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4

EMULSIFYING CAPACITY AND STABILITY OF FREEZE-DRIED BEEF MUSCLE AND MEAT BY-PRODUCTS

A Dissertation

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ABSTRACT

The relationship of emulsifying capacity and emulsion stability with bovine tissue parameters (total protein, salt-soluble protein, total lipid, neutral lipid, polar lipid and ash) was studied. Beef muscle (Psoas major) and meat by-products (liver, kidney and heart) were chopped, freeze-dried, ground and screened through No. 10 and No. 20 mesh size sieves, respectively. The samples were analyzed for emulsion capacity and stability, and the tissue parameters were determined. Based on the results of this study, the following conclusions were made:

Of the four tissues, liver had the highest emulsion capacity, although there were no significant differences among the means of muscle, liver and kidney ($P<0.05$). Heart tissue had significantly lower emulsion capacity than the other tissue powders.

Liver possessed the highest emulsion stability, followed by kidney and muscle. Emulsions of heart tissue were the least stable.

A negative nonlinear relation was established graphically between total protein and emulsion capacity and stability ($r = -.63$ and $r = -.78$, respectively). [As the concentration of protein increased, emulsion capacity and stability decreased.]

In liver, salt-soluble protein constituted the greatest proportion of total protein, followed by heart and kidney. Regardless of the amount of salt-soluble protein, a relatively weak relation was observed

with emulsifying capacity (r = 0.56). However, a stronger relation was found between emulsion stability and salt-soluble protein (r = 0.75).

Total lipid content appeared to be one of the factors with most influence on emulsion capacity and stability. Strong linear correlations were observed (r = 0.99 and r = 0.97, respectively). By its biological nature, liver tissue contained the highest amount of total lipids, which was assumed to be directly responsible for its higher emulsion capacity and stability.

Neutral lipid content did not seem to exert any effect on either emulsion capacity or stability and exhibited weak relations with them (r = 0.55 and r = 0.32, respectively).

Along with total lipid content, polar lipid content also seemed to be an important factor in emulsion capacity and stability. Polar lipid content exhibited a moderate correlation with emulsion capacity (r = 0.77), but was highly correlated with emulsion stability (r = 0.91).

Ash content of muscle and tissue powders did not exhibit any strong correlation with either emulsion capacity or stability (r = 0.39 and r = 0.59, respectively.

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CHAPTER I

INTRODUCTION

Protein deficiency is now recognized as a major nutritional problem in most of the underdeveloped and developing countries. Besides the shortage of high quality foods, the population in these areas is increasing at an exponential rate (45). "Within 15 years the world will not tolerate wasting and destroying foods. The over-abundance of food in this country gives many of us a false sense of security. World-wide starvation is certain before the end of the century," said Ezra Levin (36).[1]

Presently, large quantities of edible meat by-products such as blood, bone, fat, liver, kidney, heart, lungs, brain, stomach, intestines and various glandular tissues of potential value as human food are either processed as animal feed or wasted. The use of these meat by-product concentrates (MPC) in sausage would not only increase the nutritional value but decrease the cost and help curb environmental pollution (35, 36, 37, 38, 41, 43).

Saffle (49) reported that MPC added to sausage and other processed foods as emulsifying agents have a tendency to increase emulsion capacity (ED), emulsion stability (ES), water-holding capacity and to influence

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[1]The numbers in parentheses represent similarly numbered references in the Literature Cited.

color, flavor and peelability. At the present time, there is little research to support these assumptions. Acton and Saffle (1, 2), Satterlee and Free (51) investigated the emulsifying capacity of muscle and various tissue powders and found a positive correlation between EC and salt-soluble protein content.

In this study, an attempt was made to investigate EC and ES of various freeze-dried bovine tissues (muscle, liver, kidney and heart) and to determine if parameters (total protein, salt-soluble protein, total lipid, neutral lipid, polar lipid and ash) of the tissues are correlated with and can be used as indicators of EC or ES.

CHAPTER II

REVIEW OF LITERATURE

I. DEFINITION AND CONCEPT OF MEAT EMULSION

Meat emulsion may be defined as a heterogeneous system in which a normally immiscible solid (fat) is intimately dispersed in another liquid (water) in the form of small droplets or globules (8, 13, 49).

In most emulsions, the most widely involved liquids are oil and water. Pure oil and pure water seldom form emulsions. The oil phase may consist of oils, hydrocarbons, waxes, resins and other substances which behave like oil (hydrophobic materials), whereas the water phase may include solutions of salts, sugars or other organic and collodial materials (hydrophilic materials). In order to form a stable emulsion, a third substance called an emulsifier or emulsifying agent, including proteins, phospholipids, sterols and a wide range of synthetic materials, is required (13, 19, 60).

