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Browning of Heated Ground Beef Containing Added Phospholipid and Reducing Sugar

Carolyn McCord
University of Tennessee, Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Carolyn McCord entitled "Browning of Heated Ground Beef Containing Added Phospholipid and Reducing Sugar." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Ada Marie Campbell, Major Professor

We have read this thesis and recommend its acceptance:

Curtis C. Melton, Grayce E. Goertz

Accepted for the Council:
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Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Bruce E. Baker

Accepted for the Council:

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BROWNING OF HEATED GROUND BEEF CONTAINING ADDED PHOSPHOLIPID AND REDUCING SUGAR

A Thesis

Presented for the

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Degree

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Carolyn McCord

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ABSTRACT

Knowledge concerning reactions contributing to browning of cooked ground beef patties and the cooking drip is limited. The possible contribution of carbonyl-amine reaction(s) involving phospholipids and reducing sugar to color of cooked meat and drip was investigated.

Glucose was added at 2 and 4 percent levels to ground beef to which commercial bovine phospholipid had been added. The concentrations of reducing sugar and phospholipid and distribution of phospholipids among the three major classes were determined in raw and cooked meat. Light reflectance determinations were made on the cooked meat surface, and absorbance readings and reducing sugar determinations were made on the drip.

Phospholipid content of raw and cooked meat lipid did not differ significantly. Distribution of phospholipid classes was similar in raw and cooked meat. Drip phospholipids could not be separated by thin layer chromatography. Reducing sugar values for the cooked meat plus drip were less than those of the raw meat. Browning of the meat did not increase with increasing glucose concentrations. Increasing glucose concentration did result in increased browning of the drip. Both caramelization and carbonyl-amine reactions could have contributed to drip browning. A possible role of phospholipids in browning could not be established in the absence of drip phospholipid data.

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CHAPTER I

INTRODUCTION

Color is one criterion used by the consumer to evaluate the quality of cooked meat. The browning of uncured meat during cooking produces desirable changes in color, odor, and flavor. Part of the brown color of cooked meat depends upon the types and amounts of myoglobin derivatives and decomposition products that are present. Brown color in cooked meat may be caused also by changes in other muscle constituents. Pearson et al. (1962, 1966) studied browning in heated pork and found that the carbonyl-amine reaction and caramelization of the natural meat sugars contributed to the brown color. Wasserman and Spinelli (1970) reported that the carbonyl-amine reaction and caramelization contributed to browning in beef diffusate and in model systems simulating the composition of beef muscle tissue.

Research concerning the role of other muscle constituents in browning has been limited. Some muscle phospholipids, for example the cephalins phosphatidylserine and phosphatidylethanolamine, contain free amino groups which can react with aldehydes as do the amino groups of amino acids and proteins to produce brown colors (Lea, 1957). Although phospholipids form approximately 10-40 percent of the total lipid of the muscle, lipid browning reactions of this type have been reported only in dried eggs (Kline et al., 1951) and commercial soybean lecithin (Scholfield and Dutton, 1954). In both products the undesirable browning resulted from a reaction involving phosphatidylethanolamine and glucose.

In reported studies involving meat phospholipids, cephalin appeared to be very reactive. When Nakanishi and Suyama (1969) heated phospholipids from beef liver and muscle with saturated aldehydes, they observed the formation of α, β unsaturated aldehydes. Cephalin was identified by infrared and ultraviolet spectroscopy as the specific phospholipid class reacting with the saturated aldehydes. In studies conducted at The University of Tennessee (Roberts, 1966; Campbell and Turkki, 1967; Lackey, 1973), phospholipids in the drip from cooked ground beef consisted primarily of cephalin. The cooked meat contained a significantly lower cephalin content than raw meat. These results suggested the possibility of a partitioning of phospholipids and/or a reaction of phospholipids with other muscle components during heating.

The purpose of this study was to investigate the relationship between cooked ground beef color and reducing sugar-phospholipid interaction. Bowers et al. (1968) and Pearson et al. (1962) observed that the degree of browning during heating of muscle tissue or water extracts of muscle tissue increased with increasing reducing sugar content. Possible contributions of phospholipids to browning in these studies, however, were not investigated. In formulated meat products, of which glucose is a common ingredient, a browning reaction involving phospholipids and sugars may play a role in color development during heating.

CHAPTER II

REVIEW OF LITERATURE

Phospholipids

Phospholipids are found more abundantly in muscle tissue than in the surrounding adipose tissue (Lea, 1960). The neutral lipids are primarily intercellular, whereas the phospholipids form integral parts of muscle cells. Frequently proteins, carbohydrates, or both are bound to the phospholipids in the cells (Lea, 1957). Many factors have been studied in relation to their effect on muscle phospholipids, including sex, age, muscle location, diet, muscle activity, and species (Bloor, 1940; Nutt, 1963; Kuchmak and Dugan, 1963; Turkki, 1965; Hornstein and Crowe, 1967; Link et al., 1967; Lackey, 1973).

Muscle phospholipid concentration expressed as a percent of total lipid decreases as the concentration of total lipid increases (Callow, 1962; Campbell and Harrill, 1971; Keller and Kinsella, 1973). Expressed as percent of the tissue, the phospholipid content is less variable. Specific tissues have been reported to contain a characteristic phospholipid level and phospholipid composition (Bloor and Snider, 1930; Hornstein and Crowe, 1967; Link et al., 1967).

