EFFECTS OF LIPIDS ON THE OXIDATION OF PIGMENTS IN GROUND BEEF EXPOSED TO RADIANT ENERGY

by

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TABLE OF CONTENTS

Page

INTRODUCTION

Consumers associate color more than any other factor with the freshness of meat. It is the concentration and chemical state (oxygenated or oxidized) of myoglobin and hemoglobin in muscle that largely are responsible for fresh meat color. The pigment in fresh meat is in the bright cherry red form of oxymyoglobin. Metmyoglobin, the oxidized form of myoglobin, is brown. Oxidation of myoglobin to metmyoglobin may be induced by factors such as light, bacteria, lipid oxidation, packaging films, and storage temperature (Setser et al., 1973).

Using colored filters, Townsend and Bratzler (1958) demonstrated that 560-630 mu light is the region of the spectrum most damaging to meat pigments. Setser et al. (1973) found that 254 nm of radiant energy had greater effects on the oxidation of meat pigments than 405 or 577 nm light, but light, per se, was not the major cause of pigment changes in beef semitendinosus muscle. Benedict et al. (1974) found that increase in polyunsaturated lipid content of ground beef may lead to rapid oxidation of muscle myoglobin. Watts (1954) demonstrated a catalytic effect of hemoglobin and other iron porphyrins on oxidation of lipids and destruction of the pigments.

A common problem in marketing prepackaged beef is the development of an undesirable brown color after the beef has been cut. Because consumers associate a bright, cherry-red color with fresh beef, this rapid color change shortens the time that meat can be held in a display case before it is sold. To reduce waste, it would be desirable to lengthen the time before a color change occurs.

At the present time, much of the meat in retail display is packaged in air permeable films. Free exchange of oxygen takes place, which makes the meat susceptible to oxidation of lipids and pigments. Greene (1969) reported that propyl gallate and butylated hydroxyanisole inhibited oxidation of both lipids and pigments in fresh ground beef in aerobic packaging material.

The purpose of this study was to examine the effects of lipids on the oxidation of pigments (color change) in ground beef exposed to 577 nm of radiant energy.

REVIEW OF LITERATURE

Effects of display environment on color of fresh meat

Light, oxygen level, action of microorganisms, type of packaging material and storage temperature are some environmental factors that influence the shelf-life of meat in display cases. Concentration of pigments, pH, lipid content, co-catalysis by lipid oxidation and the inability of muscle tissue to reduce metmyoglobin (MetMb) to myoglobin (Mb) also may influence shelf life of meat.

The chemistry of the heme pigments responsible for fresh meat color has been reviewed elsewhere (Fox, 1966; Solberg, 1970; Govindarajan, 1973). Mb is the major pigment responsible for meat color. It is a conjugated protein that contains a heme moiety (an iron containing porphyrin compound) attached to a protein, globin. In the presence of oxygen, Mb is converted to oxymyoglobin (MbO_2) and MetMb, the oxygenated and oxidized forms, respectively. When about 60% or more of the Mb is present in the MetMb form, the meat surface takes on an undesirable brown color (Brody, 1970).

Light. Fresh meat color seems to be dependent on the type of light used in the display case. However, there are conflicting reports in the literature. Ramsbottom et al. (1951) reported there was no loss of color in meat exposed to 60 to 200 foot-candles (fc) of fluorescent light for 3 days. Rickert et al. (1957) obtained similar results with ground pork stored under 30 to 170 fc of fluorescent light. Kraft and Ayres (1954) found no difference in the color of beef samples exposed to 60 to 150 fc of fluorescent light, as compared to those stored in the dark.

Voegelli (1952) observed color changes in packaged fresh meat exposed to 215 fc from a fluorescent lamp. Marriott et al. (1967) compared the color of prepackaged beef steaks in a display case under 120 fc of soft white fluorescent light with the color of steaks held in the dark at 30°F. The color of steaks stored in the dark changed only slightly over a 10-day period; those exposed to light showed increased color degradation.

Ramsbottom et al. (1951) and Kraft and Ayres (1954) found ultraviolet light detrimental to fresh meat color. Kraft and Ayres (1954) reported rapid discoloration with ultraviolet light even though it inhibited microbial growth on the meat surface. Marriott et al. (1967) found light stimulated bacterial growth, and they postulated that there are interrelationships among the effects of light, bacterial growth and the formation of MetMb.

