

Processing Effects on Fresh and Frozen Meat Color

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Introduction

In recent years there has been a considerable amount of interest in further processing of fresh meat. The primary consideration in evaluating freshness is color. The purpose of this manuscript is to assess the present status of processed fresh meat color.

Myoglobin and color in fresh meat

Although a number of pigments are present in muscle, myoglobin is generally the only pigment present in large enough quantities to color meat. Hemoglobin constitutes 12-30% of the total muscle pigment. Since the two pigments have similar spectral properties, myoglobin alone is used as an index of fresh meat color.

The myoglobin content of muscle tissue varies not only between species but between age group as well as muscle types within a carcass. Research findings of Tang and Henrickson (1980) indicate that total pigment as well as total myoglobin content differs significantly between muscles within a carcass. The semimembranosus muscle possessed the highest total pigment and myoglobin content while the semitendinosus muscle had the lowest. Longissimus dorsi muscles were intermediate in total pigment and myoglobin content (Tables 1 and 2).

The myoglobin content of muscle tissue in veal is 1-3 mg of myoglobin per gram of wet tissue, increasing to 16-20 mg per gram in muscle of older beef animals. The quantities of myoglobin found in pork is in the same range as veal, while mutation averages somewhat higher (Bodwell and McClain, 1971).

Myoglobin, a sarcoplasmic protein, is comprised of a protein moiety, globin, which is made up of approximately 150 amino acid units, and a heme prosthetic group. The heme moiety, consists of four pyrrole rings connected by methine bridges, with an iron atom in the center. The heme structure is responsible for the color of myoglobin. Heme from which the iron has been removed is termed protoporphyrin. The protein portion of myoglobin, with the heme removed, is referred to as apomyoglobin.

Kendrew (1963) found that the polypeptide chain in sperm whale myoglobin is compactly folded in eight helical segments in such a way that the interior of the molecule is com-

pletely filled with hydrophobic groups. Deep in the hydrophobic interior is a cleft where heme is located. According to Adams (1976) the globin moiety does not assume its native conformation unless it is complexed with heme. The heme group serves to stabilize the configuration of the myoglobin molecule. The heme is oriented in such a way that the vinyl groups are buried in the hydrophobic interior and the carboxyl group of the propionic acids extend from the interior where they form part of the polar surface. Both the propionic acid side chains are believed to be hydrogen bonded to serine, histidine, and arginine amino acid residues on the globin moiety (Antonini and Brunori, 1971). According to Govindarajan (1973) the entire inner portion of the heme is surrounded by side chains of nonpolar amino acids. It is due to these nonpolar surroundings that ferrous iron (Fe^{+2}) in myoglobin is able to reversibly combine with oxygen (Govindarajan, 1973).

Table 1. Total pigment as influenced by muscle and treatment

Muscle ^a	Treatment		
	Control ^b	Stimulated ^b	Mean ^b
Semimembranosus	2.95 ^c	2.89 ^c	2.92
Longissimus dorsi	2.69 ^d	2.71 ^d	2.70
Semitendinosus	2.18 ^e	2.35 ^e	2.26

^aSix muscles per treatment.

^bMean value (mg/g wet tissue).

^{c,d,e}Values with the same superscripts are not significantly different ($P < .01$).

(Adapted from Tang and Henrickson, 1980).

Table 2. Total myoglobin as influenced by muscle and treatment

Muscle ^a	Treatment		
	Control	Stimulated ^b	Mean ^b
Semimembranosus	2.69 ^c	2.64 ^c	2.67
Longissimus dorsi	2.45 ^d	2.55 ^d	2.50
Semitendinosus	1.96 ^e	2.06 ^e	2.01

^aSix muscles per treatment.

^bmean value (mg/g wet tissue).

^{c,d,e}Values with the same superscript are not significantly different ($P < .01$).

(Adapted from Tang and Henrickson, 1980).

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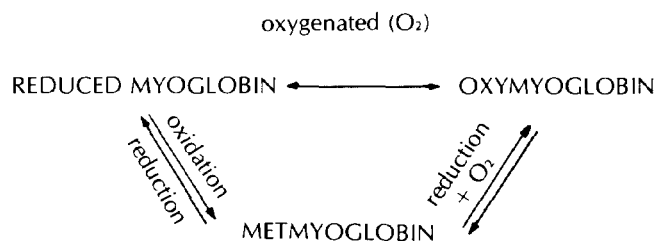
Heme iron has the ability to accept six electrons in its outer orbital, four from the nitrogen atoms of the pyrroles in the porphyrin ring, one from the nitrogen of the proximal histidine in the globin moiety, and one from the ligand bound by the heme (Govindarajan, 1973). The globin-histidine imidazole ligand enhances the ability of ferrous heme iron to form stable complexes with oxygen or other sixth ligands (Giddings, 1977; Rifkind, 1973).

In fresh meat, myoglobin can exist in three forms: reduced myoglobin (Mb), which is purple, red oxymyoglobin (MbO_2) and metmyoglobin (MMb), which is brown. The three pigments are constantly being interconverted and the relative proportions of the three pigment forms determine the color of fresh meat.

In reduced myoglobin the heme iron is in the ferrous (Fe^{+2}) state. In this state, there is no ligand in the sixth coordination position. Originally it was thought that a water molecule occupied the sixth coordination position in the absence of oxygen. However, Antonini and Brunori (1971) reported that in reduced myoglobin the sixth coordination position of the heme iron is vacant.

When a freshly cut surface of meat is exposed to air, myoglobin rapidly becomes oxygenated to a depth of a fraction of a centimeter below the cut surface (Giddings, 1977). After entering the muscle cell, oxygen diffuses through the water surrounding the myoglobin and enters the hydrophobic cleft to occupy the vacant sixth coordination site of the iron. The binding of oxygen causes an overall change in the conformation of globin which enhances binding of iron to the oxygen (Brinigar and Chang, 1974). The changes in the conformation of globin, which take place as a result of oxygen binding, brings the distal histidine of globin within interacting range of the liganded oxygen, thereby stabilizing the complex (Maxwell and Caughey, 1976).

Under suitable conditions, the pigment may also be oxidized to metmyoglobin. The oxidized or ferric form of the pigment is not able to bind oxygen, as is its ferrous counterpart (Rifkind, 1973). In fresh meat, indigenous reducing substances constantly reduce metmyoglobin to myoglobin. If oxygen is present, the myoglobin can be oxygenated. The reversible color cycle in fresh meats can be presented in the following simplified schematic representation (Bodwell and McClain, 1971):



The oxidation of myoglobin is commonly referred to as autoxidation since it is a nonenzymatic, spontaneous reaction involving free oxygen.