When oil and water are mixed, two types of emulsion may be formed. The existence of these types of emulsions was first pointed out by Clayton (19). The phase which is in the form of finely divided droplets is called the internal or discontinuous phase. The oil may become a dispersed phase and gives an oil-in-water (o/w) type emulsion. A sausage emulsion would be considered as an example for this type of emulsion.

Alternatively, the water phase which forms the matrix in which the droplets are suspended is called the external or continuous phase. The water may become the dispersed phase and gives a water-in-oil (w/o) type emulsion (3, 13).

Becher (8) introduced a third type of emulsion, called a multiple emulsion, in which both types of emulsions exist simultaneously. An oil droplet may be suspended in an aqueous phase, which in turn, encapsulates a water droplet, thus forming a w/o/w type emulsion. Jirgensons (30) stated his belief that multiple emulsion occurs at the inversion phase of emulsifications.

An o/w emulsion produces a creamy texture, can be diluted with water, can be colored by water soluble dyes and conducts electrical current, whereas, a w/o emulsion produces a greasy texture, can be diluted only with oil, can be colored by oil soluble dyes and does not conduct electrical current.

The particle size in an emulsion varies from 0.1μ to 50μ. Hansen (24) reported that fat particles in a so-called meat emulsion were much larger than 50μ. Therefore, meat emulsions could not be considered a true emulsion.

II. THEORY OF MEAT EMULSION

Formation of Emulsion

It is a fact that there is no single coherent theory of emulsion formation and stability. Researchers in this field have limited themselves to specialized systems to explain emulsion behavior. Swift et al.

(56) developed the first "model system" in which they studied the effects of pH, types of proteins and fats, chopping speed, temperature, salt concentrations, type of binders, fat particle size, prerigor, fresh and frozen meat on emulsion behavior.

Schwartz and Perry (52) have interpreted emulsion behavior and explained the fundamentals of emulsion formation. The two most important factors necessary for a stable emulsion are the existence of an interfacial film which may be regarded as an envelope around each dispersed globule, and low interfacial tension between the oil and water phases. When two immiscible liquids contact each other an interfacial tension develops due to free energy arising from an imbalance in the cohesive forces of the two liquids. This free energy causes the interface to contract to form the smallest possible interfacial area. If two liquids are completely soluble in each other, the interfacial tension is zero (8, 19). In the case of a meat emulsion, where a fat is in contact with water, the interfacial tension is quite high. It is clear that a meat emulsion is more stable if the interfacial tension is low. The internal phase of an emulsion always forms spherical droplets representing the smallest interfacial area per unit volume of liquid. Further these droplets tend to coalesce to form larger ones, again reducing the interfacial area.

The emulsification process requires considerable energy input and the addition of sodium chloride both of which increase the interfacial tensions. To reduce the interfacial tensions and, hence, increase stability, a third factor, emulsifying agents, should be introduced into the system (13, 49).

Fundamentals of Emulsifying Agents

Emulsifying agents may be classified into three major classes: surface active agents, naturally occurring materials and finely divided solids. Among these three types of emulsifying agents, the naturally occurring substances are the ones which are of most importance in meat emulsions. Most of the emulsifying agents of this sort, derived almost unchanged from natural sources, are relatively inefficient by themselves but are of considerable value in conjunction with other emulsifiers. Therefore, they are often called "auxiliary emulsifying agents." Proteins, lipids, phospholipids, sterols, fatty acids and bile acid are natural emulsifying agents and have variable emulsifying power (8, 9, 13, 30, 31, 39).

Regardless of the type of emulsifying agents, all of them perform two important functions in the emulsification process: (a) reduction of interfacial tension between the liquids; (b) protection of the emulsion by preventing the coalescence of the droplets of the internal phase.

Most emulsifying agents consist of substances with molecules containing both polar and nonpolar groups. During emulsification they are adsorbed at the interface between the phases, thus reducing the interfacial tension. When they are adsorbed, the molecules of emulsifiers orientate themselves at the interface so that the nonpolar groups, which have an affinity for the oil, will point towards the oil phase, while the polar groups, which have an affinity for the water, will point towards the aqueous phase. This type of arrangement of molecules forms

a layer called an "interfacial film" which functions as a protective coating on the droplets of the internal phase (12, 13).

Emulsification Properties of Proteins

Proteins, specifically the salt-soluble proteins of muscle, are one of the most important naturally occurring emulsifying agents. Salt-soluble proteins can be defined as those soluble in salt solutions and insoluble in pure water, e. g., actin and myosin. On the contrary, water-soluble proteins are soluble in water and insoluble in water containing salt, e. g., sarcoplasmic proteins. However, most reports on meat emulsions prefer to use the term salt-soluble protein to mean those proteins which are salt-soluble plus those proteins which are also water-soluble (49).

