Utah State University

DigitalCommons@USU

All Graduate Theses and Dissertations

Graduate Studies

5-1981

Effect of Atmospheric Oxidation on Bioavailability of Meat Iron and Liver Weights in Rats

Kathleen M. Cardon
Utah State University

Follow this and additional works at: <https://digitalcommons.usu.edu/etd>

Part of the [Food Science Commons](#), and the [Nutrition Commons](#)

Recommended Citation

Cardon, Kathleen M., "Effect of Atmospheric Oxidation on Bioavailability of Meat Iron and Liver Weights in Rats" (1981). *All Graduate Theses and Dissertations*. 5242.

<https://digitalcommons.usu.edu/etd/5242>

This Thesis is brought to you for free and open access by the Graduate Studies at DigitalCommons@USU. It has been accepted for inclusion in All Graduate Theses and Dissertations by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.

EFFECT OF ATMOSPHERIC OXIDATION ON BIOAVAILABILITY OF
MEAT IRON AND LIVER WEIGHTS IN RATS

by

Kathleen M. Cardon

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Nutrition and Food Sciences

UTAH STATE UNIVERSITY
Logan, Utah

1981

ACKNOWLEDGEMENTS

This research project was made possible through financial support from the President's Overhead Fund. I am grateful for this financial assistance.

My sincere appreciation is extended to my major professor, Dr. Arthur Mahoney. His encouragement, assistance, patience, academic guidance and friendship have been invaluable during this scholarly endeavor.

In addition, Dr. Deloy Hendricks, Dr. Von Mendenhall, Dr. Rodney Brown and Dr. Daren Cornforth receive my thanks for their responses to inquiries and for their advice. My fellow graduate students are offered thanks for intellectual comradery and assistance in research.

To my husband, Bart, I offer my love and gratitude. He has supported and sustained me through trials and success during my career as a graduate student as well as through life. To my children, Joe, Steve, David, Peter and John, I give my love and appreciation for their support. To my parents who initially guided me in the wonder of learning, I convey my love.

Kathleen M. Cardon

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	iv
LIST OF FIGURES.	v
ABSTRACT	vi
INTRODUCTION	1
Factors Affecting Absorption and Metabolism of Iron.	1
Effects of Oxidized Lipids on Growth.	3
Mechanism of Oxidation of Lipids.	5
Oxidation of Lipids and Bioavailability of Iron	6
Rationale	7
EXPERIMENT I. EFFECT OF ATMOSPHERIC OXIDATION ON IRON BIOAVAIL- ABILITY OF HAND-DEBONED TURKEY MEAT.	9
Experimental Procedure.	9
Results	15
Discussion.	17
EXPERIMENT II. EFFECT OF ATMOSPHERIC OXIDATION AND FORMALDEHYDE ON IRON BIOAVAILABILITY OF MECHANICALLY DEBONED BEEF	19
Experimental Procedure.	19
Results	22
Discussion.	25
EXPERIMENT III. A COMPARISON OF THE EFFECT OF ATMOSPHERIC OXIDA- TION ON HAND DEBONED AND MECHANICALLY DEBONED LYOPHILIZED TURKEY MEAT AND HAND DEBONED AND MECHANICALLY DEBONED LYOPHILIZED BEEF MEAT	31
Experimental Procedure.	31
Results	33
Discussion.	40
FINAL STATEMENT.	48
REFERENCES	49
APPENDICES	53
Appendix A. Micro Kjeldahl Method.	54
Appendix B. Soxhlet Method	56
Appendix C. 2-Thiobarbituric Acid Method	57
Appendix D. Iodine Number.	58

LIST OF TABLES

Table	Page
1. Vitamin mixture used in diets for iron bioavailability experiments	10
2. Mineral mixture added to diets used in determining iron bioavailability of oxidized turkey meat	13
3. Growth response of anemic rats fed diets containing lyophilized oxidized hand deboned turkey meat for 10 days . .	17
4. Hematinic responses of anemic rats fed diets containing lyophilized oxidized hand deboned turkey meat for 10 days	18
5. Mineral mixture added to diets used in determining iron bioavailability of mechanically deboned oxidized beef.	20
6. Composition of diets in Experiment 2.	21
7. Growth response of anemic male rats fed lyophilized mechanically deboned beef diets or diets containing formaldehyde.	24
8. Hematinic responses of anemic male rats fed lyophilized mechanically deboned beef diets or diets containing formaldehyde.	26
9. Summary of hematinic responses of anemic male rats fed lyophilized mechanically deboned beef diets or diets containing formaldehyde	27
10. Analytical composition of uncooked, ground, lyophilized meat before oxidation	34
11. TBA values (μg malonaldehyde/g) of meat samples before and after oxidation	34
12. Iodine number (calculated as g I_2 absorbed per 100 g sample) of meat samples before and after oxidation.	37
13. Iodine number (calculated as g I_2 absorbed per 100 g fat in sample) of meat samples before and after oxidation	37

LIST OF FIGURES

Figure	Page
1. Relationship of TBA value (expressed as malonaldehyde, mal.) and formaldehyde value to number of hours lyophilized turkey meat was exposed to air	16
2. TBA value (μg malonaldehyde/g) of meat samples at 0 hours and 336 hours oxidation.	35
3. Iodine number (calculated as g I_2 absorbed per 100 g sample) of meat samples before and after oxidation	38
4. Iodine number (calculated as g I_2 absorbed per 100 g fat in sample) of meat samples before and after oxidation. .	39

ABSTRACT

Effect of Atmospheric Oxidation on Bioavailability of
Meat Iron and Liver Weights in Rats

by

Kathleen M. Cardon, Master of Science

Utah State University, 1981

Major Professor: Arthur W. Mahoney
Department: Nutrition and Food Sciences

Iron bioavailability of diets containing oxidized turkey and oxidized beef meat was investigated in two experiments. In both studies male, weanling rats were made anemic by consumption of a low iron diet and bleeding. The experimental diets were prepared so that meat was the only source of iron. Hemoglobin regeneration served as the basis for measuring iron utilization. In experiment 1, lyophilized, uncooked turkey meat was allowed to oxidize at 20-22°C for 0, 48, 96, 144, 216, or 264 hours and then fed to the rats. The length of oxidation time of the turkey meat did not significantly affect the growth of the rats or the bioavailability of the meat iron. In experiment 2, lyophilized, uncooked beef was allowed to oxidize at 20-22°C for 14 days. Anemic rats were fed diets composed of basal, basal + iron, basal + iron + formaldehyde, 100% fresh beef, 75% fresh 25% oxidized beef, 50% fresh-50% oxidized beef, 25% fresh-75% oxidized, 100% oxidized beef and 100% fresh beef + formaldehyde. Rats fed severely oxidized beef diets had decreased body weight, enlarged livers and depressed hematinic responses. Formaldehyde did not significantly affect iron utilization in rats fed basal diets, but

depressed liver iron storage in rats fed fresh beef. A third experiment to further compare the chemical properties of uncooked, lyophilized hand deboned and mechanically deboned beef and turkey meat was performed. Analysis by the Thiobarbituric Acid Method, Formaldehyde Value, and Iodine Number indicated increased sophistication of testing methods for lyophilized meats needs to be developed. Although the Thiobarbituric Acid Method was successfully used in Experiment 1, unreliable results were obtained using this method in Experiment 3. It is assumed that this variability in results is due to using the Thiobarbituric Acid Method with a dry system. The problems encountered with the Formaldehyde Value included contamination of the water supply with aldehydes and unreliable reagent. The Iodine Number was unreliable used on meat samples. Possibly the fat could be extracted from the meat under cool nitrogen to prevent further oxidation of the fat. Then the iodine number could be determined on the extracted fat.

(58 pages)

INTRODUCTION

Factors Affecting Absorption and Metabolism of Iron

The total amount of iron in the diet does not reflect the amount of iron actually absorbed and utilized. The main control of iron absorption is at the site of the intestinal mucosa (Pike and Brown, 1975).

"When the dietary supply of absorbable iron is sufficient, the intestinal mucosa regulates iron absorption in a manner that tends to keep the body iron content consistent" (National Research Council, 1980, p. 138).

However, the mechanisms for regulating iron homeostasis are not known. Erythropoietic rate, iron stores, anemia, and intraluminal chelating and complexing agents influence iron absorption, probably via their effects on the intestinal mucosal cells (VanCampen, 1974).

Furthermore, various iron salts have different relative biological values. Mahoney and Hendricks (1976) found that ferric orthophosphate was 51% as efficient as ferrous sulfate for hemoglobin regeneration in the anemic rat. Also, ferric chloride, ferric orthophosphate, reduced iron, and sodium iron pyrophosphate are ranked in order from most efficient to least efficient in effectiveness of iron retention and hemoglobin regeneration in the anemic rat (Wadell, 1974). Likewise, the iron in various foods differs in its bioavailability. It has been found that the percent efficiency of converting iron in the following diets to hemoglobin is FeSO_4 , 51; egg diet, 45; beef diet, 43; whole wheat flour diet, 33; and enriched flour diet, 24 (Mahoney et al. 1974).