Several researchers have reported the phospholipid content of raw muscles on a wet weight basis. Hornstein et al. (1961) found the phospholipid content to range from 0.8 to 1.0 percent in beef muscle and from 0.7 to 0.9 percent in pork muscles. Other workers reported slightly

lower values ranging from 0.5 to 0.7 percent of wet weight for beef (Taylor, 1964; Turkki, 1965; Roberts, 1966; Lackey, 1973) and pork (Kuchmak and Dugan, 1963; Campbell and Turkki, 1967). Keller and Kinsella (1973) reported that ground round, ground chuck, and "ground beef" with fat contents representative of retail products contained 0.54, 0.46, and 0.33 percent phospholipid.

Phospholipid classes in raw muscle tissue have been investigated. Hornstein et al. (1961) reported that the phospholipid fraction of beef and pork lipids contained 40-45 percent lecithin, 40-45 percent cephalin, and 10-15 percent sphingomyelin. Kuchmak and Dugan (1963) found the phospholipid composition of pork to be 61, 36, and 3 percent lecithin, cephalin, and sphingomyelin, respectively. Values obtained by Turkki (1965) and Roberts (1966) for beef phospholipids were similar to those reported by Kuchmak and Dugan (1963) for pork.

The method of expressing phospholipid concentration must be considered in assessing the effect of cooking on total phospholipid content of meat. Roberts (1966), working with well-trimmed lean beef, found that little lipid was lost during cooking and the proportion of phospholipid in total lipid was essentially unchanged. In ground beef with a higher fat content (Campbell and Turkki, 1967) considerable neutral lipid was lost into the drip and the phospholipid concentration expressed as percent of total lipid was higher in cooked meat than in raw. In both studies the phospholipid content of beef expressed as percent of the meat weight was higher for the cooked than for the raw meat. Lackey (1973) did not find a significant difference between phospholipid contents of raw and cooked muscle when concentration was expressed as percent of total

lipid. The cooked muscle, however, had a higher phospholipid content than the raw on the basis of percent muscle, either dry or wet weight.

Keller and Kinsella (1973) observed that the manner of cooking, as well as type of ground meat, influenced the heating effect on meat phospholipid expressed on a wet weight basis. "Ground beef" phospholipid content increased with all three methods of cooking, teflon skillet, metal frying pan, and over charcoal. The phospholipid content of ground round increased only when the meat was cooked in a teflon skillet, and that of ground chuck decreased with all three methods of cooking.

Only small amounts of meat phospholipid are lost into the cooking drip (Roberts, 1966; Keller and Kinsella, 1973; Lackey, 1973). Changed proportions of meat phospholipid with cooking, therefore, probably reflect loss of moisture and of neutral lipids.

Information concerning the effect of heating on the relative phospholipid class distribution is limited. Macy et al. (1964) studying the effect of heating lyophilized diffusates from water extracts of beef, pork, and lamb, found that heating increased the cephalin content of diffusates. Cooked ground beef according to Roberts (1966) had a lower cephalin concentration than did the raw meat. Lecithin and sphingomyelin concentrations did not change consistently with cooking. The drip lipid had a higher cephalin content and a lower lecithin content than the corresponding cooked meat. Lackey (1973) observed that cooked ground beef had a higher percent lecithin and lower percent cephalin than raw muscle; no significant difference was found in sphingomyelin concentration in raw and cooked muscle. Drip lipid contained a lower lecithin concentration and higher cephalin concentration

than either raw or cooked muscle. A higher sphingomyelin content was found in the drip than in the cooked muscle. She was unable to show whether differences between raw and cooked meat phospholipids resulted from selective degradative changes or from partitioning. Keller and Kinsella (1973) did not find evidence of preferential degradation of the phospholipid classes during cooking.

Browning in Meat

The role of reducing sugars in browning of heated muscles has been studied by several investigators. Wood (1961) reported that browning in ox muscle extracts and in model systems resulted from the carbonyl-amine reaction. Pearson et al. (1962) found a positive relationship between the amount of reducing sugar present in pork tissue and the optical density of dried pork slurries. Bowers et al. (1968) found that the degree of browning in heated pork increased with increased reducing sugar and lipid in the tissues. According to Pearson et al. (1966) browning in heated pork extracts could be almost eliminated by removing reducing sugars with yeast. Browning in the heated pork extracts was attributed primarily to the carbonyl-amine reaction. Some brown color development, however, resulted from caramelization of the meat sugars. Similar findings were reported by Wasserman and Spinelli (1970) in heated beef diffusate.

Several sugars which can participate in browning occur naturally in meat. Glucose, glucose-6-phosphate, fructose, fructose-6-phosphate, ribose, and ribose-6-phosphate were identified by Wood (1961) as the reducing sugars in fresh ox muscle extract. In the extract from aged ox

muscle, in which autolytic processes had continued, only glucose, fructose, ribose, and traces of sugar phosphates were present. These same sugars were found by Wasserman and Spinelli (1970) in diffusates of water extracts of beef, lamb, and pork. Macy et al. (1964) found that glucose predominated, followed by fructose, ribose, and an unknown sugar. Similar concentrations of the sugars were found in the three species.

The reducing sugars in meat vary with regard to ability to enter into the carbonyl-amine reaction. Ribose-6-phosphate was the most reactive of the sugars found in extracts of fresh ox muscle, followed by ribose (Wood, 1961). Ribose was found to be the most heat labile and glucose the most heat stable in studies with diffusates from beef, pork, and lamb (Macy et al., 1964; Wasserman and Spinelli, 1970). Wasserman and Spinelli (1970) found that the rate of decomposition of ribose, fructose, and glucose in model systems followed the same trend observed with the diffusates. Reducing sugar levels in beef diffusates appeared to be the limiting factor in carbonyl-amine reactions. When excess glucose was added to beef diffusate, free amino acids disappeared completely. The molar ratio of disappearing glucose to amino nitrogen was approximately 4:1.

