30.69 ^c	11.28 ^a	0.65 ^b
Light1000	5.62 ^b	37.58 ^a	14.60 ^b	5.90 ^a	15.76 ^b	22.22 ^b	33.17 ^b	9.73 ^b	0.85 ^a
Light3000	5.66 ^a	37.19 ^b	13.14 ^c	5.47 ^b	14.26 ^c	23.24 ^a	36.03 ^a	7.77 ^c	0.93 ^a
SEM ^e	0.05	0.11	0.14	0.07	0.14	0.23	0.22	0.16	0.09

^{a,c} Least square mean values within the same column with different superscripts are significantly different ($p < 0.05$).

^d Metmyoglobin.

^e Standard error of the least square mean.

pH and surface color changes

The pH (figure 1) of normal beef was little affected by display conditions, but that of DFD beef was significantly ($p < 0.05$) increased by exposure to light (Light1000 and Light3000). The light3000 group of DFD beef at 7 days had a higher ($p < 0.05$) pH than Dark and Light1000 groups.

The L^* values (figure 2) of normal and DFD beefs significantly ($p < 0.05$) increased during the first 4 days

of cold storage whether or not the beef was light-exposed. DFD beef had significantly ($p < 0.05$) higher L^* when light-exposed (Light1000 and Light3000) than when held in the dark (Dark), unlike normal beef. Normal beef was not significantly affected by light (Light 1000 vs Dark) until 5 days of storage.

The a^* value (figure 3) of normal beef increased during the first day of display, except with the Light3000 group, then gradually decreased ($p < 0.05$)

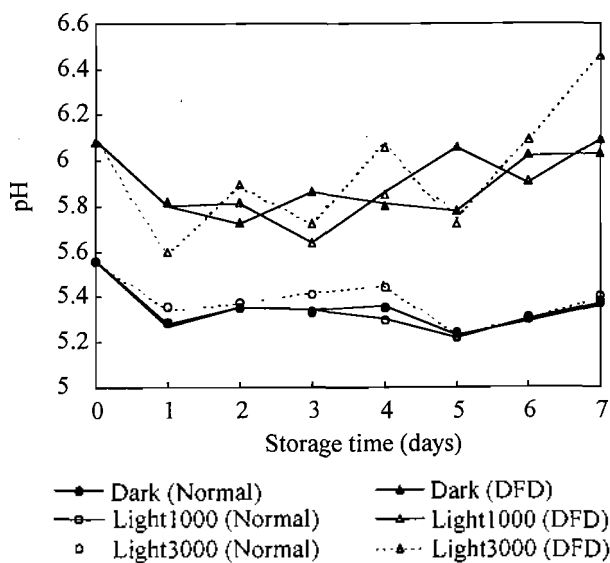


Figure 1. Effect of muscle pH and display conditions on pH in *Hanwoo* (Korean native cattle) beef during storage at $4 \pm 1^\circ\text{C}$

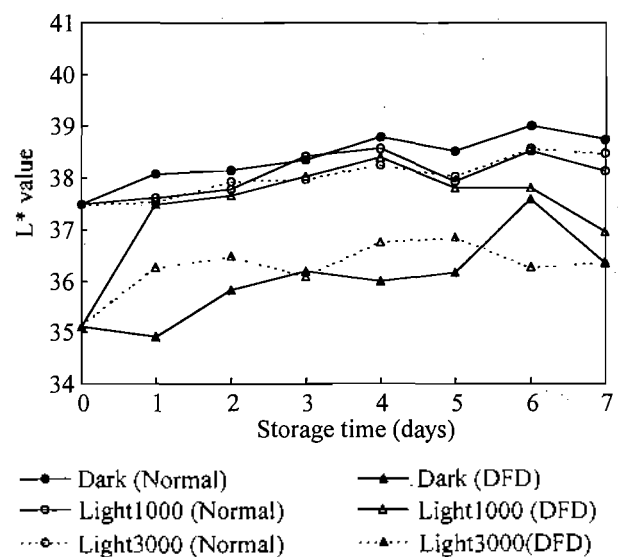


Figure 2. Effect of muscle pH and display conditions on L^* value in *Hanwoo* (Korean native cattle) beef surface during storage at $4 \pm 1^\circ\text{C}$