Solberg and Franke (1971) studied the photosensitivity of fresh meat color to the visible spectrum. They exposed bovine adductor and semitendinosus muscles to 420, 510, 540, 550, 570, 590, 632.8 and 700 nm light at 34° or 41°F for 1, 2 or 5 days. No one wavelength enhanced the production of oxidized pigment more than any other wavelength. However, an average of 5.5% more oxidized pigment was found in exposed samples than in unexposed

samples. The increase in MetMb with exposure to light was attributed to a photochemical activation of a compound such as riboflavin, which may then react with MbO_2 to form MetMb. Brown and Snyder (1969) reported that flavin mononucleotide in the presence of ethylene-diaminetetra-acetate catalyzed the photooxidation of MbO_2. They did not show riboflavin per se is capable of catalyzing photooxidation of MbO_2.

Setser et al. (1973) examined the change in fresh meat color in semitendinosus muscle during a 3-hour exposure to 254, 405 or 577 nm of radiant energy in 0, 20 or 100% oxygen. Significantly ($P<0.01$) more loss of MbO_2 in samples exposed to radiant energy than in the controls. However, the difference was considered of no practical importance. They hypothesized the increase in MetMb was a result of the oxidation of lipids by radiant energy, which in turn leads to pigment oxidation.

Satterlee and Hansmayer (1974) subjected samples of semitendinosus muscle to 250 fc of pink (540-565 nm) and white (500-590 nm) fluorescent light, 150 watt cool flood and 100 watt incandescent lights (300-1000 nm). The primary factor causing rapid loss of MbO_2 from the meat surface was the type of light used. Lower wavelengths caused more rapid oxidation of pigments. Under soft white fluorescent light, color deteriorated in only 2 1/4 days; whereas, less severe lights (incandescent flood, 100 watt incandescent, pink fluorescent) required longer for discoloration, and allowed bacteria to grow and become a factor in discoloration.

Oxygen level and pressure. The relative proportion of Mb, MbO_2 and MetMb in meat depends, to a large extent, on the partial pressure of oxygen in the muscle tissue. Brody (1970) stated that Mb is converted to MbO_2 at high oxygen levels; whereas, at relatively low concentrations, Mb is oxidized to MetMb. Formation of MetMb is accelerated by factors such as

light that also may cause denaturation of the protein, globin. Discoloration of skeletal muscle stored at low oxygen levels has been reported (Rickert et al., 1957; Snyder, 1964; Watts et al., 1966; Setser et al., 1973).

Microbial activity. The most important factor affecting shelf life of fresh meat is microbial activity (Brody, 1970). Microorganisms are introduced from the air, from handling and from tools used to cut meat. As their number increases they produce acids and protein-breakdown products. Growth is accelerated by increasing temperatures up to the optimum temperature of growth for each type of microorganism. Generally, microorganisms that contaminate meat are aerobic. The metabolic process involves an intake of oxygen and production of carbon dioxide and water. Therefore, reduction of oxygen and an increase in carbon dioxide and moisture can retard microbial activity. Oxygen-permeable packaging films may thus contribute to red-meat spoilage. A close relationship between MetMb formation and microbial activity was noted by several workers (Butler et al., 1953; Costilow et al., 1955; Marriott et al., 1967). According to evidence presented by Robach and Costilow (1961), the effect of bacteria on MetMb formation is probably a result of decreased partial pressure of oxygen in the tissues that enhances autoxidation. However, Snyder and Ayres (1961) showed Mb is subject to autoxidation that is not caused by bacteria. They (1964) also found that meat discolored quickly at -2°C, which tends to discount the role of bacteria. Satterlee and Hansmeyer (1974) stated that the primary factor causing the rapid loss of MbO_2 from the surface of meat in the display case is the type of light, not bacteria.

Temperature. Brody (1970) pointed out that many of the reactions that influence the shelf life of fresh meat are temperature dependent. Enzymatic

and non-enzymatic biochemical reactions more than double in rate with each 10°F increase up to 130° to 140°F, or up to the temperature where no more reactants are available.