Metmyoglobin reducing activity: Cutaita and Ordal (1964) reported the disappearance of initially formed metmyoglobin during a two day storage period under anaerobic conditions. Stewart et al., (1965a) found that the metmyoglobin reducing

activity in ground beef was influenced by pH, temperature, and NaCl. The reducing activity appeared to be greatest at a pH of 5.1-7.1 at 3.0-3.5°C. A concentration of 5.0% NaCl inhibited the metmyoglobin reducing activity. Brown and Snyder (1969) found that in a nonenzymatic aerobic system, the reduction of metmyoglobin by NADH or NADPH in the presence of EDTA, was accelerated. However, Ledward et al., (1977) found that when intact muscle with normal to high reducing activity was minced or ground the reducing system was destroyed and oxidation to metmyoglobin was rapid. The authors felt this was due to the loss of NADH, as earlier reported by Newbold and Scopes (1971).

Processing affects on myoglobin oxidation

Preslaughter conditions and pH: Animal breed, maturity, feed, and stress come into play when discussing preslaughter effects on the color of meat (Govindarajan, 1973). Lewis et al., (1962) noted the effects of severe exercise, cold treatment and electrical stimulation with subsequent reduction in the muscle glycogen in hogs resulted in a darker, less desirable color of the meat. Dark-cutting beef is another example illustrating the effect abnormal stress conditions prior to slaughter have on animals. It was found that during abnormal preslaughter conditions there is activation of glycogen phosphorylase with subsequent muscle glycogen depletion and abnormally high pH meat (Ashmore et al., 1972).

The pH of the muscle plays a major role in the oxidation rate of myoglobin. In 1975, Chang and Traylor reported that when pH was lowered and proton concentration increased the globin conformation was changed in such a way as to render it less effective in stabilizing the heme-oxygen complex. Therefore, at physiological pH (ca 7.4) the heme-globin association/dissociation rate is low for oxidation rendering myoglobin in the reduced state. Any condition which would cause the pH to be lowered to approximately 5.6 would cause heme dissociation, unfolding of the apoglobin, and protonation of bound oxygen resulting in the development of oxymyoglobin and/or metmyoglobin (Adams, 1976).

The pH state in meat not only affects the stability of myoglobin itself, but the mitochondria activity as well. Lawrie (1958) and Ashmore et al., (1972) have shown that oxygen utilization of enzymatic systems in meat is greater at a high pH. Therefore, in the example of dark-cutting beef and dark colored pork, the abnormal preslaughter condition created a high pH state in the meat resulting in greater oxygen utilization and consumption by the enzymic system and mitochondria, thereby maintaining myoglobin in the reduced state.

Post-rigor conditions: The changes which take place after slaughter play a profound role in determining which pigment state develops. Once slaughtered, the oxygen supply to the tissue is eliminated resulting in anaerobic glycolysis as the only source for energy production, leading to lactic acid accumulation. The physiological pH of about 7.4 rapidly declines to approximately 5.3 to 5.5, accompanied by a considerable drop in temperature from 36-39°C to 2-5°C. With this drop in pH and temperature, glycolytic enzyme activity is reduced or completely inactivated. The changes in pH not only alters the stability of myoglobin, heme-globin association/dissociation rate, oxygen utilization by the enzymic system

and mitochondria resulting in either the reduction of myoglobin (high pH) or oxidation and/or oxygenation of myoglobin (low pH), but also have a substantial effect on the water binding capacity of meat proteins which affect the perceived color. At or above the isoelectric point of actomyosin, pH 5.0, more water is bound with a decrease in unbound fluid in the muscle. This results in a tighter more compact structure and darker appearance due to a drop in scattered incident light. Normal meat with its pH of 5.3-5.5 is closer to the isoelectric point of myosin leading to a loss of bound water and an increase in scattered incident light. Hence the color appears lighter than the higher pH meat (Lawrie, 1958).

Hot boning and electrical stimulation: One method being examined to help minimize the ever increasing cost of energy is to incorporate hot-boning into an in-line post-slaughter process. It is felt that up to 50% could be saved due to the elimination of the inedible fat and bone chilling step and expedited chilling to edible lean portions.

However, when the chilling step is eliminated, meat quality may be sacrificed. Muscles hot-boned prior to rigor onset have been found to be noticeably tougher due to normal shortening but also may toughen under "cold shortening" conditions (15°C), where they are stored, (Hostetler et al., 1972; Locker and Hagyard, 1963). Cold shortening was not found to occur at a pH of 6.0 or below (Bendall and Rhodes, 1976). When the pH decline is altered by hot-boning or electrical stimulation, normal meat color development is also affected. "Heat-ring" is a phenomenon occurring in beef that has not been chilled for the proper time and is most prevalent when there is a limited quantity of subcutaneous fat. "Heat-ring" consists of either a slightly dark, slightly coarse, slightly depressed lean or a dark, coarse, textured, sunken lean near the lumbo-dorsal fascia and extending inward toward the 12th rib approximately 1.0-2.0 cm. "Heat-ring" formation is less severe in electrically stimulated beef sides. Savell et al., (1978b) hypothesized that the dark and elevated pH observed in heat-ring was due to a differential rate of chilling where the outer portion of the muscle had a faster decline in temperature. Cross et al., (1979) evaluated the benefit of covering the carcass with PVC film as an insulator (Table 3). It was felt that

PVC film may retard chilling and thus approximate the effect electrical stimulation confers on heat-ring formation. However, it was found that heat-ring formation was slightly, but not significantly reduced in PVC overwrapped sides that were not electrically shocked. Lean color was significantly improved by PVC film overwrap when sides were not electrically stimulated. In conclusion, the authors felt PVC films did not contribute much over and above the beneficial effects of electrical stimulation.

According to West, (1980) research findings indicate that electrical stimulation does accelerate color development and prevents "heat-ring" formation when carcasses are ribbed early (18-24 hr). However, after 48 hours, no differences are detected. The magnitude of effect is felt to be dependent on chill cooler conditions. The rate of chill after boning, rather than prior to boning, is critical for optimum color and tenderness development. When hot-boning results in extreme muscle contraction, lean color is reported to be consistently dark and remain dark even though pH has declined to 5.6-5.8. This latter phenomena is not totally understood.

Electrical stimulation of prerigor meat has been shown to markedly decrease the time needed for rigor mortis (de Fremery and Pool, 1959; Hallund and Bendall, 1965; Davey et al., 1975; Grusby, 1976; Gilbert and Davey, 1976; McCollum and Henrickson, 1977 (Table 4)).

Electrical stimulation combined with hot-boning seemed the logical solution to reducing energy and labor costs while improving the quality aspects of the carcass.

Both heavy-weight and light-weight beef cattle have been shown to be improved by electrical stimulation (Table 5, 6). Savell et al., (1978a) showed that the maturity score was more youthful and muscle color brighter in electrically stimulated light-weight heifers. Improved lean color uniformity, along with a decrease in heat-ring formation was reported in heavy-weight, grain-fed cattle (Savell et al., 1979).

Unpublished data as shown in Tables 1 and 2, by Tang and Henrickson (1980), indicate that electrical stimulation does not significantly alter the total pigment or total myoglobin content. However, the oxymyoglobin content was significantly higher in the electrically stimulated samples ($P < 0.01$).