over time. The decrease in the Light groups was not significantly greater than in the Dark group until after 4 days of storage. The a^* value of DFD beef was significantly ($p < 0.05$) lower in light-exposed samples (Light1000 and Light3000) than in those held in the dark. In particular, the decrease with the Light3000 group was more rapid than with the Light1000 and Dark groups.

The b^* value (figure 4) increased during the first day of display for both normal and DFD beefs. It

then decreased for normal beef in all display conditions, but in the Light1000 group of DFD beef it continued to increase during 4 days of display and then decreased. There was not a significant ($p > 0.05$) difference in b^* value between Dark and Light1000 groups of normal beef.

The C^* values (figure 5) of normal beef, except Light3000, and of DFD beef Dark group increased during the first day, then decreased. This trend was similar to that for a^* value. DFD beef declined more

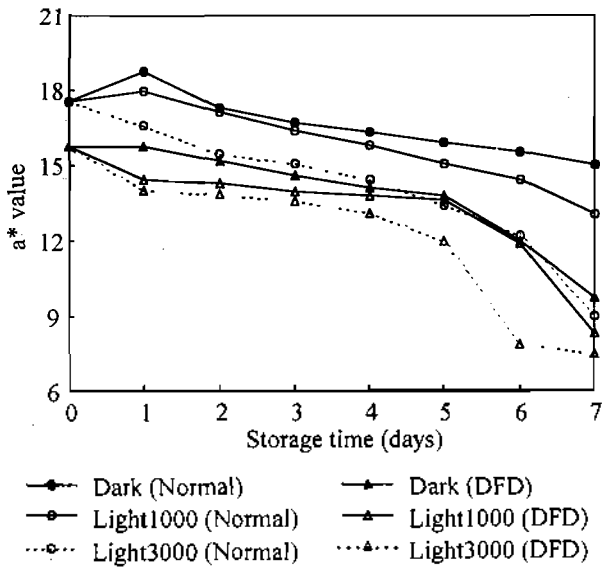


Figure 3. Effect of muscle pH and display conditions on a^* value in Hanwoo (Korean native cattle) beef surface during storage at $4 \pm 1^\circ\text{C}$

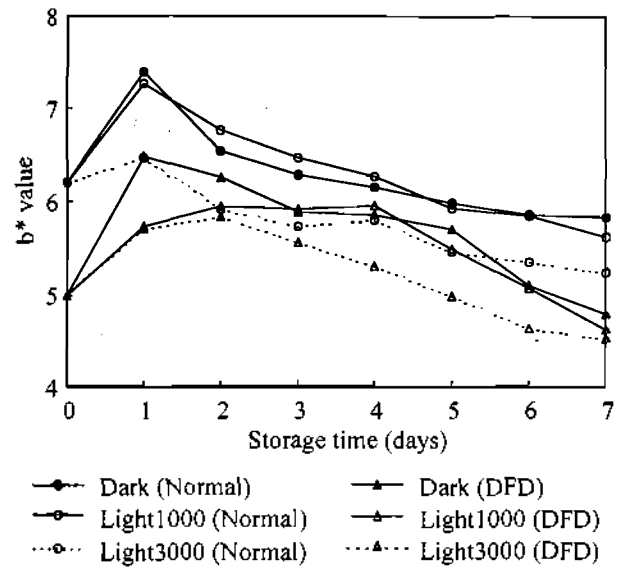


Figure 4. Effect of muscle pH and display conditions on b^* value in Hanwoo (Korean native cattle) beef surface during storage at $4 \pm 1^\circ\text{C}$