Several researchers (4, 24, 26, 28, 53) stated that salt-soluble proteins had higher emulsifying power than water-soluble proteins. Swift (58) reported that salt-soluble proteins were 30-40 percent more effective in emulsification capacity than water-soluble proteins. According to Carpenter and Saffle (16) water-soluble protein molecules had a length-to-width ratio of 4:1, whereas, those of salt-soluble proteins had a 200:1 ratio. This simply means that the salt-soluble proteins have approximately 50 times as much surface area to surround a fat particle as do the water-soluble proteins. Hegarty et al. (26) ranked the proteins of muscle based on their emulsion capacity as follows: actin, myosin, actomyosin, sarcoplasmic proteins. Figure 1

shows the schematic structure of an o/w type emulsion and the enclosure of fat globules by proteins.

The emulsion capacity of salt-soluble proteins was shown to have a maximum at pH 6.0 to 6.5 and did not change when the pH was increased to 8.0. Increasing sodium chloride content was accompanied by a significant increase in emulsion capacity (57, 62).

Solubility and Extraction of Proteins

Many investigators (16, 53, 59) have studied the solubility and extractability of soluble muscle proteins and found a sharp decrease in the solubility of fibrillar proteins at a pH below 6.5. At higher rigor temperature and lower pH, greater amounts of fibrillar components were found because of denatured sarcoplasmic proteins. This denatured precipitated protein in turn reduced the solubility of fibrillar protein (61).

While solubility is essential for extractability, meat particle size and mechanical actions are also significant limiting factors for extractability of meat proteins.

Role of Fats in Meat Emulsion

One of the major problems in sausage making is the possibility of emulsion breakdown and fat separation in the smokehouse. This is undesirable because it results in less product to sell and the products become poor in quality.

There are several factors affecting emulsion capacity, such as temperature, rate of addition of fat, amount of fat and types of fat. If temperature in the chopper exceeds 15-22°C, a breakdown of emulsion

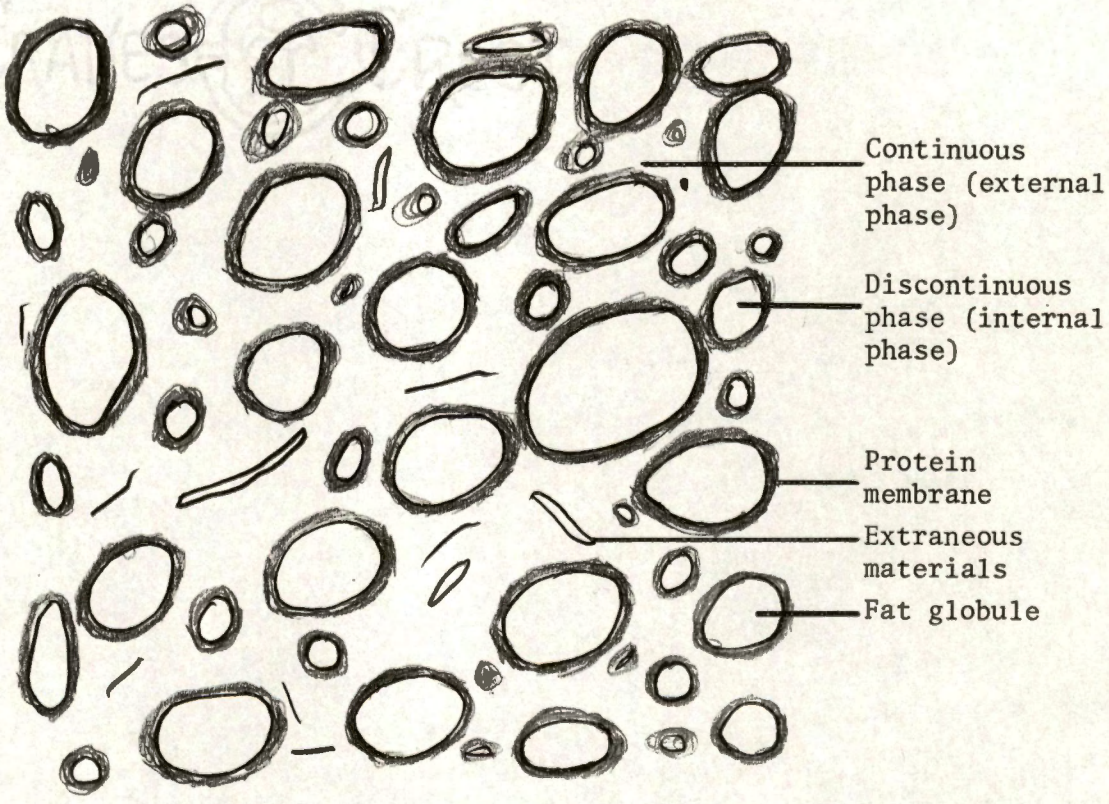

Figure 1. A schematic structure of an o/w type emulsion.

will occur. Swift et al. (56) found that the amount of fat emulsified was related inversely and linearly to the maximum temperature created during emulsification. They also found a linear relation between the rate of fat addition and the amount of fat emulsified. The explanation for this was that the protein membranes can be formed instantaneously and vigorous stirring was necessary to assure adequate dispersion of fat and intermixing of compounds.

