THE CHARACTERIZATION AND UTILIZATION OF MECHANICALLY SEPARATED BOVINE SPLEEN

by

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TABLE OF CONTENTS

Page

ACKNOWLEDGMENTS....ii

TABLE OF CONTENTS....iii

LIST OF FIGURES....vii

LIST OF TABLES....viii

1.0 INTRODUCTION....1

1.1 Background....1

1.2 Objectives....5

2.0 REVIEW OF LITERATURE....7

2.1 Spleen Description....7

2.2 Connective Tissue....8

2.21 Nutritional Aspects....8

2.22 Functional Aspects....10

2.23 Mechanical Removal....11

2.3 Iron....13

2.31 Forms and Distribution....13

2.32 Nutritional Value....18

2.4 Reported Uses of Spleen....23

3.0 MATERIALS AND METHODS....25

3.1 Spleen Evaluation....25

3.11 Mechanical Separation....25

3.12 Spleen Testing....25

3.121 Sample Preparation....25

3.122 Proximate Analysis....26

3.123 Preparation of Diets.....26
3.124 Protein Efficiency Ratio.....28
3.2 Frankfurter Evaluation.....29
3.21 Raw Material Preparation.....29
3.22 Frankfurter Formulation.....30
3.23 Frankfurter Production.....30
3.24 Frankfurter Evaluation and Analysis.....33
3.241 Proximate Analysis.....33
3.242 Physical Attributes Panel.....33
3.243 Consumer Taste Panel.....35
3.244 Laboratory Taste Panel.....35
3.245 Shear Values.....36
3.246 Objective Color Measurements.....37
3.2461 Color Meter Determinations.....37
3.2462 Pigment Determinations.....37
3.247 Microbiological Evaluation.....39
3.3 Patty Product Evaluation.....40
3.31 Patty Formulation and Preparation.....40
3.32 Patty Evaluation and Analysis.....41
3.321 Taste Panel.....41
3.322 Proximate Analysis.....41
3.4 Elemental Analysis.....41
3.41 Sample Preparation.....42
3.42 Atomic Absorption Spectrophotometry.....44

4.0 RESULTS AND DISCUSSION.....45
4.1 Spleen Evaluation.....45
4.11 Mechanical Separation.....45
4.12 Spleen Testing.....45
4.121 Proximate Composition.....45
4.122 Elemental Composition.....48
4.123 Protein Efficiency Ratio.....50
4.2 Frankfurter Evaluation.....54
4.21 Proximate Composition.....54
4.22 Elemental Composition.....54
4.23 Panel Evaluation.....56
4.231 Physical Attributes Panel.....56
4.232 Consumer Panel.....59
4.233 Laboratory Taste Panel.....61
4.24 Shear Values.....64
4.25 Objective Color Measurements.....67
4.251 Color Meter Determinations.....67
4.252 Pigment Determinations.....71
4.26 Microbiological Evaluation.....76
4.261 Mesophilic Bacteria.....76
4.262 Psychrophilic Bacteria.....78
4.263 Coliform Bacteria.....78
4.3 Patty Evaluation.....81
4.31 Cooking Yield and Proximate Compositon.....81
4.32 Elemental Composition.....81
4.33 Taste Panel.....85

5.0 SUMMARY AND CONCLUSIONS.....87

REFERENCES.....93

VITA.....100

ABSTRACT

LIST OF FIGURES

Figure	Title	Page
1	Spleen separation with a Beehive Model AU 4171 deboner and desinewing head	46
2	A close-up view of mechanically separated spleen leaving the product port and the residue exiting the discard port	46
3	Effect of mechanically separated spleen addition on the iron content of frankfurters	57
4	Effect of mechanically separated spleen addition on shear values of frankfurters measured over 6 weeks of storage	66
5	Least squares relationships between internal and external frankfurter surface Hunter L values and time of storage	70
6	Least squares relationships between total and nitroso-heme pigment concentrations and time of storage for frankfurters	74
7	Effect of mechanically separated spleen addition on the iron content of cooked meat patties	84

LIST OF TABLES

Table	Title	Page
1	Total iron content of bovine tissues	15
2	Weight of ingredients used in frankfurter formulations in kg	31
3	Frankfurter evaluation: physical attributes	34
4	Composition of whole and mechanically separated spleen raw materials	47
5	Bioassay of whole and mechanically separated spleen	51
6	Composition of frankfurters containing mechanically separated spleen	55
7	Physical attributes panel evaluation of frankfurters	58
8	Consumer panel evaluation of frankfurters	60
9	Laboratory taste panel evaluation of frankfurters	62
10	Interaction effects for Allo-Kramer shear values of frankfurters	65
11	External frankfurter color: Hunter L values	68
12	Internal frankfurter color: Hunter L values	69
13	Interaction effects for total heme pigments of frankfurters	72
14	Interaction effects for nitroso-heme pigments of frankfurters	73
15	Evaluation of mesophilic bacteria in raw emulsion and cooked frankfurters	77
16	Evaluation of psychrophilic bacteria in raw emulsion and cooked frankfurters	79
17	Evaluation of coliform bacteria in raw emulsion and cooked frankfurters	80
18	Cooked meat patty composition	82
19	Taste panel evaluation of meat patties	86

1.0 INTRODUCTION

1.1 Background

To meet present and future food needs of the world's population, all protein food sources must be utilized to their fullest potential. Livestock processing results in a considerable amount of raw by-products which are used insufficiently for edible purposes although they offer considerable potential as additional sources of animal protein for human nutrition.

Because of local customs and aversions toward certain parts of the animal body, many are discarded though they could well be used in comminuted products. A by-product which is discarded in one country is often used in another, indicating that only tradition and not considerations related to health or organoleptic characteristics are involved (Jul, 1976).

Despite high food values, most meat by-products are not used for edible purposes because no recommendations exist on their processing and utilization (Gorbatov, 1976). There is currently a need to develop technology leading to the maximum utilization of meat raw materials in food products.

The bovine spleen is a meat by-product with untapped potential for human consumption in terms of nutrition and economics. It is legally classified as a variety meat and as such is a permissible

ingredient of certain cooked sausages, including frankfurters, under defined label statements (USDA, 1973).

Beef spleen contains 17-18 percent protein, an amount equivalent to that of 80 percent lean ground beef (Watt and Merrill, 1963), approximately eight times the iron found in beef liver, and about three percent fat (Kizlaitis et al., 1962). It is also a very available raw material. Based on an average weight of 0.5 kg per head, there are approximately 381,000 kg of beef spleen produced each week in the United States, the major portion of which are currently being processed into pet food or tankage.

Although spleen is considered a great delicacy by many peoples of the world, it is rarely consumed in any form by people in the United States. There is no public information available on the amount of spleen that is used for human food in the U.S.A. Limited amounts of splenic tissue are being included in some potted meat, scrapple and "less expensive" hot dogs produced in certain areas of the country. As the world food and protein shortage worsens, an increased utilization of this high protein, high iron, low fat organ meat for human consumption may become nutritionally and economically necessary.

The food industry has been fortifying foods with iron for over 30 years in an effort to decrease the high incidence of iron deficiency anemia. The continued presence of anemia indicates that iron-fortified foods are not reaching those groups at risk in quantities sufficient to improve iron status, or that the iron sources used are not as available as assumed (Nelson and Potter, 1979). Technical problems are inherent

in iron fortification due to the many reactions which are catalyzed by iron or in which iron is a reactant. The more soluble iron compounds are recognized as having deleterious effects on wheat, milk and potato products (Bibeau and Clydesdale, 1976). Thus, the selection of iron fortification agents has been based primarily on their suitability in product formulations and economic limitations, with little consideration of the relative bioavailability of the source (Waddell, 1974).

Knowing the total iron content of a food, or mixture of foods, is not a precise measure of its value as a source of available iron. The absorption of dietary iron is dependent on the form of iron present as well as the composition of the remainder of the meal (Cook, 1977). Numerous studies have demonstrated consistently higher iron absorption from foods of animal origin than from those of plant origin. Additionally, Layrisse et al. (1973) demonstrated that meat in a meal enhances the absorption of nonheme iron represented by the sum of vegetable iron and fortification iron. These authors concluded that food iron fortification is likely to be effective only in individuals who take animal protein as part of their diet. Heme iron is absorbed directly by the mucosal cell. This process is not affected by enhancing or inhibiting substances present in the diet and can therefore be predicted from chemical measurements of the proportion of dietary iron ingested as heme iron (Cook, 1977).

Selection of a food for enrichment should consider: the availability and usage pattern of the food; the compatability of the added iron with general or anticipated properties of the food; the biological availability

of the iron and the cost per unit of available iron (Wang and King, 1973). Considering its low cost and the abundant quantities of iron present, particularly as hemoglobin; the addition of bovine spleen to comminuted and emulsion-type meat products may meet the criterion for beneficial enrichment and significantly contribute to the nutritive iron value of these products.

Bovine spleen consists of reticulo-endothelial tissue (splenic pulp) supported by a collagenous framework and encased in a thick collagenous capsule. The large amount of capsular and internal connnective tissue present in a spleen is a major drawback to its utilization. Collagen is an undesirable ingredient in ground (Cross et al., 1976) and emulsion-type meat products (Tauber, 1975). Additionally, collagen has been shown to have a negative effect on the protein nutritive value of meat (Dvorak and Vognarova, 1969; Chang and Field, 1977; Hendricks et al., 1977 and Lee et al., 1978).

Recent advances in the area of processing equipment, specifically mechanical deboners with desinewing heads, have provided the ability to remove collagenous material from poultry and red meats. This equipment presents an opportunity to economically remove the connective tissue from beef spleens and recover the splenic pulp in a state more compatible to use in ground and emulsion meat products.

There are several probable advantages to mechanically separating splenic pulp from the capsular and internal connective tissue:

1. Easier passage through further processing equipment such as grinders, choppers and emulsifiers.
2. A reduced chance of poor or broken emulsions.

3. Less offensive gristle-like material in ground products.
4. Increased biological value of the remaining protein.
5. The possibility of retrieving collagenous material for use in casings or gelatin as suggested by Gillett et al. (1976) for shank meat residue.

1.2 Objectives

The bovine spleen holds great potential as an economical source of animal protein and iron for the natural fortification of comminuted meat products. Splenic pulp appears to be ideally suited for incorporation into ground and finely comminuted meat products, but the effects of its use on sensory attributes, processing characteristics and composition have not been documented. Concern and lack of information has limited commercial adoption of products including this variety meat.

This study was designed to provide a variety of information about bovine spleen and its use potentials in ground and emulsion meat products. Scientific data on the functionality of bovine splenic pulp in product systems may promote the utilization of this valuable source of iron and protein in human nutrition.

The specific objectives of this research were:

1. To employ mechanical desinewing equipment to remove connective tissue from bovine spleens and to compare mechanically separated and whole spleen on the basis of proximate composition, certain elements and protein efficiency ratio.
2. To evaluate the physical, nutritional, organoleptic and bacteriological effects of mechanically separated spleen

(MSS) incorporation on a standardized frankfurter formulation and to measure product changes occurring during refrigerated storage.

3. To evaluate the effects of MSS incorporation on the organoleptic and nutritional attributes of a cooked meat (beef) patty.

2.0 REVIEW OF LITERATURE

2.1 Spleen Description

As the largest single collection of reticulo-endothelial cells in the body, the spleen functions in hemopoiesis, antibody production, defense against infection, destruction of erythrocytes and the storage of iron (Frandson, 1965). In the bovine, the spleen also serves as a blood storage area. The capsule is rich in smooth muscle and during adrenergic stimulation there is a massive contraction which forces the reserve blood cells into circulation (Greep, 1966).

The spleen consists of reticular tissue suspended within a collagenous framework. This framework includes a capsule and its branching continuations, called trabeculae, which penetrate the organ like a branching tree (Ham, 1969). The capsule and trabeculae consist of dense connective tissue in which there is a fairly high percentage of elastin (Bloom and Fawcett, 1968).

The reticulo-endothelial system plays a key role in the internal iron economy of the body. It is the primary organ concerned with the catabolism of hemoglobin, the supply of iron to the erythroid marrow, and the storage of iron which is not immediately required for the synthesis of metabolically active compounds. Under normal circumstances the major role is played by the reticulo-endothelial cells of the spleen, liver and bone marrow (Lynch et al., 1974).

2.2 Connective Tissue

2.21 Nutritional Aspects

The nutritive value of proteins is primarily defined by the amount and composition of essential amino acids. Collagen and elastin are relatively poor in essential amino acids compared with other meat proteins (Dvorak and Vognarova, 1969). Collagen is devoid of cystein and tryptophan and contains only small amounts of tyrosine and methionine (Hendricks et al., 1977). It has generally been recognized that inexpensive cuts of meat and meat by-products high in connective tissue have relatively poor biological value (Lee et al., 1978).

Hydroxyproline, which is absent in other muscle proteins, is a criterion for the proteins of connective tissue. A close negative relationship exists between net protein utilization and the amount of hydroxyproline in meat (Dvorak, 1972). Evaluating the protein quality of several mechanically deboned meats using amino acid analysis and protein efficiency ratio (PER), Chang and Field (1977) found that deboned meat which contained more lean and less collagen was superior in protein quality to deboned meat containing less lean and more collagen. The growth response of the rats corresponded precisely with the collagen content of the products. They concluded that protein quality and utilization is inversely related to the collagen content of a meat product.

Dvorak and Vognarova (1969) found straight line relationships when plotting the amount of individual and total available essential amino acids against the amount of hydroxyproline. Available essential amino

acids decreased with increasing amount of hydroxyproline, i.e., with increasing connective tissue protein. This relationship was determined to be the same for veal, beef and pork. The authors presented linear regression equations relating essential amino acids and hydroxyproline and suggested that protein nutritive value may be calculated from the results of nitrogen and hydroxyproline analysis.

Using the PER method to evaluate the nutritional value of all-meat and meat-soy weiners, Noda et al. (1977) found that weiners made from beef skeletal muscle yielded a higher PER (3.11) than those made with beef shank (2.18). This difference was explained by the high connective tissue content of the shank meat. Additionally, the use of beef shank caused a greater reduction in PER than the replacement of skeletal muscle with 25 percent hydrated soy protein. Other work (Anon., 1974) has demonstrated that 10 percent collagen replacement in lean beef lowered the PER by 0.3 units.

The amount of essential amino acids in meat closely parallels its PER (Alsmeyer et al., 1974). Lee et al. (1978) blended beef rounds and partially defatted chopped beef to obtain products varying in collagen content from 4 to 45.8 percent of the total protein. Total essential amino acids and rat PER decreased linearly with increasing collagen content of the products. The authors presented several highly significant linear regression equations and suggested that rat PER and the amount of essential amino acids can be effectively predicted by the chemical determination of collagen content in meat samples. The equation for rat PER was successfully tested on several red and poultry meat samples.

2.22 Functional Aspects

High levels of collagen are undesirable in sausage raw materials and formulations for functional, as well as nutritional considerations. Although unable to emulsify fat, collagen will imbibe considerable quantities of water during the chopping phase of sausage production. Upon heating, the collagen shrinks, converts to gelatin, and drains from the surface of the fat globule. This leads to the formation of a fat cap at the top and a jelly pocket at the bottom of the sausage (Kramlich et al., 1973). In addition to emulsion breakdown, high collagen levels may also cause peeling problems in frankfurters, and a type of syneresis upon storage (Tauber, 1975).

Tauber (1975) noted that a high collagen content in frankfurters creates considerable problems with shrinkage and dimensional distortion upon reheating prior to serving. High collagen frankfurters may lose 25 percent in weight and shrink one inch or more in length. To avoid problems, Tauber (1975) suggests limiting collagen type proteins to one-third of the total protein in a frankfurter formulation.

Hansen (1960) observed that the fragments of tough connective tissue formed by preliminary grinding of meat for frankfurters remain essentially unchanged during the chopping of sausage emulsions. Although thicker and tougher, the connective tissue capsule which encases the bovine spleen may be roughly likened to poultry skin. Baker et al. (1968) found that the increased firmness of chicken franks attributed to added skin was caused by small chewy skin particles which were visible at the cut surfaces.