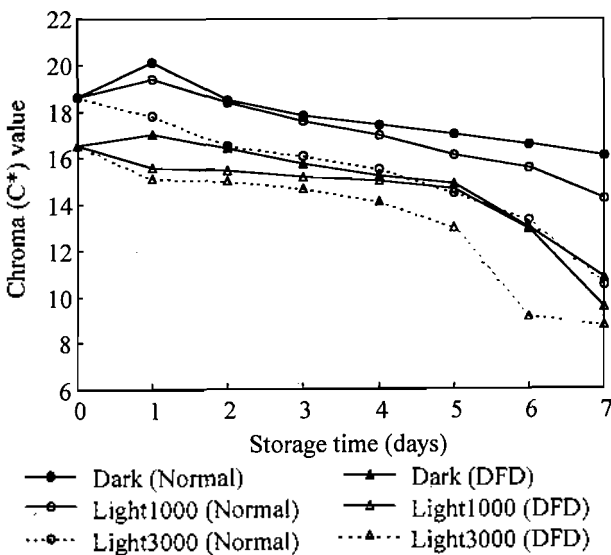


Figure 5. Effect of muscle pH and display conditions on chroma (C^*) value in Hanwoo (Korean native cattle) beef surface during storage at $4 \pm 1^\circ\text{C}$

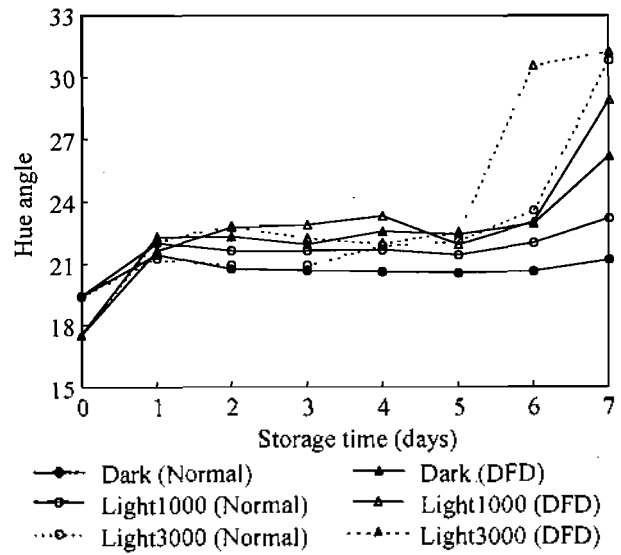


Figure 6. Effect of muscle pH and display conditions on hue angle in Hanwoo (Korean native cattle) beef surface during storage at $4 \pm 1^\circ\text{C}$

rapidly in the light-exposed sample.

The a^* value is a measure of redness, however the a^* value alone has limited meaning (Howe et al., 1982). Hue angle specifically defines the hue of the color; in meat the larger the hue angle, the less red the color. Hue angle (figure 6) increased ($p<0.05$) as display time increased, but in the Dark group of normal beef there was no significant difference in hue angle after 1 day of storage.

Surface metmyoglobin percent

Metmyoglobin of *Hanwoo* beef surface was influenced by display condition, muscle condition and display time (table 3). The metmyoglobin (%) at day 0 (before storage) was lower in DFD beef than in normal beef, but DFD beef had a higher initial rate of metmyoglobin accumulation during storage. And it increased more rapidly in the light-exposed samples. The metmyoglobin (%) of DFD beef was significantly

Table 3. Effect of muscle pH and display conditions on metmyoglobin (%) in *Hanwoo* (Korean native cattle) beef surface during storage at $4 \pm 1^\circ\text{C}$