Some information is available concerning the effect of heating on amino acids. The amino nitrogen compounds from lyophilized diffusates from beef, pork, and lamb were found by Macy et al. (1964) to be similar quantitatively and qualitatively. In all three species taurine, anserine-carnosine, and alanine were the major constituents of unheated samples. Decreases in these amino compounds accounted for 69, 72, and 45 percent of the total loss of amino compounds during heating. Larger

quantities of amino compounds were lost from beef samples than from lamb or pork. In beef diffusate Wasserman and Spinelli (1970) found that taurine, urea, carnosine, and alanine were the amino compounds present in the largest quantities. After thirty minutes of boiling, only minor changes occurred in the amino compounds except for a 60 percent decrease in arginine and 57 percent increase in cysteic acid. Further heating to produce pyrolysis resulted in large changes; there was a 40-60 percent decrease in most of the amino acids and a 77 percent decrease in asparagine-threonine complex. When an amino acid mixture containing these compounds in concentrations similar to those in beef diffusate was heated alone, no brown pigments were formed.

Browning Involving Phospholipids

Browning reactions involving phospholipids and reducing sugars in meat have not been reported. Browning reactions of this type have been reported in commercial soybean lecithin and dried whole eggs. Commercial soybean "lecithin" is a crude mixture of phospholipid, reducing sugars, and oil derived from soybeans. Evidence indicates that the brown color is a product of the carbonyl-amine reaction. Cephalin is believed to be the source of the amino group, and free and bound reducing sugars of the carbonyl group (Scholfield and Dutton, 1954). A similar browning reaction was reported by Kline et al. (1951) in whole dried eggs. The removal of glucose from the eggs before drying eliminated browning and changes in the phospholipid fraction.

CHAPTER III

PROCEDURE

Plan of Study

Commercial bovine phospholipid was added to raw ground beef to increase the phospholipid concentration. Information concerning the approximate level of adipose tissue in the ground beef was obtained from the supplier. The amount of added phospholipid needed to increase the phospholipid level to that of lean bovine muscle was calculated from the regression equation of Campbell and Harrill (1971).

The concentration of reducing sugars in the meat was increased by addition of glucose. Glucose was added to the meat at levels below (0 percent), similar to (2 percent), and above (4 percent) those found in processed meat products. Uniform distribution of the glucose within the meat was accomplished by adding the glucose in solution form.

Similar levels of sucrose in solution form were added to part of the meat. Sucrose, a nonreducing sugar, was added to the meat to compare the roles of caramelization and carbonyl-amine browning in the drip.

Meat patties with added glucose and added sucrose were cooked simultaneously in six replications. A high oven temperature was used because formulated meat products often are processed at high temperatures. Patties were elevated above the pan during cooking to enhance the degree of browning.

Values for percent lipid, percent phospholipid, phospholipid class distribution, reducing sugar content, and lightness index (surface) were

obtained for meat with varying levels of glucose. All of the above determinations except lightness index were made on raw and cooked meat; lightness index was determined only for cooked meat. Percent lipid and reducing sugar concentration were determined for cooking drip from meat with added glucose. Absorbance values were obtained as an indication of color of the cooking drip from meat with added glucose or sucrose.

Selection and Preparation of Meat Samples

Fifty pounds of ground beef were obtained from East Tennessee Packing Company. The meat was ground twice in a Hobart 4552 grinder with a disc having 0.95 cm openings. After the meat was thoroughly mixed in a Leland L-100-DA mixer, 246 g commercial bovine lecithin[1] mixed with 246 g Crystal oil[2] were distributed in the meat. Three pound portions were wrapped in two layers of medium weight aluminum foil. Nitrogen was used for flushing just prior to the final step in closure. The meat was stored at -20°C for 1-5 months.

Cooking

Two packages of the frozen meat were defrosted at 4°C beginning 48 hr prior to each replication. The contents of the two packages were thoroughly mixed and the drip was reincorporated. The meat was divided into three 750 g portions and two 125 g portions. A 0.25 M glucose solution was added at levels of 0, 2, and 4 percent glucose on a wet weight basis to each of the 750 g portions. Additional water was added

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[1]Lecithin, Animal, 60%, Nutritional Biochemicals Corporation; "contaminants" assumed to be primarily cephalin and sphingomyelin.

[2]Hunt-Wesson Foods; used in minimal amount for dispersal of phospholipids.

as needed to keep the total water level constant. Each portion was shaped into six 125 g patties with the use of an 8.4 cm diameter metal ring. The patties were placed on a wire rack elevated 9 cm above the surface of a shallow pan. A meat thermometer was inserted in the center of one patty in each pan.

A 0.13 M sucrose solution was added at levels of 2 and 4 percent sucrose, wet weight basis, to each of the 125 g portions. The level of total added water was kept constant. Each portion was shaped into a 125 g patty and placed on a wire rack elevated 9 cm above the baking pan.

After the patties reached the same temperature (approximately 2°C), they were cooked simultaneously in a Despatch rotating hearth oven at 232°C to an internal temperature of 77°C. Weights of the raw and cooked meat and cooking drip were recorded.

Three randomly selected patties for each level of added glucose were combined for lipid and reducing sugar determinations. The remaining patties were used for color measurements. To facilitate drip removal 60 and 10 ml distilled water were added respectively to the drip from meat with added glucose and the drip from meat with added sucrose. After the drip sample was heated in a water bath until the lipid liquefied, it was mixed for 1 min in a Waring Blendor. The entire drip sample from the meat with added sucrose was used for determining drip color. Samples for lipid, reducing sugar, and drip color determination were removed from drip of meat with added glucose.