Table 3. Mean values for certain carcass and longissimus muscle traits

Item ^a	Cloth shroud only		Cloth shroud PVC overwrap	
	Electric shock	No electric shock	Electric shock	No electric shock
Longissimus muscle pH	5.8 ^d	5.9 ^d	5.8 ^d	5.7 ^d
Longissimus muscle temperature (°C)	3.5 ^d	1.8 ^d	5.2 ^d	3.8 ^d
Longissimus muscle heat-ring ^b	6.5 ^d	12.3 ^e	6.3 ^d	10.3 ^e
Longissimus muscle color ^c	4.2 ^d	2.8 ^e	5.2 ^d	4.3 ^d

^aAll traits were evaluated and all measurements were obtained at 18 hr postmortem.

^b15 = extreme heat-ring; 1 = no heat-ring.

^c8 = light grayish-red; 1 = very dark red.

^{d,e}Means in the same row bearing a common superscript letter are not significantly different ($P < 0.05$).

(Adapted from Cross et al., 1979).

Table 4. pH values of selected muscles from unstimulated beef sides chilled for 24 hr and from electrically stimulated sides chilled for 5 hr (standard deviations in parenthesis)

<i>Muscle</i>	<i>Unstimulated (24 hr)</i>	<i>Stimulated (5 hr)</i>	<i>Significance of difference</i>
Longissimus	5.74 (0.15)	5.49 (0.07)	P < .01
Biceps femoris	5.59 (0.11)	5.56 (0.16)	NS
Semimembranosus	5.56 (0.10)	5.58 (0.18)	NS
Psoas	5.59 (0.12)	5.54 (0.05)	NS

(Adapted from Gilbert and Davey, 1976).

Table 5. Mean values for characteristics of longissimus muscles evaluated in intact beef sides.^a

Treatment ^b	"Heat-ring"				Incidence (%)
	Color ^c		Degree ^d		
	mean	sig. diff.	mean	sig. diff.	
Not ES	5.3	P<.01	3.1	P<.01	100
ES - 25	6.3		4.1		80
Not ES	5.8	P<0.5	3.5	P<.10	90
ES - 50	6.6		4.4		40
Not ES	5.3	P<0.5	3.3	P<.01	100
ES - 75	6.2		4.2		60

^aEvaluated by USDA personnel at the 12-13th rib interface exposed by ribbing the sides.

^bNot ES = not electrically stimulated, ES-25, 50, 75 = electrically stimulated with 25, 50, 75 impulses.

^c8 = light grayish red, 1 = very dark purple.

^dThe degree of severity of "heat-ring" formation (5 = none, 4 = slight, 3 = moderate, 2 = severe, 1 = extremely severe).

(Adapted from Savell et al., 1978a).

Table 6. Mean values for postmortem pH and certain quality-indicating characteristics of beef from electrically stimulated and control sides

<i>Trait</i>	<i>Treatment of paired sides</i>		<i>Level of prob- ability^a</i>
	<i>Electrically stimulated</i>	<i>Unstimulated (control)</i>	
	<i>Mean</i>	<i>Mean</i>	
Postmortem pH			
1 hr	6.7	7.0	P<0.01
6 hr	5.8	6.1	P<0.001
12 hr	5.8	5.9	N.S.
24 hr	5.7	5.7	N.S.
"Heat-ring" ^b	5.0	3.8	P<0.01
Lean color uniformity ^c	7.4	5.1	P<0.01

^aThe probability that the difference between treatments is statistically significant based on paired-t analysis (Steel and Torrie, 1960). P<0.05 was reported as nonsignificant (N.S.).

^b"Heat-ring" was evaluated using a 5-point scale (5 = none, 1 = extremely severe) as described by Savell et al. (1978b).

^c8 = very uniform lean color; 1 = very uneven lean color.

(Adapted from Savell et al., 1979).

Unpublished data by Nichols and Cross (1980) tested the effects of electrical stimulation combined with hot-boning on: (1) pH decline, (2) sarcomere length, and (3) color of Longissimus dorsi (LD) and Semimembranosus (SM) muscles. These authors found that the rate of pH decline of stimulated LD increased with the time the muscle remained intact on the carcass. Their data confirmed that of Davey et al., (1975) who concluded that the ultimate pH of beef muscle could be reached 5 hours after electrical stimulation (Fig. 1). Electrically stimulated LD excised at 4 hours and chilled at 3°C were at the ultimate pH in 6 hours. As shown in Table 7, electrical stimulation did not affect the color or color uniformity of the LD or SM muscles, (Nichols and Cross, 1980). However, both the color and color uniformity of the SM, but not LD, muscle were greatly effected by excision time. The authors concluded that this result could be due to the different rates of chilling between the two muscles. The LD with its lesser transverse surface area and location near the surface of the carcass would chill at a faster rate than the SM muscle which would be subject to a more severe temperature gradient. The affect that varied depth in a carcass has on the rate of postmortem glycolysis was also explained by Tarrant and Mothersill (1977). A darker muscle color was noted in muscles excised at 1 or 2 hours postmortem than those excised at either 4 hr. or 48 hr.

Unpublished data by McCafferty and Huffman, (1980) illus-

trates the potential effect hot-boned meat can have on color of a processed product. Restructured beef steaks manufactured from hot boned chunks and flaked beef showed a substantial contrast in color. Due to the incorporation of oxygen and prooxidants during the comitrol process, the flaked portion of the product turned from the dark, purplish-red, hot-boned color, to the red oxymyoglobin form. However, wherever hot-boned chunks were embedded in the steak the dark, purplish-red reduced myoglobin state existed. This produced a visually unacceptable product due to the contrast in pigment. Data reported by Cross et al., (1979), Tables 8 and 9, further exemplifies the dramatic affects hot-boning has on muscle pH as well as lean color ratings.

Refrigeration and freezer storage: The measurable myoglobin content of raw pork loin roasts was shown to be significantly increased under freezer-thaw conditions. It was noted by Deduve in 1948 and confirmed by Nocito (1973) that damage due to freezing allows myoglobin to be released more readily by meat fibers. Therefore, although freezing may decrease the overall color acceptability due to excessive thaw exudate, the method of freezing has been shown to markedly effect raw meat color acceptability. In a study reported by Tuma et al., (1975), T-bone and porterhouse steaks, cut from four matching Choice beef loins were randomly assigned to one of eight freezing treatments. After an initial bloom time steaks were wrapped in medium permeability film and frozen