Muscle pH conditions	Storage days	Display conditions			SEM ^h
		Dark	Light		
			Light1000	Light3000	
Normal	0	25.49 ^{fa}	25.49 ^{gx}	25.49 ^{gx}	0.16
	1	26.99 ^{ey}	28.64 ^{fx}	29.35 ^{fx}	0.25
	2	29.25 ^{dy}	32.00 ^{ex}	32.33 ^{ex}	0.34
	3	30.60 ^{cy}	33.04 ^{dx}	33.54 ^{dx}	0.35
	4	31.21 ^{by}	33.54 ^{cdx}	34.02 ^{cdx}	0.31
	5	31.32 ^{by}	34.16 ^{bcx}	34.76 ^{cx}	0.21
	6	31.64 ^{abz}	34.89 ^{by}	36.47 ^{bx}	0.16
	7	32.01 ^{az}	37.39 ^{ay}	41.53 ^{ax}	0.25
	SEM ⁱ	0.19	0.32	0.27	
DFD	0	22.29 ^{gx}	22.29 ^{gx}	22.29 ^{gx}	0.24
	1	28.44 ^{fx}	29.69 ^{fy}	33.46 ^{fx}	0.22
	2	29.74 ^{ez}	31.81 ^{ey}	35.29 ^{ex}	0.22
	3	31.94 ^{dz}	33.47 ^{dy}	35.68 ^{ex}	0.26
	4	32.20 ^{cdz}	35.09 ^{cy}	37.11 ^{dx}	0.24
	5	32.55 ^{cz}	35.63 ^{cy}	41.53 ^{cx}	0.39
	6	33.39 ^{bz}	38.78 ^{by}	51.35 ^{bx}	0.46
	7	37.68 ^{az}	41.95 ^{ay}	56.14 ^{ax}	0.31
	SEM ⁱ	0.22	0.27	0.40	

^{a-g} Least square mean values within the same column of the same muscle pH condition with different superscripts are significantly different ($p<0.05$).

^h Standard error of the least square mean among different display conditions within the same storage day.

ⁱ Standard error of the least square mean among different storage days within the same display condition.

^{x-z} Least square mean values within the same row with different superscripts are significantly different ($p<0.05$).

($p<0.05$) higher in light-exposed samples (Light1000 and Light3000) than in the dark sample (Dark), unlike normal beef. Zhu and Brewer (1998) reported that meat surface accumulated more metmyoglobin in the light than in the dark. Andersen et al. (1990) observed that fluorescent light increased the autoxidation rate of red oxymyoglobin to brown metmyoglobin, and greatly reduced the color stability.

R630-R580 (figure 7) of normal beef was higher than that of DFD beef. This trend was similar to those for a^* and C^* values. R630-R580 declined

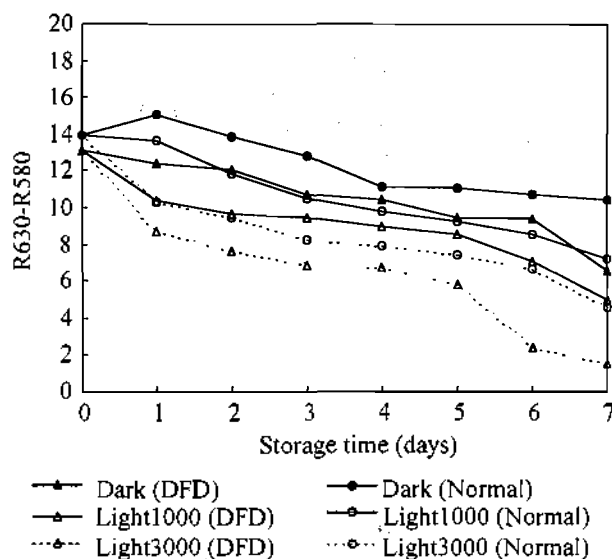


Figure 7. Effect of muscle pH and display conditions on R630-R580 in *Hanwoo* (Korean native cattle) beef surface during storage at $4 \pm 1^\circ\text{C}$