Investigators have studied the effect of various fats and oils (melted lard, cottonseed oil, soybean oil, corn oil) on emulsification. Carpenter and Saffle (15) found that more of the shorter-chain saturated fatty acids and triglycerides were amulsified than of the longer-chain saturated fatty acids and triglycerides. They found also that more of fatty acids with one double bond were emulsified than fatty acids with two double bonds. These studies indicated that smaller amounts of saturated fatty acids were emulsified than of unsaturated fatty acids with one or two double bonds.

Lipid-Protein Interaction

The interaction between lipid and meat protein is the least understood molecular association during emulsification, and no one knows with certainty how a membrane is put together around the fat particle.

Lipids may be studied in three groups: (1) nonpolar lipids (having no asymmetric electronic charge); (2) polar lipids (having one part of the molecule with electronic structure differing from the other); (3) ionic lipids (having definite positive or negative charges on part of

the molecule). Triglycerides and waxes are nonpolar lipids, whereas, glycolipids may be considered polar ones because the carbohydrate end of the molecule is more water soluble in nature than the hydrocarbon end. The hydrocarbon end is hydrophobic (water-repelling). On the other hand, phospholipids are a common example of ionic lipids, where one end is hydrophobic, the other end is an ionizable, water-soluble group.

All lipids, by virtue of their hydrophobic parts, can interact with certain functional groups of proteins. The hydrophobic groups of protein and lipid are insoluble in water. Therefore, they are forced together forming a "hydrophobic bond." Interaction between triglycerides and protein would be of this type (21, 23, 27). It has been suggested that the double bonds of the hydrocarbon chains can interact with sulfhydryl groups of protein to form a similar bonding (17, 22).

It has been proposed also that ionic interaction may take place in the same structure between phospholipids and proteins. The hydrophilic portion of the phospholipid molecule consists of a phosphate linkage to glycerol and a group such as choline, serine, etc. In an aqueous environment, the molecules tend to aggregate with the hydrophobic portions together (21, 34). Figure 2 illustrates an ionic lipid-protein bonding, where the hydrophilic heads are exposed for interaction with protein, and hydrophobic tails pointing towards phospholipids.

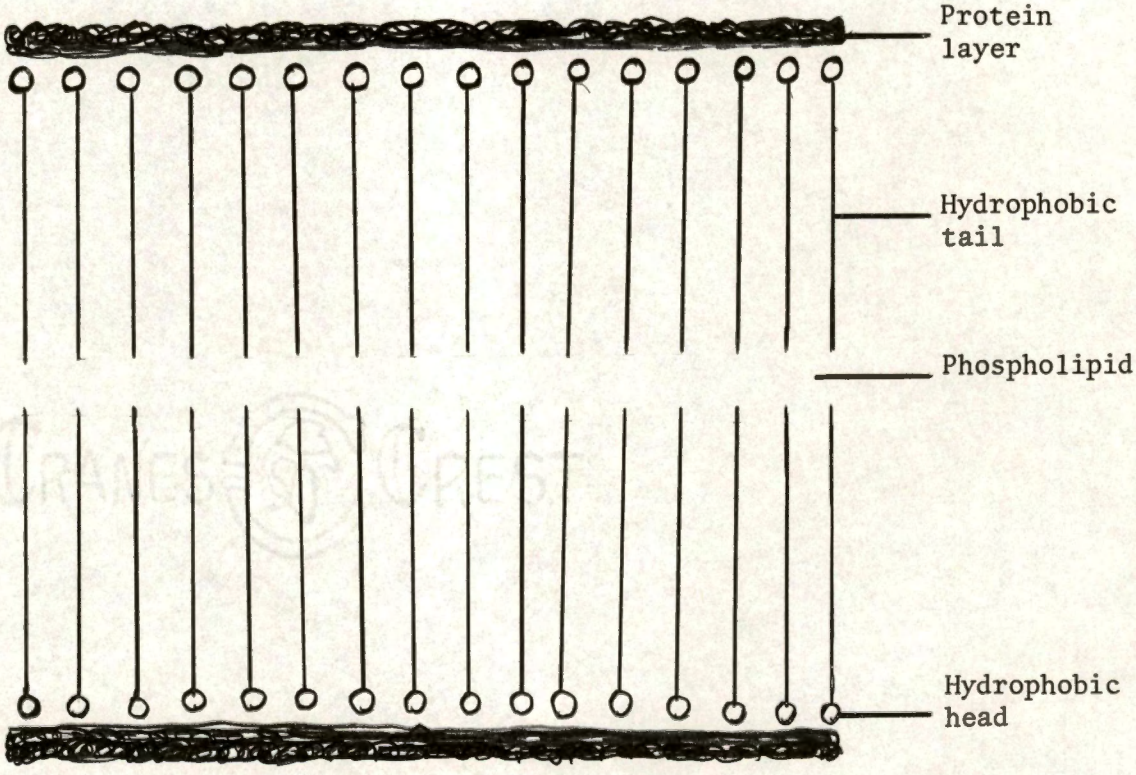

Figure 2. A simplified representation of lipid-protein membrane structure; Ionic lipid-protein bonding. Adapted from Fullington (21).