Lipid Extraction

Lipids were extracted from 40 g samples of raw and cooked meat and 60 g samples of the cooking drip by Ostrander and Dugan's (1961) modification of the Bligh and Dyer method (1959). The extraction procedure is presented in Appendix A. After extraction the filtrate was transferred into a graduated cylinder and stored at 2°C overnight. The volume of the chloroform extract, the bottom layer, was recorded after distinct separation had occurred. After the contents of the graduated cylinder were transferred into a separatory funnel and the mixture had again separated into two phases, the chloroform layer was drawn into an evaporating flask.

Total Lipid

Three 10 ml portions of the chloroform extract from the raw and three from the cooked samples were pipetted into weighed beakers. Two 25 ml portions from drip samples were treated in the same manner. The chloroform was evaporated under the hood until the sample appeared dry and then in a vacuum desiccator until the sample reached a constant weight. The weight of lipid was recorded and averaged for each sample. Percent total lipid was calculated according to the following equation:

$$\text{Percent lipid}^{3}_{\text{wet weight basis}} = \frac{10\ (\text{ml chloroform extract} \times \text{g lipid in 10 ml})}{\text{g sample extracted}}$$

The remaining chloroform was concentrated in a rotary evaporator to a volume of 15-20 ml. Samples were transferred with 30 ml chloroform

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[3] In the case of drip the multiplier 4 replaced 10 in the numerator because 25 ml of drip were used rather than 10.

into Erlenmeyer flasks; the flasks were flushed with nitrogen, stoppered, and stored at -20°C until phospholipid analyses were performed.

Phospholipid Determination

Percent phospholipid was calculated from phosphorus values determined by the micro-method of Bartlett (1959) as modified by Marinetti (1962). A standard curve was prepared from varying concentrations of monobasic potassium phosphate. A linear relationship was found between 1 and 5 µg of phosphorus. A 0.3 ml portion of the concentrated lipid extract was diluted with chloroform to 10 ml in a volumetric flask. The dilution procedure was established on the basis of preliminary work. Triplicate 0.3 ml samples of the diluted lipid extract from raw and cooked meat, duplicate phosphorus standards, and duplicate water blanks were used for each analysis. Absorbance readings were made on a Bausch and Lomb Spectronic 20 spectrophotometer with a red filter and a red sensitive phototube at a wavelength of 830 nm (Appendix B).

Three 1 ml samples of the concentrated lipid extract were removed at the time of sampling for phosphorus analysis. The 1 ml samples were pipetted into preweighed beakers and dried in the manner described for total lipid. For each sample the weight of the lipid was recorded and used in determining phospholipid concentration as percent of total lipid (Appendix C).

Phospholipid Classes

Separation of neutral lipids from the phospholipids of raw and cooked meat and fractionation of the phospholipids into lecithin, cephalin, and sphingomyelin were accomplished by the thin layer

chromatographic procedure described by Wood and Kinsell (1963). The procedure for preparing the plates is presented in Appendix D. Each channel was spotted with a standard phospholipid solution or with two applications, totaling 10 µl, of concentrated lipid extract. Spotting was done under a stream of nitrogen. The spotted plates were placed in a developing chamber saturated with a chloroform-methanol-water mixture in a ratio of 80:35:5 (v/v/v). After the plates had developed 1 hr, they were air dried for 10 min, sprayed with a 50 percent (v/v) sulfuric acid solution, and heated in an air oven at 180°C for 25 min. Plates were stored in a drying box with desiccant until scanned.

A Photovolt Densitometer with Varicord recorder and integrator was used to scan the plates. Peaks for lecithin, cephalin, and sphingomyelin in samples were identified by comparison with those for standards. Percentage of each phospholipid class was estimated from the pen strokes under each peak in relation to the total pen strokes under the three peaks.

Reducing Sugar Determination

Water extracts were prepared from the raw and cooked meat and the corresponding drip by a modification of the AOAC (1970) method. Sample size was reduced as the percent of added glucose increased; approximately 10, 5, and 2.5 g samples were used respectively for 0, 2, and 4 percent added glucose. Procedure for the extraction is presented in Appendix E.

Reducing sugar determinations were made on the water extracts by a modification of the method described by Nelson (1944). Since the protein had been precipitated during the extraction, this portion of Nelson's

procedure was omitted (Appendix F). A standard curve was prepared from varying concentrations of glucose standard. A linear relationship was found between 0.1 and 0.4 mg glucose. Sample size was determined on the basis of preliminary work. Three samples, two standards, and two blanks were used for each series of samples. Absorbance readings were made on a Bausch and Lomb Spectronic 20 spectrophotometer with a blue sensitive phototube at a wavelength of 500 nm.

Light Reflectance

The Kollmorgan Color Eye readings X, Y, Z, and X' were taken on the top surface of the patties. Three randomly selected patties from meat of each level of added glucose were wrapped in aluminum foil and stored in a styrofoam container to reduce heat and moisture loss prior to color measurement.

Percent reflectance of the patties at 610 (X), 550 (Y), 445 (Z), and 435 nm (X') was measured in relation to a white vitrolite tile standard. A 7.6 x 3.8 x 0.2 cm microscope slide was placed between the aperture and each sample (or standard) prior to each measurement. The readings were converted to CIE chromaticity coordinates x and y and to lightness index ($L = Y^{1/2}$). The x and y values were plotted on a CIE chromaticity diagram to determine dominant wavelength of the sample color.