Evaluating the potential usefulness of various parts of several classes of poultry in sausages, Maurer and Baker (1966) found significant negative correlations between the collagen content of the meat and its emulsifying capacity. The significant correlations were largely due to the amount of skin in each part. Hudspeth and May (1969) reported that of the poultry tissues tested, skin was the least desirable with respect to emulsification properties.

Investigating the functional properties of mechanically deboned poultry meat in frankfurter formulations, Schnell et al. (1973) found that increasing amounts of skin resulted in decreased stability of the raw emulsions and increased heating loss values. Fat caps were observed on the finished product at the higher levels of skin.

2.23 Mechanical Removal

Several workers have reported the ability of mechanical deboning equipment to remove much of the connective tissue from red and poultry meat (Field, 1976). While mechanically deboning broiler backs, Satterlee et al. (1971) visually observed that large amounts of skin tissue passed through the deboner and were expelled with the bone. As the skin content of the broiler backs was increased from 0 to 44 percent, the collagen content of the mechanically deboned meat product decreased slightly.

To determine the exact mode of action of the deboner on skin, Satterlee et al. (1971) passed isolated skin through the deboner. The whole skin had a collagen content of 29.5 mg/g sample. After passage through the deboner, the product contained 2.5 mg collagen/g sample and the residue had 30.3 mg collagen/g sample.

Field and Riley (1974) found the hydroxyproline content of mechanically deboned lamb breasts to be lower than that of hand-boned lamb breasts. This finding along with that of less glycine and proline, confirmed that some connective tissue was removed by the deboner. The authors noted that machine deboning thereby increases the nutritional value of lamb breasts.

In a comparison study, Field et al. (1974) noted improved emulsion stability, texture score, and reduced shrink in bologna due to the lower connective tissue content of machine deboned mutton.

Gillett et al. (1976) used a Beehive deboner head specially designed to permit the desinewing of meat cuts having heavy connective tissue. This head had larger perforations than the typical deboning head. Connective tissue and fat decreased an average of 46 and 12.8 percent, respectively, when beef shanks, plates, chucks, and pork shoulders were desinewed. The effects of desinewed meat on the processing and palatability of cooked salami formulations were evaluated. Salami prepared with nondesinewed meat broke down and formed jelly pockets. In contrast, the salami made from desinewed meat exhibited reduced shrinkage, improved panel ratings, and the absence of jelly pockets.

Cross et al. (1976) reported that connective tissue is a major problem associated with the acceptance of ground beef. They found that meat from U.S. Utility or lower quality or from minor cuts of any grade, produced a product that was unacceptably high in connective tissue. Cross et al. (1978) evaluated the effect of desinewing versus grinding on the textural properties of comminuted beef. Patties from

desinewed beef were rated more tender and lower in detectable connective tissue than ground patties. The effect of desinewing was greatest on cuts from older carcasses which usually have large amounts of tough connective tissue. Meat desinewed through the 0.19 cm head was superior to that desinewed through the 0.25 and 0.32 cm heads.

Hendricks et al. (1977) studied the effect of desinewing on the protein quality of beef shank muscle. The desinewed meat had 40 percent less hydroxyproline and a 16 percent higher chemical score than the control. Desinewing improved the tryptophan level sufficiently so that it was no longer the first limiting amino acid. The sulphur amino acids became most limiting in the desinewed meat. These investigators noted that any processing procedures which decrease connective tissue in meats would be expected to increase the biological value of the meat protein.

2.3 Iron

2.31 Forms and Distribution

Biological iron in the mammalian body may be chemically classified into two groups: 1.) Heme compounds, such as hemolglobin, myoglobin, cytochrome, catalase and peroxidase; and, 2.) Non-heme compounds, such as the ferroflavin enzymes, transferrin, and the storage compounds ferritin and hemosiderin (Moore and Dubach, 1962). Most of the body iron is present in circulating hemoglobin (66%) (Lynch et al., 1974); about 3 to 5 percent is in myoglobin, while less than 1 percent is in heme-containing enzymes or is in transit through the plasma attached to transferrin (Bothwell and Finch, 1962). The iron storage compounds account for approximately 25 to 30 percent of iron in the body (Bothwell

and Finch, 1962). They are predominantly present in the liver, spleen and bone marrow, but are also found in smaller amounts in other parts of the body (Weinfeld, 1970; Underwood, 1971).

Among the body organs the liver and spleen usually carry the highest iron concentrations, followed by the kidney, heart, skeletal muscles and brain, which contain only one-half to one-tenth the levels in the liver and spleen. Individual variation in the iron levels of liver, kidney and spleen can be very high (Underwood, 1977).

Studying iron absorption in human subjects, Martinez-Torres et al. (1974) stated that liver is the best food in terms of nutritive iron value because of its high iron content, its high absorbability and its effect on the absorption of vegetable iron.

The data presented in Table 1 demonstrate that significantly greater quantities of iron are contained in bovine spleens than in bovine livers. Investigations of the absorption of iron from bovine spleen have not been conducted. However, comparisons of spleen and liver with regard to the amount and forms of iron present may be of value in predicting the bioavailability of splenic iron.

Martinez-Torres et al. (1974) performed chemical and radioactive determinations of the various iron compounds present in veal liver. Their results indicated that most of the iron is present in the form of heme as hemoglobin and that about 25 to 30 percent is in the form of ferritin and hemosiderin. The same assay performed on cow liver showed that 54 percent of the iron was heme iron, 33 percent was ferritin and 13 percent was hemosiderin.

Table 1. Total Iron Content of Bovine Tissues

	Tissue Type	Spleen	Liver	Kidney	Heart	Muscle
Ammerman et al. (1967)	dry	1,067	233	479	223	85
Standish et al. (1969)	dry	1,219	185	315	291	91
		2,671	269	360	291	81
		8,941	605	410	329	98
		5,479	496	326	287	87
Standish et al. (1971)	dry	1,521-6,725	256-552	320-400	261-327	73-94
Ammerman et al. (1974)	dry	750-2,763	227-445	331-358	251-261	127-152
Kizlaitis et al. (1962)	wet	750	91	85	50	
Clement et al. (1972)	wet					
steak						30
veal						28.6
Jenkins (1977) cow	wet					24.5
steer						20.3
heifer						20.6

[a]Expressed as ppm on a wet or dry tissue basis.

In a study of 60 kg pigs, Furugouri (1973) found that heme iron accounted for 33 percent of the total iron in liver and 47 percent in spleen. Ferritin accounted for 60 percent of the nonheme iron of liver and 41.3 percent in spleen.

The amount of storage iron in the body, its distribution between hemosiderin and ferritin, and its relative concentration within a given tissue is dependent upon a number of factors including the species, sex, age, diet and health of the subject.

The main factor affecting the relative distribution of iron between ferritin and hemosiderin in mammals is the total storage iron concentration. Up to certain levels and rates of storage, iron is deposited in the liver and spleen readily and in roughly the same amounts as ferritin and hemosiderin (Underwood, 1977). It is generally stated that, under normal physiological conditions, there is a slight preponderance of ferritin iron over hemosiderin iron in storage. With increasing concentrations of iron this ratio is reversed and hemosiderin increases relative to ferritin (Bothwell and Finch, 1962; Moore and Dubach, 1962; Harrison et al., 1974).

Over a wide range of iron depletion and storage rates and levels, the distribution between ferritin and hemosiderin remains relatively constant, and the iron moves readily from one storage form to the other (Underwood, 1977).

Studying the relative distribution between ferritin and hemosiderin in the splenic and hepatic storage iron of 130 human necropsies, Morgan and Walters (1963) found that in normal subjects somewhat more than half of the storage iron was present as ferritin. Considerable similarity

was found in the behavior of the storage iron in the liver and spleen. The authors (Morgan and Walters, 1963) noted that this is probably due to the fact that much of the storage iron is in cells of the reticulo-endothelial system, and that these cells behave in a similar way in both organs. It is also possible that iron is distributed between ferritin and hemosiderin in much the same way in hepatic parenchymal cells as in reticulo-endothelial cells.

Standish et al. (1969) microscopically observed iron stained sections of livers and spleens from beef calves (steers) fed graded levels of dietary iron for 84 days. A lesser amount of hemosiderin was observed in the liver than the spleen at each level of dietary iron. Small and moderate amounts of hemosiderin were observed in sections of spleens from animals fed 0 and 400 ppm iron, respectively. Liver sections of the animals fed 0 or 400 ppm iron rations did not contain hemosiderin. Much hemosiderin was seen as large accumulations in the spleens of cattle given 1,600 ppm iron diets, whereas liver sections from these same animals contained only a moderate amount. Noting that the iron content of almost all tissues studied increased as dietary iron was increased, Standish et al. (1969) suggested that the mucosal block mechanism of the abomasum, if one exists in ruminants, was overcome by as little as 400 ppm supplemental iron.

Standish et al. (1971) found that increasing the phosphorous content from 0.23 to 0.46 percent in a 1,000 ppm iron diet caused a 50 percent decrease in microscopically observed hemosiderin of spleens and livers from beef calves fed for 77 days.

Iron absorption as well as the total iron status of a bovine animal depends upon the amount of iron in the diet (Standish et al., 1969), the chemical form of the iron present (Ammerman et al., 1967), the quantity of other elements present (Standish et al., 1971), and the amounts and proportions of other components in the whole diet (Underwood, 1966). These facts can, in part, account for the wide range of iron content reported for bovine tissues under varied experimental conditions. Listed in Table 1 are the ranges of iron content reported by several authors. Although no author gives specific information as to the form of iron present in any tissue, it can be observed that the greatest amounts and ranges of iron were reported for those tissues known to hold the major portion of storage iron.

2.32 Nutritional Value

Iron deficiency is probably the most prevalent deficiency state affecting human populations (Underwood, 1977). In contrast with other dietary deficiencies, iron deficiency is not closely linked with socioeconomic status but is widely prevalent both in developing countries and in highly industrialized nations. The normal diet contains more than five times the total amount of iron needed to maintain iron balance, the problem is not so much inadequate intake but rather poor availability of dietary iron (Cook, 1977).

The absorption of iron is affected by the age, iron status and state of health; by conditions within the gastrointestinal tract; by the amount and chemical form of iron ingested; and by the amounts and proportions of various other components of the diet, both organic and inorganic (Prasad, 1978).

The assimilation of dietary iron occurs from two independent pools: heme and nonheme (Layrisse et al., 1973). The heme chelate of iron is absorbed directly into the mucosal cell where the porphyrin ring is split to make the iron available to the body. This process is not affected by enhancing substances such as ascorbic acid or inhibiting substances such as phytates, oxalates and desferrioxamine and other factors which have profound influences on the absorption of nonheme iron (Linder and Munro, 1977 and Prasad, 1978). Inorganic forms of iron and iron-protein complexes must be reduced to the ferrous state and released from conjugation for effective absorption (Underwood, 1977). All forms of nonheme iron appear to enter a common pool, the availability of which is greatly influenced by a variety of dietary substances that either facilitate or impair absorption (Layrisse et al., 1973).

Numerous workers have shown that iron absorption is significantly lower, and dietary iron requirement therefore higher, in vegetable diets than in foods from animal sources, mixed diets, and iron salts. Hussain et al. (1965) examined iron absorption from wheat, hemoglobin, ferritin and various iron salts. Wheat iron was less available than iron from iron salts, hemoglobin, or ferritin. Investigating iron absorption from different animal and vegetable commodities fed to human subjects, Layrisse et al. (1969) determined that the iron in foods of plant origin was consistently absorbed to a lesser degree than that from foods of animal origin.

The ingredients of meals and diets clearly affect dietary iron requirements profoundly through their effects on absorption (Underwood, 1977). Martinez-Torres and Layrisse (1971) used the technique of

simultaneous administration of two foods tagged with different radio-isotopes of iron to reveal food interactions in iron absorption. They reported that absorption of iron from veal muscle was not affected by ascorbic acid, but was significantly reduced by desferrioxamine. Absorption from veal muscle is slightly reduced when it is administered with vegetable foods in a meal. However, veal muscle approximately doubles the absorption of vegetable iron. Hemoglobin (purified) iron absorption is enhanced approximately twice when it is administered with meat in a meal. Martinez-Torres and Layrisse (1971) suggested that the digestion of meat yields protein degradation substances which increase food iron absorption.

Measuring the dietary absorption of iron from complete meals, Layrisse and Martinez-Torres (1972) demonstrated the importance of meat for the nutritive iron value of a diet. The absorption of ferric chloride was reduced by vegetable foods, whereas heme iron absorption was not affected. Ferric chloride absorption was almost twice as high when it was administered with veal and maize than when it was given with maize alone. The ratio of hemoglobin to meat iron absorption was close to unity and was not affected by maize or a meal containing three vegetable foods, each carrying high concentrations of substances that inhibit iron absorption. Meat was responsible for 80 percent of the nutritive iron value of a complete meal consisting of three vegetable foods and one-third meat.

In a study of iron fortification, Layrisse et al. (1973) determined that fortification iron provided in an available form mixes with the

nonheme iron pool of the diet. The absorption of meat iron was not affected by the dose of nonheme iron present either as a vegetable food or an iron salt. However, the presence of meat in a meal increased appreciably the absorption of nonheme iron represented by the sum of vegetable and fortification iron. Fortification iron added to a vegetable food showed a very limited absorption, reaching 0.3 mg with an intake of 60 mg of fortification iron. By contrast, a supplement of 5 mg of iron eaten with veal muscle resulted in absorption of 0.85 mg of iron. The authors concluded that food iron fortification is likely to be effective only in individuals who take animal protein as part of their diet.

Employing the extrinsic tag model to study iron absorption in humans, Layrisse et al. (1974) demonstrated that the amount of dietary iron does not reflect the net amount of iron absorbed and utilized by individuals. Also, that the ingredients of a meal such as beef, fish and fruits are paramount in order to obtain a reasonable utilization of the nonheme iron. In one test, heme iron accounted for 62 and 42 percent of the total iron absorbed despite the fact that the meat iron intake was only 20 and 12 percent of the total dietary iron. Overall, the iron absorption from vegetable foods was approximately doubled by the effect of 50 g of meat, nearly thrice by 100 g of fish, and almost five times by 66 mg of ascorbic acid or 150 g of papaya containing a like amount of ascorbic acid. Although fish enhanced the absorption of nonheme iron, it was noted that most of the iron in the fish was in the form of ferritin and the absorption of this iron compound is reduced when administered with vegetable foods.

Martinez-Torres et al. (1974) investigated veal liver and concluded that it is the best food in terms of nutritive iron value because of its high iron content, its high absorbability and its effect on the absorption of vegetable iron. Veal liver iron was absorbed as well as veal muscle iron. Absorption was not affected by ascorbic acid, but there was a marked reduction with desferrioxamine or maize. Since heme iron absorption is not affected by these chelating substances (Layrisse and Martinez-Torres, 1972), the authors noted that they may affect the absorption of ferritin and hemosiderin iron from liver. The interaction of liver and maize resulted in a three fold enhancement of the absorption of the vegetable iron and a reduction of the absorption of liver iron.

Iron absorption from liver represents the sum of iron absorption from heme, present in hemoglobin, and from nonheme, present in ferritin and hemosiderin which may be absorbed independently or with interaction between them and other liver proteins (Martinez-Torres et al., 1974).

Layrisse et al. (1975) conducted a series of experiments to evaluate the absorption of ferritin iron by man and to determine which iron pool it could be identified with. They showed that purified ferritin has a very low absorbability. However, absorption from ferritin was markedly increased when it was given with either muscle or liver. The mean absorption from ferritin was 12 or 6.6 percent when administered with veal muscle or liver, respectively. Ferritin iron did not exchange with the heme pool and was not absorbed as efficiently as heme iron. Iron absorption from purified ferritin and ferritin in liver was decreased by

desferrioxamine and vegetables. Ascorbic acid elevated ferritin iron absorption from 5.3 to 11.5 percent. Kizlaitis et al. (1962) found beef livers and spleens to contain 22 and 46 mg ascorbic acid per 100 g on a fresh basis, respectively.

Iron absorption of ferritin is affected by substances that enhance or reduce iron absorption in the same proportion as vegetable iron. However, this compound is not entirely mixed with vegetable food iron when they are administered together. The completed study (Layrisse et al., 1975) did not elucidate whether ferritin is incompletely miscible with a nonheme iron pool or if it really forms a third iron pool.