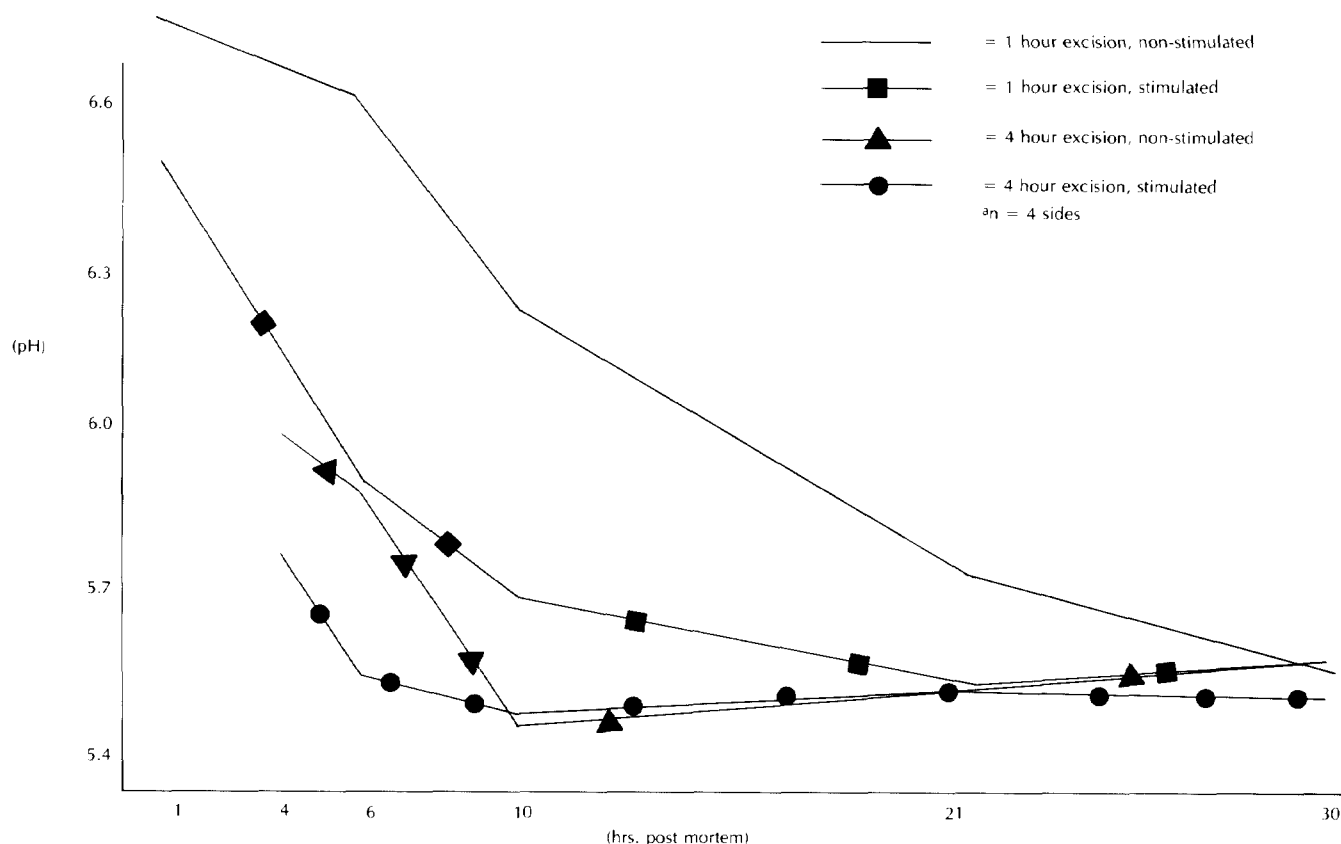


Figure 1: Decline of pH in LD muscles chilled at 3°C for 5 days and stored.^a (Adapted from Nichols and Cross, 1930).

Table 7. Mean values for appearance parameters of LD and SM muscles over 5-day evaluation period^a

Parameter	Muscle	Days				
		1	2	3	4	5
Color uniformity ^b	LD	5.5	5.3	5.0	4.9	4.7
	SM	4.8	4.5	4.0	3.8	3.4
Fat cover appearance ^c	LD	4.8	4.6	4.3	4.0	3.7
	SM	4.5	4.1	3.6	3.2	2.8
Muscle color ^d	LD	5.5	5.3	5.1	4.9	4.7
	SM	5.3	5.1	4.8	4.6	4.2
Percent reflectance	LD	38.3	—	—	—	47.3
	SM	41.8	—	—	—	48.0

^aChilling at 3°C for 5 days.^b6 = uniform, 5 = very slightly non-uniform (1-10%) and 1 = extremely non-uniform (41-50%).^c6 = very fresh, 5 = fresh, 4 = normal, and 1 = severe or extreme discoloration.^d9 = very light cherry red, 5 = slightly dark red and 1 = black.

(Adapted from Nichols and Cross, 1980).

Table 8. Ratings for lean color of hot- and cold-boned beef primals

Primal cut ^b	Lean color ^a	
	Hot boned ^c	Cold boned ^d
	Initial	Initial
Ribeye	2.58	6.0 ^e
Strip	2.68	6.0 ^e
Tenderloin	2.38	5.3 ^e
Inside round	2.88	4.5 ^e
Average	2.88	5.5 ^e

^a8 = light greyish red; 1 = very dark red or purple.^bn = 10 observations per mean.^cBoned 1 h postmortem.^dBoned 48 h postmortem.^{e,f,g}Means in the same row with different superscripts are significantly different (P<.05).

(Adapted from Cross et al., 1979).

to an internal temperature of 25°F, then held 12-14 hours at -20°F. Visual and objective evaluations indicated that treatments 1, 2, 3, and 8 developed unsatisfactory dark color while a brighter red satisfactory color was observed in treatments 4, 5, 6 and 7 where a rapid short-term freezing system using -70°F or lower were utilized.

Hanging beef in refrigerated storage and/or during transit creates environmental conditions optimum in many aspects for the discoloration of the exposed surfaces. The following factors, as summarized by Lanier et al., (1977), contribute to discoloration of shipped hanging beef: the partial pressure of oxygen in the environment as influenced by meat wraps,

Table 9. The pH of hot- and cold-boned beef cuts after removal from the carcass

Primal cut ^a	pH	
	Hot-boned 1 h	Cold-boned 48 h
Ribeye	6.1 ^b	5.7 ^c
Strip	6.0 ^b	5.7 ^c
Tenderloin	6.1 ^b	5.9 ^b
Inside round	6.1 ^b	5.7 ^c
Average	6.1 ^b	5.8 ^c

^an = 10.^{b,c}Means in the same row with different superscripts are significantly different (P<.05).

(Adapted from Cross et al., 1979).

bacterial growth or artificial atmospheres, temperature, tissue lipid oxidation, and possibly drying of the meat surface.