Besides, there are numerous dietary influences upon iron absorption. Hegsted et al. (1949) concluded that the absolute amount of iron and/or phosphorus in the diet as well as the iron-phosphorus ratio influences

the amount of iron absorbed. They concluded that a low level of phosphorus in the diet led to excessive iron stores in the liver. Conversely, addition of phosphorus salts decreased the iron deposited in the liver. In addition, the inclusion of phytate in the diet decreases iron absorption. Bjorn-Rasmussen (1974) concluded that the addition of 7% bran to wheat bread decreased iron absorption by a factor of two. The marked reduction in iron absorption was ascribed to the content of phytate in bran. Layrisse et al. (1968) concluded that the interaction of vegetable with animal food during digestion may change the pattern of iron absorption if these foods were given alone. Further, he suggests that a certain proportion of animal food should be included in the diet to enhance iron absorption from the vegetable food. Cook and Monson (1976) determined that neither protein nor animal protein per se enhances iron absorption. They found that the inclusion of beef, lamb, pork, chicken, liver and fish in the diet increases the rate of non-heme iron absorption. In contrast, milk, cheese and eggs do not increase iron absorption and may decrease iron availability. In addition, the addition of vitamin C to the diet may increase the absorption of non-heme iron by a four fold factor (Bjorn-Rasmussen and Hallberg, 1974; Cook and Monson, 1977). Increased iron absorption and iron deposition in the liver was found when rats were fed a low-protein, high fat diet (Kaufman et al., 1958). Amine and Hegsted (1975) found that diets high in fat favored iron utilization. In fact, iron absorption was greater in diets in which the fat was supplied as coconut oil rather than corn oil. Hemoglobin concentration was used as the criteria for iron utilization. Conversely, Farmer (1977) found that high fat diets decrease iron absorption when iron

bioavailability data is interpreted on the basis of hemoglobin regeneration. When the data is interpreted on the basis of percent efficiency in converting dietary iron into hemoglobin, the effect of lipid source and level on iron utilization was found to be negligible.

Hence, the amount of metabolizable iron is dependent upon the organism's present supply of iron, the amount of iron present in the diet, the efficiency of utilization of the iron source, and numerous dietary factors.

Effects of Oxidized Lipids on Growth

The consumption of oxidized fats by animals has been reported to have various physiological effects (Kaunitz et al., 1965; Kaunitz et al., 1966). Consumption of oxidized fats by rats has affected longevity. Andrews et al. (1956) showed that rats consuming a severely oxidized soybean oil diet (1200 peroxide number) died within approximately three weeks. Kaunitz et al. (1955) demonstrated that rats fed mildly oxidized cottonseed oil, chicken fat and beef fat in a long term nutritional study showed a higher death rate than those fed the corresponding fresh fats. Kaunitz et al. (1956) continued their studies and discovered that feeding mildly heated and aerated butter and lard did not significantly reduce the life span of rats. With similarly treated soybean and corn oils, survival was as good as with fresh animal fat. In contrast, feeding fresh vegetable oils led to significantly shorter life spans.

In addition, oxidized fat in the diet has been reported to reduce the rate of growth. Nolen et al. (1967) fed rats diets consisting of 15% fat which had been used for frying until it was unfit for further use. In a two year study the rats fed the oxidized fat had slower

growth rates than rats fed fresh fat diets. However, there were no clinical, metabolic or pathological criteria to suggest that the oxidized fats adversely affected the rats consuming them. Nolen (1973) also reported decreased growth in dogs when hydrogenated soybean oil was used for deep fat frying under commercial conditions until it became unfit for further use. Nakamura et al. (1973) found that autoxidized safflower oil administered daily by intubation to rats caused reduced growth.

Enlarged livers are commonly reported in animals fed oxidized fat. Kaunitz et al. (1955) found enlarged livers in rats fed refined cottonseed oil which had been aerated and heated to 95°C for 200-300 hours. A diet containing 10% rancid soybean oil (peroxide number 530-550) supported almost normal growth in rats, although they did have enlarged livers reported Greenberg and Frazer (1953). Likewise, Miller and Landes (1975) reported enlarged livers in rats fed oxidized or heated soybean oil. In addition, Andia and Street (1975) have reported enlarged livers in rats fed thermally oxidized fat.

When autoxidized safflower oils were fed to rats, Nakamura et al. (1973) found no significant changes in the liver, but found marked changes in the endoplasmic reticulum. Also, activity of the enzymes serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase were increased. Furthermore, Andia and Street (1975) reported that rats fed thermally oxidized fats had increased smooth endoplasmic reticulum proliferation plus an induction of complex microsomal enzymes in the livers.

Mechanism of Oxidation of Lipids

Fats may become rancid as a result of lipolysis, a reaction wherein the ester linkages of lipids are subject to hydrolysis resulting from enzymes, from thermal stress, or from chemical action. Fats may also become rancid as a result of oxidation. Oxidation usually proceeds through a free radical chain reaction mechanism which involves (1) initiation, formation of the free radical, (2) propagation, free radical chain reaction and (3) termination, formation of nonradical products. The major initial products of reactions between fatty acids and oxygen are peroxides. Initially an unsaturated hydrocarbon loses a hydrogen to form a radical, $RH \rightarrow R\cdot + H\cdot$. Then oxygen adds at the double bond to form a diradical. During propagation, the chain reaction continues as follows:

The result is the formation of peroxy radicals, hydroperoxides, and new hydrocarbon radicals. Termination occurs when two radicals interact.

Hydroperoxides are easily decomposed by a number of factors including high energy radiation, thermal energy, metal catalysis, and enzyme activity. Iron may act as a metal prooxidant in hydroperoxide decomposition which results in the formation of additional radicals. The

reaction which takes place is:

Hydroperoxides decompose easily in the presence of multivalent metal ions such as iron to form both $\text{RO}\cdot$ and $\text{ROO}\cdot$ radicals as the metal ions undergo oxidation-reduction.

Heme compounds have been attributed to being oxidation catalysts. The heme compounds may initiate peroxidation or may function as peroxide decomposers. However, when heme compounds are present at two to four times the amount needed for the most effective oxidation, the heme compounds exert an antioxidant effect and no oxidation takes place (Fennema, 1976).

Another aspect of lipid oxidation and metal catalysts which is of importance is water activity. Water activity can influence the rate of reaction in a lipid phase. With a decrease in water activity, the rate of oxidation first decreases. However, with a further decrease of water activity, the rate of oxidation increases. This particular effect is attributed to several factors, among them changes in hydration of trace metal catalysts which become more active as they lose water (Labuza, 1980).

Oxidation of Lipids and Bioavailability of Iron

A great deal of research has been done on factors affecting absorption and metabolism of iron. Also, several research papers have been published on the growth retardation effects of including oxidized

lipids in the diet. However, very little information exists on the effects of oxidation on iron bioavailability. "Cotton-fur" syndrome in mink fed diets of Pacific hake (Merluccius productus) is due to an induced iron deficiency anemia that is responsive to intramuscular iron supplementation (Stout et al., 1960a). The iron deficiency could be prevented by cooking the hake thoroughly (Stout et al., 1960b). The flesh of hake may contain 15-300 ppm of formaldehyde depending on the tissue assayed and the duration of the storage (Mendenhall, 1970; Amano and Yamada, 1964). It has been shown that formaldehyde accumulates in some meats as oxidation takes place (Andrews et al., 1977). Miller and Landes (1975) reported that rats fed diets high in oxidized or damaged oil absorbed and utilized iron for hemoglobin synthesis less efficiently than rats fed diets containing fresh oil.

Rationale

Iron deficiency anemia is recognized as a major nutritional disorder particularly among infants, children and women during the female reproductive period and during pregnancy (AMA, 1968; National Research Council, 1980). As the prevalence of this disorder is so wide spread among several populations, it is important to ascertain any factors which may lead to this disorder. A few studies have implicated oxidized fats (Stout et al., 1960a; Stout et al., 1960b; Miller and Landes, 1975) as causing anemia or decreased hemoglobin synthesis. Hence, it was decided to further investigate this possibility.

Freeze drying or lyophilizing food allows a system wherein reaction rates are low and structural changes are minor. Due to the decreased moisture the food could be exposed to air for the oxidation period without

contamination from bacterial growth. Further, in using meat as the product tested, both factors to be tested were present; that is, iron and fat.

EXPERIMENT I
EFFECT OF ATMOSPHERIC OXIDATION ON IRON BIOAVAILABILITY
OF HAND-DEBONED TURKEY MEAT

Experimental Procedure

Meat Preparation

Meat was gleaned from turkey frames by hand, ground, and lyophilized. The lyophilized, uncooked, turkey meat was spread evenly to a depth of approximately 5 mm on plastic trays and allowed to oxidize at 20-22°C for 0, 48, 96, 144, 216 or 264 hours. After portions of turkey meat were exposed to air for the specified number of hours, they were vacuum packed and stored at -40°C until the experimental diets were prepared.

Preparation of Diets

A low-iron diet and six experimental diets were prepared using stainless steel equipment which was cleaned and rinsed with deionized water before each diet was prepared. The diet ingredients were mixed in an electric mixer with an epoxy coated bowl and paddle.

The low-iron diet prepared to aid in developing iron deficiency contained (in g/kg diet): casein, 327g; dextrose, 418 g; rendered turkey fat, 170 g; α -cellulose, 25g; vitamin mixture, 20 g (Table 1); and mineral mixture, 40 g (Table 2). The quantity of casein used was based upon the assumption that casein contains 85% protein. All ingredients were weighed to the nearest gram.

Six experimental diets were prepared, each differing from the others only in terms of the number of hours the meat was exposed to the air. The diets were formulated to contain 27.9% protein, 18.1%

Table 1. Vitamin mixture¹ used in diets for iron bioavailability experiments

Vitamin	g/kg mixture ²	mg/kg diet
Vitamin A concentrate (200,000 IU retinyl acetate/g)	4.5	90
Vitamin D concentrate (400,000 IU calciferol/g)	0.25	5
Alpha-tocopherol	5.0	100
Ascorbic acid	45.0	900
Inositol	5.0	100
Choline Chloride	75.0	1500
Menadione	2.25	45
p-Aminobenzoic acid	5.0	100
Niacin	4.5	90
Riboflavin	1.0	20
Pyridoxine hydrochloride	1.0	20
Thiamine hydrochloride	1.0	20
Calcium pantothenate	3.0	60
Biotin	0.020	0.4
Folic acid	0.090	1.8
Vitamin B ₁₂	0.00135	0.27

¹Vitamin Diet Fortification Mixture supplied by Nutritional Biochemicals Corporation, Cleveland, Ohio.