It has been stated (Martinez-Torres and Layrisse, 1971) that different patterns of iron absorption from various types of meat may be found because of the varied proportions of hemoglobin, myoglobin and ferritin. The iron absorption from hemosiderin has not been determined (Layrisse et al., 1975).

2.4 Reported Uses of Spleen

Speaking at the 22nd European Meeting of Meat Research Workers, Duda (1976) stated that several by-products, which could potentially be used for manufacture of food products for human consumption including spleen, are widely wasted. Later, Gorbatov (1977) noted that despite a high food value, most by-products are not used for edible purposes because no recommendations exist on their processing and utilization. No literature dealing with the use of fresh bovine spleen in the United States has been located. Limited work has been conducted by European workers. However, only abstract reports are available.

Skrabka-Blotnicka and Maskos (1976) employed customary procedures and spices to produce pates with 15 or 30 percent beef, pig or calf spleen or liver. At 15 percent incorporation, the product with beef spleen was assessed tastiest; and at 30 percent, the decreasing order was beef spleen, calf and pig spleen and calf liver, beef liver, and pig liver. Products in jars retained their organoleptic quality for 6 months. It was concluded that, owing to its relative low price, spleen is a desirable replacement for liver in pate manufacture. Oreshkin (1977) investigated the partial substitution of spleen for liver in liver sausage. However, no additional details are available.

Farstad (1977) conducted a hygenic and nutritional evaluation of bovine and porcine spleens. With reference to tables of literature data, it was concluded that the nutritional value of spleen is slightly inferior to that of liver. Microbiological studies on 100 samples of bovine spleen showed that 35 percent of samples were sterile. The microflora of the nonsterile samples consisted mainly of Bacillus spp., Micrococcus spp., non-haemolytic coliforms, and α-haemolytic streptococci. No salmonellae or residues of inhibitory substances were detected in either bovine or porcine spleens. It was concluded that there are no hygenic objections to the use of spleens as food.

3.0 MATERIALS AND METHODS

3.1 Spleen Evaluation

3.11 Mechanical Separation

Employees of Valleydale Packers, Inc. (Salem, VA) collected 136 kg (300 lb) of beef spleens from a day's kill of 363 to 454 kg (800 to 1000 lb) slaughter cattle. Chilled, boxed spleens were placed in insulated transport containers, iced, and driven to Shen-Mar Food Products Corp. (Bridgewater, VA).

At the Shen-Mar facility, 113 kg of beef spleen were passed through a Beehive model AU 4171 deboner with a desinewing head containing 0.19 cm perforations. Product and residue were collected in pre-weighed containers to allow calculation of yield percentage. The mechanically separated splenic pulp, residue and remaining whole spleens were placed in white opaque, 30.5 x 50.8 cm polyolefin type L348, Cryovac bags (W. R. Grace and Co., Simpsonville, SC) and packed in ice for transport to the VPI&SU Meat Laboratory.

3.12 Spleen Testing

3.121 Sample Preparation

Within 18 hours of mechanical separation, two bags of mechanically separated spleen (MSS) were randomly selected and opened. The contents, approximately 5.4 kg/bag, were placed in a hand operated, 8.2 kg capacity, stainless steel sausage stuffer (Vogt Model KV-9), and extruded onto 65 x 45 x 3 cm, cellophane-lined trays. The MSS was smoothed to approximately 2 cm thickness, covered with cellophane and

frozen at -21°C. As the trays were being loaded, quart-size plastic freezer bags were filled at random intervals. Three of six plastic bags were selected as triplicate samples for proximate and elemental (Sect. 3.4) analysis. The remaining three bags were frozen and retained as backup samples.

Approximately 16 kg of whole spleen were cut into 1.3 cm cubes and homogenized in a Hobart Model 8142 food cutter. Chopping was continued until a consistency similar to that of MSS was obtained. All connective tissue collected on the comb and blade shaft of the food cutter was returned to the spleen mass. Homogenized whole spleen was placed in the stuffer, extruded onto cellophane-lined trays and into plastic bags and treated exactly as was the MSS.

3.122 Proximate Analysis

Triplicate 150 g samples of whole spleen, MSS and the separation residue were weighed into 150 x 25 mm, plastic, tissue culture dishes (Falcon, No. 3025). Moisture was determined by lyophilization of the samples. Each freeze dried sample was then finely divided in a Waring blender and distributed into three-118 ml glass jars. The samples were then freeze dried and held in desiccators until needed for proximate and elemental (Sect. 3.4) analysis. Crude protein, ash and ether extract determinations were made on the dry samples by AOAC (1975) methods.

3.123 Preparation of Diets

The MSS and homogenized whole spleen which had been frozen in cellophane-lined trays was removed from the freezer and cut on a band

saw into appropriately sized blocks for freeze drying. Cut blocks of spleen were double packaged in custom-sized bags made from a polyethylene Cryovac film and held at -29°C until approximately 7.5 kg of each spleen material had been freeze dried.

Freeze dried whole spleen and MSS were separately blended in a Waring blender, thoroughly mixed, placed in an open Cryovac film bag and allowed to air equilibrate for 24 hours. At this point, triplicate 3 g samples of each were collected and analyzed for crude protein (AOAC, 1975). A second set of samples was taken for the determination of dry matter by freeze drying and ether extract by AOAC (1975) methods. The remaining spleen material was double packaged in Cryovac film and placed in a -29°C freezer until shipment.

When all analyses were completed, the freeze dried MSS and whole spleen were shipped to Purina Test Diets (Ralston Purina Co., Richmond, IN) for formulation into diets to be used in the determination of protein efficiency ratio (PER).

Diets were prepared from whole spleen, MSS and a reference casein. Each was standardized to 10 percent protein. Fat required to bring each diet to the 8 percent level was obtained by adding corn oil and lard in a 1:1 ratio. Mineral mix was added to all diets at the 5 percent level, neglecting the ash in the protein source (Staub, 1978). The diets contained 3 percent Solka Floc and 2 percent vitamin mix. A 50:50 mixture of sucrose and Dextrin was added to make the total of all ingredients in each diet equal to 100 percent.

Diet materials were extruded to form standard rabbit pellet-sized units. This modification was added to improve accuracy in the retrieval

and weighing of daily refusal over that possible with powdered diets. Complete diets were packed in plastic bags and shipped to VPI&SU. Upon arrival, they were sealed in enameled No. 303 cans and placed in a -21°C freezer until required for daily feeding.

3.124 Protein Efficiency Ratio

Weanling male, Sprague-Dawley rats, 21 days of age were obtained from a commercial supplier (Flow Labs, Inc., Dublin, VA). Rats were weighed to the nearest 0.1 g at arrival and individually housed in hanging, 24.5 x 17.5 x 18 cm, stainless steel cages with mesh floors and fronts. A commercial rat chow and water were fed ad libitum during a five day acclimation period.

At the completion of the acclimation period, rats weighing 42.8 to 58.5 g were divided into groups of three by ascending order of weight. The rats within each group were then randomly assigned to a test diet and location on the cage rack. Assay groups were assembled in lots of 10 rats so that the mean weight between lots was within 1.4 g.

Cardboard dividers were placed between cages to isolate the drop area beneath each cage. This system was used to allow identification of dropped pellets by animal and thereby increase the accuracy of consumption records. Rats were fed the experimental diets for a total of 28 days. A weighed quantity of fresh feed was presented daily. Each rat's allotment of feed was based on the weight of feed consumed during the previous 24 hours. Water was available ad libitum. Individual weight gains were determined on a bi-weekly basis.

Upon conclusion of the 28-day feeding study, PER values were calculated for each rat based upon individual weight gain and protein

consumption. Analysis of variance and Duncan's multiple range tests (Barr et al., 1976) were performed on the actual PER values before adjustment to a casein value of 2.5.

3.2 Frankfurter Evaluation

3.21 Raw Material Preparation

The beef used in the frankfurter production phase of this experiment was obtained from a U.S. Utility cow carcass supplied by the Meat Laboratory, VPI&SU. Two hind quarters and one forequarter were hand boned, ground through a 1.27 cm plate, mixed in a paddle-ribbon mixer and then ground through a 0.48 cm plate. The second grind was followed by mixing and sampling for fat and moisture determinations. The ground beef was then weighed into type L348 Cryovac bags (4.54 kg/each) and stored in a -21°C freezer.

Boneless pork shoulders and regular pork trim were purchased from Valleydale Packers, Inc. The pork shoulders were cut into cubes and ground through a 1.27 cm and a 0.48 cm plate. Each grind was followed by thorough mixing in a paddle-ribbon mixer. Regular pork trim was ground through the 1.27 cm plate only. Pork raw materials were sampled for fat and moisture analysis, packaged in type L348 Cryovac bags (4.54 kg/bag) and stored in a -21°C freezer.

Meat raw materials were analyzed for fat and moisture prior to formulation. Fat was determined by a modification of the Modified Babcock procedure (Ockerman, 1974) wherein 11 ml of a 2:98 mixture of alkylbenzyldimethylammonium chloride (Baker) and concentrated sulfuric acid was used as the digestion agent. Moisture was determined by oven drying.

3.22 Frankfurter Formulation

Beef and pork frankfurters were formulated with 0, 5, 10 and 15 percent of the meat block being MSS. Substitutions of MSS were made at the expense of the pork portion of the control formula. The formulations in Table 2 were designed to yield final products with similar proximate compositions from 90.72 kg (200 lb) of raw emulsion. Frankfurters were formulated to contain 25 percent fat, no more than 10 percent added water, and 3.5 percent nonfat dry milk (NFDM) in the final products.

A commercial seasoning (Regal Peerless Frank Seasoning, bag No. 1, Griffith Laboratories, Union, NJ) was supplemented with ground white pepper and paprika. Savortex brand of calcium reduced NFDM was obtained from Western Dairy Products. Prague Powder N.N. (Griffith) was used to supply 156 ppm sodium nitrite.

The salt (1.82 kg), white pepper (82.64 g), paprika (82.64 g), and Prague Powder (165.3 g) were blended and packaged for use with each batch of frankfurters. Savortex (2.87 kg) and the commercial frankfurter seasoning (4.33 kg) were packaged individually for use in each formulation. Liquid smoke (44 ml, Griffith) was added to the water and ice at the time of frankfurter production.

3.23 Frankfurter Production

Frozen meat raw materials were removed from the freezer and defrosted in a 2°C cooler for 48 hours prior to use. The MSS was used within 48 hours of mechanical separation and was not frozen. All meat and dry ingredients were transported to the facilities of Green Hill Inc. (Elliston, VA), where frankfurters were produced under commercial conditions.

Table 2. Weight of ingredients used in frankfurter formulations in kg.

Ingredient	Level of Spleen (%)			
	0	5	10	15
Beef	33.08	33.08	33.08	33.08
Lean pork trim	13.63	8.34	3.04	0
Regular pork trim	19.45	21.43	23.42	23.15
MSS	0	3.31	6.62	9.92
Water & ice	15.21	15.21	15.21	15.21
Seasoning, cure & NFDM[a]	9.35	9.35	9.35	9.35

[a]Seasoning ingredients: Griffith Regal Peerless frank seasoning, bag #1, 4.33 kg; sodium chloride, 1.82 kg; ground white pepper, 82.64 g; paprika, 82.64 g; liquid smoke, 44 ml. Cure ingredient: Prague Powder N.N., 165.3 g. Calcium reduced NFDM, 2.87 kg.

Ingredients were mixed in a paddle-ribbon mixer. The beef, salt-cure mix and MSS (where required) were allowed to mix approximately 5 min before the spice, lean pork trim and half the water and ice were added. After an additional 3 min of mixing, the regular pork trim, NFDM and remaining water and ice were added.

Emulsification was completed by passing each meat mix through a dual-plate Mince Master (Griffith) emulsifer. Final emulsion temperatures were below 18°C. The finished emulsions were stuffed and linked in 22-23 mm diameter, clear, Precision Nojax E-Z Peel cellulose casings (Union Carbide Corp., Chicago, IL) by a Townsend Frank-a-Matic (Townsend Engineering Co., Des Moines, IA). Stuffing rate was adjusted to yield 10 frankfurters per pound.

All frankfurters were simultaneously cooked in an air conditioned smoke house operated to produce 54.4°C for 20 min; 71.1°C for 15 min; 76.7°C for 20 min; and 87.8°C for 10 minutes. Two minutes of steam completed the cooking process. Frankfurters reached an internal temperature of 72°C in the smokehouse.

Cooked frankfurters were showered with cold water to an internal temperature of 24°C and placed in a 17-min brine shower where the internal temperature was reduced to 2°C. Chilled franks were immediately peeled with a Townsend Ranger peeler and vacuum packaged in Cryovac Surlyn film by a Hooper 1000 (W. R. Grace) vacuum packaging machine. Packaged frankfurters were packed in shipping boxes and transported to VPI&SU where they were stored in a 2°C cooler until required for further testing.

3.24 Frankfurter Evaluation and Analysis

3.241 Proximate Analysis

Five frankfurters from each of three randomly selected packages of each spleen addition level were cut into 2 cm lengths, mixed and ground through a 0.5 cm plate into a gallon-size plastic food bag. Franks were ground with a Kitchenaid Model C-4 (Hobart Corp., Troy, OH) home food mixer and grinder attachment. After thorough mixing, triplicate samples were obtained by weighing approximately 100 g of ground frankfurter into each of three 150 x 25 mm plastic, tissue culture dishes (Falcon, No. 3025).

Moisture was determined by lyophilization. Each freeze dried sample was then finely broken in a Waring blender and distributed into three-118 ml glass jars. The samples were then re-freeze dried and held in desiccators until needed for proximate and elemental (Sect. 3.4) analysis. Crude protein, ash and ether extract determinations were made on the dry samples by AOAC (1975) methods. Carbohydrate was calculated by difference.

3.242 Physical Attributes Panel

On the third day after frankfurter production, an untrained panel of five persons experienced in evaluating processed meats was assembled to score the physical attributes of each product. Randomly selected frankfurters from each treatment were coded and displayed on white butcher paper in a well lighted room. Franks were displayed both whole and split longitudinally for inspection by the panel.

The physical attributes evaluated and descriptive terms employed are shown in Table 3. Frankfurter physical properties were scored as

Table 3. Frankfurter evaluation: physical attributes

Description	Score
External Color:	
Deep smoked/cured	5
Slightly deep smoked/cured	4
Smoked/cured	3
Average cured	2
Pale	1
Firmness: resistance to pressing	
Very firm	5
Firm	4
Soft	3
Very soft	2
Mushy	1
Resilience: ability to return to original shape after pressing	
Excellent - rapidly	5
Good - moderately rapid	4
Fair - slowly or only partially	3
Poor - slight suggestion of return	2
Very poor - mushiness	1
Binding: resistance to tearing	
Excellent	5
Good	4
Fair/average	3
Poor	2
Very poor	1
Internal Color:	
Deep red	5
Red	4
Pink	3
Pale pink	2
Pale	1
Texture:	
Excellent	5
Good	4
Fair/average	3
Poor	2
Very poor	1
Overall Physical Acceptability:	
Excellent	5
Good	4
Fair/average	3
Poor	2
Very poor	1

follows: external color (5 = deep smoked-cured, 1 = pale); internal color (5 = deep red, 1 = pale); firmness (5 = very firm, 1 = mushy); resilience, binding, texture and overall physical acceptability (5 = excellent, 1 = very poor). Panel responses were averaged and reported as descriptive statistics.

3.243 Consumer Taste Panel

Frankfurters were evaluated by an 11-member consumer panel of persons unfamilar with the nature of the study. They received a 1 lb, coded package of frankfurters at four separate times during the first 2 weeks of the study. The order of presentation was random with the constraint that no panel member received all four treatments in an ascending or descending order of spleen addition. Panel members were instructed to prepare the frankfurters as they normally would, with no special considerations.

Products were evaluated and scored for flavor and color on a 5-point hedonic scale (5 = very desirable, 1 = very undesirable) while texture was rated from 5 (very firm) to 1 (very soft). As an alternative to the overall satisfaction question, the participants were asked what price they would pay for each product relative to the average price of frankfurters. Purchase price was scored on a 7-point hedonic scale where 7 = high premium (30 cents/lb above average priced frankfurters), 4 = average price, and 1 = substantial discount (30 cents/lb below average priced frankfurters). Data were subjected to analysis of variance and the Duncan procedure (Barr et al., 1976).