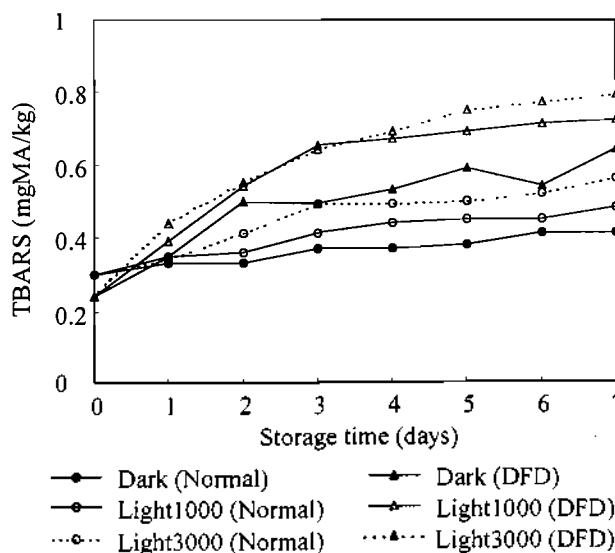


Figure 8. Effect of muscle pH and display conditions on TBARS in *Hanwoo* (Korean native cattle) beef during storage at $4 \pm 1^\circ\text{C}$

Table 4. Correlation coefficients among quality characteristics of *Hanwoo* (Korean native cattle) beef

	pH	L^*	a^*	b^*	Chroma	Hue angle	MetMb ¹	R630-R580	TBARS
pH	1.0000								
L^*	-0.6437***	1.0000							
a^*	-0.4445***	0.1445**	1.0000						
b^*	-0.5210***	0.2628***	0.7627***	1.0000					
Chroma	-0.4584***	0.1580**	0.9979***	0.8013***	1.0000				
Hue angle	0.2437***	-0.0069	-0.8085***	-0.2767***	-0.7704***	1.0000			
MetMb ¹	0.2308***	0.0483	-0.8244***	-0.4882***	-0.8075***	0.8363***	1.0000		
R630-R580	-0.2580***	0.0174	0.8576***	0.6475***	0.8552***	-0.6896***	-0.8874***	1.0000	
TBARS	-0.4368***	0.4676***	-0.3166***	-0.2818***	-0.3217***	0.1947**	0.3614***	-0.4698***	1.0000

* p<0.05, ** p<0.01, *** p<0.001.

¹ Metmyoglobin.

gradually during storage and the decline was more rapid in the light-exposed samples.

Lipid oxidation

TBARS (figure 8) which represents fat rancidity tended to increase as display time increased, and it increased more rapidly in the light-exposed samples. Consequently, light promoted not only discoloration but also lipid oxidation. TBARS of day 0 was lower in DFD beef than in normal beef, but DFD beef had a higher initial rate of lipid oxidation during storage. Andersen et al. (1990) provided evidence for the pigment oxidation process as an initiator of lipid oxidation.

Correlation among quality characteristics

The a^* value correlated highly ($p<0.001$) with b^* , chroma (C^*) values and R630-R580 ($r=0.7627$, 0.9979 , and 0.8576 , respectively) (table 4). Also, a^* value correlated highly ($p<0.001$) with hue angle and metmyoglobin ($r=-0.8085$ and -0.8244 , respectively). TBARS was correlated with metmyoglobin ($r=0.3614$). Faustman et al. (1992) provided evidence for a strong relationship between pigment oxidation and lipid oxidation in fresh ground veal.

CONCLUSIONS

Color characteristics of *Hanwoo* beef surface were influenced by muscle pH condition (Normal, DFD) and display condition (Dark, Light1000, Light3000). *Hanwoo* beef surfaces accumulated more metmyoglobin in the light (Light100 and Light3000) than in the dark (Dark). Also, the rate of decrease in redness during refrigerated storage was enhanced by light exposure and by an increase in light intensity which promoted not only discoloration but also lipid oxidation. The surface color of DFD beef was more affected by display conditions, and color changed more rapidly in the light-exposed sample. Therefore, storage in the

dark is effective in retarding the formation of a brown color in *Hanwoo* beef. Also, to prevent excessive discoloration during display, retail meat markets should avoid using DFD beef with abnormally high pH and avoid using excessive light intensity during display.