²These amounts were made up to 1 kg with dextrose.

fat and 20.1 ppm iron. The meat was the sole source of iron. These diets contained (in g/kg) lyophilized turkey meat, 473 g; dextrose, 442 g; α -cellulose, 25 g; vitamin mixture, 20 g; and mineral mixture, 40 g. The vitamin and mineral mixtures were identical to those used in the low-iron diet. All diets were stored at refrigeration temperatures.

Animals and Animal Care

Sixty weanling, male Sprague Dawley rats (obtained from Simonsen Laboratories, Gilroy, California) were housed individually in stainless steel cages in a temperature controlled room. The rats were fed ad libitum the low-iron casein diet (11.6 ppm Fe) for 14 days. About 0.7 cc blood was taken by capillary tube from the retro-ocular capillary bed on the third and eighth days to increase the anemic condition.

On the fourteenth day the rats were weighed and hemoglobin measurements taken. The rats were then assigned to six groups of ten each so that mean hemoglobin concentration and mean body weights were similar; 4.60-4.76 g/dl and 68-74 g, respectively. Eight to ten grams of the experimental diets were fed daily to each rat for 10 days according to the following schedule: 8 g on day 1, 10 g on days 2-3, 9 g on days 4-7, and 8 g on days 8-10. The feed schedule was adjusted to minimize the amount of unconsumed diet. The diet allowance was weighed out daily and any remaining diet in food cups weighed and subtracted from the daily intake. Demineralized water was allowed ad libitum during the entire experiment.

At the conclusion of the 10-day experimental feeding period, body weights and hemoglobin values were again measured. The rats were killed by decapitation and the livers and spleens weighed. The livers were

Table 2. Mineral mixture added to diets used in determining iron bio-availability of oxidized turkey meat

Salt	g salt/kg mixture	Mineral	mg mineral/kg diet
CaHPO ₄ · 2H ₂ O	610.75	Ca	5,690
		P	4,397
KCl	105.15	K	2,206
NaCl	38.125	Na	600
MgCO ₃	34.675	Mg	400
MnSO ₄ · H ₂ O	4.275	Mn	55.6
ZnSO ₄ · 7H ₂ O	1.25	Zn	11.4
CuSO ₄ · 5H ₂ O	0.55	Cu	5.6
KI	0.0055	I	0.17
Na ₂ SeO ₃	0.0023	Se	0.04

individually packaged in cellophane, labeled and frozen for later iron analysis.

Analytical

The micro-Kjeldahl method was used for determining the nitrogen content of the meat (Appendix A). Crude protein was then determined by assuming that protein contains 16% nitrogen. Hence, the percent nitrogen is multiplied by a factor of 6.25 to determine the crude protein. The lipid content was analyzed using the Soxhlet method (Appendix B). Meats, diets, and livers were charred in porcelain crucibles and then ashed at 500-600°C for 24 hours. The ash residue of each was solubilized with 10 ml 6N HCl, heated to boiling, transferred to acid washed plastic bottles, and diluted to 25 ml with demineralized water. These samples were analyzed for iron content by atomic absorption spectrophotometry

at 2483 Å⁰ using an air-acetylene flame. Triplicate samples were used in all the above determinations with the exception of the livers. The oxidized turkey meat was analyzed for malonaldehyde using the 2-Thiobarbituric Acid Method (Sinnhuber and Yu, 1958) (see Appendix C). In addition, the oxidized turkey meat was analyzed for formaldehyde (Andrews et al., 1977) (see Appendix D). The above chemical analysis were done in duplicate.

Hemoglobin (Hb) concentrations were measured in duplicate samples of fresh blood using the cyanmethemoglobin method of Crosby et al. (1954). Hemoglobin regeneration was calculated in four ways: (1) hemoglobin gain (g/dl) is computed as the difference between final and initial hemoglobin concentrations; (2) hemoglobin gain (g) is the difference between final and initial hemoglobin levels. Final and initial hemoglobin levels are calculated assuming that 6.7% of the body weight reflects blood volume. Hence, hemoglobin (g) is calculated by multiplying 6.7%, the body weight and the hemoglobin concentration; (3) hemoglobin iron gain (mg) is calculated based on the assumption that the iron concentration of hemoglobin is 3.35 mg/g. The hemoglobin iron gain (mg) is therefore the product of hemoglobin gain (g) and 3.35 per cent iron; (4) hemoglobin iron gain per unit iron intake (mg/mg) is the hemoglobin iron gain (mg) divided by the food iron consumption (mg) (Mahoney et al., 1974).

Statistical Analysis

The data were analyzed by analysis of variance. When treatment "F" was statistically significant ($P < .05$), least significant difference (LSD) values were calculated (Carmer and Swanson, 1973).

Results

Meat Composition

The lyophilized meat (less than 5% moisture) contained 53 mg Fe, 383 g fat and 589 g protein per kg.

The turkey meat diets contained approximately 30 ppm Fe (Tables 3 and 4). (Every attempt was made to prevent iron contamination during the preparation of the diets. However, the analyzed values, approximately 30 ppm Fe, exceeded the formulated value, 20.1 ppm Fe.)

The thiobarbituric acid (TBA) and formaldehyde values for the turkey meat are plotted in Figure 1. The relationship between oxidation time in hours (X) and TBA value (Y) was $Y = 0.0729 X + 10.27$; $r = 0.97$. The relationship between oxidation time (X) and formaldehyde value (Y) was $Y = 0.0690 X + 1.6428$; $r = 0.9997$.

Animal Responses

The growth responses of the anemic rats fed the lyophilized turkey meat which had been allowed to be oxidized for varying lengths of time are reported in Table 3. Body weight gains and liver weights did not differ significantly among groups. Although significant ($P < 0.05$) differences among treatments were found for spleen weights and liver as a percent of body weight, the differences were random in relationship to the dietary variables.

The hematinic responses of the rats are shown in Table 4. There were no significant ($P < 0.05$) differences among groups except in liver iron (μg). Again the differences were random in relationship to the dietary variables.

Figure 1. Relationship of TBA value (expressed as malonaldehyde, mal.) and formaldehyde value to number of hours lyophilized turkey meat was exposed to air

Table 3. Growth response of anemic rats fed diets containing lyophilized oxidized hand deboned turkey meat for 10 days

	Oxidation Time (Hours)						LSD 05/01
	0	48	96	144	216	264	
Dietary Fe, $\mu\text{g/g}$ ¹	30.4	30.7	30.3	29.8	29.2	31.2	--
No. of rats	10	10	10	10	9 ²	10	--
BW initial, g	70	68	70	70	74	68	NS
BW gain, g	29	30	27	29	26	27	NS
Liver, g	2.99	2.94	2.80	2.95	3.03	3.02	NS
Liver, % of BW	3.0	3.0	2.9	3.1	3.0	3.2	0.2/0.3
Spleen, g	0.40	0.41	0.38	0.45	0.30	0.36	.08/.11

¹Analyzed values.

²One rat in this diet group died within 24 hours of receiving the experimental diet.

Discussion

Miller and Landes (1975) reported rats fed diets high in oxidized soybean oil absorbed and utilized iron for hemoglobin synthesis less efficiently than rats fed fresh oil. Stout et al. (1960a, 1960b) have associated iron deficiency with formaldehyde in feed in mink. It has been shown that formaldehyde accumulates in some meats as oxidation takes place (Andrews et al., 1977). However the results of this experiment do not confirm a relationship between iron deficiency and oxidized turkey meat. The varying degrees of oxidation of the lyophilized, uncooked turkey meat did not affect either animal growth or bioavailability of meat iron for the rats in this experiment.

Table 4. Hematinic responses of anemic rats fed diets containing lyophilized oxidized hand deboned turkey meat for 10 days

	Oxidation Time (Hours)						LSD 05/01
	0	48	96	144	216	264	
Dietary Fe, $\mu\text{g/g}$ ¹	30.4	30.7	30.3	29.8	29.2	31.2	--
No. of rats	10	10	10	10	9 ²	10	--
Fe intake, mg	2.61	2.64	2.64	2.64	2.65	2.64	NS
Hb initial, g/dl	4.65	4.64	4.63	4.67	4.76	4.60	NS
Hb gain, g/dl	3.77	3.73	3.69	3.68	4.23	3.74	NS
Hb-Fe gain, mg	1.13	1.12	1.08	1.13	1.22	1.08	NS
Hb-Fe/Fe intake, mg/mg x 100	43	42	41	43	46	41	NS
Liver Fe, $\mu\text{g/g}$	33.8	34.9	33.9	37.3	39.4	37.0	NS
Liver Fe, μg	103	102	95	112	119	112	12/16

¹Analyzed values.

²One rat in this diet group died within 24 hours of receiving the experimental diet.

EXPERIMENT II
EFFECT OF ATMOSPHERIC OXIDATION AND FORMALDEHYDE ON IRON
BIOAVAILABILITY OF MECHANICALLY DEBONED BEEF

Experimental Procedure

Meat Preparation

Mechanically deboned uncooked beef was lyophilized. A portion of this meat was frozen until the diets were formulated. An additional portion of mechanically deboned, lyophilized, uncooked beef was spread evenly to a depth of approximately 5 mm on plastic trays and allowed to oxidize at 20-22°C for 14 days. This meat was then used in the preparation of the experimental diets.

Preparation of Diets

A low-iron diet and thirteen experimental diets were prepared. Stainless steel equipment which was cleaned and rinsed with deionized water was utilized in the preparation of the diets. The diet ingredients were mixed in an electric mixer with an epoxy coated bowl and paddle. Ingredients were measured to the nearest gram.

A low-iron diet was prepared to again aid in developing iron deficiency. It contained (in g/kg diet): casein, 200 g; dextrose, 485 g; rendered beef fat, 200 g; α -cellulose, 25 g; vitamin mixture, 20 g (Table 1); NaHPO_4 , 42.8 g; CaCO_3 , 15.6 g; and trace mineral mixture, 11.6 g (Table 5). The experimental diets were prepared, differing in percentages of fresh meat, oxidized meat, casein (basal) diet, FeSO_4 and formaldehyde (Table 6).