3.244 Laboratory Taste Panel

An untrained, 12-member panel of persons experienced in the

sensory evaluation of foods was used to evaluate the frankfurters at 2 week intervals during a 6-week shelf life period.

Two packages of frankfurters from each spleen addition level were randomly selected for organoleptic evaluation after 0, 2, 4 and 6 weeks storage. Frankfurters were heat-sealed in boilable plastic bags (5/bag) with 150 ml of tap water and placed in a 75°C water bath for 15 min. After heating, the ends were removed from the frankfurters and the remainder was cut in half. The frankfurter halfs were placed in coded, 118 ml white plastic cups and immediately served to the panelists. All evaluations were conducted in a specially designed taste panel facility.

Products were evaluated and scored on 5-point hedonic scales for flavor, color (5 = very desirable, 1 = very undesirable), texture (5 = very firm, 1 = very soft) and overall acceptability (5 = very acceptable, 1 = very unacceptable). Panel scores were subjected to analysis of variance and the Duncan procedure (Barr et al., 1976).

3.245 Shear Values

The effect of level of spleen and time of storage on the textural qualities of the frankfurters was evaluated by shear resistance after 0, 2, 4 and 6 weeks storage. Shear values were determined using an Allo-Kramer shear press, model S2HE (Precision Metals Engineering, Inc., Rockville, MD), with the 454 kg (1000 lb) compression ring and the standard, 10-blade, shear-compression cell. Determinations were made using a 25-sec downstroke and a range setting of 10.

At each test period, 10 frankfurters from each treatment were obtained by removing five frankfurters from each of two randomly selected packages. A 6.5 cm sample was cut from the center of each frankfurter

and weighed to the nearest 0.01 g. The cut and weighed sections were individually sheared after placement in the center of the shear cell at right angles to the slots. Maximum force values were used to compute shear values as pounds of force required to shear per gram of frankfurter (Baker et al., 1968). Shear values were evaluated by analysis of variance and the Duncan procedure (Barr et al., 1976). Mean shear values for each treatment, disregarding test periods, were calculated and plotted.

3.246 Objective Color Measurements

The effect of level of spleen and time of storage on the color of the frankfurters was objectively evaluated by Hunter color meter L value and total and nitroso-heme pigment determinations made after 0, 2, 4 and 6 weeks of storage.

3.2461 Color Meter Determinations

A Hunter Lab Model D25 Color and Color Difference Meter (Hunter Associates, McLean, VA) was used to objectively evaluate the color of each product over time. Hunter L values were determined on the internal and external surfaces of 10 frankfurters from each treatment at every test period. Frankfurters were obtained by removing five from each of two randomly chosen packages of each treatment. Color values were evaluated by analysis of variance and the Duncan procedure (Barr, et al., 1976). Least squares lines relating L value and time of storage were plotted for each frankfurter surface and level of spleen.

3.2462 Pigment Determinations

Total and nitro-heme pigments were determined by the method of Hornsey (1956). Packages of frankfurters were foil wrapped

before removal from the 2°C storage cooler. All determinations were made in a darkened room. Two randomly selected packages of frankfurters from each treatment were tested separately. Five frankfurters from each package were cut into 2 cm lengths, mixed and ground through a 0.5 cm plate into a gallon-size plastic food bag. Frankfurters were ground with a Kitchenaid Model 4-C home food mixer and grinder attachment.

Nitroso-heme pigment determinations were made in duplicate on each ground frankfurter sample. Ten grams of ground sample were weighed into a 125 ml erlenmeyer flask. After adding 50 ml of a 40:4 (v/v) acetone-water solution, the flasks were stoppered, mixed, and held in the dark for 5 minutes. The slurry was then filtered through two layers of Whatman No. 4 filter paper. Filtrate was collected in an aluminum foil-wrapped 125 ml erlenmeyer flask. The absorbance of each filtrate was measured in a 1 cm cell at 540 nm using a Perkin-Elmer, Coleman Model 124 double beam spectrophotometer. A 40:10 acetone-water solution was used as a reference blank. The concentration of nitroso-heme pigment, expressed as ppm of hematin, was calculated by multiplying the absorbance of 540 nm by a factor of 290.

Total heme pigment determinations were made in duplicate on each ground frankfurter sample. The procedure was identical to that described for nitroso-heme pigments, except that the samples were mixed with a 40:3:1 (v/v) acetone-water-concentrated HCl solution and held in the dark for 60 minutes. Absorbance was measured at 640 nm against a reference 40:9:1 acetone-water-HCl solution. The concentration of total heme pigment, expressed as ppm hematin, was computed by multiplying the absorbance by a factor of 680.

Pigment data were subjected to analysis of variance and the Duncan procedure (Barr et al., 1976). Least squares lines relating ppm hematin and time of storage were plotted for each pigment type and level of spleen.

3.247 Microbiological Evaluation

Mesophilic, psychrophilic and coliform bacterial counts were determined on the raw emulsions. Similar counts were made on the cooked frankfurters after 0, 2, 4 and 6 weeks storage at 2°C.

Duplicate raw emulsion samples were obtained from each treatment by aseptically cutting individual links from separate casings immediately after stuffing. The raw frankfuters were placed in Whirl-Pak bags, packed on ice and transported to VPI&SU. The microbiological testing was conducted by the methods described for cooked frankfurters.

Two packages of each frankfurter treatment were randomly selected at each sampling interval. Each package was aseptically opened to allow the transfer of one entire frankfurter to a sterile, polyethylene, 18 x 30 cm, Stomacher Bag (Cooke Laboratory Products, Alexandria, VA). After the addition of 180 ml of 0.1 percent sterile peptone, each frankfurter was homogenized for 2 min by a Stomacher Lab-Blender 400 (Cooke Laboratory Prod.).

Appropriate 10-fold dilutions were made in 99 ml 0.1 percent sterile peptone dilution blanks prior to pour-plating with Trypticase soy agar (TSA; BBL, Cockeysville, MD). Duplicate plates of each dilution were incubated 48 hr at 30°C for total mesophilic plate counts and 10 days at 5°C for psychrophilic plate counts.

The determination of viable coliform bacteria was carried out according to the methods of Speck et al. (1975). Dilutions were spread-plated in duplicate on TSA. After being held at 25°C for 1 hr, the plates were overlaid with 10-12 ml of violet red bile agar (BBL). This plating technique was employed to allow repair by coliforms which may have been injured during the frozen storage of meat raw materials or the cooking of frankfurters, before introduction of the selective media. All plates were incubated 48 hr at 35°C, after which all pink-to-red colonies were counted. Following a positive presumptive test on the agar medium, a proportionate number of representative colonies from each plate was picked into 2 percent brilliant green lactose bile broth. Gas production after 48 hr at 35°C was taken to be confirmatory evidence that the colonies were coliforms.

3.3 Patty Product Evaluation

3.31 Patty Formulation and Preparation

Ground meat patties were produced from ground beef and MSS to contain 0, 5 and 10 percent spleen. Approximately 9.1 kg of boneless beef chuck containing an estimated 20 percent fat was ground through a 1.27 cm plate. Units of ground chuck weighing 2.27, 2.15 and 2.04 kg were thoroughly mixed with 0, 113.4 and 226.8 g of MSS, respectively. One percent salt (22.7 g) was included in each product. The hand-mixed products were ground through a 0.32 cm plate, remixed and chilled. Patties were formed with the aid of a 10.5 x 1.5 cm plastic mold, weighed and oven broiled for 7 min per side. Final weights were taken and shrink losses were calculated.

3.32 Patty Evaluation and Analysis

3.321 Taste Panel

Immediately after cooking, the meat patties were cut into quarters, coded and served to an untrained 18-member taste panel. Patties were evaluated and scored on 5-point hedonic scales for juiciness (5 = very juicy, 1 = very dry), flavor and mouth feel (5 = very desirable, 1 = very undesirable) and overall acceptability (5 = very acceptable, 1 = very unacceptable). Panel scores were evaluated by analysis of variance and the Duncan procedure (Barr et al., 1976).

3.322 Proximate Analysis

Four cooked patties from each spleen addition level were ground through a 0.5 cm plate into a quart-size plastic food bag and mixed. Triplicate 100 g samples were weighed into 150 x 25 mm, plastic, tissue culture dishes (Falcon) and frozen.

Moisture was determined by lyophilization. Each freeze dried sample was then finely broken in a Waring blender and distributed into three-118 ml glass jars. The samples were then freeze dried and held in desiccators until needed for proximate and element (Sect. 3.4) analysis. Crude protein, ash and ether extract determinations were made on the dry samples by AOAC (1975) methods.

3.4 Elemental Analysis

Iron, copper and zinc were determined using the freeze dried samples of spleen, frankfurter and cooked patty described in Sections 3.122, 3.241 and 3.322, respectively.

3.41 Sample Preparation

Each of the triplicate samples of all spleen, frankfurter and patty products was hydrolyzed in duplicate. This plan allowed a total of 6 determinations for each spleen material and product treatment.

Approximate 1 g, weighed to the nearest 0.0001 g, freeze dried samples were weighed into 100 ml beakers. An inital 20 ml of concentrated, A.C.S. Reagent grade nitric acid (HNO_3, Fisher) was added. Beakers were covered with a watch glass and placed on a hot plate. Samples were refluxed until the total volume in the beaker was reduced to 8-10 ml. (Note: The volume of HNO_3 should not fall below 5 ml while boiling off the first 20 ml. There is usually enough lipid remaining that violent boil-overs occur if the volume is reduced too low too early.) At this point, an additional 10 ml of HNO_3 were added and refluxing continued. This step was repeated so that a total of 40 ml of HNO_3 had been added to each sample. When the volume of HNO_3 was reduced to 5 ml, the beakers were removed from the hot plate and allowed to cool.

After the addition of 4 ml of perchloric acid ($HClO_4$, Baker), the samples were refluxed until the sample solution was light yellow to colorless and dense white fumes of $HClO_4$ had been evolved. Beakers were removed from the hot plate and allowed to cool. All samples were clear and colorless at this point. (Note: As the last of the HNO_3 is boiling off in the presence of $HClO_4$, the solution goes into a frothy "high-boil". As this vigorous boiling collapses, the solution may turn dark or appear charred. Continued refluxing yields a clear to faint yellow

solution which becomes colorless on cooling. One can usually observe a progressive clearing of a charred sample with each refluxed drop falling from the watch glass. Time of refluxing with HNO_3 seems to be as important as the total volume used. The longer it takes to reach a final volume of 5 ml of HNO_3, the less frequent or intense the charring upon heating with $HClO_4$.)

Cooled hydrolysates were filtered through Whatman #41 ashless filter paper into volumetric flasks. Distilled, deionized water was used to wash the watch glass, beaker and filter paper until the appropriate volume had been collected. Spleen hydrolysates were collected in 50 ml volumetric flasks, whereas the frankfurter and patty samples were collected in 25 ml volumetric flasks.

This extensive nitric acid treatment was employed to destroy the considerable amount of fat contained in the freeze dried samples, particularly those of frankfurter and patty products. In a preliminary trial, the wet ashing method of Perkin-Elmer (1971) was used. Nitric and perchloric acids (5 parts HNO_3: 2 parts $HClO_4$) were added to the samples before heating for about 30 min. As these hydrolysates were filtered, large amounts of fat were collected in the filter paper. Results indicated that the fat may have caused depressed element recovery. As a follow-up, attempts were made at fat destruction by dropwise adding 30 percent H_2O_2 while boiling the sample with HNO_3. This method proved to be dangerous as well as extremely time and reagent consuming. Through trial and error, it was learned that a very rigorous treatment with HNO_3 satisfactorily destroyed the fat. When followed by

$HClO_4$, the hydrolysates left filter papers that were free of any traces of fat and produced clear, colorless filtrates.

3.42 Atomic Absorption Spectrophotometry

All element analyses were performed with a Perkin-Elmer Model 403 atomic absorption spectrophotometer. A 4-in burner head, standard air-acetylene flame and single element hollow cathode lamps were used for all elements. The instrument settings and other experimental conditions were in accordance with the manufacturer's specifications (Perkin-Elmer, 1976). Element values were expressed as μg element/g wet tissue or product. Data were evaluated by analysis of variance and the Duncan procedure (Barr et al., 1976).

4.0 RESULTS AND DISCUSSION

4.1 Spleen Evaluation

4.11 Mechanical Separation

The feasibility of using mechanical desinewing equipment for the removal of capsular and internal connective tissue from beef spleen was investigated. A total of 113.4 kg of beef spleen were passed through a Beehive Model AU 4171 deboner with a desinewing head. The effectiveness of this operation is graphically illustrated in Figures 1 and 2.

Mechanically separated spleen (MSS) leaving the product port of the desinewing head was deep red in color and free of visually apparent connective tissue. The MSS was a physically homogeneous and rather viscous, yet fluid material. The thick, tough connective tissue capsules were effectively removed from the splenic pulp and exited the machine at the discard port. The separation process yielded 79.1 percent MSS and 20.9 percent residue (Table 4). Calculation showed that the equipment retained 9.1 kg of spleen at the completion of the run.

4.12 Spleen Testing

4.121 Proximate Composition

The proximate composition of whole spleen, MSS and the residue are presented in Table 4. Mechanical separation did not greatly alter the percent protein or ash, however, the MSS contained 52 percent less fat than whole spleen. Moisture levels were higher in the MSS than whole spleen due to the reduced fat levels. The residue fraction, comprised primarily of connective tissue, was higher in protein and fat and lower in moisture and ash than either the whole spleen or MSS.

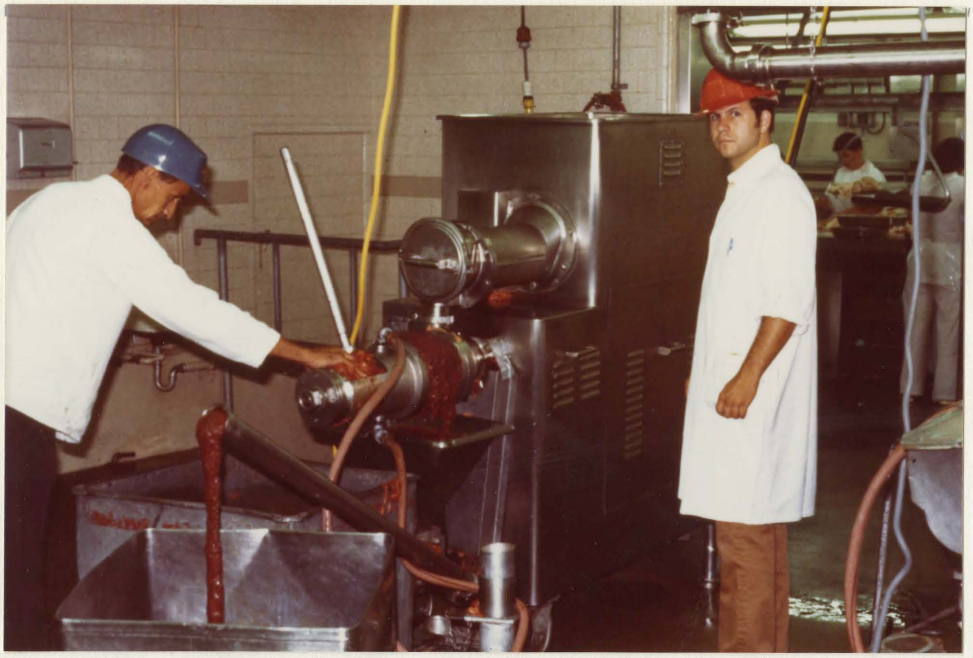

Figure 1. Spleen separation with a Beehive Model AU4171 deboner and desinewing head.

Figure 2. A close-up view of mechanically separated spleen (left) leaving the product port and the residue exiting the discard port.

Table 4. Composition of whole and mechanically separated spleen raw materials.

	Whole Spleen	MSS[a]	Residue[b]
Separator Yield (%)		79.1	20.9
Moisture[c]	74.2	77.6	67.1
Fat[c]	6.0	2.9	11.1
Protein[c]	17.6	17.0	20.7
Ash[c]	1.3	1.4	0.9
Fe[d]	518.9 f	762.0 e	245.8 g
Cu[d]	1.6 e	1.9 e	1.2 e
Zn[d]	20.9 f	19.7 g	25.0 e

[a]MSS: Mechanically separated spleen.