Drip Color

The degree of color development in the drip was measured on a water extract. Approximately 25 ml of drip from meat with added glucose were filtered through a Büchner funnel covered with Whatman No. 1 filter paper. All of the drip from meat with added sucrose was filtered in the

same manner. A 0.2 ml sample of each filtrate was diluted to 10 ml in a volumetric flask. Absorbance was measured against a distilled water blank on a Bausch and Lomb Spectronic 20 spectrophotometer with a blue sensitive phototube at a wavelength of 375 nm.

Statistical Analysis

Data for percent lipid, percent phospholipid, phospholipid class distribution, lightness index, and drip color were subjected to analysis of variance. The Student-Newman-Keuls Test was applied where significant differences among means were found (Sokal and Rohlf, 1969).

CHAPTER IV

RESULTS AND DISCUSSION

The lipid content of raw and cooked meat and the corresponding drip is shown in Table I. On a wet weight basis the cooked meat had a significantly ($P < 0.05$) higher lipid content than the raw meat. Nutt (1963), Taylor (1964), Roberts (1966), Campbell and Turkki (1967), and Lackey (1973) reported similar findings in percent lipid of raw and cooked muscles.

The phospholipid concentrations in extracted lipid from raw and cooked meat are compared in Table II. The average phospholipid content did not differ significantly between raw and cooked meat lipids. Roberts (1966) and Lackey (1973), working with lean beef samples, reported similar findings. When beef samples with higher lipid concentrations were studied (Campbell and Turkki, 1967), the phospholipid concentrations were higher in the cooked than in raw meat lipids.

The average percent distribution of phospholipids among the three major classes is presented in Table III. The addition of bovine phospholipid to the raw meat slightly altered the proportions of lecithin, cephalin, and sphingomyelin from those normally reported for muscle tissue. The values of 49.4, 27.5, and 22.4 percent for lecithin, cephalin, and sphingomyelin, respectively, were somewhat low for lecithin and cephalin and high for sphingomyelin as compared with those reported by Kuchmak and Dugan (1963), Turkki (1965), and Roberts (1966).

TABLE I

PERCENT LIPID IN RAW AND COOKED GROUND BEEF AND DRIP[1]

	Raw			Cooked			Drip		
	% Added Glucose			% Added Glucose			% Added Glucose		
Replication	0	2	4	0	2	4	0	2	4
1	11.9	11.3	12.2	18.5	16.6	18.4	4.4	3.4	4.4
2	11.5	11.7	11.7	19.6	18.4	17.5	1.8	2.9	3.0
3	11.9	11.8	11.7	19.1	18.4	17.5	2.3	1.7	2.6
4	11.8	11.7	11.3	16.6	18.4	17.2	5.0	5.4	4.9
5	11.6	11.3	11.0	16.7	16.6	16.3	5.0	2.6	1.8
6	11.1	11.6	11.2	17.0	16.5	14.1	4.4	4.0	3.8
Mean[2]	11.6^{b}	11.6^{b}	11.5^{b}	17.9^{a}	17.5^{a}	16.8^{a}	3.8^{c}	3.3^{c}	3.4^{c}
Std. Dev.	0.3	0.2	0.4	1.3	1.0	1.5	1.4	1.3	1.1

[1]Wet weight basis.

[2]Means with different superscripts differ at the $P < 0.05$ level.

TABLE II

PERCENT PHOSPHOLIPID IN EXTRACTED LIPID FROM RAW AND COOKED GROUND BEEF

	Raw			Cooked		
	% Added Glucose			% Added Glucose		
Replication	0	2	4	0	2	4
1	8.08	13.07	8.69	9.08	9.48	9.44
2	8.31	8.00	7.35	8.03	8.66	9.21
3	9.17	7.13	8.26	9.24	8.50	8.28
4	8.46	9.59	8.32	9.02	9.14	9.06
5	8.27	7.78	7.16	9.57	7.69	8.90
6	8.08	8.16	7.94	8.90	8.84	10.03
Mean[1]	8.40	8.96	7.95	8.97	8.72	9.15
Std. Dev.	0.72	4.71	0.35	0.27	0.38	0.34

[1]Differences are not significant at the $P < 0.05$ level.

TABLE III

AVERAGE PERCENT DISTRIBUTION OF PHOSPHOLIPID CLASSES IN COOKED AND RAW GROUND BEEF PHOSPHOLIPID[1,2]

% Added Glucose	Lecithin		Cephalin		Sphingomyelin	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
0	49.4 ± 3.1	52.0 ± 3.8	27.5 ± 2.9	26.4 ± 5.4	22.4 ± 1.1	21.6 ± 3.6
2	50.6 ± 5.2	51.0 ± 5.1	27.3 ± 7.5	28.4 ± 7.7	22.0 ± 4.5	20.7 ± 3.6
4	50.1 ± 6.3	52.4 ± 4.2	25.6 ± 7.0	26.9 ± 7.2	23.8 ± 2.5	20.9 ± 4.3

[1]Mean and standard deviation for 6 replications.

[2]Differences are not significant at the $P < 0.05$ level.

The relative proportions of the phospholipid classes in meat lipids were not significantly affected by cooking (Table III). Roberts (1966) and Lackey (1973) reported a significantly lower cephalin content in cooked than in raw beef lipid, and higher cephalin levels in the drip lipid. Although the phospholipids in the drip were not analyzed in this study, the data for raw and cooked meat suggest no partitioning of phospholipid classes. The possibility of degradative change in drip phospholipids exists.

Reducing sugar values for raw and cooked meat and drip are presented in Table IV. The reducing sugar accounted for in the cooked meat and drip was less than that found in the raw meat at each level, thus indicating that reducing sugar participated in some reaction(s). The fluctuation in percent "recovery" of reducing sugar suggests a possible problem in the analysis. However, when the results for raw meat (to which known amounts of glucose were added) were corrected for added water, the analyses were found to give higher values than the theoretical. The loss of glucose apparently was real.