In a model system utilizing a wind tunnel to control temperature, humidity, and air velocity, Lanier et al., (1977) found that metmyoglobin formation was accelerated under conditions of increased temperature and air velocity. Figure 2 shows that for short-term storage (<2 days), lower relative humidity (85% RH) increased metmyoglobin formation while during prolonged storage (>2 days) high relative humidity (95% RH) increased metmyoglobin formation. It was postulated that 95% RH, for a prolonged period of time created the ideal moist surface allowing for bacteria to proliferate thus

Freezing Cycle

Treatment No.	Liquid nitrogen systems	Results
1	45 min -15°F	Dark, not satisfactory
2	10 min -40°F and equilibrate -5°F case	Dark, not satisfactory
3	30 min -40°F	Dark, not satisfactory
4	20 min -70°F	Bright red satisfactory
5	2 min 15°F, 2 min -40°F, -100°F till the end point	Bright red satisfactory
6	½ min 0°F, ½ min -50°F, 1 min -100°F, 1 min -200°F, and 1 min temper	Bright red satisfactory
7	-200°F till the end point	Bright red satisfactory
8	Mechanical air blast -20°F for 24 hr.	Dark, not satisfactory

*All satisfactory had longer shelf lives in retail case than the unsatisfactory ones. (Tuma et al., 1975).

accelerating pigment oxidation. In conclusion, the authors predicted that lean beef surfaces would be best preserved during extended storage with exposed air at 0.5 (mps), and 85-90% RH, and temperature close to 0°C. Beef from older animals where there exists a higher pigment concentration would show a faster decrease in color acceptability than younger animals under identical storage conditions. Loading a trailer with carcasses that show an internal temperature $\geq 15^{\circ}\text{C}$ risks the development of a warm, high RH environment around the meat surfaces initiating bacterial growth and surface discoloration (Lanier et al., 1978).

Metal ions: Certain metallic ions affect the oxidation of oxymyoglobin and thus the color of fresh meat. Copper is extremely active in promoting the autoxidation of oxymyoglobin to metmyoglobin while Fe, Zn, and Al are less active (Clydesdale and Francis, 1976). Castro (1971) proposed a theory to explain the prooxidant effect of metal ions in which he stated that complete electron transfer from heme iron to bound oxygen only occurs upon approach of a second metal ion or a proton. With the addition of ethylenediamine tetraacetic acid (EDTA), catalytic metal ions are chelated and in the bound form are less available for pigment oxidation.

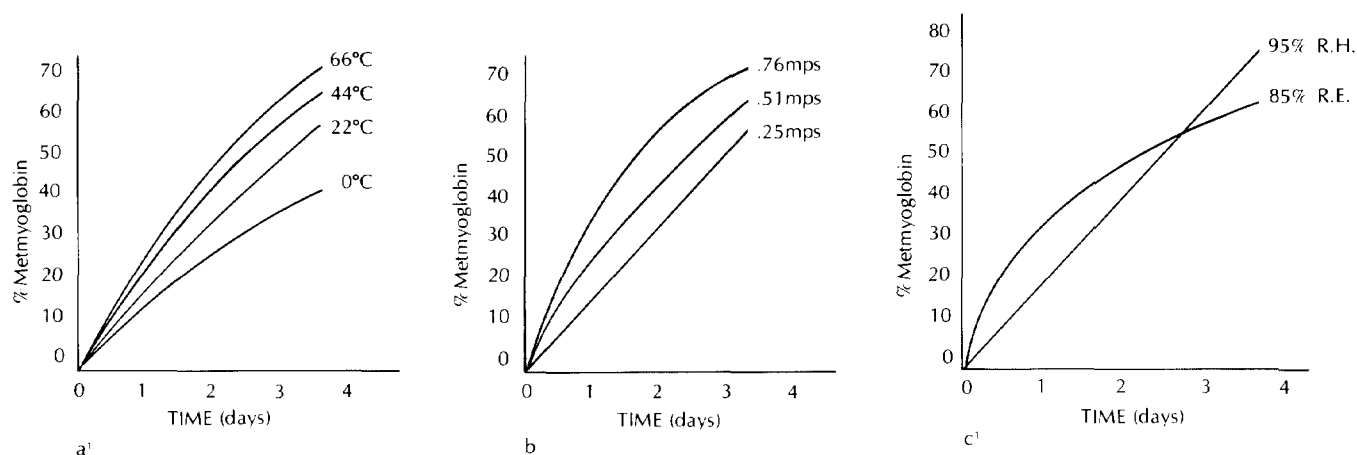


Figure 2: Rates of metmyoglobin formation as affected by storage (a) temperature (at air velocity = 0.51 mps, RH 90%); (b) air velocity (at temperature = 3.3°C, RH 90%); and (c) relative humidity as predicted (3.3°C, air velocity = 0.51 mps).

¹mps = meters per second

(Adapted from Lanier et al., 1977).

Many processing techniques may inadvertently affect pigment oxidation by introducing metal ions into the meat. Copper water lines, hard water, and grinding or mechanical tenderization are a few sources of potential metal ion inoculation.

Ascorbic acid: Ascorbic acid, a universally accepted antioxidant, has more recently been claimed as a meat color stabilizer by acting as a metmyoglobin reducing agent. Some muscles, e.g. *M. psoas major* and *M. gluteus medius* are believed to be more susceptible to discoloration than *M. longissimus dorsi* and *M. semitendinosus*. These latter muscles, which exhibit good color stability, are thought to have a higher metmyoglobin reducing activity (MRA) (Hood, 1971). Fox et al., (1975) suggest that the key to the effect ascorbic acid has on fresh-meat color lies in a nitrogenous base—ascorbate interaction. Rikert et al., (1957) reported that ascorbic acid protected and stabilized the meat color in ground meat where the reducing agent would be better distributed throughout the meat. This has not been shown to occur when whole slices of meat are treated with ascorbic acid either by spraying the surface or dipping the meat. Preliminary tests, reported by Hood, (1957), indicate that spraying 2.5% or 5% ascorbate onto the surface of prepackaged steaks resulted in inhibition of metmyoglobin formation but also impaired the formation of the characteristic "bloom" associated with fresh meat color.

A study was conducted whereby massive doses of sodium ascorbate (500 ml of a 50% w/v solution) were intravenously injected into beef animals 5 to 10 min before slaughter. This time period allowed for complete distribution of the ascorbate throughout the musculature. Carcasses were chilled for 48 hr. to 5°C, cut into primal cuts, vacuum packaged and stored at 0°C for 10 days. Controls containing no ascorbate were treated likewise. Steaks were then sliced, packaged on polystyrene trays and overwrapped in PVC oxygen-permeable film. At 5°C, the ascorbate treated meat showed less color deterioration and metmyoglobin accumulation than controls in three of the four muscles tested. When held at 5°C, the shelf life of control meat from *M. psoas major* and *M. gluteus medius* were 1 and 4 days, respectively. Shelf life of the same muscles for ascorbate treated meat was at least 6 days for both muscles. A 1-2 day increase in shelf life was observed from

ascorbate treated *M. semimembranosus* muscle as well. The color stable meat from *M. longissimus dorsi* was acceptable after 6 days storage in both control and ascorbate treated samples (Table 10). In conclusion it was felt that the ascorbate treated meat showed great potential in extending the shelf life of meat and making centralized prepackaging of meat a commercially viable proposition (Hood, 1975).