[b]Residue: Spleen material exiting the discard port.

[c]Means of triplicate analyses. Expressed as percent on a wet basis.

[d]Means of 6 hydrolysates. Expressed as µg/g wet tissue.
Means in the same row with different letters are significantly different ($P < 0.05$).

The lower moisture, higher protein content in the residue was likely caused by the concentration of lower moisture connective tissue and fat in that fraction. The general trend effects of mechanical separation on the proximate components of whole spleen parallel those reported for a variety of cuts from beef and pork by Gillett et al. (1976).

The moisture and protein levels in MSS were very similar to those given for raw beef heart (Watt and Merrill, 1963). The MSS had a moisture:protein ratio of 4.1:1, substantially higher than that of typical sausage meats (Ockerman, 1974).

4.122 Elemental Composition

The copper content of MSS (Table 4) was not significantly different from that of whole spleen or the residue. When expressed on a dry basis, the copper values of whole spleen (6.2 ppm) and MSS (8.5 ppm) are in agreement with those of Standish et al. (1969) and Standish et al. (1971) who reported 5.1 and 8.6 ppm, respectively, for whole spleen. On a dry basis, spleen contains about the same level of copper as beef muscle (Standish et al., 1969 and 1971). However, on a wet basis MSS contained less copper than the 2.7 ppm value given by Doyle and Spaulding (1978) for beef muscle.

Zinc concentration (Table 4) was greatest in the residue, followed by whole spleen and MSS. The higher level of zinc in the residue may be explained by the role of this essential element in collagen synthesis. The zinc values for whole spleen (20.9 ppm) and MSS (19.7 ppm) are in agreement with the value of 20 ppm reported for beef spleen by Hansard et al. (1968). Whole spleen and MSS contained less zinc than the 23 ppm reported for beef muscle (Hansard et al., 1968).

Mechanically separated spleen had a significantly greater iron content than did the whole spleen or residue (Table 4). The increased iron value of MSS was attributed to the concentrating effect of removing the low-iron connective tissue from the spleen mass.

The iron content of MSS (762 ppm) is in agreement with the concentration of 750 ppm reported for fresh spleen by Kizlaitis et al. (1962). However, the iron levels reported for spleens of normal cattle vary greatly (Ammerman et al., 1974). This point is evidenced by the data presented in Table 1. Preliminary studies of splenic pulp samples obtained from several individual bovine spleens produced iron values ranging from 500 to 1000 ppm, wet basis.

Although the MSS appeared physically homogeneous, it was deemed expedient to investigate the degree of homogeneity in the MSS by making a series of determinations in addition to the original analyses. Five, 5.4 kg bags (Cryovac type L348) of MSS, which had been frozen at -29^{o} C upon return from the mechanical separation site, were sampled, without defrosting. Moisture determinations were made on each sample by lypholization. Element determinations were made by hydrolyzing wet and freeze dried samples from each bag in duplicate.

The five backup samples had moisture contents ranging from 77.5 to 77.9 percent with a mean of 77.7 percent. These results were in agreement with the value of 77.6 percent determined for the original samples. The mean values obtained from the 20 zinc and copper determinations were 20.1 and 1.8 ppm, respectively. The comparable values from the original samples were 19.7 ppm Zn and 1.9 ppm Cu. The close agreement between the original and backup samples in terms of moisture,

zinc and copper was evidence of the homogeneity of the MSS material.

The results of the iron analyses demonstrated that the apparently homogeneous MSS was heterogeneous in terms of iron. The average iron values determined for the five bags were 1,226.1, 838.0, 775.5, 673.3 and 842.4 ppm, thus demonstrating the biological variability of iron in bovine spleen. The value of 1,226.1 ppm obtained for the first bag appears high, yet it is within the range of values presented in Table 1. The mean of the 16 determinations made on the last four bags was 782.3 ppm, which indicated that the value obtained from the original sampling (762.0 ppm) was acceptably representative of the MSS produced in this study.

4.123 Protein Efficiency Ratio

The effect of mechanical separation on spleen protein quality was assessed by protein efficiency ratio (PER). The results of this study are presented in Table 5. The PER determined for the casein control (3.16) is within the normal limits reported for the growing rat. Hegarty (1975) reviewed the literature with reference to the PER values reported for casein diets and noted that 10 papers adhering exactly to AOAC methodology reported a mean casein PER of 3.08 with a range of 2.60 to 3.59. Seven papers reported 28 day PERs with Sprague-Dawley rats. The mean of these was 3.07. Burnette and Rusoff (1978) noted the importance of the PER for casein falling within a standard range and reported that the Protein Quality Subcommittee of the Grocery Manufacturers of America was considering setting a range of 2.8 to 3.2 for a test to be certified.

Table 5. Bioassay of whole and mechanicaly separated spleen[a].

	Diet		
	MSS[b]	Whole Spleen	Casein
Ave. Initial Weight (g)	49.7	49.9	51.1
Ave. Final Weight (g)	192.7 d	187.9 d	166.8 e
Ave. Total Gain (g)	143.0 d	138.0 d	115.7 e
Ave. Total Consumption (g)	481.4 d	451.9 d	385.3 e
Ave. Daily Gain (g)	5.1	4.9	4.1
Ave. Daily Consumption (g)	17.2	16.1	13.8
Raw PER[c]	2.94 e	3.05 de	3.16 d
Corrected PER	2.3	2.4	2.5

[a]Ten rats for each treatment.
Means in the same category bearing different letters are significantly different ($P < 0.05$).

[b]MSS: Mechanically separated spleen.

[c]PER: Protein efficiency ratio.

Rats fed the spleen diets ate well, grew rapidly and exhibited significantly greater final weights and total gains than those fed the casein control. The PER values for whole spleen and MSS were not significantly different from each other. Whole spleen and casein gave similar PER values, but the MSS diet yielded a PER lower than the casein control. This study failed to demonstrate the beneficial effect of connective tissue removal from spleen that was predicted from the work of Hendricks et al. (1977), Chang and Field (1977) and Lee et al. (1978), all of which reported increased PER values with decreased connective tissue in meat products.

The casein-adjusted PER values of whole spleen (2.4) and MSS (2.3) were lower than the value of 2.85 reported for lean beef by Happich et al. (1975) and 2.7 reported for 79 percent lean ground beef by Hegarty and Ahn (1976). Chang and Field (1977) reported unadjusted PER values of 2.47 and 2.69 for mechanically deboned beef that was typical of commercial production and concluded that these products were high in protein quality. The use of MSS at the 5 to 15 percent level in formulated products with high proportions of skeletal muscle such as frankfurters and meat patties would yield food products with satisfactory protein quality.

The experimentally determined PER for whole spleen and MSS may misrepresent the true biological quality of spleen protein. Since samples are analyzed for nitrogen rather than protein, the standard AOAC procedure (AOAC, 1975) specifies that diets supply 1.6 percent nitrogen and that a factor of 6.25 x N be used to calculate protein in the final diet. However, the factor of 6.25 has not been verified

for spleen and it may be too high. Although no determinations were included in this study, spleen would be expected to contain substantial quantities of non-protein nitrogen, present as nucleic acids and hemoglobin. Including non-amino nitrogen in the calculation of protein would result in a lowering of the calculated PER.

Burnette and Rusoff (1978) noted several factors that can affect PER including non-protein nitrogen level. Hegarty (1975) listed urea, creatinine, creatine, uric acid and ammonia as sources of non-amino nitrogen and noted that they have caused doubts about the use of the factor N x 6.25 for calculation of the protein content of certain foods. Sarwar et al. (1973) claimed that because of the high non-protein nitrogen content of oilseed meals, a more realistic conversion factor would be N x 5.5. Use of this factor had the effect of decreasing the percentage of protein but increasing the PER value.

Lee et al. (1978) developed regression equations relating PER, collagen content and percent essential amino acids for beef. They stated that 32.2 percent essential amino acids were required to give a PER of 2.50. Schweigert et al. (1954) and Olson (1970) reported data that show beef spleen to contain 35.4 to 37.8 percent of the same essential amino acids. These values would tend to indicate that the experimentally determined PER for spleen may be low.

4.2 Frankfurter Evaluation

4.21 Proximate Composition

Beef and pork frankfurters were produced under commercial conditions with 0, 5, 10 and 15 percent of the meat block being MSS. Since the objective of this study was to determine the effect of MSS incorporation on a standardized frankfurter product, raw materials were carefully formulated to yield a series of products which varied only in their spleen level and not proximate composition. As shown in Table 6, there were no significant differences among the frankfurter treatments in proximate composition, except that the control product contained more protein than the 5 and 15 percent MSS frankfurters.

4.22 Elemental Composition

The elemental contents of the frankfurters are presented in Table 6. Although significant differences were found, level of spleen did not consistently affect the copper content of the frankfurters. At the levels determined (0.7 - 0.9 ppm), two frankfurters (90 g) would provide 3.2 to 4.1 percent of the 2 mg U.S. recommended dietary allowance (RDA) for copper (Schmidt, 1976).

Zinc concentration significantly decreased with increased level of MSS. This effect may be attributed to the slightly lower level of zinc in MSS than muscle (Section 4.122). Two control frankfurters (90 g) would provide 13.4 percent of the 15 mg RDA for zinc (NAS/NRC, 1974), whereas an equal serving of the 15 percent MSS product would provide 12.5 percent.

Table 6. Composition of frankfurters containing mechanically separated spleen.

	Level of Spleen (%)			
	0	5	10	15
Moisture[ad]	51.7 e	53.2 e	52.3 e	52.3 e
Fat[ad]	24.7 e	24.1 e	24.2 e	25.0 e
Protein[ad]	14.9 e	14.0 f	14.5 ef	14.1 f
Ash[ad]	3.5 e	3.4 e	3.5 e	3.4 e
Carbohydrate[bd]	5.2 e	5.3 e	5.5 e	5.2 e
Fe[cd]	16.5 h	36.4 g	64.8 f	80.4 e
Cu[cd]	0.7 g	0.9 e	0.8 f	0.7 g
Zn[cd]	22.4 e	21.6 f	21.5 f	20.8 g

[a]Means of triplicate analyses. Expressed as percent on a wet basis.

[b]Calculated by difference.

[c]Means of 6 hydrosylates. Expressed as μg/g wet frankfurter.

[d]Means in the same row with different letters are significantly different ($P < 0.05$).

Increasing levels of MSS resulted in significantly increased iron concentrations in the frankfurters. The iron data listed in Table 6 is graphically presented in Figure 3. This graph illustrates that the iron value of the frankfurters was linearly related to the level of MSS incorporated. The concentration of iron in the control frankfurter (16.5 ppm) was in agreement with the values of 15 to 19 ppm tabulated by Watt and Merrill (1963).

Frankfurters made with 5, 10 and 15 percent MSS contained 2.2, 3.9 and 4.9 times more iron than the control, respectively. Two frankfurters (90 g) from the 0, 5, 10 or 15 percent MSS treatment would provide 14.9, 32.8, 58.3 and 72.4 percent, respectively, of the 10 mg RDA for children 4 to 10 and males over 19 years of age (NAS/NRC, 1974). Males 11 to 18 and females 11 to 51 years of age would receive 8.3, 18.2, 32.4 and 40.2 percent of their 18 mg RDA (NAS/NRC, 1974) from a 90 g serving of the 0, 5, 10 and 15 percent MSS frankfurters, respectively. These data illustrate the potential value of MSS as a natural source of iron for the fortification of commonly consumed comminuted meat products such as frankfurters.

4.23 Panel Evaluations

4.231 Physical Attributes Panel

Statistical analyses were not performed on the results of the physical attributes panel evaluation of the frankfurters (Table 7) but inferences can be made by consideration of the numerical scores. With the exception of the external color score comparison between the 10 and 15 percent products, mean panel scores indicated that the cured meat color of the external surface and interior of the frankfurters intensified

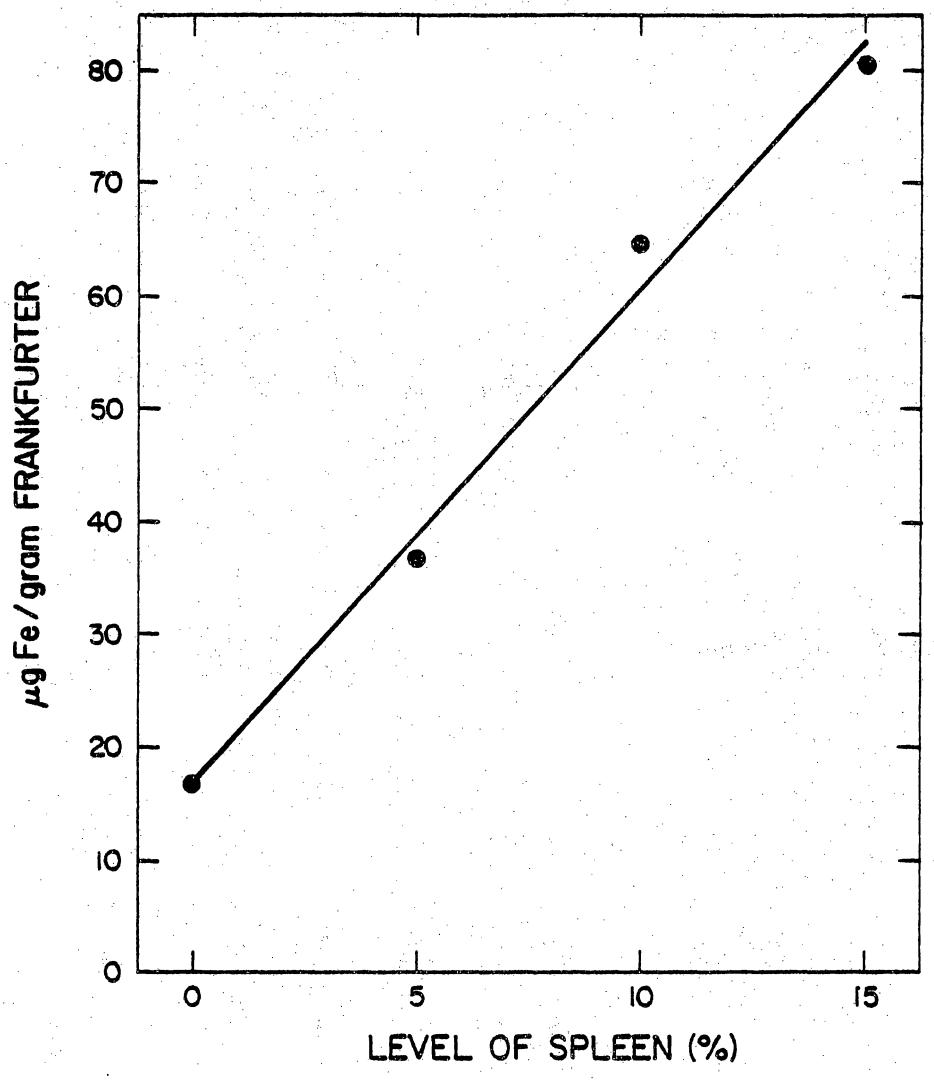

Figure 3. Effect of mechanically separated spleen addition on the iron content of frankfurters.

Table 7. Physical attributes panel evaluation of frankfurters[a].

	Level of Spleen (%)			
	0	5	10	15
External Color[b]	2.2	3.4	4.4	4.2
Interior Color[c]	2.6	3.2	4.2	4.4
Firmness[d]	4.4	4.0	4.0	4.0
Resilience[e]	4.2	4.0	3.6	3.4
Binding[e]	4.2	3.8	3.6	2.6
Texture[e]	4.2	3.8	3.6	2.6
Overall Acceptability[e]	4.2	3.8	3.6	2.4

[a]Mean scores from 5 panelists.
[b]External color: 5 = deep smoked/cured, 1 = pale.
[c]Internal color: 5 = deep red, 1 = pale.
[d]Firmness: 5 = very firm, 1 = mushy.
[e]Resilience, binding, texture and overall: 5 = excellent, 1 = very poor.

with increasing levels of MSS. The 10 and 15 percent MSS frankfurters exhibited a smoked sausage appearance, even though no external smoke was applied.