Table 5. Mineral mixture added to diets used in determining iron bioavailability of mechanically deboned oxidized beef

Salt	g salt/kg mixture ¹	Mineral	mg mineral/kg diet
KCl	296.9	K	1,806
MgCO ₃	121.0	Mg	404
ZnSO ₄ ·7H ₂ O	38.0	Zn	100
MnSO ₄ ·H ₂ O	12.7	Mn	47.9
CuSO ₄	1.0	Cu	3.0
KI	0.8	I	2.2
CoCl ₂ ·6H ₂ O	0.7	Co	2.01

¹Dextrose was added to bring the total volume to 1 kg.

Animals and Animal Care

A total of 126 weanling, male Sprague Dawley rats (obtained from Simonsen Laboratories, Gilroy, California) were housed individually in stainless steel cages in a temperature controlled room. The rats were fed the low-iron casein diet (12.8 ppm Fe) ad libitum for ten days. About 0.7 cc blood was taken by capillary tube from the retro-ocular capillary bed on the third and eighth days to increase the anemic condition.

On day 10 the rats were weighed and hemoglobin measures taken. Then the rats were assigned to 14 groups of nine each so that mean hemoglobin concentration and mean body weight were similar. The experimental diets were fed to the rats according to the following schedule: 8 g on day 1, 7 g on day 2, 6 g on days 3-10. Any food not consumed was weighed back daily. The total diet consumed was calculated and recorded. Demineralized water was allowed ad libitum

during both the low-iron casein diet and the experimental diets.

Table 6. Composition of diets in experiment 2

Diet	Fe Added ¹	CH ₂ O Added ²	Fe ppm ³
Basal (casein)	-	-	12.8
Basal	10 ppm	-	23.6
Basal	20 ppm	-	35.2
Basal	20 ppm	40 ppm	32.3
Basal	20 ppm	80 ppm	30.3
Basal	40 ppm	-	48.2
Basal	40 ppm	80 ppm	49.1
100% fresh beef	-	-	26.0
75% fresh beef 25% oxidized beef	-	-	24.1
50% fresh beef 50% oxidized beef	-	-	23.7
25% fresh beef 75% oxidized beef	-	-	23.6
100% oxidized beef	-	-	22.6
100% fresh beef	-	40 ppm	25.8
100% fresh beef	-	80 ppm	26.0

¹Iron added as FeSO₄·7 H₂O.

²Formaldehyde

³Iron content by analysis

Body weights and hemoglobin values were again measured at the conclusion of the ten day experimental feeding period. The rats were killed by decapitation. The livers were weighed, individually packaged in cellophane, labelled, and frozen for later iron analysis.

Analytical

The lipid content of the meat was determined by using the Soxhlet method (Appendix B). Meats, diets, and livers were charred in porcelain crucibles and then ashed at 500-600°C for 24 hours. The ash residue of each was solubilized with 10 ml 6N HCl, heated to boiling, transferred to acid washed plastic bottles, and diluted to 25 ml with demineralized water. These samples were analyzed for iron content by atomic absorption spectrophotometry at 2483 Å using an air-acetylene flame. Triplicate determinations were analyzed on meat and diet samples.

Hemoglobin (Hb) concentrations were measured in duplicate samples of fresh blood using the cyanmethemoglobin method (Crosby et al., 1954). The amount of iron gained as hemoglobin was calculated assuming that 6.7% of the body weight reflects blood volume and that the iron concentration of hemoglobin is 3.35 mg/g.

Statistical Analysis

The data were analyzed by analysis of variance. When treatment "F" was statistically significant ($P < 0.05$), least significant difference (LSD) values were calculated (Carmer and Swanson, 1973).

Results

Meat Composition

The lyophilized fresh mechanically deboned beef (less than 5% moisture) contained 56 mg Fe and 598 g fat per kg. The lyophilized oxidized beef contained 55 mg Fe and 573 g fat per kg.

Animal Responses

The body weight gain and the liver weights of the anemic rats fed

the lyophilized oxidized beef diets and the diets containing formaldehyde are shown in Table 7. The body weight gain increased significantly ($P < 0.05$) with the addition of iron to the basal diet (groups 1-3, 6). However, the addition of formaldehyde to the basal diet did not significantly affect body weight gain (groups 3-7). The body weight gain was decreased ($P < 0.01$) as oxidized beef was combined with fresh beef in increasing proportions (groups 8-12). Although the addition of formaldehyde to the fresh beef did cause a decrease in body weight, the decrease was not statistically significant (groups 8, 13, 14).

Liver size increased ($P < 0.05$) as the proportion of oxidized beef in the diet increased (groups 8-11) with the exception of the rats in group 12 fed the 100% oxidized beef diet (Table 7). The animals in this diet group had livers that did not differ significantly in weight from the animals fed the 100% fresh beef diet. However, the animals fed the 100% oxidized beef diet (group 12) lost an average of 14.4 g of body weight during the experiment. When liver size expressed as percent body weight is used as the criteria for an enlarged liver, the addition of oxidized beef to fresh beef in the diet resulted in significantly ($P < 0.01$) larger livers, including the rats fed the 100% oxidized beef diet. The addition of formaldehyde to the fresh beef diets (groups 8, 13, 14) resulted in a decrease ($P < 0.05$) in liver size. When liver size was calculated as percent of body weight, there was likewise a significant decrease in size. However, the pattern of decreased liver size did not continue as the amount of formaldehyde was increased. The addition of formaldehyde to the basal diets (groups 3-7) did not usually significantly alter the liver size. If significance occurred, no pattern to match the experimental design was evident.

Table 7. Growth response of anemic male rats fed lyophilized mechanically deboned beef diets or diets containing formaldehyde

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	LSD .05/.01 ²
	Basal (Casein)	Basal + 10 ppm Fe	Basal + 20 ppm Fe	Basal + 20 ppm Fe + 40 ppm CH ₂ O ¹	Basal + 20 ppm Fe + 80 ppm CH ₂ O	Basal + 40 ppm Fe	Basal + 40 ppm Fe + 80 ppm CH ₂ O	100% Fresh Meat	75% Fresh Meat 25% Oxidized	50% Fresh 50% Oxidized	25% Fresh 75% Oxidized	100% Oxidized	100% Fresh + 40 ppm CH ₂ O	100% Fresh + 80 ppm CH ₂ O	
Dietary Fe, µg/g ³	12.8	23.6	35.2	32.3	30.3	48.2	49.1	26.0	24.1	23.7	23.6	22.6	25.8	26.0	
No. of rats	9	9	9	9	9	9	9	9	9	9	9	9	9	9	
BW initial, g	91.1	87.9	88.4	86.0	85.4	90.4	88.1	86.6	87.6	88.7	85.4	88.0	86.7	86.2	NS
BW gain, g	7.2	9.9	14.4	13.7	11.1	13.6	14.5	14.3	15.3	8.2	0	-14.4	15.9	10.2	5.7/7/6
Liver, g	3.2	3.0	3.1	3.2	3.0	3.0	3.0	3.0	3.2	3.4	3.4	2.8	2.8	2.6	0.3/0.4
Liver, % of BW	3.3	3.1	3.0	3.2	3.1	2.9	2.9	3.0	3.2	3.5	3.9	3.7	2.7	2.8	0.2/0.3

¹Formaldehyde.

²Mean differences must exceed the values indicated to be significantly different at the 5% or 1% levels of probability.

³Analyzed values.

The hematinic responses of the rats fed the lyophilized oxidized beef diets or the diets containing formaldehyde are shown in Table 8 and summarized in Table 9. The gain in hemoglobin concentration (g/dl) was not significantly affected as oxidized beef was added to fresh beef (groups 8-12, see Tables 8 and 9). However, the iron gained as hemoglobin (Hb-Fe, mg) decreased ($P < 0.01$) as oxidized beef replaced fresh beef in the diet. In addition, the efficiency of converting dietary iron to hemoglobin iron (Hb-Fe/Fe intake, mg/mg $\times 100$) decreased ($P < 0.05$) as oxidized beef replaced fresh beef in the diet. The liver iron content was not significantly affected in a consistent pattern as oxidized beef replaced fresh beef.

When formaldehyde was added to the fresh beef (groups 8, 13, 14), the gain in hemoglobin, the gain in hemoglobin concentration and the efficiency of conversion of food iron to hemoglobin iron were not significantly affected (Tables 8 and 9). However, liver iron concentration decreased significantly ($P < 0.05$) as did total liver iron ($P < 0.01$).

When iron was added to the basal diet (groups 1-3, 6), the hematinic responses were increased ($P < 0.01$) (Tables 8 and 9). When formaldehyde was added to the basal diet (groups 3-7), the hematinic responses were not significantly affected.