III. DETERMINATION OF EMULSION CAPACITY

The most critical point during the determination of emulsion capacity is detection of the exact break point. Webb et al. (64) found the electrical resistance method to be reliable for determining the end-point. Haq (25) reported that emulsions with relatively high total lipid content had lower alternating current conductivities than did those with low lipid content.

Meat protein under various concentration, pH and ionic strength conditions, exhibits a characteristic net charge. When an electrical field is applied to a continuous phase, the positively charged particles migrate to the negative electrode and vice versa. When this happens, the flow of current is impeded by the presence of the particles collected near the electrodes. These changes in the flow of current are monitored by an ohmmeter during emulsion formation and breakdown. During the emulsion collapse, the viscosity abruptly decreases due to inversion of emulsion (20). When the end point is reached, the pointer of the ohmmeter suddenly drops, indicating the breakdown.

IV. CHEMICAL COMPOSITION OF BEEF MUSCLE, LIVER, KIDNEY AND HEART

Lean muscle consists of approximately 20 percent protein, 70 percent water, 9 percent fat, and 1 percent ash. Muscle proteins may be classified according to their locations, as intracellular (myofibrillar, sarcoplasmic) and extracellular proteins (connective tissue). The major

protein in muscle is actomyosin, a globulin that consists of two proteins, actin and myosin, combined in a 1 to 3 ratio. It is the structural component that gives muscle the power of movement and is quite significant from the meat emulsion point of view because of the ability to emulsify more fat than other muscle proteins. Actin and myosin are salt-soluble proteins and have a length of 470°A and 1600°A, respectively (7, 14, 46).

Lipids of muscle include neutral fat, phospholipids, glycolipids, cholesterol and cholesterol esters. The quantity varies widely in different muscles of the same animal, and in different species, e. g., the total lipid of striated muscle of chicken averages about 7 percent (on a dry-weight basis), and of hog about 22 percent. These differences are due mainly to differences in the amount of neutral fat deposition in the tissues (5, 33, 40, 50, 55).

Beef liver contains about 19.7 percent protein, most of which is extracellular (66). Total lipid content of liver is about 3.2 percent (on a fresh-water basis), and is composed primarily of phospholipids and neutral lipids. Bloor (11) found the neutral fats of beef liver to be considerably more unsaturated. The fatty acid percentage of liver fat was 70 while the yield of fatty acid from true fat would be about 95 percent (11).

Beef kidney and heart contain 15 percent protein, 8.1 percent lipid, 16.9 percent protein, 3.7 percent lipid, respectively. As an emulsifying agent, the proteins are the main elements influencing the emulsion capacity and stability of a meat emulsion. When the percentage of

protein of muscle and meat by-products are to be considered, one would reasonably expect a higher emulsion capacity from muscle than the meat by-products. However, in the first phase of the present experiments, this was not the case; a higher emulsion capacity was obtained from liver and kidney than from muscle. Therefore, an additional attempt was made to investigate other possible factors, such as salt-soluble proteins, total lipid, neutral lipid, polar lipid and ash, which could be affecting emulsion capacity and stability of the samples.

CHAPTER III

MATERIALS AND METHODS

I. PREPARATION AND TREATMENT OF SAMPLES

Four pounds each of beef liver, kidney, heart and muscle tissues (Psoas major) were used. They were obtained from a local meat packing company. The beef muscle and meat by-products were randomly selected and quality grade, age, and sex of animal were disregarded.

The samples were trimmed of all external fat and loose connective tissue. They were cut into small pieces and chopped in an Osterizer blender for 1 minute. Distilled water was added during chopping in an amount sufficient to make the samples viscous and to ease homogenization. Immediately after this process, the meat samples were freeze-dried in a VirTis freeze-dryer (The VirTis Company, Gardiner, N. Y.) at 105 mμ of Hg for about 3 days without the application of heat.

The freeze-dried products were ground in a porcelain mortar and screened through No. 10 and No. 20 mesh size sieves, respectively. The coarse fibrous material that remained in the sieves was discarded. The finely ground samples were put in plastic bags, placed in a desiccator and kept in a cold room at 10°C throughout the experiment. The samples were analyzed for emulsion capacity, emulsifying stability, total protein, salt-soluble protein, total lipid, neutral lipid, polar lipid, moisture and ash.

II. METHODOLOGY AND INSTRUMENTATION

Determination of Emulsion Capacity

The emulsion capacity (EC) of each tissue powder was determined by a modified technique based on the method described by Satterlee and Free (51) and Tsai et al. (63). The experimental setup utilized in this study is shown in Figure 3.