Control frankfurters received a higher mean firmness score than those containing MSS. However, all MSS-containing products were scored as "firm". Resilience, binding, texture and overall physical acceptability score decreased with increased level of MSS. In general, the greatest decreases occurred between the 10 and 15 percent products. The only below average physical scores recorded were for binding, texture and overall acceptability of the 15 percent MSS frankfurters.

These results indicate that the addition of 15 percent MSS had a detrimental effect on the physical characteristics of the frankfurters, particularly binding and texture. After heat processing, no fat caps were observed on any of the products. Each treatment peeled well and had adequate skin formation. However, the 15 percent MSS frankfurters had softer, less compact interiors than the other products. Warmed 15 percent frankfurters had a slightly more open texture and a tendency to release grease and water upon biting. They also lacked the "snap" and "bite" of the more physically acceptable frankfurters.

4.232 Consumer Panel

All frankfurters were rated acceptable for flavor, texture and color by the consumer panel (Table 8). Increasing levels of MSS did not significantly alter the acceptability of frankfurter flavor and color. Consumer panel texture scores reflected the trend toward softer frankfurters with increased MSS noted by the physical attributes panel. The purchase level responses favored the 5 and 10 percent MSS products,

Table 8. Consumer panel evaluation of frankfurters

	Level of Spleen (%)			
	0	5	10	15
Flavor Scores[ad]	3.5 e	3.7 e	3.5 e	4.0 e
Texture Scores[bd]	4.0 e	4.0 e	3.5 ef	3.0 f
Color Scores[ad]	3.5 e	3.7 e	4.0 e	3.8 e
Purchase Level[cd]	3.8 e	4.1 e	4.1 e	3.9 e

[a]Flavor and color: 5 = very desirable, 1 = very undesirable.

[b]Texture: 5 = very firm, 1 = very soft.

[c]Purchase level: 7 = 30¢/lb premium, 4 = average price/lb, 1 = 30¢/lb discount.

[d]Means in the same category with different letters are significantly different ($P < 0.05$).

but the differences were not significant. Overall, these results indicated that as much as 15 percent MSS can be incorporated in frankfurters without seriously altering or decreasing consumer acceptability.

4.233 Laboratory Taste Panel

The interaction effects for the laboratory taste panel ratings of the frankfurters are given in Table 9. The flavor of all products, except those containing 15 percent MSS, was rated above average acceptability after each storage period. Taste panel scores indicated that the flavor of the 15 percent product was significantly less desirable than that of the other treatments, regardless of storage time.

During the first 4 weeks of storage, no significant differences in flavor were noted between the 0, 5 and 10 percent MSS products. The 0 and 5 percent products received similar flavor scores throughout. Only the 10 percent product demonstrated a significant decrease in flavor desirability after 6 weeks storage.

Texture was rated acceptable for all frankfurters except those with 15 percent MSS and the 10 percent product after 6 weeks storage. In general, frankfurter texture scores decreased with increased level of MSS.

Frankfurter color was rated acceptable for all products except the 15 percent product after 2 weeks of storage. With reference to overall acceptability, all frankfurters were rated above average except the 15 percent product and the 10 percent product after 6 weeks storage. During the first 4 weeks, no significant differences in overall acceptability scores were noted between the 0, 5 and 10 percent MSS

Table 9. Laboratory taste panel evaluation of frankfurters

Storage Time, Weeks	Level of Spleen (%) 0	5	10	15
	Flavor Scores[ad]			
0	3.7 Ae	3.7 Ae	3.8 Ae	2.5 Be
2	3.8 Ae	3.8 Ae	3.8 Ae	2.5 Be
4	3.9 Ae	3.9 Ae	4.2 Ae	2.2 Be
6	4.1 Ae	3.8 Ae	3.1 Bf	2.0 Ce
	Texture Scores[bd]			
0	4.3 Ae	4.0 Ae	3.2 Bef	2.1 Ce
2	4.3 Ae	3.8 Ae	3.1 Bef	1.7 Cef
4	4.7 Ae	3.8 Be	3.3 Be	1.5 Cf
6	4.3 Ae	3.6 Be	2.7 Cf	1.4 Df
	Color Scores[ad]			
0	3.6 Ae	3.7 Ae	3.6 Aef	3.3 Ae
2	3.8 Ae	4.0 Ae	3.8 Ae	2.3 Bf
4	3.8 Ae	4.0 Ae	3.6 Aef	2.3 Bf
6	3.8 Ae	3.8 Ae	3.1 Af	2.2 Bf
	Overall Acceptability Scores[cd]			
0	3.6 Ae	3.8 Ae	3.8 Ae	2.6 Be
2	4.0 Ae	3.8 Ae	3.7 Ae	2.1 Bef
4	3.8 Ae	4.1 Ae	3.6 Ae	1.8 Bf
6	4.3 Ae	3.8 Ae	2.9 Bf	1.7 Cf

[a]Flavor and color: 5 = very desirable, 1 = very undesirable.

[b]Texture: 5 = very firm, 1 = very soft.

[c]Overall acceptability: 5 = very acceptable, 1 = very unacceptable.

[d]Means in rows and columns within each category with different letters are significantly different ($P < 0.05$).

frankfurters. The 0 and 5 percent products were rated similarly throughout. The 10 and 15 percent products demonstrated a significant decrease in overall acceptability after 6 and 4 weeks, respectively.

Overall, taste panel results indicated that the 15 percent MSS frankfurters were significantly less desirable than those containing 0, 5 or 10 percent MSS. There was also evidence to suggest that the shelf life of frankfurters with 10 percent MSS may be shorter than those with 0 or 5 percent MSS.

The consumer and taste panel assessments of the level of acceptability achieved by the 15 percent MSS frankfurters were not in agreement. In general, taste panelists found the 15 percent product to be below average acceptability for all characteristics evaluated whereas the consumer panelists scored this product average or above.

The consumer panel gave the 15 percent frankfurters the numerically highest flavor score. Taste panel flavor scores for this product were significantly lower than those of any other product evaluated. This obvious discrepancy in the acceptability level of the 15 percent product between the consumer panel and taste panel may be explained by the methods of preparation and the absence of condiments in the latter.

Overall, the three panel evaluations indicated that up to 10 percent MSS may be successfully incorporated in frankfurter formulations without significantly altering or decreasing consumer acceptability. Results of the consumer panel suggest that frankfurters with 15 percent MSS may be acceptable in some markets or as components of mixed dishes such as beans and franks.

Several panelists commented that the higher MSS-containing frankfurters (10 and 15 percent) had a spicier, more intense flavor and a softer, pasty-like texture, somewhat similar to that of liver sausage. It was observed that panelists who preferred or enjoyed spicier, ethnic-type foods preferred the 10 and 15 percent MSS frankfurters whereas panelists who were more accustomed to bland foods preferred the 0 or 5 percent products.

4.24 Shear Values

The effect of level of spleen and time of storage on the textural qualities of the frankfurters was evaluated by Allo-Kramer shear resistance after 0, 2, 4 and 6 weeks storage at 2°C. Interaction effects for the shear values, expressed as pounds of force required to shear per gram of frankfurter, are presented in Table 10.

Frankfurter shear resistance decreased linearly with increased level of MSS within each test period. Minor inconsistencies were evident for shear values within each treatment during the 6 weeks of storage. Although statistically significant, these differences did not appear to be of major importance.

Since no trend toward increased or decreased shear resistance with time of storage was noted, shear values for each treatment were pooled and plotted (Figure 4). These pooled averages, representing 40 frankfurters of each treatment sheared over a 6 week period, indicated that the shear resistance of the frankfurters decreased linearly with increased level of MSS. The 5, 10 and 15 percent MSS frankfurters exhibited 15.3, 32.9 and 46.1 percent less shear resistance, respectively, than the control frankfurters.

Table 10. Interaction effects for Allo-Kramer shear values of frankfurters[a].

Storage Time, Weeks	Level of Spleen (%)			
	0	5	10	15
0	3.30 Ad	2.94 Bb	2.36 Cb	1.83 Dbc
2	3.66 Ab	3.00 Bb	2.39 Cb	1.76 Dc
4	3.48 Ac	2.91 Bb	2.21 Cc	1.92 Db
6	3.44 Acd	2.91 Bb	2.38 Cb	1.96 Db

[a]Values expressed as lbs force/g frankfurter. Means in rows and columns with different letters are significantly different ($P < 0.05$).

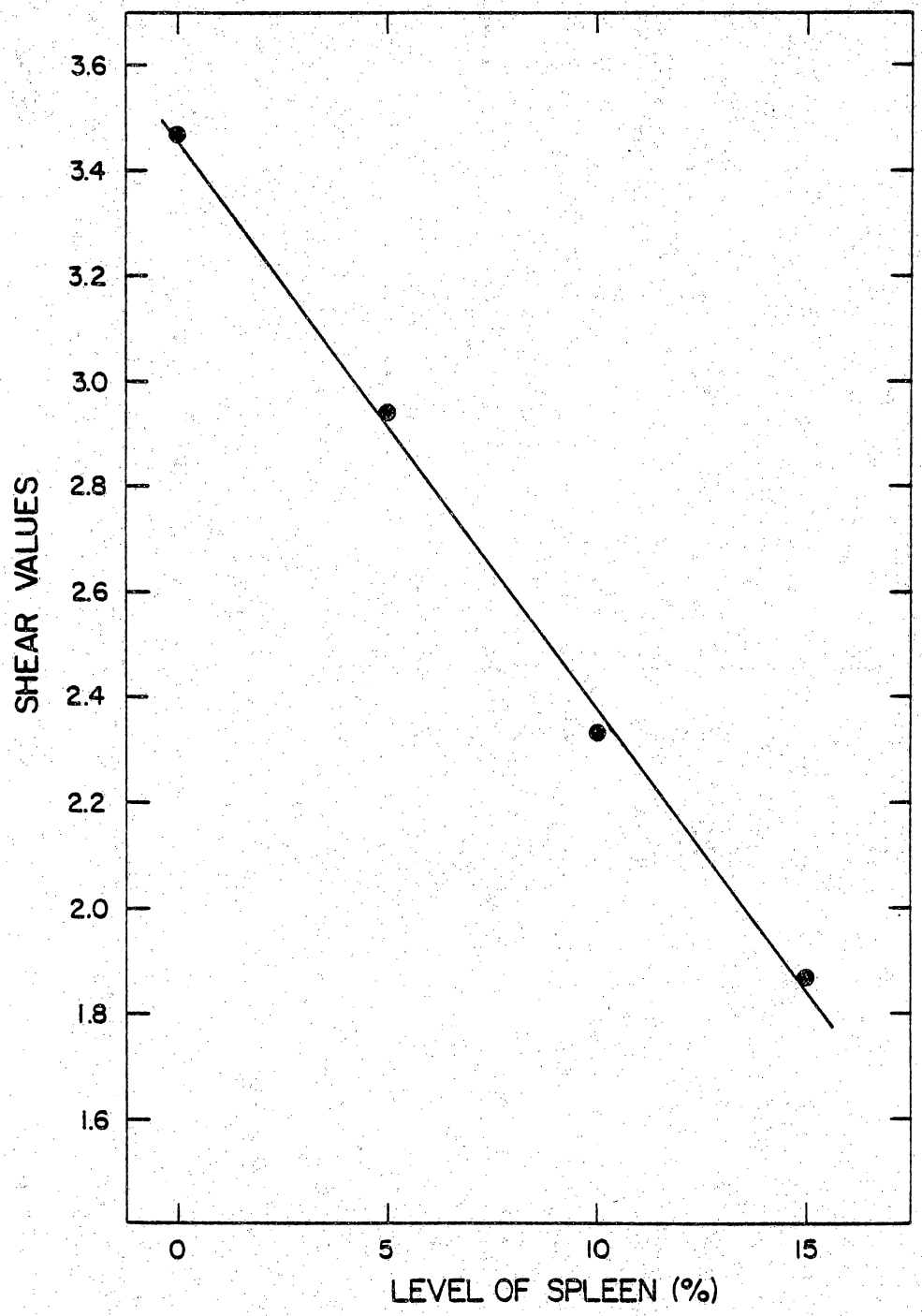

Figure 4. Effect of mechanically separated spleen addition on shear values of frankfurters measured over 6 weeks of storage.

The observed and measured loss in firmness of frankfurters due to increased levels of MSS in the formulations is most probably a result of the absence of skeletal muscle protein in splenic tissue. Non-skeletal proteins contribute only slightly to binding in emulsion meat products. They react much like fat and must be bound up by the matrix (Carpenter, 1976). Since the proteins of spleen lack the desired emulsion-forming and binding properties (Satterlee et al., 1973), increasing levels of MSS dilute the myofibrillar proteins of the formulation and cause losses in firmness.

4.25 Objective Color Measurements

4.251 Color Meter Determinations

Hunter Color and Color Difference Meter L Values were determined on 10 internal and external surfaces of each frankfurter treatment after 0, 2, 4 and 6 weeks of storage at 2^{o} C. A larger numeric L value denotes a lighter product surface. The interaction effects for external and internal surface Hunter L values are presented in Tables 11 and 12, respectively. Least squares lines relating Hunter L value and time of storage for each frankfurter surface and treatment are presented in Figure 5.

External surface Hunter L values determined at the initial test indicated that increased levels of MSS resulted in significantly darker frankfurters. Each product showed significant fading of the external surface color during storage. As shown by the regression lines in Figure 5, the rate of exterior surface lightening increased with increased level of MSS. After 2 weeks storage, the external color of the 15 percent MSS product was not significantly different from that of the 10 percent

Table 11. External frankfurter color: Hunter L values[a].

Storage Time, Weeks	Level of Spleen (%)			
	0	5	10	15
0	50.6 Ac	45.6 Bc	40.5 Cc	38.5 Dc
2	51.8 Ab	47.4 Bb	42.7 Cb	41.8 Cb
4	50.6 Ac	47.0 Bb	42.9 Cb	41.9 Db
6	52.0 Ab	47.2 Bb	43.0 Cb	42.7 Cb

[a]Means in rows and columns with different letters are significantly different ($P < 0.05$).

Table 12. Internal frankfurter color: Hunter L values[a].

Storage Time, Weeks	Level of Spleen (%)			
	0	5	10	15
0	56.0 Ab	51.0 Bb	45.3 Cbc	42.2 Dd
2	55.6 Abc	51.0 Bb	45.1 Cc	43.1 Dc
4	55.2 Ac	50.8 Bb	45.3 Cbc	43.8 Db
6	55.3 Ac	51.1 Bb	45.7 Cb	44.2 Db

[a]Means in rows and columns with different letters are significantly different ($P < 0.05$).