In general, temperatures of 30° and 32°F were more beneficial than 38° and 42°F in retarding discoloration of fresh meat (Butler et al., 1953; Jaye et al., 1962; Fellers et al., 1963; Snyder, 1964). Snyder (1964) stated that the decreased discoloration with decreased temperature was dependent on contact of meat surfaces with air and decreased respiratory activity of the meat at the lower temperatures. Increased storage temperature accelerated enzymatic reduction of MetMb, but it also accelerated Mb autoxidation.

Brown and Dolev (1963) examined the rate of oxidation in highly purified tuna and beef MbO_2 solutions at 0°, -5°, -10°, -15° or -18°C. Oxidation rates decreased with decreasing temperatures until the solutions solidified (-10°C); then autoxidation rates increased sharply. They assumed the results were caused by a more suitable Mb - O_2 environment on solidification. Repeated freezing and thawing did not have a strong effect on oxidation rate (Brown and Dolev, 1963). However, Townsend and Bratzler (1958) concluded repeated freezing and thawing in an oxygen impermeable wrapper has a marked effect on frozen meat color.

Effects of muscle composition on color of fresh meat

Myoglobin. Myoglobin is a complex protein present in the sarcoplasmic fraction of skeletal muscle. In a live animal it functions (with hemoglobin) in the transport and storage of oxygen required for the metabolic activity of the animal (Govindarajan, 1973).

Lawrie (1953) found a parallel relationship between myoglobin content and the extent of muscular activity that was a response to oxygen demand.

Broumand et al. (1958) reported a relationship between the concentration of MetMb and the observed color in beef and lamb as follows: 20 to 30% MetMb, dull red; 40 to 50% MetMb, greyish red; 60 to 70% MetMb, reddish grey; and 80+%, grey or brown. Brody (1970) also reported that when 60% or more of the Mb is present as MetMb, the meat becomes an undesirable brown color.

Lipid content and lipid oxidation. Rickert et al. (1957) demonstrated a relationship between fat content and "redness" in muscle. The co-catalytic effect of heme and other iron porphyrin oxidations and the oxidation of lipids was discussed by Watts (1954), Younathan and Watts (1959) and Greene (1969). Younathan and Watts (1959) demonstrated myoglobin-catalyzed lipid oxidation in meat. Their data suggest that the ferric form of the pigment is the active catalyst in muscle tissue rancidity.

Early studies on lipid oxidation in meat were concerned with adipose tissue lipids, and cellular lipids were ignored. Cellular lipids do not occur as globules, but they are integral parts of various subcellular organelles such as microsomes. Those lipids are polar in nature because of the presence of phospholipids and often are associated with proteins. Lipid oxidation in lean meats may involve cellular lipids. The addition of cellular lipids to freshly ground beef accelerated lipid oxidation; whereas, the addition of adipose fat had little or no effect on the oxidation of lipid or Mb (Govindarajan, 1973). Hornstein et al. (1961) reported cellular lipids are more unsaturated than adipose tissue lipids and so are more susceptible to oxidation. Benedict et al. (1974) stated that increased in polyunsaturated content of beef may lead to rapid oxidation of muscle Mb.

Hutchins et al. (1967) found positive correlations between MetMb content and thiobarbituric acid values in stored ground beef. Their data were not clear as to whether pigment oxidation caused lipid oxidation or vice versa. Ledford and Macfarlane (1971) found that initial treatment of frozen beef such as freeze-thawing, delayed freezing or mincing increased both Mb and lipid oxidation. Higher initial MetMb concentrations had no effect on the rate of lipid oxidation. This disagreed with results of Younathan and Watts (1959) who found that the oxidized form of the pigment is the active catalyst in tissue rancidity.

Greene (1969) reported that butylated hydroxyanisole and propyl gallate protected against lipid oxidation and MetMb formation up to 8 days during refrigerated storage. Also, ascorbic acid temporarily inhibited the formation of MetMb. Liu and Watts (1970) suggested that ascorbic acid keeps Mb in reduced form, and thus, indirectly inhibits lipid oxidation. Their theory is based on the assumption that the oxidized or ferric form of the pigment is the active catalyst of lipid oxidation. Benedict et al. (1974) studied the effectiveness of five antioxidant treatments on the stability of Mb in ground beef with about 3 or 10% polyunsaturated lipid. The antioxidants included two lipid antioxidants (α-tocopherol and L-ascorbyl stearate, two water soluble antioxidants (ascorbic acid and ascorbic acid/sodium bicarbonate) and an antioxidant synergist (citric acid). Citric acid and ascorbyl stearate were effective antioxidants at both fat levels. α-Tocopherol showed no antioxidative effect, but ascorbic acid alone or with bicarbonate accelerated oxidation of lipids and Mb. This is opposite to Greene's (1969) findings.