Discussion

Reduced weight gains were observed only in those rats fed severely oxidized beef (Table 7, groups 10-12). Miller and Landes (1975) and Greenberg and Fraser (1953) reported that rats fed severely oxidized soybean oil had reduced weight gains. Both groups of researchers also noted that relative liver weights were increased when severely

Table 8. Hematinic responses of anemic male rats fed lyophilized mechanically deboned beef diets or diets containing formaldehyde

Group	Basal (Casein)	Basal + 10 ppm Fe	Basal + 20 ppm Fe	Basal + 20 ppm Fe + 40 ppm CH ₂ O ¹	Basal + 20 ppm Fe + 80 ppm CH ₂ O	Basal + 40 ppm Fe	Basal + 40 ppm Fe + 80 ppm CH ₂ O	100% Fresh Meat	75% Fresh Meat 25% Oxidized	50% Fresh 50% Oxidized	25% Fresh 75% Oxidized	100% Oxidized	100% Fresh + 40 ppm CH ₂ O	100 Fresh + 80 ppm CH ₂ O	LSD .05/.01 ²
Dietary Fe, µg/g ³	12.8	23.6	35.2	32.3	30.3	48.2	49.1	26.0	24.1	23.7	23.6	22.6	25.8	26.0	
No. of rats	9	9	9	9	9	9	9	9	9	9	9	9	9	9	
Fe intake, mg	.73	1.43	2.12	1.99	1.83	3.00	2.96	1.69	1.54	1.48	1.38	1.16	1.68	1.64	0.10/0.14
Hb initial, g/dl	5.27	5.45	5.65	5.33	5.32	5.35	5.35	5.58	5.50	5.47	5.67	5.33	5.32	5.28	NS
Hb gain, g/dl	1.78	2.97	4.99	5.86	5.60	7.80	8.09	2.88	3.00	2.84	2.77	2.93	2.93	2.89	0.82/1.09
Hb/Fe gain, mg	0.48	0.78	1.36	1.47	1.34	1.98	2.05	0.83	0.88	0.72	0.54	0.35	0.87	0.76	0.21/0.27
Hb-Fe/Fe intake, mg/mg X 100	66.6	54.1	61.1	73.7	73.4	65.9	69.3	49.3	56.8	48.8	38.6	32.4	51.9	38.5	13.1/17.5
Liver Fe, µg/g	44	51	64	67	55	67	64	67	52	53	57	60	59	55	11.0/15.0
Liver Fe, µg	138	154	199	208	169	198	193	204	162	176	190	168	180	149	36/47

¹Formaldehyde.

²Mean differences must exceed the values indicated to be significantly different at the 5% or 1% levels of probability.

³Analyzed values.

Table 9. Summary of hematinic responses of anemic male rats fed lyophilized mechanically deboned beef diets or diets containing formaldehyde

Hematinic Measure	Basal Diet Increase Fe	Basal Diet Increase CH_2O^1	Meat Diet Increase % Oxid. ²	Meat Diet Increase CH_2O
Hb gain, g/dl	↑ (.01)	No pattern	NS	NS
Hb-Fe gain, mg	↑ (.01)	NS	↓ (.01)	NS
Hb-Fe/Fe intake	No Change (NS)	NS	↓ (.05)	NS
Liver Fe, $\mu\text{g/g}$	↑ (.01)	NS	No pattern	↓ (.05)
Liver Fe, μg	↑ (.01)	NS	No pattern	↓ (.01)

¹Formaldehyde.

²Mechanically deboned, uncooked beef was lyophilized. A portion of the mechanically deboned, lyophilized, uncooked beef was allowed to oxidize at 20^o-22^oC for 14 days. The beef diets were then made in the following proportions: 100% fresh, beef, 75% fresh - 25% oxidized beef, 50% fresh - 50% oxidized beef, 25% fresh - 75% oxidized beef, and 100% oxidized beef.

oxidized soybean oil was fed, although not significantly in the Greenberg and Fraser (1953) study. In the present study, rats fed the severely oxidized beef meat (Table 7, groups 9-12) had increased relative liver weights. Formaldehyde addition to the basal diet did not affect relative liver weight. However, the addition of formaldehyde to the fresh meat diet resulted in decreased relative liver weight in rats.

The assay of iron bioavailability based upon the efficiency of converting food iron into hemoglobin iron enabled the detection of oxidation effects of meat iron bioavailability in this study (Tables 7, 8 and 9). When a low-iron basal diet is supplemented with iron

(groups 1, 2, 3, and 6), the gain in hemoglobin concentration and the gain in hemoglobin increased as expected (Mahoney and Hendricks, 1976). Further, in agreement with earlier reports (Mahoney et al., 1974, Mahoney and Hendricks, 1976), the efficiency of the conversion of dietary iron to hemoglobin iron remained practically constant over the range of dietary iron levels; this is a characteristic of a valid assay. With this procedure, it was found that the bioavailability of iron of oxidized meat was depressed (group 12 versus 8). Adding 80 ppm formaldehyde to the fresh meat diet (group 14 versus 8 and 13) also depressed meat iron bioavailability. These differences are not apparent if changes in hemoglobin concentration are used as criteria for the evaluation of iron bioavailability. As pointed out earlier (Mahoney et al., 1974), when differences in animal growth occur as in this study, the efficiency of converting food iron into hemoglobin iron would be the criterion of choice.

Only those rats fed lyophilized beef that had been oxidized at 20-22°C for 14 days had impaired iron metabolism (Table 8, group 12). The liver iron content of these rats was also reduced. This finding is in agreement with those of Miller and Landes (1975) who observed that rats fed either aerated or heated soybean oil had reduced hemoglobin concentrations, hematocrit values, and decreased liver iron stores.

Although the addition of formaldehyde to the fresh lyophilized beef diet reduced the efficiency of converting meat iron to hemoglobin iron and reduced the iron content of the liver (Table 8, group 14 versus 8), this did not occur in the rats fed the basal diet supplemented with ferrous sulfate (Table 8, groups 7 versus 6 and 5 versus 3). A possible reason for this effect is that fresh lyophilized beef

may have contained some endogenous formaldehyde. Thus, the formaldehyde content of the meat diet fed group 14 would include that naturally present in the product plus that added, while the formaldehyde content of the basal diets would have reflected only that added. An alternative reason for this effect is that other oxidation products (Gray, 1978) in the meat, such as aldehydes, ketones, glycols and dicarboxylic acids potentially present in the lyophilized beef, might have contributed to reduced bioavailability of meat iron. But the formaldehyde added to the basal diets might have interacted with the casein present making it unavailable to affect iron metabolism in rats fed these diets. Formaldehyde readily interacts with free amino groups and has been used industrially to harden casein (Haurowitz, 1963). On the other hand, added formaldehyde might have interacted with the organic iron compounds in meat to decrease iron bioavailability but did not interact with the inorganic ferrous sulfate in the casein diets.

Formaldehyde values were determined for the turkey meat in experiment 1. An attempt was made to determine formaldehyde values for the beef and basal diets in experiment 2. Even when formaldehyde was added to the basal or beef diets, little formaldehyde was recovered analytically. This might be due to formaldehyde reacting with free amino groups (Haurowitz, 1963). The formaldehyde contents of the oxidized beef diets were 5.6, 15, 26.3, 35.8 and 41 ug/g ($y = 0.0269x - 0.1662$, $r = 0.99$), respectively, for groups 8-12. The formaldehyde values for the oxidized beef were lower than for the oxidized turkey if the turkey formaldehyde data are extrapolated to a similar length of oxidation time. As turkey fat has a higher percentage of unsaturated fat than beef fat, one would expect the formaldehyde values for

the turkey to be higher than for the beef. Thus far, it has been our hypothesis that increased oxidation of meat resulted in decreased bioavailability of iron. Oxidation, as measured by formaldehyde values, does not seem to be an appropriate predictor of decreased bioavailability of iron across meat diets from different species.

Although formaldehyde has been associated with oxidized fat, it is not likely that it is the only chemical moiety affecting hematinic responses and relative liver size. Some hematinic responses were depressed when rats were fed fresh, lyophilized beef diets to which formaldehyde had been added (Table 8, group 8 versus groups 13-14 and Table 9). However, different hematinic responses were depressed when rats were fed diets high in oxidized beef (Table 8, groups 9-12 and Table 9). Furthermore, the addition of formaldehyde to fresh, lyophilized beef diets resulted in rats with decreased relative liver weights (Table 8, groups 13-14). In contrast, rats fed diets high in oxidized beef had increased relative liver weights (Table 8, groups 9-12 and Table 9). As formaldehyde is not the only aldehyde formed as a result of oxidation, it may be that other aldehydes may be more involved in affecting iron bioavailability than formaldehyde. Possibly this may explain the above results.

In this experiment mechanically deboned, lyophilized, uncooked beef diets caused decreased body growth, enlarged livers, and a depression of some measures of hematinic response in growing rats.

EXPERIMENT III

A COMPARISON OF THE EFFECT OF ATMOSPHERIC OXIDATION ON HAND DEBONED
AND MECHANICALLY DEBONED LYOPHILIZED TURKEY MEAT AND HAND DEBONED
AND MECHANICALLY DEBONED LYOPHILIZED BEEF MEATExperimental ProcedureMeat Preparation

Hand deboned dark turkey (HDT) meat was obtained from the Moroni Processing Co., Moroni, Utah. The dark turkey meat was taken directly off the boning line at the meat processor's plant. It was frozen, sliced in approximately 1.9 cm slices and lyophilized.

Mechanically deboned turkey (MDT) meat was likewise obtained from the Moroni Processing Co., Moroni, Utah. Whole turkey frames were used in the mechanical deboning. A Beehive AU-4171 Mechanical Deboner with a 0.031 inch (0.787 mm) perforated cylinder was used for the deboning. After mechanical deboning, the turkey meat was frozen, sliced into approximately 1.9 cm slices, and lyophilized.

Hand deboned beef (HDB) was obtained through the Utah State University Meat Laboratory, Logan, Utah. It was ground, frozen, sliced, and lyophilized.

Mechanically deboned beef (MDB) was obtained from the University of Wyoming Meat Laboratory, Laramie, Wyoming. The beef bones with adhering meat were fed through a BA-8 Beehive Bone Cutter. An AUX-705 Beehive Mechanical Deboner equipped with an AUX-705 0.018 inch head (0.457 mm perforations) was used for the deboning. The mechanically deboned beef was frozen, sliced and lyophilized.

The above lyophilized meat samples were ground after lyophilization.

The hand deboned beef and mechanically deboned turkey were ground using a plate with 2 mm to 3 mm perforations. The hand deboned turkey and mechanically deboned beef were ground using a plate with 6 mm perforations. The coarser grinding plate was used for the last two samples ground, because the mechanically deboned turkey had become stuck in the machinery, causing a power failure and the meat grinder to lock.

The uncooked, lyophilized, ground meat was spread evenly to a depth of approximately 5 mm on plastic trays and allowed to oxidize at 20-22°C for 0 hours and 336 hours. After the meat was exposed to the air the proper length of time, it was vacuum packed and stored in nitrogen atmosphere at -20°C until the chemical analyses were completed.