NaCl: NaCl has a substantial and important influence on meat proteins and pigments. However, the prooxidant properties of NaCl on meat has been, and is, a perplexing and puzzling question for food scientists according to Ellis et al., (1968). Salt, a key ingredient in curing, has been associated with the freezing problems encountered in bacon, and similar products. A more recent encounter with the effect salt has on lipid and pigment oxidation is in restructured fresh pork and beef products (Ly and Huffman, 1979; McCafferty and Huffman, 1980). Preliminary findings indicate that when a level as low as 0.50% to 0.75% NaCl is added to a restructured beef or pork mixture a significantly greater amount of discoloration results with the addition of salt. This doesn't seem to be as much of a problem with pork as with beef, possibly indicating a direct NaCl-pigment interaction rather than an indirect involvement of pigments through a NaCl-lipid-pigment interaction. Govindarajan et al. (1977) reported that a 3.0% NaCl concentration resulted in more rapid pigment oxidation in raw meat than was found in the control. MRA was reported to be decreased with the addition of 5.0% NaCl (Stewart et al., 1965). Salts have been shown to inhibit, to various degrees, some of the respiratory enzymes of meat (Grant, 1956). The darker color initially observed in NaCl treated meat may also be due to the affect NaCl has on the water binding capacity of the meat. NaCl increases the water-retaining capacity and swelling of meat on the alkaline side of the isoelectric point (Swift and Ellis, 1956). This would result in a tighter more compact structure and darker appearance due to a drop in scattered incident light, similar to the effect observed in dark cutting beef.

Other salts besides NaCl have been tested for their prooxidant activity, however a review of the literature seems to indicate wide disagreement. Chang and Watts, (1950) reported no effect from potassium chloride and sodium sulfate, however, Hills and Conochie (1946) proposed that the

Table 10. Effect of ascorbate treatment on metmyoglobin accumulation ($\nabla(K/S)_{572}(K/S)_{525}$) in four beef muscles at 5°C temperature.^a

Muscle	Treatment means day 2		Treatment means day 4		Treatment means day 6	
	Ascorbate	Control	Ascorbate	Control	Ascorbate	Control
<i>M. psoas major</i>	.09**	.23	.10***	.33	.14***	.40**
<i>M. gluteus medius</i>	.05*	.08	.05***	.16	.09***	.28
<i>M. semimembranosus</i>	.04	.04	.04	.07	.08*	.17
<i>M. longissimus dorsi</i>	.05	.04	.04	.05	.12	.11

^an = 10.

*P<.05.

**P<.01.

***P<.001.

(Adapted from D. E. Hood, 1975).

chloride ion was the culprit in increased oxidation. This theory is now felt to be incorrect.

A study on the stability of margarine showed that metal ion contamination, (principally copper and iron) in the water and salt that was added was large enough to increase the lipid oxidation in the margarine. This problem could be controlled with purer water and salt supplies as well as with the addition of chelating agents (Mertens et al., 1971). More research is needed in the area of salt and its effect on lipid and pigment oxidation in fresh and cured meat.

Beef and Pork Blends: Unpublished data by Parizek et al., (1980) has shown that when beef and pork were blended in manufacturing patties, blends were judged more acceptable in raw appearance and palatability traits than pure beef or pure pork. A blend of 70% beef, 30% pork and 75% beef, 25% pork were found to be most acceptable. Such beef/pork mixtures are easily made and could support the consumption of beef during shortages, reduce patty costs and present an alternative to non-meat products.

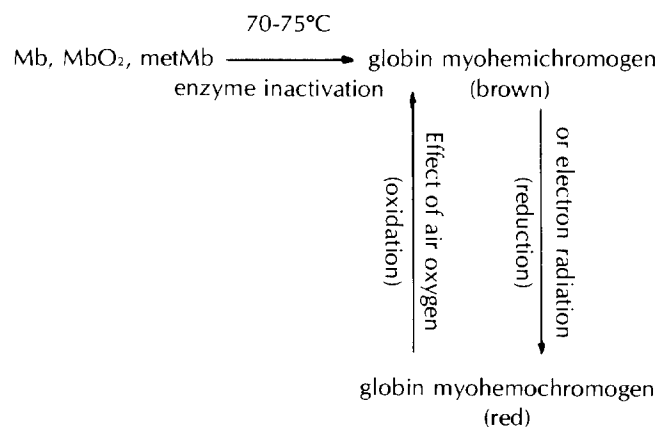
Ionizing Radiation: The area of preserving meat by ionizing radiation was pioneered in the 1940's and 1950's. The process has not been without problems, but none the less, it does have potential. Under anaerobic conditions, when raw meat is irradiated, the myoglobin is in the form of oxymyoglobin, resulting in a desirable bright red color. Enzymes are not completely inactivated by irradiation, therefore, for long-term storage it is necessary to cook the meat to an internal temperature of 70°C prior to irradiation. Cooked irradiated meat, under anaerobic conditions, is bright red in color due to the reduction of hemichrome to hemochrome upon radiation treatment. Once air comes in contact with the irradiated raw or cooked meat, the color changes to brown metmyoglobin or a gray brown cooked color, respectively (Hultin, 1976).

When meat undergoes ionizing radiation, various lipids and proteins are broken down to undesirable odiferous compounds. The degree of unpleasantness varies between species of animals, highest in beef to mildest in pork and chicken. It has been noted that bitter taste may appear in irradiated beef steaks due to the conversion of ATP to hypoxanthine. A drop in the temperature during irradiation to -80°C or below has

been found to lessen the odor and flavor side effects (Urbain, 1971).

Satterlee et al., (1972) showed that a new MbO₂ consisting of a mixture of five MbO₂ molecules, each with a different isoelectric point are formed upon irradiation. These authors felt that irradiation altered the apomyoglobin moiety rather than the heme group as earlier reported by Satterlee et al., (1971).

Kamarei et al., (1979) concluded that radiation reduces the brown pigment of cooked meats and in the presence of air the reduced red pigment oxidizes back to the original brown compound. The suggested oxidation-reduction mechanism is shown in the following diagram:



Restructured Fresh Meat: Color problems have been noted in restructured beef steaks. Discoloration occurring both in the raw and cooked state have been attributed to the addition of salt and other processing effects (Huffman and Cordray, 1979; Booren et al., 1979). Slight discoloration has been noted to occur during mechanical tenderization at the needle puncture points on the meat. McCafferty, (1980) noted some unacceptable overall color scores by the sensory panel due to the contrast effect from chunked and flaked portions of the steak. This was more noticeable when hot-boned meat was used in the formulation.

Table 11. Effect of vacuum treatment on subjective and objective surface color

	Vacuum mixing	No vacuum mixing
Subjective color score ^c	2.28 ^a	1.78 ^b
% R_{630} -% R_{580}		
Lean fraction	.31	.33
After mixing	.37	.40
Finished steaks	.32	.36
% Metmyoglobin		
Lean fraction	20.40	18.15
After mixing	6.95	5.72
Finished steaks	12.20	10.60

^{a,b}Means with different superscript letters in the same row are significant ($P < .05$).

^c1 = highly desirable fresh meat color; 5 = unacceptable fresh meat color.

n = 3.

(Adapted from Booren, 1980).