A small plastic blender jar was used for emulsification experiments. Two small holes were drilled through the jar where copper electrodes were inserted. One larger hole, for the addition of oil, was also drilled in the jar. The jars were placed on the blender upside down to facilitate emulsification while the addition of oil continued at the rate of three drops per second.

The sample to be emulsified was blended with 5 percent NaCl and 0.02 M $NaHCO_3$ buffer (pH 6.5) in an Osterizer blender. To extract salt-soluble proteins, 0.05 g of sample was blended with 30 ml of buffer at 12,000 RPM for 30 seconds. After the extraction process, 30 ml of Wesson vegetable oil was added and chopping started at 12,000 RPM with the addition of more oil. Emulsion formation breakdown was measured by a volt-ohm meter (Micronta Multitester). The DC resistance increased sharply during emulsion breakdown.

Emulsion capacity (EC) was expressed as the amount of oil that could be emulsified by 100 mg of protein (47, 49). The emulsification experiment was performed in a cool room (10°C), and samples, buffer, oil and equipment were equilibrated to 10°C before experimentation started. The experiment was conducted in two replications.

Determination of Emulsion Stability

The stability of beef muscle and meat by-products emulsions were measured according to methods described by Korschgen and Baldwin (32). As in the determination of EC, 0.05 g of sample was emulsified with Wesson vegetable oil. The addition of oil was stopped just before the emulsion breakpoint occurred (5 percent less oil was added). The emulsions were kept at 10°C for approximately 24 hours prior to centrifugation. After this period of time, they were weighed and then centrifuged at 2800 X G for 10 minutes. The supernatant was decanted and the pellet was reweighed. The percent ES was calculated according to the following formula:

$$\text{percent emulsion stability} = \frac{\text{g remaining in the centrifuge tube X 100}}{\text{g original weight of sample}}$$

Four determinations were made in each of two replications.

Determination of Total Protein

Total nitrogen was determined by the Kjeldahl method (6). For each test, 0.5 g of freeze-dried sample was used. Total apparent protein was calculated by using a conversion factor of 6.25. Results were expressed on a dry-weight basis.

Determination of Salt-Soluble Protein

For the determination of salt-soluble protein a similar method to those of Wierbicki et al. (65), and Saffle and Galbreath (48) was used. One and a half grams of freeze-dried samples was blended with 100-125 ml of 5 percent NaCl and 0.02 M $NaHCO_3$ (pH 6.5) in an Osterizer blender at

12,000 RPM for 1 minute. The sides of the blender jar were swabbed, and the mixture was allowed to stand for 3 minutes, then blended for an additional minute. The pH of the samples was adjusted to 6.0 before extraction. The slurry was transferred to centrifuge tubes and centrifuged for 10 minutes at 3020 X G. The clear portion of the solution was decanted and recentrifuged for another 10 minutes at 3020 X G. Twenty-five ml. of the supernatant were pipeted into a Kjeldahl flask and analyzed to determine salt-soluble protein content. The centrifugation process was done in a Sorvall centrifuge (Model RC2-B) at 0±1°C.

The mg of protein in the supernatant divided by the mg of total protein in the 1.5 g freeze-dried sample times 100 was equal to the percent salt-soluble protein. Salt-soluble protein was expressed as percent of the total protein.

Total Lipid Extraction

The extraction procedure of Bligh and Dyer (10) as modified by Ostrander and Dugan, Jr. (42) was used. The extraction solvent used was composed of chloroform: methanol: water (4:2:1, v:v:v:). Lipids were extracted from 5 g samples of beef muscle, liver, kidney or heart. Since the samples were lyophilized, they were rehydrated with 49.5 ml. of distilled water and macerated in an Osterizer blender at high speed (12,000 RPM) for 2 minutes. A chunk of dry ice, approximately one cubic inch in size, and 130 ml of absolute methanol were added and the samples were blended at 12,000 RPM for 5 minutes. After adding 65 ml

of chloroform, the mixture was reblended for 5 minutes at the same speed. Another 65 ml of chloroform were added and blended for 30 seconds. Then 65 ml of distilled water containing 1.0 g of zinc acetate (to precipitate the protein) was added and blended for 10 seconds. The extraction mixture was filtered through a No. 3 Buchner funnel and Whatman No. 1 filter paper with suction. The residue with the filter paper was reblended with 100 ml of chloroform for 2 1/2 minutes. The mixture was filtered as above and the residue on the funnel was rinsed with 50 ml of chloroform. The filtrate was transferred into a 500-ml graduated cylinder, stored overnight at approximately 10°C and allowed to separate into two layers. The volume of the chloroform-lipid layer in the bottom of the cylinder was recorded and the entire contents were transferred into a 500-ml separatory funnel. After the formation of two layers, the chloroform-lipid portion was drained into a 500-ml Erlenmeyer flask. Portions of 4 to 10 ml were pipeted immediately into preweighed 50 ml beakers and placed under the hood until the evaporation of chloroform was completed. After the evaporation of solvent, the beakers were placed in a vacuum desiccator. The beakers containing lipids were reweighed and the percent total lipid calculated:

$$\text{percent total lipid} = \frac{\text{ml } CHCl_3 \text{ extract X g lipid in 10 ml sample X 100}}{\text{g meat sample extracted X 10}}$$