REFERENCES

- Andersen, H. J., G. Bertelsen, and L. H. Skibsted. 1990. Colour and colour stability of hot processed frozen minced beef: Results from chemical model experiments tested under storage conditions. *Meat Sci.* 28:87.
- Cornforth, D. P. 1994. Color: Its basis and importance. In: *Quality Attributes and their Measurement in Meat, Poultry and Fish Products* (Ed. A. M. Pearson and T. R. Dutson). Blackie Academic & Professional, London. pp. 34-39.
- Demos, B. P., D. E. Gerrard, R. W. Mandigo, X. Gao and Tan, J. 1996. Mechanically recovered neck bone lean and ascorbic acid improve color stability of ground beef patties. *J. Food Sci.* 61:656.
- Faustman, C. and R. G. Cassens. 1990. The biochemical basis for discoloration in meat: a review. *J. Muscle Foods* 1:217.
- Faustman, C., S. M. Specht, L. A. Malkus and D. M. Kinsman. 1992. Pigment oxidation in ground veal: Influence of lipid oxidation, Iron and zinc. *Meat Sci.* 31:351.
- Govindarajan, S. 1973. Fresh meat color. *CRC Critical Reviews in Food Technology.* 4:117.
- Greene, B. E., I. Hsin and M. W. Zipser. 1971. Retardation of oxidative color changes in raw ground beef. *J. Food Sci.* 36:940.
- Hedrick, H. B. 1981. Preventive treatments during the pre-slaughter period. In: *The Problem of Dark-Cutting in Beef* (Ed. D. E. Hood and P. V. Tarrant). Martinus Nijhoff, Hague, Netherlands, pp. 213-228.
- Howe, L., E. A. Gullett and W. R. Osborne. 1982. Development of pink color in cooked pork. *Can. Inst. Food Sci. Technol. J.* 15:19-23.
- Kropf, D. H. 1980. Effects of retail display conditions on meat color. *Pro. 34th Ann. Recip. Meat Conf.* 33:15.
- Kryzwicki, K. 1979. Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the

- surface of the beef. *Meat Sci.* 3:1.
- Ledward, D. A. 1971. Metmyoglobin formation in beef muscles as influenced by water content and anatomical location. *J. Food Sci.* 36:138.
- Little, A. C. 1975. Off on a tangent. *J. Food Sci.* 40:410.
- Livingston, D. J. and W. D. Brown. 1981. The chemistry of myoglobin and its reactions. *Food Technol.* 35:244.
- Marriott, N. G., H. D. Naumann, W. C. Stringer and H. B. Hedrick. 1967. Color stability of pre-packaged fresh beef as influenced by pre-display environments. *Food Technol.* 21:104.
- Newton, K. G. and C. O. Gill. 1981. The microbiology of DFD fresh meats: A review. *Meat Sci.* 5:223-232.
- Rennerre, M. 1987. Biochemical factors influencing metmyoglobin formation in beef muscles. *Meat Sci.* 19:151.
- Rennerre, M. 1990. Factors involved with the discoloration of beef. *Internat. J. Food Sci. and Technol.* 25:613.
- SAS Institute, Inc. 1993. SAS User Guide. SAS Institute, Inc., Cary, N.C.
- Sinnhuber, R. O. and T. C. Yu. 1977. The 2-thiobarbituric acid reaction, An objective measure of the oxidative deterioration occurring in fats and oils. *J. Jpn. Soc. Fish. Sci.* 26:259.
- Strange, E. E., R. C. Benedicts, R. E. Gugger, V. G. Metzger and Swift, C. E. 1974. Simplified methodology for measuring meat color. *J. Food Sci.* 39:988.
- Zhu, L. G. and M. S. Brewer. 1998. Discoloration of fresh pork as related to muscle and display conditions. *J. Food Sci.* 63:763.