The dominant wavelength for all patties ranged between 543C and 545C. Lightness index values for the cooked ground beef patties are presented in Table V. Differences in lightness index values among meats with 0, 2, and 4 percent added glucose were not significant. Pearson et al. (1962, 1966) and Bowers et al. (1968) reported that the degree of browning in heated pork increased with increasing reducing sugar concentration in the raw tissue. It should be noted that samples were subjected to more severe heating conditions in the Pearson et al.

TABLE IV

AVERAGE TOTAL REDUCING SUGAR IN RAW AND COOKED GROUND BEEF AND DRIP

	Reducing Sugar (mg)[1]				
% Added Glucose	Raw	Cooked	Drip	Cooked + Drip	% "Recovery"[2]
0	1306	795	457	1,252	94
2 (theor.)[3]	16,093 (15,120)	7,589	6,043	13,632	85
4 (theor.)	29,502 (28,435)	16,321	12,103	28,424	94

[1]Totals for 6 patties and the drip therefrom are averaged for the 6 replications.

[2]Percent of reducing sugar of raw meat accounted for in cooked meat plus drip.

[3]Calculation shown in Appendix G.

TABLE V

LIGHTNESS INDEX VALUES FOR COOKED GROUND BEEF PATTIES[1]

Replication	% Added Glucose		
	0	2	4
1	3.02	3.16	2.79
2	3.75	3.92	3.69
3	3.31	3.76	3.43
4	3.37	3.26	2.86
5	3.20	2.97	3.03
6	3.71	3.28	3.35
Mean[2]	3.39	3.39	3.19
Std. Dev.	0.29	0.37	0.35

[1]Each value is an average of three determinations.

[2]Differences are not significant at the $P < 0.05$ level.

and Bowers et al. studies than in the present study. The relatively large standard deviations in this study may be attributed to the accumulation of drip on the patty surface.

Absorbance values for the cooking drip are shown in Table VI. The degree of browning increased with increased glucose or sucrose concentration in the meat ($P < 0.05$). Pearson et al. (1962, 1966) reported increased absorbance with increased reducing sugar concentration; however, no reports of studies involving browning in drip were found in the literature. Drip from meat with 2 and 4 percent added glucose was significantly ($P < 0.05$) darker than that from meat with the corresponding levels of added sucrose. These data suggest that the carbonyl-amine reaction contributed to browning in the glucose-containing drip. The extent of the carbonyl-amine reaction in the drip containing glucose, however, cannot be determined from absorbance values because glucose caramelization is possible even in the presence of amino compounds (Wasserman and Spinelli, 1970).

If browning of meat and/or drip had increased with increasing glucose concentrations but not with increasing concentrations of sucrose, it could have been assumed that carbonyl-amine reactions were primarily responsible for browning. Increased browning of meat surfaces was not shown, possibly because of the accumulation of drip on the surfaces during cooking and the consequent unevenness of browning. Another factor that interfered with the reflectance measurements was the appearance of surface cracks, apparently because the water that was used for uniform distribution of glucose reduced the cohesiveness of the meat mixture.

Increasing glucose concentration did result in increased browning of the drip but increasing sucrose concentration also increased drip browning. Whereas the browning effect of the nonreducing sugar, sucrose

TABLE VI

ABSORBANCE AT 375 nm OF DRIP FROM COOKED GROUND BEEF WITH VARYING LEVELS OF ADDED SUGAR

Replication	% Added Glucose			% Added Sucrose	
	0	2	4	2	4
1	0.296	0.345	0.348	0.400	0.450
2	0.288	0.303	0.320	0.290	0.298
3	0.203	0.327	0.350	0.295	0.335
4	0.285	0.395	0.520	0.390	0.470
5	0.213	0.295	0.400	0.280	0.350
6	0.232	0.317	0.470	0.279	0.410
Mean[1]	0.253[e]	0.330[c]	0.401[a]	0.322[d]	0.386[b]
Std. Dev.	0.041	0.035	0.078	0.057	0.068

[1]Means with different superscripts differ at the $P < 0.05$ level.

should involve caramelization, that of glucose could involve a combination of caramelization and carbonyl-amine browning. Thus it became necessary to look to changes in meat components for explanation of the browning effects.

It had been hoped that if initial levels of both phospholipid and reducing sugar in the meat were elevated and a moderately severe heat treatment were applied, changes in meat constituents would be measurable. Loss of reducing sugar with cooking is indicated; the reducing sugar accounted for in the cooked meat and drip was less than that found in the raw meat at each level. The fate of the glucose could have involved caramelization, carbonyl-amine reactions involving amino groups of proteins, and/or carbonyl-amine reactions involving amino groups of phospholipids. Phospholipid content of the drip lipid might have provided a clue as to whether the latter type of browning reaction occurred. Unfortunately the phospholipid that was added to the raw meat for the purpose of possibly accentuating the effects of heating and/or some other condition(s) unique to this study resulted in a cooking drip that presented apparently insurmountable analytical difficulties. Separated phospholipids did not appear on the developed thin layer chromatograms. This was true regardless of the level of added glucose.

The results of this study indicate that added glucose resulted in increased browning of the drip; however, the question as to whether phospholipid participated in the browning was not answered. The problems encountered might be avoided in further work by the following procedures:

1. Use of lean muscle tissue comminuted with the desired levels of added glucose. Phospholipid concentration would be relatively high

without the necessity of adding lipid; glucose would be well dispersed without the necessity of adding water.