Determination of Neutral Lipids

The petroleum ether extract method (Goldfish) was used for the determination of neutral lipids (6). About 1.5 g of moisture-free samples were placed in thimbles and extracted with petroleum ether for 10.5 hours. The percent neutral lipids was calculated as follows:

$$\text{percent neutral lipids} = \frac{\text{g lipid extracted}}{\text{g sample used}} \times 100$$

Determination of Polar Lipids

The percentage of polar lipids was obtained by simply subtracting the means of neutral lipids from the means of total lipids.

Moisture Determination

The moisture content of the freeze-dried samples was determined by A. O. A. C. (6) methods. About 2 g of samples were dried in a vacuum oven at 70°F for 12 hours and percent moisture was calculated as follows:

$$\text{percent moisture} = \frac{\text{g dried sample}}{\text{g original sample}} \times 100$$

Ash Determination

Ash content of the samples was also determined by the A. O. A. C. (6) method. About 1.5 g of samples were ashed in a furnace at 600°C for 3 hours and percent ash calculated.

III. STATISTICAL ANALYSIS

Data on EC, ES, total protein, salt-soluble protein, total lipid, neutral lipid, polar lipid and ash were analyzed in a two-way classification. Gross correlations between variables were determined. Differences between individual means were examined by means of Duncan's Multiple Range Test (54).

CHAPTER IV

RESULTS AND DISCUSSION

I. COMPOSITION OF FREEZE-DRIED MATERIALS

Total Protein and Salt-Soluble Protein

The analysis of variance of percent total protein and salt-soluble protein of freeze-dried muscle, liver, kidney and heart powder is shown in Table 1. The differences in percent total protein of individual tissues were highly significant at the 0.01 probability level. At the same level of probability, the differences between replications also were highly significant. The reasons for the occurrence of this high level of significance between the replications could be that the samples were selected randomly disregarding quality grades, age and sex, and different animals were used for each replicate. Another possible cause may include variation between replications in sampling.

Total protein composition of the freeze-dried tissue powders is presented in Table 2. The protein concentration varied between muscle and each tissue powder, the means ranging from 62.76 percent to 79.43 percent. The differences among the means of total protein were highly significant ($P<0.01$). Heart tissue had a higher protein content than either muscle or kidney, while liver possessed the lowest concentration of protein. This relatively large variation was expected because of known differences in biological and chemical compositions of these

TABLE 1

ANALYSES OF VARIANCE OF TOTAL PROTEIN AND SALT-SOLUBLE PROTEIN OF FREEZE-DRIED BEEF MUSCLE, LIVER, KIDNEY AND HEART POWDER

Source	D.F.	Mean Squares	
		Total Protein	Salt-Soluble Protein
Tissues	3	406.23**	2144.57**
Replications	1	105.34**	236.91**
Residual Error	27	2.36	17.01

** $P<0.01$

TABLE 2

PERCENT TOTAL PROTEIN OF FREEZE-DRIED BEEF MUSCLE, LIVER, KIDNEY AND HEART POWDER 1/

Replication	Muscle	Liver	Kidney	Heart
	80.86	69.13	79.30	76.33
I	74.11	70.25	77.57	79.96
	79.27	70.52	76.77	79.30
	77.02	69.13	74.78	78.49
	73.88	57.13	73.33	79.72
II	77.28	57.39	74.69	80.97
	77.41	57.89	74.28	79.45
	79.18	57.01	74.01	81.11
Mean 2/	77.43[a]	62.76[b]	75.42[c]	79.43[d]
Standard Error	2.64	6.76	2.17	1.63

1/ Percent total protein was calculated on a dry-matter basis (it was determined on ground freeze-dried tissue which passed through a No. 20 sieve).

2/ Mean of 8 observations.

2/ Means within row followed by the same letter are not significantly different at the 0.05 level of probability.

tissues. Satterlee and Free (51) reported the concentrations of protein in beef muscle, liver, kidney and heart tissue powders to be 21.90, 79.73, 76.53 and 85.32 percent, respectively. It is not logical, however, to compare their percent total protein of muscle (21.90%) with findings of the present study, since theirs was reported on a fresh-weight basis. However, a close similarity was observed between the total protein concentrations of other tissues, with the exception of liver. Other reports (4, 5, 14, 46) indicated the concentration of muscle tissue and tissue powders to be 20.0 (on a fresh-weight basis), 19.7, 15.0 and 16.9 percent, respectively. If they are examined closely, one can see that the observed differences between the concentrations of tissues vary. These discrepancies are probably due, to some extent, to the fact that the different samples and techniques were utilized for the determinations of proteins. The samples were obtained from different animals in each replicate and freeze-dried. After the freeze-drying step, the tissues were ground and screened through No. 10 and No. 20 mesh size sieves. The coarse fibrous materials that remained in the sieves were discarded and were not utilized in the analysis. Therefore, the exclusion of these parts, much of which was probably collagen protein, may have caused some variation in total protein and salt-soluble protein concentrations.