Analytical

The micro-Kjeldahl method was used for determining the nitrogen content of the meat (Appendix A). The crude lipid analysis was made using the Soxhlet method (Appendix B). Malonaldehyde content of meat samples was analyzed using the Thiobarbituric Acid Method (Sinnhuber and Yu, 1958) (Appendix C). The Iodine Number (Daniel and Neal, 1967) (Appendix D) was used to measure the degree of unsaturation of the lipid in the meats. The above determinations were done using triplicate samples.

Statistical Analysis

The data were analyzed by analysis of variance. When treatment "F" was statistically significant ($P < .05$), least significant difference (LSD) values were calculated (Carmer and Swanson, 1973).

Results

The moisture, lipid, nitrogen and iron content of the uncooked, lyophilized ground meat samples are reported in Table 10. The hand deboned turkey contained 2.3% moisture, 15.9% crude lipid, 74.8% crude protein, and 32.3 ppm iron. The mechanically deboned turkey contained 3.5% moisture, 39.2% crude lipid, 51.2% crude protein and 35.1 ppm iron. The hand deboned beef contained 2.4% moisture, 46.5% crude lipid, 45.7% crude protein, and 28.4 ppm iron. The mechanically deboned beef contained 2.4% moisture, 42.3% crude lipid, 45.9% crude protein and 85.0 ppm iron.

The TBA values (μg malonaldehyde/g) of the meat samples are shown in Table 11 and Figure 2. There was a significant difference ($P < .01$) between 0 hours oxidation time and 336 hours oxidation time. Using the LSD it is noted that the TBA value increases significantly only for the mechanically deboned turkey. There is no significant difference in TBA values for varying oxidation times of hand deboned turkey, hand deboned beef, or mechanically deboned beef.

The turkey samples had significantly higher ($P < .01$) TBA values than the beef samples as noted in Table 11 and Figure 2.

Significant differences for TBA values occurred between mechanically deboned and hand deboned samples. The mechanically deboned turkey oxidized for 336 hours had a significantly higher ($P < .05$) TBA value than the hand deboned samples oxidized for 0 hours. There was no significant difference between TBA values for hand and mechanically deboned meat for turkey at 0 hours oxidation or beef at 336 hours oxidation time.

Table 10. Analytical composition of uncooked, ground, lyophilized meat before oxidation

Meat	Moisture (%)	Crude Lipid (%)	Crude Protein (%)	Iron (ppm)
Hand Deboned Turkey	2.3	15.9	74.8	32.3
Mechanically Deboned Turkey	3.5	39.2	51.2	35.1
Hand Deboned Beef	2.4	46.5	45.7	28.4
Mechanically Deboned Beef	2.4	42.3	45.9	85.0

Table 11. TBA values (μg malonaldehyde/g) of meat samples before and after oxidation

Meat Treatment	0 Hour Oxidation	336 Hours Oxidation	LSD .05/.01
Hand Deboned Turkey	7.1	7.8	0.9/1.2
Mechanically Deboned Turkey	7.0	9.8	
Hand Deboned Beef	1.7	2.6	
Mechanically Deboned Beef	2.9	2.8

Figure 2. TBA values (µg malonaldehyde/g) of meat samples at 0 hours and 336 hours oxidation

The iodine number was calculated in two ways. First it was calculated as I_2 absorbed per 100 g sample (Table 12 and Figure 3). Second it was calculated as I_2 absorbed per 100 g fat (Table 13 and Figure 4).

When the iodine number was calculated as g I_2 absorbed per 100 g sample, there were no significant differences between samples oxidized 0 hours and 336 hours.

There were significant differences ($P < .01$) in iodine numbers between the turkey and beef meat. The mechanically deboned beef oxidized for 0 hours had a significantly lower ($P < .01$) iodine number than the turkey samples oxidized for 0 hours. Likewise, the mechanically deboned beef oxidized for 336 hours had a significantly lower ($P < .01$) iodine number than the turkey samples oxidized similarly. However, there were no significant differences between the iodine numbers for hand deboned beef at 0 hours or 336 hours oxidation time and the turkey samples oxidized identically.

There were no significant differences in iodine number of hand deboned or mechanically deboned turkey at either 0 hours oxidation or 336 hours oxidation time. Likewise, there was no significant difference in iodine number between hand deboned and mechanically deboned beef that had been allowed to oxidize 0 hours. However, oxidized mechanically deboned beef had an iodine value which was significantly lower ($P < .01$) than the iodine value for oxidized hand deboned beef.

When the iodine number was calculated as g I_2 absorbed per 100 g fat (Table 13 and Figure 4), there were no significant differences between samples oxidized 0 hours and 336 hours. Again there were significant differences ($P < .01$) in iodine values between the turkey

Table 12. Iodine number (calculated as grams I_2 absorbed per 100 g sample) of meat samples before and after oxidation

Meat Treatment	Iodine Number 0 Hours Oxidation	Iodine Number 336 Hours Oxidation	LSD .05/.01
Hand Deboned Turkey	41.0	41.5	4.1/5.8
Mechanically Deboned Turkey	43.5	40.5	
Hand Deboned Beef	38.3	39.6	
Mechanically Deboned Beef	34.5	31.0	

Table 13. Iodine number (calculated as g I_2 absorbed per 100 g fat in sample) of meat samples before and after oxidation

Meat Treatment	Iodine Number 0 Hours Oxidation	Iodine Number 336 Hours Oxidation	LSD .05/.01
Hand Deboned Turkey	257	263	17/24
Mechanically Deboned Turkey	111	104	
Hand Deboned Beef	83	86	
Mechanically Deboned Beef	82	74

Figure 3. Iodine number (calculated as g I₂ absorbed per 100 g sample) of meat samples before and after oxidation

Figure 4. Iodine number (calculated as g I_2 absorbed per 100 g fat in sample) of meat samples before and after oxidation

and beef meat. The turkey samples had significantly higher ($P < .01$) iodine numbers than the beef samples.

The hand deboned turkey had significantly higher ($P < .01$) iodine numbers than the mechanically deboned turkey. However, there were no significant differences between the iodine numbers for hand deboned and mechanically deboned beef.

In addition to the results reported above, there were some very observable changes in the meat as oxidation time increased. No attempt was made to measure these changes. As oxidation time increased the coloring of the meat became brownish. With decreased oxidation periods, the meat remained reddish in color. In addition, with increased oxidation time, the meat increased in odor.

Discussion

The analytical composition of the uncooked, lyophilized, ground meat varied interestingly. The hand deboned beef and mechanically deboned beef had similar moisture, lipid, and protein values. However, the mechanically deboned beef had much higher iron values (85 ppm) than the hand deboned beef (28.4 ppm) (Table 10). As a comparison, mechanically deboned beef in experiment 2 contained 55 ppm iron. Farmer et al. (1977) found the iron content of mechanically deboned shank beef to be 93.1 ppm and suggested that the increased iron values for mechanically deboned meat might be attributed to the incorporation of bone marrow into the final product during the deboning process or to iron accumulated in the mechanically deboned meat from the equipment during the deboning process. At any rate increased levels of iron in mechanically deboned beef have been confirmed.

Both hand deboned turkey and mechanically deboned turkey had low percentages of moisture, 2.3% and 3.5% respectively. However, the lipid and protein content of the turkey samples varied greatly. The hand deboned turkey contained much less fat (15.9%) than the mechanically deboned turkey (39.2%). As the turkey was being hand deboned, the leaner meat from the breast and legs would be removed. When the whole turkey frames are used for mechanical deboning, much of the fat remains on the frame. Thus, hand deboned turkey would have a lower percentage of fat and higher percentage of protein. Conversely, mechanically deboned turkey would have a higher percentage of fat and lower percentage of protein. Allred (1976) found hand deboned turkey to have 10.8 ppm iron and mechanically deboned turkey to have 18.8 ppm iron. Our data showed a much higher iron level; hand deboned turkey 32.3 ppm iron and mechanically deboned turkey 35.1 ppm iron. In both instances the mechanically deboned turkey had higher iron levels. This might be attributed to the same reasons described above for the mechanically deboned beef having increased iron levels.

The mechanically deboned meat had increased levels of iron over the hand deboned meat. However, the mechanically deboned beef had a much greater increase in iron over the hand deboned beef than the mechanically deboned turkey had over the hand deboned turkey. As the bones of beef are much larger than turkey bones, more abrasion takes place when they are processed in the bone cutter. Consequently, more iron would be accumulated from the equipment during the deboning process for beef than for turkey.

The presence of malonaldehyde in food products has been associated with rancidity or oxidation in foods (Sinnhuber and Yu, 1958).

Malonaldehyde has been detected in a variety of food including raw ground beef (Hutchins et al., 1967) and cooked meats (Tarladgis et al., 1960; Shamberger et al., 1977). During the oxidation process, unsaturated fatty acids are broken down to a variety of low molecular weight compounds including malonaldehyde. As meat contains varying degrees of unsaturated fatty acids, it would be expected that during an increased oxidation period, degeneration of the unsaturated fatty acids would result in increased amounts of malonaldehyde. However, in this experiment an increased oxidation period resulted in significantly higher levels of malonaldehyde as measured by the TBA value for only mechanically deboned turkey. Increased oxidation time did not significantly increase TBA values in hand deboned turkey, hand deboned beef, or mechanically deboned beef. It is possible that oxidative degeneration had taken place preceeding the experimental oxidation period and had stabilized. Also, as malonaldehyde forms compounds with free amino groups (Fennema, 1976), this may have occurred in this experiment.