2. Application of a more severe heat treatment than was used in the present study. Ideally the treatment would be such that browning effects would be exaggerated and there would be one heated product (rather than cooked meat plus drip) to compare analytically with the raw samples.

CHAPTER V

SUMMARY

The degree of browning in ground beef patties and the corresponding drip was studied. Commercial bovine phospholipid and varying concentrations of glucose were added to raw ground beef. Sucrose was added to raw meat from the same lot at the same concentrations as glucose. The meat, shaped in patty form, was cooked in a Despatch rotating hearth oven at 232°C to an internal temperature of 77°C. Values for percent lipid, percent phospholipid, phospholipid class distribution, reducing sugar content, and light reflectance (surface) were obtained for meat with varying levels of glucose. All of the above determinations except lightness index were made on raw and cooked meat; lightness index was determined only for cooked meat. Percent lipid and reducing sugar concentration were determined for cooking drip from meat with added glucose. Absorbance values were obtained as an indication of the color of the cooking drip from meat with added glucose or sucrose.

Significant differences were not found between the phospholipid content of the raw and cooked meat lipid. The distribution of phospholipid classes in raw and cooked samples was not significantly different. Drip phospholipids could not be separated by thin layer chromatography. Surface browning did not differ significantly among cooked samples with 0, 2, and 4 percent added glucose. Browning in the drip increased with increasing concentrations of glucose or sucrose. The drip from meat containing glucose was darker than that from meat containing sucrose.

Some of the reducing sugar in the raw meat was not accounted for in the cooked meat plus drip. Although something apparently happened to the glucose, the relative contribution of carbonyl-amine browning and caramelization to the color of the drip containing glucose could not be established by absorbance values. Whether phospholipids contributed to browning could not be determined because of lack of drip phospholipid data. Further work should involve leaner meat subjected to more severe heating conditions.

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APPENDICES

APPENDIX A

LIPID EXTRACTION[1] (OSTRANDER AND DUGAN, 1961)

1. Transfer weighed sample into blender jar.
2. Wash sample beaker with 130 ml distilled methanol. Transfer the methanol from the beaker into the blender jar and blend 5 min.
3. Add 65 ml distilled chloroform and blend 5 min.
4. Add 65 ml distilled chloroform and blend 20 sec.
5. Add 65 ml distilled water containing 1.5 g zinc acetate and blend 10 sec.
6. Transfer contents of blender to a Büchner funnel covered with Whatman No. 1 filter paper. Filter under suction, maintaining a stream of nitrogen over the funnel.
7. Transfer residue, filter paper, and 1/2 facial tissue used for wiping the funnel into the blender jar. Rise funnel with 100 ml chloroform; add rinse to blender jar.
8. Blend sample 2 1/2 min.
9. Filter sample following procedure used previously. Rinse with 75 ml chloroform.
10. Transfer filtrate into a graduated cylinder. Wash container with 25 ml methanol and add methanol to graduated cylinder.
11. Store at 2°C until two clear phases appear.

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[1]For drip lipid all solvents and zinc acetate were increased by 50 percent.

APPENDIX B

PHOSPHORUS ANALYSIS (MARINETTI, 1962)

1. Three diluted lipid samples, two phosphorus standards, and two blanks are analyzed simultaneously.
2. Place the following in micro-Kjeldahl flasks:

Samples	0.3 ml diluted extract
	0.7 ml demineralized water
	1.2 ml 70% perchloric acid
	2 glass beads
Blanks	1.0 ml demineralized water
	1.2 ml 70% perchloric acid
	2 glass beads
Standards	1.0 ml phosphorus standard[2]
	1.2 ml 70% perchloric acid
	2 glass beads

3. Digest for 30 min on micro-Kjeldahl digestion rack.
4. Cool and add 7 ml demineralized water and 1.5 ml 2.5% ammonium molybdate solution.[3]

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[2]Stock solution is prepared by bringing 5.624 g potassium phosphate dibasic (K_2HPO_4) to 1 liter volume with distilled water. Standard solution is prepared by diluting 5 ml stock solution to 1 liter. The standard phosphorus solution contains 5 µg/ml.

[3]Ammonium molybdate solution is prepared by dissolving 5.0 g $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in 195 ml demineralized water.

5. Mix samples; add 0.2 ml Fiske Subbarow reagent.[4]
6. Heat flasks in boiling water for 7 min.
7. Cool 20 min in cold water. Pour into cuvettes and read absorbance at 830 nm.

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[4]Fiske Subbarow reagent is prepared by combining 3.75 g sodium bisulfite ($NaHSO_3$) with 25 ml demineralized water. Add 0.0635 g 1-NH_2 naphthol-4-sulfonic acid [1,2,4-$H_2N(HO)C_{10}H_5SO_3H$] and 0.125 g anhydrous sodium sulfite (Na_2SO_3). Filter and store at 4°C. Make fresh weekly.

APPENDIX C

CALCULATION OF PERCENT PHOSPHOLIPID IN EXTRACTED LIPID

1. Calculate µg phosphorus (P) in volume of diluted extract used.

$$\mu g \text{ in } 0.3 \text{ ml extract} = \frac{\text{absorbance of sample } (\mu g \text{ P/ml standard})}{\text{absorbance of standard}}$$

2. Convert µg P to µg phospholipid (PL).

$$\mu g \text{ PL in } 0.3 \text{ ml diluted extract} = (\mu g \text{ P})(25)$$

3. Convert to grams phospholipid.

$$g \text{ PL in } 0.3 \text{ ml diluted extract} = \frac{\mu g \text{ PL}}{10^6}$$

4. Calculate g phospholipid per ml concentrated extract.

$$g \text{ PL/ml concentrated extract} = \frac{g \text{ PL/0.3 ml diluted extract}}{0.009^{(5)}}$$

5. Percent phospholipid in total lipid.

$$\% \text{ PL in total lipid} = 100 \left(\frac{g \text{ PL/ml concentrated extract}}{g \text{ lipid/ml extract}}\right)$$

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[5] Dilution factor (see p. 13).