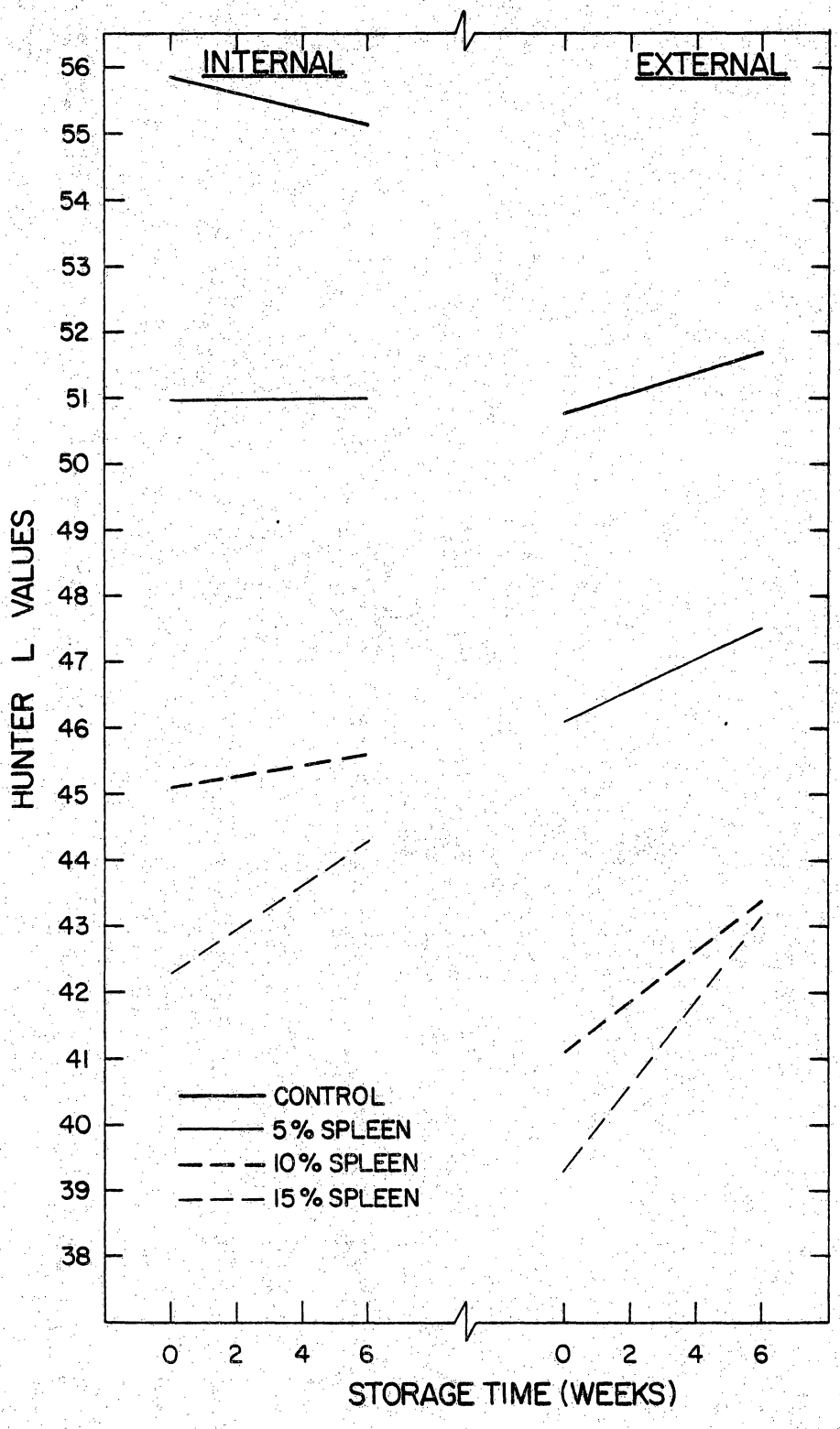

Figure 5. Least squares relationships between internal and external frankfurter surface Hunter L values and time of storage.

product.

Internal surface Hunter L values verified that darker frankfurter interiors were obtained with increased levels of MSS. This trend was significant after each storage interval. During storage, the interior of the control frankfurters darkened slightly, the 5 and 10 percent products remained unchanged and the 15 percent product faded significantly.

4.252 Pigment Determinations

The interaction effects for the total and nitroso-heme pigment determinations made after 0, 2, 4 and 6 weeks storage are presented in Tables 13 and 14, respectively. Least squares lines relating ppm of hematin and time of storage for each pigment type and level of MSS are presented in Figure 6.

The initial total pigment concentrations increased linearly with increased level of MSS in the frankfurters. This relationship was not valid after 2 weeks of storage. Significant losses in total pigment concentration were recorded after 2 weeks for the 10 and 15 percent products and after 4 weeks in the 0 and 5 percent treatments. Within 2 and 6 weeks of storage, the total pigment concentration of the 15 percent product had fallen significantly below that of the 10 and 5 percent products, respectively.

The regression lines shown in Figure 6 for the total pigments of the 0, 5, 10 and 15 percent products had slopes of -2.2, -3.2, -5.6 and -10.3, respectively. These lines indicated that the rate of decrease in total pigment concentration increased with increased level of MSS.

Table 13. Interaction effects for total heme pigments of frankfurters[a].

Storage Time, Weeks	Level of Spleen (%)			
	0	5	10	15
0	118.15 Db	144.16 Cb	172.89 Bb	189.38 Ab
2	121.04 Cb	142.97 Bb	155.72 Ac	145.01 Bc
4	111.01 Dc	128.52 Cc	148.07 Ad	141.95 Bc
6	107.10 Dc	127.50 Bc	137.87 Ae	122.06 Cd

[a]Expressed as ppm of hematin. Means in rows and columns with different letters are significantly different ($P < 0.05$).

Table 14. Interaction effects for nitroso-heme pigments of frankfurters[a].

Storage Time, Weeks	Level of Spleen (%)			
	0	5	10	15
0	78.37 Dc	107.45 Cb	122.02 Bbc	152.11 Ab
2	75.84 Dc	107.23 Cb	126.08 Bb	132.31 Ad
4	82.58 Db	108.90 Cb	123.11 Bbc	140.07 Ac
6	77.21 Dc	97.44 Cc	119.55 Ac	112.52 Be

[a]Expressed as ppm of hematin. Means in rows and columns with different letters are significantly different ($P < 0.05$).

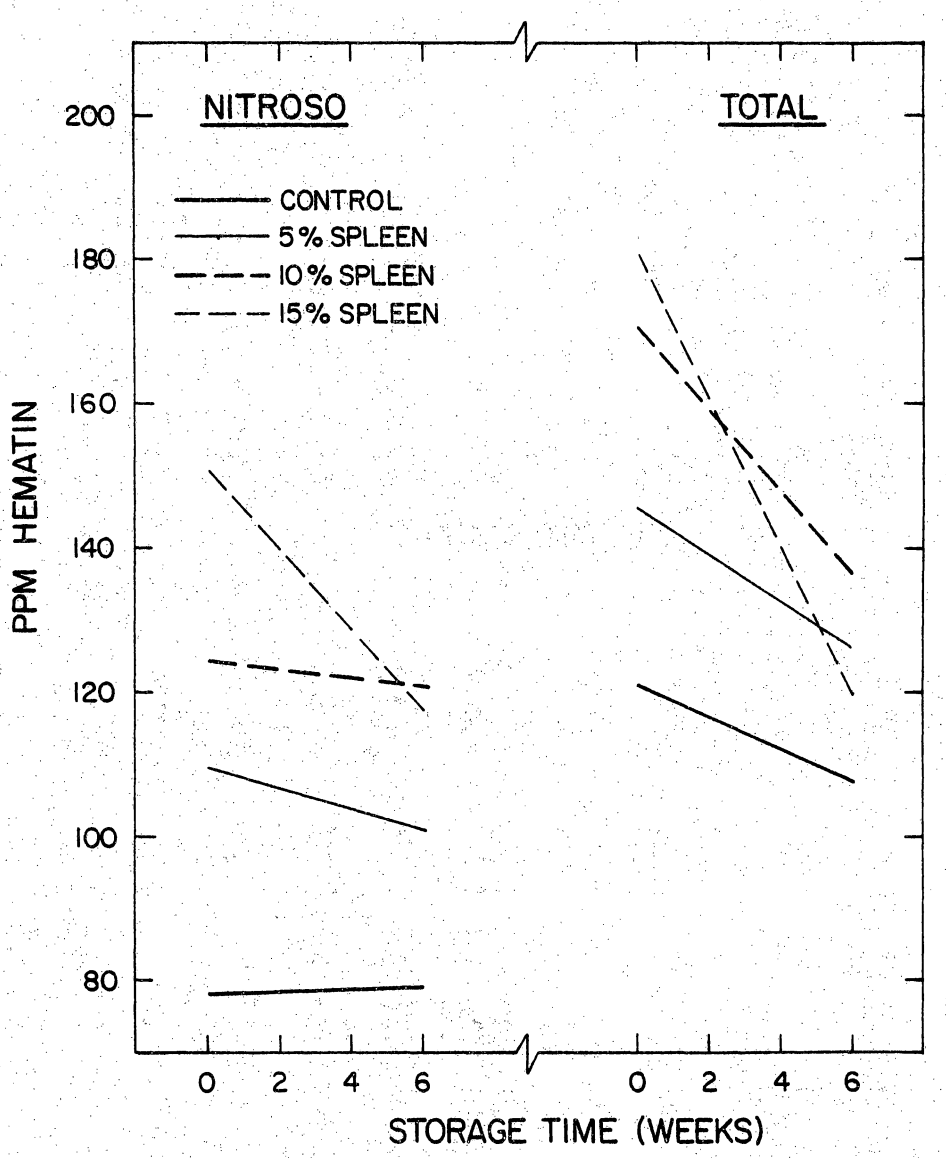

Figure 6. Least squares relationships between total and nitroso-heme pigment concentrations and time of storage for frankfurters.

Results obtained during the first 4 weeks of the storage period indicated that increased levels of MSS yielded increased concentrations of nitroso pigments in the frankfurters. During storage, the nitroso pigment concentrations of the 0 and 10 percent products showed some inconsistencies but remained essentially unchanged. After 6 weeks of storage, the 5 and 15 percent MSS frankfurters showed a significant loss in nitroso pigment. As shown in Figure 6, the 16 percent product demonstrated the fastest rate of nitroso pigment decrease. The 15 percent MSS frankfurters contained significantly less nitroso pigment than the 10 percent product at the completion of the study.

As the level of MSS in the formulations increased, frankfurter color increasingly resembled that of smoked sausage. This pigmentation was particularly apparent in the 10 and 15 percent products. These frankfurters had a dark, brown-red smoked sausage appearance even though no external smoke was included in their processing. The increased intensity of brown-red pigmentation of frankfurters with increased level of MSS was most probably a result of the abundant quantity of hemoglobin in the MSS (see Figures 1 and 2).

The fading of cured meat products over time is a well known occurrence. Both objective methods of color measurement indicated that color fading and pigment losses occurred during prolonged storage of the frankfurters. The only exceptions noted were for the internal surfaces of the 0, 5 and 10 percent products and the nitroso pigment concentrations of the 0 and 10 percent products. In general, the loss of darkness and pigment concentration increased with increased level of MSS. The greatest amounts and rates of change were recorded for the 15 percent

MSS frankfurters.

It should be noted that even after the degree of fading recorded here, each of the MSS-containing products remained darker on each surface and contained more pigment than the control product. These objectively measured color losses were not visually apparent. During informal sessions, all persons asked were able to place packages of the frankfurters in order of MSS addition level on the basis of external color after 6 weeks of storage.

4.26 Microbiological Evaluation

4.261 Mesophilic Bacteria

Results of the mesophilic bacterial counts are presented in Table 15. Level of MSS had no apparent effect on the number of mesophiles detected in the stuffed, uncooked emulsion. Heat processing decreased mesophilic numbers in each product by approximately 3 log cycles. This level of thermal destruction and the recorded number of survivors is in agreement with data reported by Palumbo et al. (1974) for frankfurters processed to 71.1°C.

Post processing (week 0) mesophilic counts were similar, approximately 10^3/g, for each treatment. The largest increase in mesophilic population was observed between the fourth and sixth week. During the 6 weeks of storage at 2°C, total mesophilic counts had increased approximately 3 log cycles over the 0 week counts. A trend toward increased numbers with increased level of MSS was noted after 6 weeks of storage.

The data presented here for mesophilic bacteria were in agreement with those reported by Palumbo et al. (1977) for laboratory processed

Table 15. Evaluation of mesophilic bacteria in raw emulsion and cooked frankfurters[ab].

	Level of Spleen (%)			
	0	5	10	15
Emulsion[c]	5.1×10^6	6.9×10^6	8.0×10^6	5.1×10^6
Week 0[d]	8.8×10^2	3.8×10^3	1.5×10^3	6.5×10^3
Week 2	1.6×10^3	1.3×10^2	1.0×10^3	1.5×10^3
Week 4	2.5×10^3	3.8×10^3	2.5×10^3	1.5×10^4
Week 6	3.0×10^5	1.5×10^6	1.7×10^6	7.5×10^6

[a]Mean number of colony forming units/gram for duplicate plating of duplicate samples.

[b]Incubated 48 hours at 30°C.

[c]Stuffed, uncooked frankfurter emulsion.

[d]Time of storage at 2°C for cooked frankfurters.

and stored frankfurters. The final mesophilic counts compared favorably with those reported for commercial frankfurters. Duitschaever (1978) investigated the bacteriological quality of 180 units of frankfurters obtained from grocery stores in Canada and found 21 percent of the samples had aerobic plate counts (APC, 32°C, 48 hr) of 10^5 - 10^6/g; 10 percent had APCs of 10^6 - 10^7/g; and 69 percent had APCs from 10^7 - 10^{9+}/g.

4.262 Psychrophilic Bacteria

Results of the psychrophilic bacterial counts are presented in Table 16. Level of MSS had no apparent effect on the number of psychrophilic bacteria in the raw emulsions or cooked products. Upon refrigerated storage psychrophilic counts were reduced below minimum detection levels (<500/g). The decrease in cell numbers during low temperature storage may be explained as being a heat injury phenomenon. Psychrophiles are known to be heat sensitive (Jay, 1978). Initial counts (week 0) were taken before a prolonged period of storage and may have detected an injured population. A decrease in survivors might have occurred during the storage period.

4.263 Coliform Bacteria

Results of the coliform bacteria counts are presented in Table 17. Level of MSS had no consistent effect on the number of viable coliforms confirmed in the uncooked frankfurter emulsions. Coliform numbers were greatly reduced by heat processing as indicated by the week 0 counts. During low temperature storage coliform counts from the 0 and 5 percent products fell below the minimum detection level of <50/g after 2 weeks. This same decrease occurred in the 10 and 15

Table 16. Evaluation of psychrophilic bacteria in raw emulsion and cooked frankfurters[ab].

	Level of Spleen (%)			
	0	5	10	15
Emulsion[c]	1.5×10^6	1.9×10^6	2.3×10^6	1.7×10^6
Week 0[d]	2.8×10^3	2.3×10^3	2.5×10^3	1.4×10^3
Week 2	<500[d]	<500	<500	<500
Week 4	<500	<500	<500	3.7×10^3
Week 6	<500	<500	<500	<500

[a]Mean number of colony forming units (CFU)/gram for duplicate plating of duplicate samples.

[b]Incubated 10 days at 5°C.

[c]Stuffed, uncooked frankfurter emulsion.

[d]Time of storage at 2°C for cooked frankfurters.

[e]Less than minimum detection level of 500 CFU/gram.

Table 17. Evaluation of coliform bacteria in raw emulsion and cooked frankfurters[a].

	Level of Spleen (%)			
	0	5	10	15
Emulsion[b]	1.6×10^5	3.0×10^5	2.7×10^5	2.5×10^5
Week 0[c]	8.8×10^1	<50	<50	3.0×10^2
Week 2	<50[d]	<50	2.3×10^2	6.3×10^1
Week 4	<50	<50	<50	<50
Week 6	<50	<50	<50	<50

[a]Mean number of pink-to-red colony forming units (CFU)/gram for duplicate plating of duplicate samples. Confirmed in brilliant green lactose bile broth.

[b]Stuffed, uncooked frankfurter emulsion.

[c]Time of storage at 2°C for cooked frankfurters.

[d]Less than minimum detection level of 50 CFU/gram.

percent products after 4 weeks of storage.

Overall, results of the microbiological evaluations indicated that the inclusion of MSS in frankfurter formulations did not increase bacterial numbers in the raw emulsions. MSS did not adversely affect the microbiological quality of the finished frankfurters or shorten their useful shelf life. At the completion of the shelf life study, the frankfurters were microbiologically acceptable and showed no signs of spoilage ie., slime formation, off-odor or discoloration.

4.3 Patty Evaluation

4.31 Cooking Yield and Proximate Composition

Ground meat patties were produced to contain 0, 5 and 10 percent MSS. Patties were oven broiled for 7 min per side before sampling. As shown in Table 18, level of MSS did not affect cooking yield. There were no significant differences among the cooked meat patties in proximate composition, except that the control patties contained more fat than those with 5 or 10 percent MSS.

4.32 Elemental Composition

The elemental contents of the cooked meat patties are presented in Table 18. Patties made with 5 and 10 percent MSS contained more copper than the control. This result may be explained by the lower fat content of the treatment patties and the close similarity between spleen and muscle in copper concentration. The copper levels in the patties were nearly identical to those determined for the frankfurters. At the levels determined (0.7 - 0.9 ppm), 100 g of cooked patty would provide 3.5 to 4.5 percent of the 2 mg RDA for copper (Schmidt, 1976).

Table 18. Cooked meat patty composition[a].

	Level of Spleen (%)		
	0	5	10
Cooking Yield (%)	69.2	69.1	69.0
Moisture[bd]	53.9 e	53.6 e	54.3 e
Fat[bd]	19.1 e	17.8 f	17.4 f
Protein[bd]	25.4 e	26.3 e	26.0 e
Ash[bd]	2.1 e	2.0 e	2.1 e
Fe[cd]	30.8 g	79.1 f	118.2 e
Cu[cd]	0.7 f	0.9 e	0.9 e
Zn[cd]	45.6 g	49.1 e	46.9 f

[a]Oven broiled 7 min/side.