The turkey samples had significantly higher ($P < .01$) TBA values than the beef samples (Table 11 and Figure 2). Poultry lipids have been found to be more unsaturated than those of red meat (Lea, 1938). Hence, it would be expected that the turkey meat would have higher TBA values than the beef. Shamberger et al. (1977) have published malonaldehyde contents of beef and turkey. Although it is difficult to compare TBA values with certainty from laboratory to laboratory, Shamberger found high levels of malonaldehyde in uncooked turkey (10.8 $\mu\text{g/g}$) and generally

more moderate amounts in varying cuts of uncooked beef (1.2 $\mu\text{g/g}$ - 7.2 $\mu\text{g/g}$). Our findings would be consistent with these. In addition, the hand deboned, uncooked, lyophilized turkey samples used in Experiment 1 had initial TBA values (Figure 1) similar to the TBA values for the hand deboned, uncooked, lyophilized turkey in this experiment.

It would be assumed that with the pulverizing that takes place during mechanical deboning, increased surface areas would be available to oxidation, plus oxygen would be introduced during the mechanical deboning process. Hence, it would be expected that mechanical deboning would make fatty acids in the meat more susceptible to oxidation and result in degeneration of fatty acids to aldehydes, peroxides, dycols, ketones, and decarboxylic acids. Therefore, higher TBA values would be expected for mechanically deboned meat. In this experiment TBA values for mechanically deboned meats were higher for turkey oxidized 336 hours, beef oxidized 0 hours, and beef oxidized for 336 hours (Table 11 and Figure 2), although significantly for only the turkey oxidized for 336 hours ($P < .01$) and beef oxidized 0 hours ($P < .05$). As the original hand deboned and mechanically deboned beef samples and turkey samples did not come from the same source, it is difficult to evaluate which other factors such as freshness, aging of beef, lipid content of feed, storage temperatures, etc. may have affected the initial oxidation and production of malonaldehyde for these meats. Consequently, an evaluation of mechanical versus hand deboning on TBA values is difficult to ascertain with the number of samples that we used, the possible diversity of initial handling, and the variability in the limited statistics

in this study.

The TBA values obtained for uncooked, lyophilized, hand deboned turkey in Experiment 1 initially were higher than in Experiment 3. This is partly because the TBA values in Experiment 1 were calculated by the formula Optical Density minus Blank times the factor (108) divided by the weight of the meat equals TBA value. Had the samples in Experiment 3 been calculated by the same formula, the TBA value for the samples in Experiment 3 would have been much higher. In Experiment 3 malonaldehyde standards were prepared, run through the method, optical densities read, a regression line calculated, and TBA values for the meats were calculated based on the malonaldehyde standards. It was expected that more significant differences between our meat samples would occur. It has been suggested that TBA value is not a reliable indicator in dry systems (Fennema, 1976). The procedure appears to have been successful for Andrews et al. (1977) and in Experiment I. However, the results in Experiment III are questionable and might be related to the fact that a dry system was being tested.

Iodine or equivalent amounts of other halogens will add at double bonds of unsaturated fatty acids. If a known amount of a halogen is added at the beginning of a determination and time is allowed for the reagent to add at all double bonds in the fat molecule, then a determination of the remaining halogen will, by difference, give the amount of halogen combining with the fat. Hence, a highly unsaturated fat will have a high iodine number and a saturated fat will have a low iodine number. In using the iodine number, it was felt that the initial iodine number would give an indication of the degree of saturation of the fat. The meat samples

would be allowed to oxidize for 336 hours at which time they would again be tested with the iodine number. If oxidation had taken place, there would be a decrease in the number of double bonds available for combination with the halogen. Hence, the meat sample would have a lower iodine number.

When the iodine number was calculated either as grams I_2 absorbed per 100 g sample or per 100 g fat, there was no significant difference between samples oxidized 0 hours and 336 days. Possibly oxidation had taken place during the handling and treatment of the meat preceeding the experimental oxidation period, so that a state of stability had been reached. This seems unlikely. It is more likely that the halogen had combined with other compounds in the meat such as porphyrin, phenylalanine and tyrosine, which contain double bonds. Hence, the iodine number does not give a true reflection of oxidative changes in meat because of possible complexing of the halogen with compounds other than unsaturated fatty acids.

With both methods of calculating the iodine number, turkey samples had significantly higher iodine numbers than beef samples. This would be expected as turkey has been shown to contain more unsaturated fat than beef (Lea, 1938). Hence, turkey would be expected to have higher iodine numbers. When calculating the iodine number as g I_2 absorbed per 100 g sample there were no significant differences between hand deboned turkey and beef samples. Other factors such as initial handling may have affected the oxidation state of the meat. Furthermore, the fact that it is suspected that the halogen is combining

with other compounds makes this data suspect.

When the iodine number was calculated as g I_2 absorbed per 100 g fat, there was a significant difference between hand deboned turkey and mechanically deboned turkey. However, this could be in part attributed to the fact that the hand deboned turkey contained 15.9% lipid and the mechanically deboned turkey contained 39.2% lipid. A 0.2 g sample of meat was used when testing for iodine number. Hence, the amount of lipid in the hand deboned sample was much less. The high iodine number is a reflection of the method of calculation. In addition, the fact that iodine numbers for hand deboned turkey were very similar to values for mechanically deboned turkey when calculated as g I_2 absorbed per 100 g sample gives substance to the possibility of I_2 combining with other compounds.

The iodine number calculated of 257 for fat from hand deboned turkey oxidized 0 hours is very unrealistic. An iodine number of 122.6 is listed in Weast (1968) for corn oil. The iodine number for corn oil was determined to be 117.2 for a sample in our laboratory. The iodine number for fresh chicken fat was determined in our laboratory to be 98. This determination was completed within three hours of the slaughter of the chicken. (The chicken fat was obtained from the Utah State Poultry Farm, Logan, Utah). Although the iodine number is usually expressed as g I_2 absorbed per 100 g lipid, this appears highly inaccurate on our meat samples.

In conclusion, using both the TBA value and the iodine number as measures of oxidation on turkey and beef samples proved frustrating. The TBA value has proved unreliable in dry systems. The iodine number appears to have the fault of the halogen combining with other compounds

when a meat sample instead of a lipid is tested.

FINAL STATEMENT

Two studies were conducted to determine the effect of atmospheric oxidation on bioavailability of meat iron and liver weights in rats. The first experiment did not show a relationship between iron deficiency and oxidation time of turkey meat. The second experiment demonstrated that only rats fed lyophilized beef that had been oxidized at 20-22°C for 336 hours had impaired iron metabolism. Rats fed fresh lyophilized beef diets with added formaldehyde did not exhibit the same hematinic responses as rats fed oxidized beef.

As the results of the first two experiments varied, a third experiment on lyophilized turkey and beef meat was conducted to try to account for the variability in results between Experiment 1 and 2. Although every attempt was made to duplicate the meats used in Experiment 1 and 2, the percentage meat composition of protein, lipid and iron varied greatly in the hand deboned turkey and mechanically deboned beef. Furthermore, attempts to reproduce formaldehyde values TBA values, and iodine numbers met with obstacles. Further testing will be necessary to develop reliable techniques for testing oxidation in lyophilized meats.

The turkey and beef meats which had been allowed to oxidize were unacceptable for human consumption because of the extremely rancid odor. As the degree of oxidation in lyophilized meat must be very high before a significant depression of growth and hematinic response occurs in laboratory animals, it is not likely that humans would eat enough severely oxidized meat to affect the bioavailability of iron in the diet.

REFERENCES

- AMA (American Medical Association, Committee on Iron Deficiency). 1968. Iron deficiency in the United States. J. Am. Med. Assoc. 203:407.
- Allred, L. 1976. Some effects of mechanical deboning on the composition and the bioavailability of protein and iron in turkey frame meat. MS thesis, Utah State University.
- Amano, K. and K. Yamada. 1964. A biological formation of formaldehyde in the muscle tissue of gadoid fish. Bull. Japanese Soc. Scientific Fisheries 30:430.
- Amine, E. K. and D. M. Hegsted. 1975. Effect of dietary carbohydrates and fats on inorganic iron absorption. J. Agr. Food Chem. 23:204.
- Andia, A. M. G. and J. C. Street. 1975. Dietary induction of hepatic microsomal enzymes by thermally oxidized fats. Ag. Food Chem. 23:173.
- Andrews, J. S., J. F. Mead and W. H. Griffeth. 1956. Toxicity of lipid peroxides in the rat. Fed. Proc. 15:918.
- Andrews, S. J., C. G. Ponce and V. T. Mendenhall. 1977. Recovery of formaldehyde from mechanically deboned turkey. J. Food Sci. 42:1168.
- Bjorn-Rasmussen, E. 1974. Iron absorption from wheat bread -- influence of various amounts of bran. Nutr. Metabol. 16:101.
- Bjorn-Rasmussen, E. and L. Hallberg. 1974. Iron absorption from maize. Nutr. Metabol. 16:94.
- Carmer, S. G. and M. R. Swanson. 1973. An evaluation of 10 pairwise multiple comparison procedures by Monte Carlo methods. J. Am. Stat. Assoc. 68:66-74.
- Cook, J. D. and E. R. Monson. 1976. Food iron absorption in human subjects. III. Comparison of the effects of animal protein on nonheme iron absorption. Am. J. Clin. Nutr. 29:859.
- Cook, J. D. and E. R. Monson. 1977. Vitamin C, the common cold, and iron absorption in man. Am. J. Clin. Nutr. 30:235.
- Crosby, W. H., S. I. Munn and F. W. Furth. 1954. Standardized method for clinical hemoglobinometry. U.S. Armed Forces Med. J. 5:693.
- Daniel, L. J. and A. L. Neal. 1967. Laboratory experiments in biochemistry. New York: Academic Press.