APPENDIX D

PREPARATION OF THIN-LAYER CHROMATOGRAPHY PLATES

(WOOD AND KINSELL, 1963)

1. Acid wash 20 x 20 cm glass plates.
2. Mix 30 g silica gel G and 60 ml distilled water in a mortar. Coat plates with 0.3 mm layer of silica gel with a Camag applicator.
3. Dry plates 10 min.
4. Activate by heating at 120°C for 1 hr.
5. Store plates in a closed container with desiccant. If not used within 48 hr, reactivate by heating at 120°C for 1 hr.
6. Divide area on each plate into 10 channels, each 2 cm wide (marked with a Camag spotting guide in the direction in which the coating was applied).

APPENDIX E

REDUCING SUGAR EXTRACTION (AOAC, 1970)

1. Place weighed sample in 250 ml centrifuge bottle.
2. Add 25 ml petroleum ether, mix thoroughly, decant, and discard supernatant.
3. Repeat with two additional 25 ml portions of petroleum ether.
4. Add 100 ml distilled water, 5 ml freshly prepared zinc acetate solution[6] and 5 ml potassium ferricyanide solution.[7] Stopper tightly and let stand 15 min shaking vigorously several times during this period.
5. Centrifuge 15 min at 1500 x G.
6. Decant supernatant into Büchner funnel covered with Whatman No. 1 filter paper.
7. Add 25 ml freshly prepared washing solution, containing 1 ml zinc acetate solution, 1 ml potassium ferricyanide solution, and 200 ml distilled water, to the residue in the centrifuge bottle. Let stand 10 min shaking vigorously several times during this period.
8. Centrifuge 10 min at 1500 x G.
9. Decant and filter supernatant through the same filter paper.
10. Repeat steps 7, 8, and 9.

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[6]Reagent is prepared by mixing 12 g zinc acetate [$Zn(OAc)_2 \cdot H_2O$] in distilled water and diluting to 100 ml.

[7]Reagent is prepared by mixing 6 g potassium ferricyanide [$K_4Fe(CN_6) \cdot 3H_2O$] in distilled water and diluting to 100 ml.

APPENDIX F

REDUCING SUGAR CONCENTRATION (NELSON, 1944)[8]

1. Pipette sample filtrate into a Folin-Wu blood sugar tube.
2. Add 1 ml distilled water to two tubes to serve as blanks.
3. Add 1 ml glucose standard[9] to each of two tubes.
4. Add 1 ml freshly prepared solution containing 25 parts of copper reagent A[10] to 1 part of copper reagent B[11] to each tube.
5. Heat tubes, covered with marbles, in a boiling water bath for 20 min.
6. Cool tubes in cold water.
7. Add 1 ml arsenomolybdate color reagent[12] to each tube.

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[8]Barium hydroxide precipitation of protein was omitted because zinc acetate was used during extraction.

[9]Glucose stock standard is prepared by dissolving 200 mg anhydrous glucose ($C_6H_{12}O_6$) in distilled water and diluting to 500 ml. Dilutions are made as needed.

[10]Copper reagent A is prepared by dissolving 25 g sodium carbonate (Na_2CO_3), 25 g potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$), 20 g sodium bicarbonate ($NaHCO_3$), and 200 g sodium sulfate (Na_2SO_4) in 800 ml distilled water, diluting to 1 liter, and filtering.

[11]Copper reagent B is prepared by dissolving 15 g cupric sulfate ($CuSO_4 \cdot 5H_2O$) in 85 ml distilled water and adding one drop concentrated sulfuric acid (H_2SO_4).

[12]Arsenomolybdate color reagent is prepared by dissolving 25 g ammonium molybdate [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$] in 450 ml distilled water, adding 21 ml concentrated sulfuric acid (H_2SO_4), mixing, and adding 3 g sodium arsenate, dibasic ($Na_2HAsO_4 \cdot 7H_2O$) dissolved in 25 ml distilled water, mixing, and incubating at 37°C for 24 to 48 hr.

8. Dilute mixture in tubes to 25 ml mark with distilled water.
9. Mix contents in the tubes thoroughly.
10. Pour into cuvettes and read absorbance at 500 nm.

TABLE VII

CALCULATION OF THEORETICAL AMOUNTS OF REDUCING SUGAR IN RAW MEAT WITH ADDED GLUCOSE

% Added Glucose	Mixed[a]	Original Meat in 750 g of Mixture	Reducing Sugar in Original Meat, mg	Reducing Sugar Added, mg	Reducing Sugar, Theoretical, mg
0	750 g meat[b] 48 g water	93.98% or 704.8g	1306 (analytical)	--	--
2	750 g meat 48 g water 15 g glucose	92.25% or 691.9g	1282 $[1306(\frac{691.9}{704.8})]$	13,838 $(0.02 \times 691.9 \times 10^3)$	15,120
4	750 g meat 48 g water 30 g glucose	90.58% or 679.4g	1259 $[1306(\frac{679.4}{704.8})]$	27,176 $(0.04 \times 679.4 \times 10^3)$	28,435

[a]Used 750 g of each mixture.

[b]Added phospholipid is included in meat.

VITA

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