Booren et al., (1979) compared vacuum treatment and conventional mixing in sectioned and formed beef steaks. Oxidative and colorimetric changes due to vacuum were determined during processing and storage of the finished product. Results indicate that vacuum mixing produced less desirable surface color in finished steaks than no vacuum at all (Table 11). Oxy-myoglobin and metmyoglobin contents were not found to be significantly different between treatment groups as indicated by spectrophotometric analysis. Mixing time did not seem to alter subjective or objective color scores. The authors felt that possible denaturation of the globin moiety resulted from low oxygen tension during vacuum mixing, producing the less desirable color. Changes in color between the treatments were small, yet significant. Highly desirable color was noted in all samples examined.

Discussion

A. M. Mullins, *Louisiana State University*: Dale, did you say anything about vacuum packaging and the subsequent effect of color?

D. L. Huffman: The question, if I understand it correctly, is the influence of vacuum packaging on consumer packaged retail cuts. I did not review the literature in that particular area. However, as you're well aware, this does result in the reduced myoglobin form and apparently there is some acceptance of this color by the retailer and by the consumer. I think this is slow in coming, but I think we will see some more acceptance in the future.

References

- Adams, P. A. 1976. The kinetics and mechanism of the recombination reaction between apomyoglobin and haemin. *Biochem. J.* 159:371.
- Antonini, E. and M. Brunori. 1971. Hemoglobin and myoglobin in their reactions with ligands, North-Holland Publication Co., New York.
- Ashmore, C. R., W. Parker and L. Doerr. 1972. Respiration of mitochondria isolated from dark cutting beef: Post-mortem changes. *J. Anim. Sci.* 34:46.
- Bendall, J. R. and D. N. Rhodes. 1976. Electrical stimulation of beef carcass and its practical application. *European Meats Conf. London*. B2:3.
- Bodwell, C. E. and P. E. McClain. 1971. Proteins. Pages 78-133 in J. F. Price and B. S. Schweigert, (Eds). *The Science of Meat and Meat Products*. W. H. Freeman and Co., San Francisco.
- Booren, A. M. 1980. Binding of meat pieces into sectioned and formed beef steaks. Ph.D. Dissertation. University of Nebraska. Lincoln, Neb.
- Booren, A. M., K. W. Jones, R. W. Mandigo and D. G. Olson. 1979. Processing factors influencing binding of meat pieces into restructured, fresh beef steaks. (abstract). *Proc. 71st Annual Meeting of the Am. Soc. of Anim. Sci.* Tuscon, AZ.
- Brinigar, W. S. and C. K. Chang. 1974. Simple dioxygen heme complexes formed in N, dimethylformamide. *J. Am. Chem. Soc.* 6:5595.
- Brown, B. J. R., D. L. Harrison and C. Setser. 1978. Ground beef exposed to radiant energy: Effects of fat and BHA on color. *J. Food Sci.* 43:827.
- Brown, W. D. and H. E. Snyder. 1969. Non-enzymatic reduction and oxidation of myoglobin and hemoglobin by NAD and flavins. *J. Biol. Chem.* 244:6702.
- Castro, C. E. 1971. Theory of heme protein reactivity. *J. Theor. Biol.* 33:475.
- Chang, C. K. and T. G. Traylor. 1975. Kinetics of oxygen and carbon monoxide binding to synthetic analogs of the myoglobin and hemoglobin active sites. *Proc. Nat'l Acad. Sci. U.S.A.* 72:1166.
- Chang, I. and B. M. Watts. 1950. Some effects of salt and moisture on rancidity in fats. *Food Research*. 15:313.
- Clydesdale, F. M. and F. J. Francis. 1976. Pigments. Pages 385-426 in O. R. Fennema, (Ed). *Principles of Food Science*. Part 1. Marcel Dekker Inc., New York.
- Cross, H. R. 1979. Effects of electrical stimulation on meat tissue and muscle properties—a review. *J. Food Sci.* 44:509.
- Cross, H. R., G. C. Smith, A. W. Kotula and D. A. Muse. 1979. Effects of electrical stimulation and shrouding method on quality and palatability of beef carcasses. *J. Food Sci.* 44:1560.
- Cutaia, A. J. and Z. J. Ordal. 1964. Pigment changes in anaerobically packaged ground beef. *Food Technol.* 18:757.
- Davey, C. L., K. V. Gilbert and W. A. Carse. 1975. Carcass electrical stimulation to prevent cold shortening toughness in beef. *New Zealand J. Agr. Res.* 19:13.
- de Fremery, D. and M. J. Pool. 1959. Biochemistry of chicken muscle as related to rigor mortis and tenderization. *Food Res.* 28:73.
- DeDuve, C. 1948. A spectrophotometric method for the simultaneous determination of myoglobin and hemoglobin in extracts of human muscle. *Acta. Chem. Scand.* 2:264.
- Dutson, T. R., G. C. Smith and Z. L. Carpenter. 1979. Lysosomal enzyme distribution in electrically stimulated ovine muscle. *J. Food Sci.* 44:335.
- Ellis, R., G. T. Currie, F. E. Thornton, N. C. Bollinger and A. M. Gaddis. 1968. Carbonyls in oxidizing fat. II. The effect of the pro-oxidant activity of sodium chloride on pork tissue. *J. Food Sci.* 33:555.
- Fox, J. B., Jr., S. Keyser and R. A. Nicholas. 1975. Pyridine catalysis of ascorbate reduction of metmyoglobin. *J. Food Sci.* 40:435.
- Giddings, G. G. 1977. The basis of color in muscle foods. *CRC Crit. Rev. Food Technol.* 9:81.
- Govindarajan, S. 1973. Fresh meat color. *CRC Crit. Rev. Food Tech.* 4:117.
- Grant, N. H. 1956. The respiratory enzymes of meat III. The influence of various ions on beef succinoxidase. *Food Research*. 21:326.
- Grusby, A. H. 1976. Effects of electrical stimulation and high temperature conditioning on bovine muscle tenderness characteristics. Masters Thesis. Univ. of Fla.
- Hallund O. and J. R. Bendall. 1965. The long-term effect of electrical stimulation on the post-mortem fall of pH in the muscles of Landrace pigs. *J. Food Sci.* 30:296.
- Hills, G. L. and J. Conochie. 1946. The mechanism of the oxidant effects of commercial salt and water in butterfat. *J. Council Sci. Ind. Res. (Australia)* 19:414.
- Hood, D. E. 1971. *Proc. 17th Meeting European Meat Res. Workers*. Bristol. p. 677.
- Hood, D. E. 1975. Preslaughter injection of sodium ascorbate as a method of inhibiting metmyoglobin formation in fresh beef. *J. Sci. Food Agric.* 26:85.
- Hostetler, R. L., B. A. Link, W. A. Landman and H. A. Fitzhugh. 1972. Effect of carcass suspension on sarcomere length and shear force of some major bovine muscles. *J. Food Sci.* 37:132.
- Huffman, D. L. and J. C. Cordray. 1979. Restructured fresh meat cuts from chilled and hot processed pork. *J. Food Sci.* 44:1564.
- Hultin, H. O. 1976. Characteristics of muscle tissue. Pages 577-614. in O. R. Fennema, (Ed). *Principles of Food Science*. Part 1. Marcel Dekker, Inc. New York.
- Kamarei, A. R., M. Karel and E. Wierbicki. 1979. Spectral studies on the role of ionizing radiation in color changes of radappertized beef. *J. Food Sci.* 44:25.
- Kendrew, J. C. 1963. Myoglobin and structure of protein. *Science*. 139:1259.
- Lanier, T. C., J. A. Carpenter, R. T. Toledo and J. O. Reagan. 1978. Transportation and color maintenance of hanging beef. *J. Food Sci.* 43:168.
- Lanier, T. C., J. A. Carpenter and R. T. Toledo. 1977. Effects of cold storage environment on color of lean beef surfaces. *J. Food Sci.* 42:860.
- Lawrie, R. A. 1958. Physiological stress in relation to dark-cutting beef. *J. Sci. Food Agr.* 9:721.
- Ledward, D. A., G. C. Smith, H. M. Clarke and M. Nicholson. 1977.