pH. Changes that take place in muscle following slaughter affect myoglobin, and hence meat color. When the animal dies, the oxygen supply is

cut off and the muscle system resorts to anaerobic glycolysis for energy production. This leads to the production of lactic acid and a decrease in pH. MbO_2 oxidizes faster at low pH than at high pH values (Cutaia and Ordal, 1964). As cellular breakdown occurs, the pigments come in contact with compounds such as fats in adipose tissues by diffusion. This interaction may lead to mutual co-oxidation of the fat and the pigment. Also, the drop in pH may induce Mb oxidation, because the oxidation of Mb is rapid at low pH values (Govindarajan, 1973).

The color of meat is darker at a high pH (6.5) than at a low pH (5.3 to 5.5). The higher the pH the more water that is bound to the protein of muscle and results in a tightly packed muscle structure that appears dark in color, because its surface scatters little incident light. For this reason dark cutting beef (pH 6.5 or more) has a dark color. Normal muscle (pH 5.3 to 5.5) has a loose structure because of the loss of water holding capacity of the protein. Because the muscle is not packed tightly, more incident light is scattered on the surface and the color is lighter than for dark cutting beef.

Measurement of meat color by reflectance spectrophotometry

The measurement of meat color is a difficult task because of two factors; the complexity of Mb distribution in muscle and the dynamic nature of the pigment. Also, complicating the process is the presence of intramuscular fat, which tends to interfere with color measurement. The reflectance value of muscle tissue increases with increasing muscular fat, independent of wavelength (Elliot, 1967). The arrangement of fibers on the surface of the meat also appears to affect the reflectance values (Elliot, 1967). Snyder (1968) summarized the principle of the reflectance technique. He stated

that absorption peaks for Mb, MbO_2 and MetMb occur at the same wavelengths for both reflectance measurements on meat and transmission measurements on solutions of the pigments. However, the absorption maxima and minima are defined less sharply with reflectance measurements.

Efforts to put reflectance data on a quantitative basis use the Kubelka-Munk function. Reflectivity of an optically thick sample ($R\infty$) depends on the ratio of the absorption coefficient (K) and the scattering coefficient (S) of the colorant layer as follows: $\frac{K}{S} = \frac{(1 - R\infty)^2}{2\ R\infty}$. $R\infty$, the light that is not absorbed, is measured spectrophotometrically.

The indedent light absorbed by the pigment in solution relative to that scattered by the matrix of meat solids decreases as percentage reflectance increases. The Kubelka-Munk relationship was shown in work with a model system of Mb derivatives (Snyder and Armstrong, 1967). If only reflectance absorbancy values (R_a) were used, the amount of light absorbed was not directly proportional to concentration of pigment according to Beer's law, although the deviation was small (Snyder, 1965). However, Franke and Solberg (1971) found a linear relationship by plotting R_{a632} against percentage MetMb. They speculated that 632 nm light minimizes scatter because of its relatively low energy.

To follow rapid changes in pigment composition of fresh meat, it is necessary to make measurements directly on intact muscle rather than first extracting meat pigments. Extraction of pigments is cumbersome, destroys the sample being analyzed, and is likely to change the relative portions of Mb, MbO_2 and MetMb. The ideal sample for reflectance spectrophotometry is flat, homogeneously pigmented, opaque and light diffusing. Nevertheless reflectance spectrophotometry is used to evaluate pigment changes in muscle

tissue which is translucent and inhomogeneously pigmented with an uneven surface (Little and Mackinney, 1969).

Snyder (1965) stated that the variation among samples is an important factor in the precision obtainable with reflectance measurements. Percentage reflectance depends on pigment concentration, amount of intramuscular fat, amount of moisture at the surface of the meat and the oxidation of oxygenation state of the pigment. To minimize the effect of nonhomogeneitys of the sample, ratios of reflectance at two different wavelengths often are used (Snyder, 1968).