[b]Means of triplicate analyses. Expressed as percent on a wet basis.

[c]Means of 6 hydrolysates. Expressed as µg/g cooked patty.

[d]Means in the same row with different letters are significantly different ($P < 0.05$).

Level of spleen did not consistently affect the zinc content of the cooked patties. A 100 g serving of the 0, 5 and 10 percent MSS cooked patties would provide 30.4, 32.7 and 31.3 percent of the 15 mg RDA for zinc (NAS/NRC, 1974), respectively. All patty treatments had greater than twice the zinc concentration determined for the frankfurters. This higher level of zinc may be attributed to the nearly two times higher level of protein in the patties as compared to the frankfurters.

Increasing levels of MSS resulted in significantly increased iron concentrations in the cooked patties. The iron data listed in Table 18 is graphically presented in Figure 7. This graph illustrates that the iron value of the patties was linearly related to the MSS addition level. The concentration of iron determined for the ground beef control (30.8 ppm) is in agreement with the value of 32 ppm tabulated for cooked ground beef by Watt and Merrill (1963).

Cooked meat patties made with 5 and 10 percent MSS contained 2.6 and 3.8 times more iron than the cooked ground beef control, respectively. A 100 g serving of the 0, 5 or 10 percent MSS products would provide 30.8, 79.1 and 118.2 percent, respectively, of the 10 mg RDA for children 4 to 10 and males over 19 years of age. Males 11 to 18 and females 11 to 51 years of age would receive 17.1, 43.9 and 65.7 percent of their 18 mg RDA of iron from a 100 g serving of the 0, 5 and 10 percent MSS cooked patties, respectively. These data indicate the great potential value of MSS as a natural source of iron for the fortification of ground meat products.

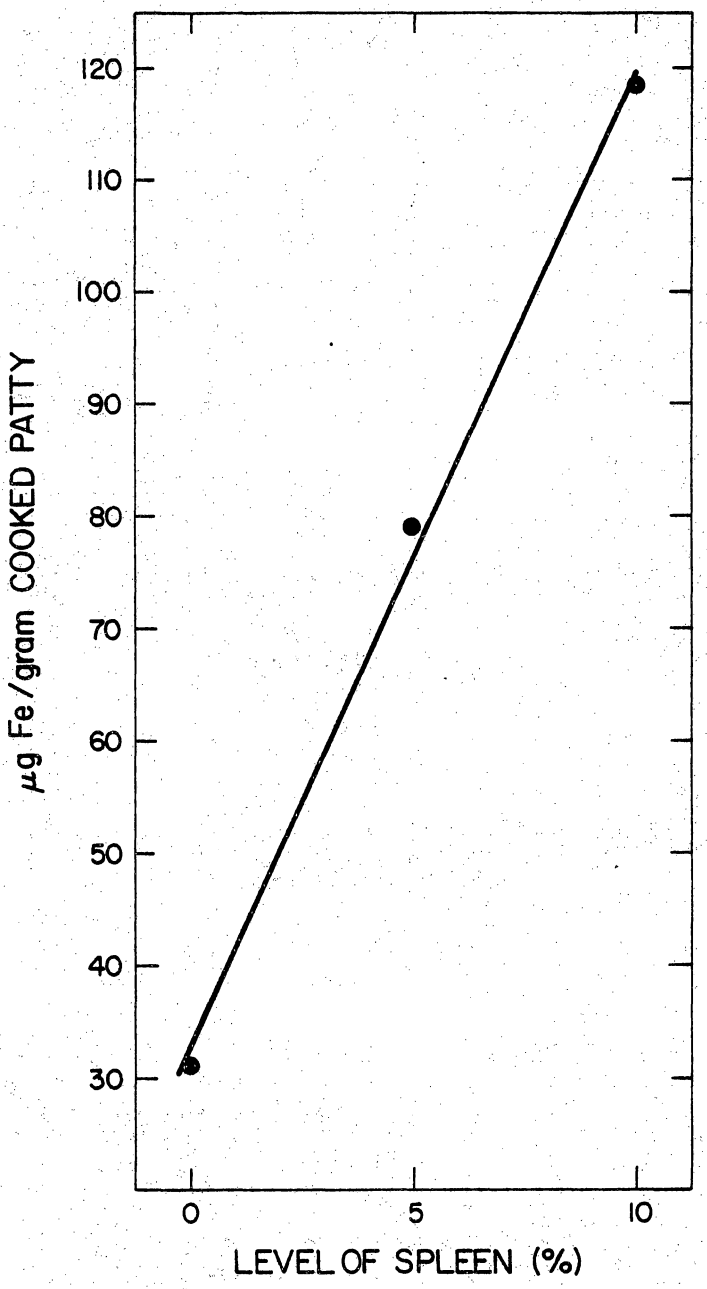

Figure 7. Effect of mechanically sepatated spleen addition on the iron content of cooked meat patties.

4.33 Taste Panel

The taste panel evaluation of the cooked meat patties did not indicate any significant differences between the products with respect to juiciness, flavor, mouth feel or overall acceptability (Table 19). Although the results were not statistically significant, the mean values indicated that juiciness and mouth feel increased with increasing level of MSS and that flavor and overall acceptability were somewhat lower in the MSS-containing products.

Overall, these results indicate that up to 10 percent MSS can be added to ground beef without adversely affecting consumer acceptability of the cooked product. It should be stressed that these patties were served without bread or condiments.

Table 19. Taste panel evaluation of meat patties

	Level of Spleen (%)		
	0	5	10
Juiciness[ad]	2.9 e	3.1 e	3.4 e
Flavor[bd]	3.7 e	3.6 e	3.4 e
Mouth Feel[bd]	3.2 e	3.2 e	3.4 e
Overall Acceptability[cd]	3.6 e	3.4 e	3.4 e

[a]Juiciness: 5 = very juicy, 1 = very dry.

[b]Flavor and mouth feel: 5 = very desirable, 1 = very undesirable.

[c]Overall acceptability: 5 = very acceptable, 1 = very unacceptable.

[d]Means in the same category with different letters are significantly different ($P < 0.05$).

5.0 SUMMARY AND CONCLUSIONS

The feasibility of using mechanical desinewing equipment for the removal of capsular and internal connective tissue from bovine spleen was investigated. Mechanically separated spleen (MSS) leaving the product port of the desinewing head was deep red in color, physically homogeneous and free of visually apparent connective tissue. The separation process yielded 79.1 percent MSS.

Although mechanical separation did not alter the percent protein or ash, the MSS contained 52 percent less fat and 4.4 percent more moisture than whole spleen. Whole spleen and MSS had similar copper concentrations. Zinc content was statistically lower in the MSS than whole spleen, however the difference (1.2 ppm) is probably not nutritionally significant.

The MSS product was shown to be homogeneous with respect to moisture, copper and zinc but proved to be somewhat variable with respect to iron concentration. Random sampling indicated that MSS had an average iron concentration of 762 ppm on a wet basis.

The effect of mechanical separation on spleen protein quality was assessed by protein efficiency ratio (PER). Rats fed the spleen diets ate well, grew rapidly and exhibited significantly greater final weights and total gains than those fed the casein control. The PER values for whole spleen (2.4) and MSS (2.3) were not significantly different from each other. The PER value for casein (2.5) was significantly higher than that of MSS but not whole spleen.

The PER study failed to demonstrate a beneficial effect of connective tissue removal on the protein quality of bovine spleen. However, the use of MSS at the 5 to 15 percent level in formulated products with high proportions of skeletal muscle would yield food products with satisfactory protein quality.

The experimentally determined PER for whole spleen and MSS may have been negatively influenced by the convention of multiplying nitrogen by the factor 6.25 to calculate protein. Spleen potentially has a substantially higher nonprotein nitrogen content than most foods which, if included in the calculation of protein, would result in a lowering of the calculated PER. Future studies should include a determination of nonprotein nitrogen and verification or modification of the conventional factor N x 6.25 for use with splenic tissues.

Chemical analyses indicated that MSS is a high protein (17.0 percent), low fat (2.9 percent), potentially valuable source of iron for the natural fortification of comminuted meat products. Although mechanical separation did not improve spleen protein quality as measured by PER, it was visually apparent at the time of separation that removal of the capsular connective tissue was an important step in the preparation of spleen for inclusion in comminuted meat products.

Beef and pork frankfurters were produced under commercial conditions with 0, 5, 10 and 15 percent of the meat block being MSS. After heat processing, no fat caps were observed on any of the products. Each treatment peeled well and had adequate skin formation. There were no significant differences among the frankfurters in proximate composition, except that the control contained more protein than the 5 and 15 percent

MSS frankfurters. Level of spleen did not consistantly affect the copper content of the frankfurters but zinc concentration decreased with increased level of MSS. Although statistically significant, these differences were probably not nutritionally important. Increasing levels of MSS resulted in significantly increased iron concentrations in the frankfurters. Products made with 5, 10 and 15 percent MSS contained 2.2, 3.9 and 4.9 times more iron than the control, respectively.

A physical attributes panel noted intensified cured meat color and decreased resilience, binding and overall physical acceptability with increased level of MSS. The greatest decrease in physical score occurred between the 10 and 15 percent MSS products. The only below average physical scores recorded were for binding, texture and overall physical acceptability of the 15 percent MSS frankfurters.

Consumer panel results indicated that as much as 15 percent MSS can be incorporated into frankfurters without seriously decreasing consumer acceptability. All frankfurters were rated acceptable for flavor, texture and color. Texture scores reflected a trend toward softer frankfurters with increased MSS.

A laboratory taste panel, conducted after 0, 2, 4 and 6 weeks of storage, rated the 15 percent MSS frankfurters below average for flavor, color, texture and overall acceptability. During the first 4 weeks, no significant differences in flavor, color and overall acceptability scores were noted between the 0, 5 and 10 percent products. Flavor and overall acceptability scores obtained after 6 weeks storage suggested that the shelf life of frankfurters with 10 percent MSS may be shorter than those with 0 or 5 percent MSS. Taste panel results indicated a general decrease

in texture score with increased levels of MSS.

Allo-Kramer shear values determined after 0, 2, 4 and 6 weeks storage decreased linearly with increased level of MSS within each test period. Shear values pooled by treatment showed that the 5, 10 and 15 percent MSS frankfurters offered 15.3, 32.9 and 46.1 percent less shear resistance, respectively, than the control product. This objective measure of texture verified the trend toward softer frankfurters with increased MSS indicated by the three sensory panels.

External and internal frankfurter color was objectively evaluated by Hunter color meter L values after 0, 2, 4 and 6 weeks of storage. Total and nitroso pigments were also determined. Initial (week 0) external and internal surface darkness and total and nitroso pigment concentration increased with increased level of MSS. Each product showed significant fading of the external surface color during storage. The internal color of all but the 15 percent product was stable. Statistically significant loses in nitroso pigment concentration were recorded for the 5 and 15 percent products, but not the 0 or 10 percent products. The total pigment concentration of each treatment decreased significantly during storage. The loss of external surface darkness and total pigment concentration increased with increased level of MSS. The greatest amounts and rates of change were recorded for the 15 percent product. These objectively measured color losses were not visually apparent.

Level of spleen had no apparent effect on the number of mesophilic, psychrophilic or coliform bacteria in the stuffed, uncooked frankfurter

emulsions. Bacterial numbers were reduced during thermal processing. Post processing (week 0) bacterial counts were similar for each treatment. During the 6 week storage period, mesophiles increased in number, whereas psychrophiles and coliform bacteria decreased below their respective detection limit. No signs of spoilage were present after 6 weeks storage. Most commercial products have a 4 to 6 week shelf life. The inclusion of MSS did not adversely affect the microbial quality of the finished frankfurters or shorten shelf life.

The results of this study indicate that the use of up to 10 percent MSS in a frankfurter formulation may be an acceptable means of increasing the iron content of frankfurters while allowing the better utilization of beef spleen protein and the reduction of product cost. Consumer panel data suggest that frankfurters with 15 percent MSS may be acceptable in some markets or as components of mixed dishes. Further studies are necessary to understand and overcome the texture and color fading problems associated with the use of MSS in frankfurters, especially at the 15 percent level.

Ground meat patties containing 0, 5 and 10 percent MSS yielded 69 percent after oven broiling. Control patties contained more fat and less copper and zinc than the 5 and 10 percent MSS products. Increasing levels of MSS resulted in significantly increased iron concentrations in the cooked patties. Products made with 5 and 10 percent MSS contained 2.6 and 3.8 times more iron than the control, respectively. Taste panel data did not indicate significant differences between the patties with respect to juiciness, flavor, mouth-feel or overall acceptability.

Results indicated that up to 10 percent MSS can be added to ground beef without adversely affecting consumer acceptance of the cooked product.

This study has shown that mechanically desinewing equipment can modify bovine spleen to produce a usable raw material of animal origin for inclusion in human food. Mechanically separated spleen is a potentially valuable source of protein capable of substantially elevating the iron level of commonly eaten meat products such as frankfurters and ground beef while maintaining consumer acceptability.

According to Whitlock (1978) there is some indication that iron deficiency anemia causes decreased ability to concentrate and therefore interferes with the learning process in young children. Products containing mechanically separated beef spleen may be a worthwhile addition to the school lunch program as well as to the general market place.

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THE CHARACTERIZATION AND UTILIZATION OF MECHANICALLY SEPARATED BOVINE SPLEEN

by

Ralph J. Bittel

(ABSTRACT)

Studies were conducted to provide information about bovine spleen and its potential uses in comminuted meat products. Scientific data may generate interest and lead to the better utilization of this valuable source of iron and protein in human nutrition.

An effective, economical means of removing capsular and internal connective tissue from splenic pulp was found. Beef spleens were passed through a Beehive deboner with a desinewing head. The process yielded 79.1 percent mechanically separated spleen (MSS) containing 17.0 percent protein, 2.9 percent fat and 762 ppm iron.

The effect of mechanical separation on protein quality was determined by protein efficiency ratio (PER). Rats fed MSS and whole spleen diets exhibited significantly greater weight gains than those fed casein. PER values for whole spleen (2.4) and MSS (2.3) were not significantly different. The PER value of casein (2.5) was significantly higher than that of MSS but not whole spleen.

Beef and pork frankfurters were produced with 0, 5, 10 and 15 percent of the meat block being MSS. Substitutions of MSS were made at the expense of the pork portion of the formula. There were no major differences in proximate composition among the treatments. No fatting or peelability problems were experienced. Vacuum packaged frankfurters were held at 2^{o} C and evaluated at 2 week intervals for 6 weeks.

Initial frankfurter color, as indicated by internal and external Hunter L values and total and nitroso-heme pigments, intensified linearly with increased MSS. Each product showed significant fading of the external surface and loss of total pigment concentration. Rate and degree of change increased with increased MSS. Internal color of all except the 15 percent product was stable. Significant losses in nitroso pigment occurred in the 5 and 15 percent products.

A physical attributes panel noted intensified cured meat color and decreased resilience, binding and overall physical acceptability with increased MSS. Consumer panelists rated all products acceptable for flavor, texture and color. A bi-monthly laboratory taste panel evaluated all frankfurters, except those containing 15 percent MSS, acceptable in flavor, texture, color and overall acceptability during storage. Allo-Kramer shear values verified the trend toward softer frankfurters with increased MSS indicated by the sensory panels.

Frankfurters with 5, 10 and 15 percent MSS had 2.2, 3.9 and 4.9 times more iron than the control, respectively. Level of MSS did not influence bacterial numbers in the emulsions or cooked frankfurters. During storage, mesophiles increased whereas psychrophiles and coliforms decreased. No signs of spoilage were observed after 6 weeks storage.

Meat patties containing 0, 5 and 10 percent MSS yielded 69 percent after oven broiling. Taste panelists rated all patties acceptable for juiciness, flavor, mouth-feel and overall acceptability. Cooked patties with 5 and 10 percent MSS contained 2.6 and 3.8 times the iron of the all-beef control, respectively.

MSS is a valuable source of protein capable of elevating the iron value of comminuted meat products while maintaining consumer acceptability.