- Relative role of catalysts and reductants in the formation of metmyoglobin in aerobically stored beef. *Meat Sci.* 1:149.
- Lewis, P. K., C. J. Brown and M. C. Heck. 1962. Effect of preslaughter treatments on certain chemical and physical characteristics of beef muscle. *J. Anim. Sci.* 21:433.
- Locker, R. H. and C. J. Hagyard. 1963. A cold-shortening effect in beef muscles. *J. Sci. Food and Agr.* 14:787.
- Ly, Alexander and D. L. Huffman. 1979. Effect of salt concentration on quality restructured pork chops. Personal communication.
- Maxwell, J. C. and W. S. Caughey. 1976. An infrared study of NO bonding to heme and hemoglobin β . Evidence for inositol hexaphosphate indirect cleavage of proximal histidine to iron bonds. *Biochemistry* 15:388.
- McCafferty and D. L. Huffman. 1980. Effect of hot boning and method of comminution of restructured beef steaks. Personal Communication.
- McCollum, P. D. and R. L. Henrickson. 1977. The effect of electrical stimulation on the rate of post-mortem glycolysis in some bovine muscles. *J. Food Qual.* 1:15.
- Mertens, W. G., C. E. Swindells and B. T. Teasdale. 1971. Trace metals and the flavor stability of margarine. *J. Amer. Oil Chem. Soc.* 48:544.
- Nacito, J. S., B. H. Bayne, M. P. Penfield and B. H. Meyer. 1973. Myoglobin content and color of raw pork loin roasts as affected by freezing at two rates. *J. Anim. Sci.* 37:1339.
- Newbold, R. P. and P. K. Scopes. 1971. Post-mortem glycolysis in ox skeletal muscle: Effect of mincing and of dilution with and without the addition of orthophosphate. *J. Food Sci.* 36:209.
- Nichols, J. E. and H. R. Cross. 1980. Effects of electrical stimulation and early post-mortem muscle excision on pH decline, sarcomere length, and color in beef muscles. *J. Food Sci.* (Accepted for Publication).
- Parizek, E. A., C. B. Ramsey, J. D. Tatum and T. L. Hoes. 1980. Acceptability of beef/pork hamburger patties. Personal communication.
- Rifkind, J. M. 1973. Hemoglobin and myoglobin. Page 832 in G. Eichhorn, (Ed). *Inorganic Biochemistry*. Vol. 2. Elsevier, Amsterdam.
- Rikert, J. A., L. Bressler, C. O. Ball and E. F. Stier. 1957. Factors affecting quality of prepackaged meat. *Food Technol.* 11:567.
- Savell, J. W., G. C. Smith, T. R. Dutson, Z. L. Carpenter and D. A. Suter. 1977. Effect of electrical stimulation on palatability of beef, lamb and goat meat. *J. Food Sci.* 42:702.
- Savell, J. W., G. C. Smith, Z. L. Carpenter and F. C. Parrish, Jr. 1979. Influence of electrical stimulation on certain characteristics of heavy-weight beef carcasses. *J. Food Sci.* 44:911.
- Savell, J. W., G. C. Smith and Z. L. Carpenter. 1978a. Effect of electrical stimulation on quality and palatability of light-weight beef carcasses. *J. Anim. Sci.* 46:1221.
- Savell, J. W., T. R. Dutson, G. C. Smith and Z. L. Carpenter. 1978b. Structural changes in electrically stimulated beef muscle. *J. Food Sci.* 43:1606.
- Savell, J. W., G. C. Smith, T. R. Dutson, Z. L. Carpenter and D. A. Suter. 1976. Effect of electrical stimulation on beef palatability. *J. Anim. Sci.* 43:246.
- Smith, G. C., T. R. Dutson, H. R. Cross and Z. L. Carpenter. 1979. Electrical stimulation on hide-on and hide-off calf carcasses. *J. Food Sci.* 44:335.
- Sorinmade, S. O., H. R. Cross and K. Ono. 1978. The effect of electrical stimulation on lysosomal enzyme activity, pH decline and beef tenderness. 24th European Meeting of Meat Research Workers, (Kulmbach, Germany).
- Stewart, M. R., B. K. Hutchins, M. W. Zipser and B. M. Watts. 1965. Enzymatic reduction of metmyoglobin in ground beef. *J. Food Sci.* 30:487.
- Satterlee, L. D., W. D. Brown and C. Lycometros. Stability and characteristics of the pigment produced by gamma irradiation of metmyoglobin. *J. Food Sci.* 44:25.
- Satterlee, L. D., M. Vilhelm and H. M. Barnhart. 1971. Low dose gamma irradiation of bovine metmyoglobin. *J. Food Sci.* 36:549.
- Swift, C. E. and R. Ellis. 1956. The action of phosphates in sausage products. I. Factors affecting the water retention of phosphate-treated ground meat. *Food Technol.* 10:546.
- Tang, B. H. and R. L. Henrickson. 1980. The effect of post-mortem electrical stimulation on bovine myoglobin and its derivatives. (Accepted for Publication).
- Tarrant, P. V. and C. Mothersill. 1977. Glycolysis and associated changes in beef carcasses. *J. Sci. Food Agr.* 28:739.
- Tuma, H. J., D. H. Kropf, D. B. Erickson, D. L. Harrison, S. E. Trieb, A. D. Dayton. 1975. Frozen meat: Its distribution costs, acceptance, and cooking and eating qualities. *Kansas State University Agri. Exp. Sta. Res. Bull.* 166.
- Urbain, W. M. 1971. Meat preservation. Page 426 in J. F. Price and B. S. Schweigert, (Ed). *The Science of Meat and Meat Products*. 2 Ed. W. H. Freeman and Company, San Francisco.
- Westervelt, R. G. and J. R. Stouffer. 1978. Relationships among spinal cord severing, electrical stimulation and post-mortem quality characteristics of the porcine carcass. *J. Anim. Sci.* 46:5.