MATERIALS AND METHODS

Sample preparation

Three ground beef products differing in fat level (approximately 15, 30, or 45%) were formulated by the Department of Animal Science and Industry, Kansas State University. Those products were prepared from carcasses (550-600 lb.) of "long fed" beef animals (14 weeks, grain sorghum after being on grass). Meat scientists in the Department of Animal Science and Industry estimated the carcasses would have graded U.S. Good or U.S. Choice.

Ether extract was measured on aliquots of each product by the Analytical Laboratory, Department of Animal Science and Industry. Triplicate samples (approximately 2 g) were dried, extracted with ethyl ether for 16 hours on a Soxhlet extraction apparatus; the ether was evaporated, and the percentage ether extract was calculated.

Chlortetracycline (30 ppm, Greene, 1969) was mixed thoroughly with five pounds of each ground beef product to retard bacterial spoilage. Meat from each product (15, 30 or 45% fat) was divided into 80-g portions that were

used as experimental samples. During all direct handling of the meat, sterile disposable plastic gloves were worn to reduce possible contamination. Butylated hydroxyanisol (BHA) was added dry by weight (0.01%; Greene, 1969) to two of the four experimental samples from each product (Table 1).

Table 1—Treatment combinations for six replications

Trtm't comb. code	Fat, approx. %	Storage dark, -29°C (-20°F)	Exposure 577 nm, air -1°C (+30°F)	Antioxidant BHA, 0.01%
A	15[a]	+	-	-
B	15[a]	+	+	-
C	15[a]	+	+	+
D	15[a]	+	-	+
E	30[b]	+	-	-
F	30[b]	+	+	-
G	30[b]	+	+	+
H	30[b]	+	-	+
J	45[c]	+	-	-
K	45[c]	+	+	-
L	45[c]	+	+	+
M	45[c]	+	-	+

[a] 16.32% fat as determined by ether extract
[b] 30.19% fat as determined by ether extract
[c] 45.50% fat as determined by ether extract
+, treated, with BHA, stored in dark or exposed
-, untreated, without BHA or unexposed

All samples were wrapped in aluminum foil, frozen at -29°C (-20°F), held at that temperature until used (1 to 16 weeks).

For each replication, the four 20-g samples from each product were packed in stainless steel containers after thawing 4 hours at 4.4°C (40°F), covered with an air permeable film, packaged in Whirl-pak freezer bags and frozen at -29°C (-20°F) in the dark and stored under those same conditions for at least 24 hours before spectrophotometric measurements were taken on the experimental samples.

Exposure, spectral reflectance and subjective evaluation

For those samples exposed to radiant energy, the order of exposure was randomized within each replication (Table 2). Exposed samples were subjected to radiant energy at 577 nm and a temperature of -1°C (30°F) for 2 hours in an atmosphere of 20% oxygen. The cooling system consisted of forcing compressed air (20% oxygen) through a moisture condensing trap into a copper coil suspended in liquid nitrogen, and then into the sample chamber where exposure occurred. The source of radiant energy was a 500-watt medium pressure mercury lamp fitted with a standard mercury line interference filter (577 nm) for the selective absorption of unwanted spectral components (Figure 1).

Reflectance values were recorded for each experimental sample at 30 minute intervals using a Bausch and Lomb 600 recording spectrophotometer with a magnesium oxide reference standard. To reduce variation among spectrophotometric readings, all samples were measured at the same location on the surface of the sample. Reflectance of light was read on the transmission scale across the spectrum from 403 to 695 nm. Readings were taken at 474, 525, 571, 614 and 630 nm. Data for 474, 525, 571, and 630 nm were converted to K/S values. A compensating polar planimeter was used to measure the

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Fig. 1—Light exposure and cooling system

A. Power supply
B. Variable power regulator
C. Housing for mercury lamp
D. Sample chamber
E. Sample holder
F. Whirlpak bags
G. Aerobic packaging film
H. Ground beef sample
I. Cooling system
J. Blower to cool filter