- Farmer, B. R. A. 1977. The iron bioavailability of mechanically-deboned meats. M.S. thesis, Utah State University.
- Farmer, B. R., A. W. Mahoney, D. G. Hendricks and R. A. Gillett. 1977. Iron bioavailability of hand-deboned and mechanically deboned beef. J. Food Sci. 42:1630.
- Fennema, O. R. 1976. Principles of Food Science. Part 1. Food Chemistry. New York: Marcel Dekker, Inc.
- Gray, J. I. 1978. Measurement of lipid oxidation: a review. J.A.O.C.S. 55:539.
- Greenberg, S. M. and A. C. Frazer. 1953. Some factors affecting the growth and development of rats fed rancid fat. J. Nutr. 50:421.
- Haurowitz, F. 1963. The chemistry and function of protein, 2nd ed., New York: Academic Press.
- Hegsted, D. M., C. A. Finch and T. D. Kinney. 1949. The influence of diet on iron absorption. II. The interrelation of iron and phosphorus. J. Exp. Med. 90:147.
- Hutchins, B. K., T. H. P. Liu and B. M. Watts. 1967. Effects of additives and refrigeration on reducing activity, metmyoglobin and malonaldehyde of raw ground beef. J. Food Sci. 32:214.
- Kaufman, N., J. V. Klavis, and T. D. Kinney. 1958. Excessive iron absorption in rats fed low-protein, high-fat diets. Lab. Invest. 7:369.
- Kaunitz, H., R. E. Johnson and L. Pegues. 1965. A long-term nutritional study with fresh and mildly oxidized vegetable and animal fats. J.A.O.C.S. 42:770.
- Kaunitz, H., R. E. Johnson and L. Pegues. 1966. Longer survival time of rats fed oxidized vegetable oil. Proc. Soc. Exp. Biol. Med. 123:204.
- Kaunitz, H., C. A. Slanetz and R. E. Johnson. 1955. Antagonism of fresh fat to the toxicity of heated and aerated cottonseed oil. J. Nutr. 55:577.
- Kaunitz, H., C. A. Slanetz, R. E. Johnson, H. B. Knight, D. H. Saunders and D. Swern. 1956. Biological effects of the polymeric residues isolated from autoxidized fats. J.A.O.C.S. 33:630.
- Labuza, T. P. 1980. The effect of water activity on reaction kinetics of food deterioration. Food Technol. 34, No. 4:36.
- Layrisse, M., C. Martinez-Torres and M. Roche. 1968. Effect of interaction of various foods on iron absorption. Am. J. Clin. Nutr. 21:1175.

- Lea, C. H. 1938. Rancidity in edible fats. Food Investigation Special Report No. 46. Great Britain Dep. Sci. and Ind. Res.
- Mahoney, A. W. and D. G. Hendricks. 1976. Effects of dietary iron level on efficiency of converting food iron into hemoglobin by the anemic rat. *Nutr. Metab.* 20:222.
- Mahoney, A. W., C. C. Van Orden and D. G. Hendricks. 1974. Efficiency of converting food iron into hemoglobin by the anemic rat. *Nutr. Metab.* 17:223.
- Mendenhall, V. T. 1970. The isolation and identification of a microbial inhibitor for Pacific hake (Merluccius productus). Ph.D. dissertation, Oregon State University, Corvallis.
- Miller, J. and D. R. Landes. 1975. Effects of feeding oxidized or heated soybean oil on tissue composition and hematological status of rats. *J. Food Sci.* 40:545.
- Nakamura, M., H. Tanaka, Y. Hattori and M. Watanabe. 1973. Biological effects of autoxidized safflower oils. *Lipids* 8:566.
- National Research Council. 1980. Recommended Dietary Allowances, 9th ed. Washington, D.C.: National Academy of Sciences.
- Nolen, G. A. 1973. A feeding study of a used, partially hydrogenated soybean oil, frying fat in dogs. *J. Nutr.* 103:1248.
- Nolen, G. A., J. C. Alexander and N. R. Artman. 1967. Long-term rat feeding study with used frying fats. *J. Nutr.* 93:337.
- Pike, R. L. and M. L. Brown. 1975. Nutrition: An Integrated Approach, 2nd ed. New York: John Wiley & Sons, Inc.
- Shamberger, R. J., B. A. Shamberger and C. E. Willis. 1977. Malonaldehyde content of food. *J. Nutr.* 107:1404.
- Sinnhuber, R. O. and T. C. Yu. 1958. 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. II. The quantitative determination of malonaldehyde. *Food Technol.* 12:9.
- Stout, F. M., J. E. Oldfield and J. Adair. 1960a. Aberrant iron metabolism and the "cotton-fur" abnormality in mink. *J. Nutr.* 72:46.
- Stout, F. M., J. E. Oldfield and J. Adair. 1960b. Nature and cause of the "cotton-fur" abnormality in mink. *J. Nutr.* 70:421.

- Tarladgis, B. G., B. M. Watts, M. T. Younathan and L. Dugan, Jr.
1960. A distillation method for the quantitative determination
of malonaldehyde in rancid foods. J.A.O.C.S. 37:44.
- Van Campen, D. 1974. Regulation of iron absorption. Fed. Proc.
33:100.
- Waddell, J. 1974. The bioavailability of iron sources and their
utilization in food enrichment. Fed. Proc. 33:1779.
- Weast, R. C. ed. 1968. Handbook of Chemistry and Physics, 49th
ed. Cleveland: The Chemical Rubber Co.

APPENDICES

APPENDIX A

Micro Kjeldahl Method

Weigh by difference a finely ground, homogenous, dried food sample. Use 0.1000 to 0.1500 g of meat. Weigh the sample on to a small piece of waxed paper and transfer to the Kjeldahl flask. Weigh the paper.

Run two reagent blanks through all the steps of the procedure and subtract the blank titration from the sample titrations.

Add approximately 1 g of the catalyst mixture (9:1 Na_2SO_4 : CuSO_4) with a small measuring scoop followed by 4 ml concentrated H_2SO_4 . Wash down neck with small amount of distilled water. Add henegar granule to flask.

Place the flask on the digestion apparatus under a hood with the exhaust fan on. Cook until the solution is a clear aqua (approximately 5 hours). Swirl sample occasionally.

Allow the flasks to cool. Wash the neck of the flask down with a wash bottle to dilute the sample to about 10 ml. Transfer the clear solution to a distillation flask with a series of 3-4 small rinses with distilled water. Place the flask in an ice bath to cool before distilling.

The steam distillation apparatus should be heating while preparing samples. When boiling vigorously, turn on cold water in the condensor. Place 10 ml of the boric acid solution (4% boric acid + 5 ml 0.4% bromocresol green in ethyl alcohol) in a 50 ml Erlenmeyer flask and set it under the condenser with the tip below the surface of the solution. The distillation flask should be clamped into position.

Add 20 ml NaOH (45% solution) and quickly turn on the steam. Let the distillation proceed for 5 minutes after the product appears, or until 10-15 ml of distillate have been collected. Let the flask down below the condensor and let it drip for an additional minute. Turn off the steam, remove the distillation flask, and rinse the tip of the condensor into the collection flask.

Titrate the sample with standardized 0.05 N HCl to a faint green end point.

Calculation

$$\% \text{ Nitrogen} = \frac{(\text{ml acid titrated} - \text{ml blank titrated}) (\text{acid normality}) \times 0.014 \times 100}{\text{wt. of sample in g}}$$

$$\% \text{ crude protein} = \% \text{ nitrogen} \times 6.25$$

APPENDIX B

Soxhlet Method

Weigh filter paper. Weigh 1.0 - 1.5 g sample on to filter paper. Wrap sample in filter paper, close securely, label, and record total weight.

Dry overnight in vacuum oven at 60°C.

Cool in dessicator. Weigh. Determine percentage moisture.

Extract lipid from sample in Soxhlet apparatus using petroleum ether as the solvent for approximately 10-12 hours.

Dry packaged sample in 60°C oven overnight. Cool in dessicator. Record weight. Determine percentage lipid.

APPENDIX C

2-Thiobarbituric Acid Method (TBA)

Weigh a 250 ml boiling flask and record weight. Add a 1 g sample of meat. Record weight.

Add 5 ml water, 6 ml TBA, 5 ml pyridine HCl solution (300 ml pyridine and 700 ml 6N HCl), and 10 ml of TCA solution (20% Trichloroacetic Acid). Connect to condensor and cook for 30 minutes exactly.

Add 70 ml of 0.6N HCl. Swirl gently. Heat another 10 minutes. Cool for 10 minutes or to room temperature.

Pour 30 ml of sample into 50 ml centrifuge tube and centrifuge for 10 minutes at 17.65 G. Pipet 10 ml of petroleum ether into a centrifuge tube. Pipet 15 ml of sample into tube containing petroleum ether. Shake by hand for 1 minute. Centrifuge for 5 minutes at 17.65 G.

Read OD immediately in Beckman DB Spectrophotometer at 535 mμ against a water blank.

Place 0, 1, 2, 4, 6, 10, 15, 20 ml 1 ppm malonaldehyde standard solution in 250 ml boiling flask. Follow steps above. Calculate regression line for malonaldehyde. Calculate μg malonaldehyde per gram sample.

APPENDIX D

Iodine Number

Rinse glass stoppered 250 ml Erlenmeyer flasks with dry chloroform. Weigh flasks when they are completely dry. Add sample to flask. Weigh it again.

Add 10 ml anhydrous chloroform to each flask.

From an automatic buret under the hood, add exactly 25 ml of pyridine sulfate dibromide to each flask. (Space flasks about 15 minutes apart). Stopper flasks and place them in the dark (in a drawer) for exactly 30 minutes.

Add 10 ml of 15% potassium iodide to the flasks. Mix the contents well by shaking the flasks.

Add 75 ml of H_2O to each flask.

Titrate the I_2 with approximately 0.1 N standardized sodium thiosulfate. Titrate to a light yellow color, then add 5 ml 1% starch indicator solution and complete the titration while shaking. Note the volume on the buret just after one drop has changed the solution from blue to colorless.

Preparation of pyridine sulfate dibromide. Prepare this in an ice bath in the hood:

- a. 8 g pure pyridine in 20 ml glacial acetic acid.
- b. 10 g conc. H_2SO_4 in 20 ml glacial acetic acid.

Combine a and b. Then add 8 g Br_2 in 20 ml glacial acetic acid. Make to 1 liter with glacial acetic acid. The resulting solution is 0.1 N.