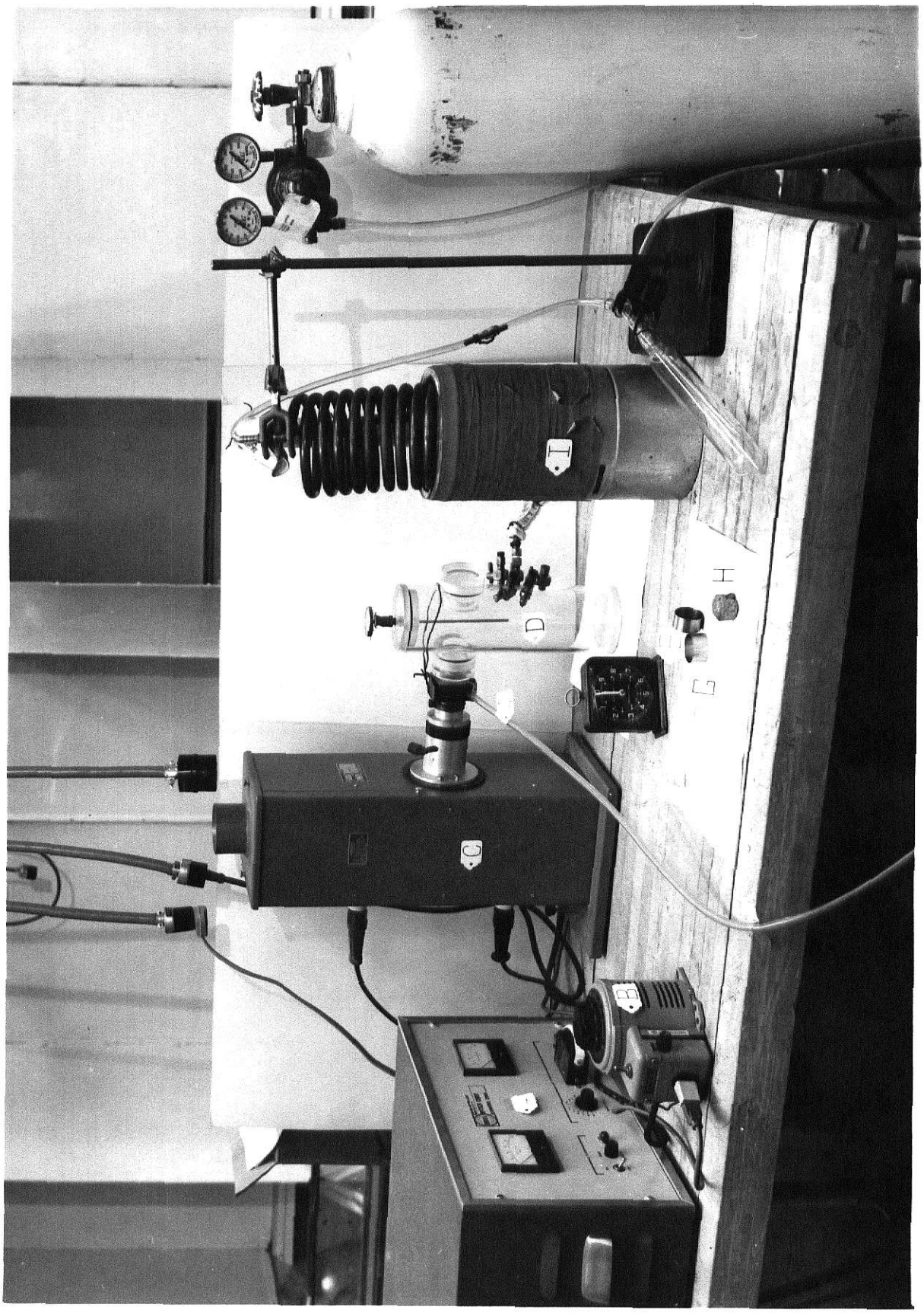

Table 2—Experimental design for samples exposed to radiant energy

Replication No.	Order of exposure					
	1	2	3	4	5	6
	Treatment combinations					
I	G	B	K	L	C	F
II	G	L	K	F	C	B
III	K	F	C	L	B	G
IV	C	B	K	G	F	L
V	L	B	K	C	G	F
VI	F	C	K	L	B	G

Treatment combination code:

B, 15% fat
C, 15% fat, BHA
F, 30% fat
G, 30% fat, BHA
K, 45% fat
L, 45% fat, BHA

total area (sq cm) under the curve, and to measure the area under the curve of the red portion (630 to 695 nm) of each spectrophotometric curve.

Visual color scores also were assessed by the experimentor or the lab assistant using a scale of 1-6 with one indicating very bright red and 6 indicating very grey-brown. Color photographs in "Beef Color" (Dept. An. Sci., KSU, undated) were used as color standards. The scale was expanded to include scores of 7, 8 and 9 to provide a more exact description of pale samples. For example, the appearance of a sample scored 7, 8 or 9 was similar to that of a sample scored 4, 5 or 6, respectively, but less intensly colored (pale).

After exposure, samples were stored in the dark at -29°C (-20°F) for seven days, then they were re-exposed, and reflectance and visual color were measured again.

pH determination

Triplicate measurements were made on aliquots of meat from each percentage fat level after 20 weeks frozen storage. The frozen meat from each fat level was thawed at 5°C for approximately 18 hr., then ground twice in an electric food grinder. A Beckman pH meter (Model 76) was used to obtain the pH of a slurry prepared from 10 g ground muscle and 50 ml distilled water (Rogers et al., 1967).

Statistical analyses

To analyze effects of exposure to radiant energy, data were divided into three groups. Group 1 data were from samples stored (1 - 7 days) in the dark at -29°C and then exposed to radiant energy for two hours with spectrophotometric readings taken every 30 min. After exposure, the samples from which group 1 data were obtained were stored in the dark for one week, then re-exposed to radiant energy. Group 2 data included spectrophotometric readings and visual scores for those samples during storage and re-exposure (at 30 min. intervals for two hours). Group 3 was composed of data from six readings taken on each unexposed sample held in dark storage at -29°C for two weeks.

Variance (σ^2) among data in each group was calculated for visual scores, percentage reflectance, K/S values, ratios of K/S values, and areas under the spectrophotometric curve.

Bartlett's test for homogeneity of variance was used to determine significant differences between variances for exposure groups in each treatment combination.

Visual scores, percentage reflectance, K/S values, and areas under the spectrophotometric curve (sq cm) for group 3 (unexposed samples) were analyzed by analysis of variance:

Source of Variation	df
Replications	5
Fat level	2
BHA	1
Fat level x BHA	2
Error	25
Sub/sampling	172
Total	207

Two samples from replication V were destroyed accidently after two days' readings had been recorded. Thus, n = 64 for 30% fat and n = 72 for 15 and 45% fat treatment combinations. Because of the unequal numbers, separate LSDs were calculated when F-values attributable to fat level in the meat were significant.

RESULTS AND DISCUSSION

Ether extract and pH

Percentage ether extract and pH for the three ground beef products used in the study are given in Table 3. Ether extract was measured to determine the accuracy of the calculated values for fat and analytical values were close to the calculated percentage of fat in the products. Ground beef for all products was from the same source, so pH was not expected to differ among fat levels. The pH values were those usually found for beef.

Table 3—Ether extract and pH values for ground beef products on the basis of calculated fat level

Calculated fat, approx %	Ether extract, %	pH
15	16.32	5.30
30	30.19	5.35
45	45.50	5.35

Variance within treatments and between exposure groups

Variances for all measurements made on samples from all treatment combinations are shown in Table 4. Data were separated into groups according to the amount of exposure samples received. Group 1 included those samples that were exposed to two hours of radiant energy. Group 2 involved measurements taken on samples after an original two hour exposure. That included readings during storage and a re-exposure period of two hours. Group 3 samples were stored in the dark for two weeks and were not exposed to radiant energy. Only treatment combinations for which variances differed ($P \leq 0.05$) between any two groups will be discussed (Table 4).

Variances for visual scores were small and showed no consistent trends. Group 2 samples with 30% fat without BHA, showed significantly ($P \leq 0.05$) larger variance than groups 1 or 3 containing 30% fat without BHA. In 30% fat with BHA, unexposed samples (group 3) showed less variation ($P \leq 0.05$) than the other groups. However, for 45% fat without BHA, the variance was greatest for unexposed samples (group 3), but significantly greater ($P \leq 0.05$) only for group 1. Mean visual scores for exposed samples after 0 and 2 hrs. and storage plus 2 additional hrs. exposure (577 nm) are given in Table 5. In general, scores increased after samples were re-exposed. Decreased