Table 1 shows also the analysis of variance of salt-soluble protein. The differences in concentrations of salt-soluble protein and between tissues and replications were highly significant ($P<0.01$).

The concentration of salt-soluble protein is presented in Table 3. Duncan's multiple range test showed significant ($P<0.05$) differences among the means of all tissue powders. Liver tissue gave the highest concentration of salt-soluble protein (69.68%), while beef muscle had only 33.91 percent. Muscle and heart tissue powders possessed a close range of protein concentration (33.91% and 43.64%, respectively), which was in agreement with the salt-soluble protein content of beef muscle and heart (34.04% and 41.24%, respectively) reported by Saffle (47).

Total Lipid, Neutral Lipid and Polar Lipid

The analysis of variance of total lipid and neutral lipid of freeze-dried beef muscle and tissue powders is presented in Table 4. The differences in percent total lipid of beef muscle and each tissue were highly significant at the 0.01 level of probability. Because of the reasons explained earlier in this chapter, such as random selection of samples disregarding quality grades, age, and sex. The differences between the replications were also highly significant ($P<0.01$).

Table 5 shows percent total lipid of freeze-dried tissue powders. The concentration of total lipid ranged from 16.65 to 22.64 percent. Duncan's multiple range test showed no significant differences among the mean total lipid of muscle, liver and kidney, while heart was significantly lower ($P<0.05$).

Ostranger and Dugan, Jr. (42) analyzed lyophilized raw pork muscle and found total lipid content to be 38.6 percent with a 1.19 percent variation. Bligh and Dyer (10) reported that the results of total lipid

TABLE 3

PERCENT SALT-SOLUBLE PROTEIN OF FREEZE-DRIED BEEF MUSCLE, LIVER, KIDNEY AND HEART POWDER 1/ 2/

Replication	Muscle	Liver	Kidney	Heart
	33.27	65.79	42.78	46.69
I	31.83	63.45	49.91	47.93
	29.16	64.04	66.64	45.95
	31.57	63.16	66.14	43.98
	36.11	73.69	67.42	41.47
II	36.11	74.58	68.49	40.45
	36.75	76.06	66.89	41.47
	36.44	76.66	65.56	41.21
Mean 3/	33.91[a]	69.68[b]	61.73[c]	43.64[d]
Standard Error	2.85	6.07	9.72	2.90

1/ Percent salt-soluble protein was expressed as percent of total protein.

2/ Percent salt-soluble protein was calculated on a dry-matter basis.

3/ Mean of 8 observations.

3/ Means within row followed by the same letter are not significantly different at the 0.05 level of probability.

TABLE 4

ANALYSES OF VARIANCE OF TOTAL LIPID AND NEUTRAL LIPID OF FREEZE-DRIED BEEF MUSCLE, LIVER, KIDNEY AND HEART POWDER

Source	D.F.	Mean Squares	
		Total Lipid	Neutral Lipid
Tissues	3	62.97**	21.16**
Replications	1	249.09**	17.46**
Residual Error	27	9.91	0.69

**P<0.01

TABLE 5

PERCENT TOTAL LIPID OF FREEZE-DRIED BEEF MUSCLE, LIVER, KIDNEY AND HEART POWDER 1/

Replication	Muscle	Liver	Kidney	Heart
I	25.94	28.99	26.93	16.34
	12.57	25.60	23.50	19.88
	29.21	27.00	28.06	18.29
	23.52	23.00	27.40	18.04
II	18.27	17.99	24.30	14.40
	15.99	20.62	15.33	16.46
	17.70	19.61	18.39	13.16
	21.28	17.60	17.27	16.62
Mean 2/	20.51[a]	22.54[a]	22.64[a]	16.65[b]
Standard Error	4.58	4.20	4.58	1.85

1/ Percent total lipid was calculated on a dry-matter basis.

2/ Mean of 8 observations.

2/ Means within row followed by the same letter are not significantly different at the 0.05 level of probability.

determinations on a sample of cod muscle were reproducible to within a ± 2 percent deviation. Due to highly significant differences between replications, a range of 10 to 20 percent variations were found in total lipid content of tissue powders.

The total lipid of muscle includes neutral lipid, phospholipids, glycolipids and cholesterol. The quantity of these varies widely in different muscles and in comparable muscles of different species; e.g., the total lipid content of white muscle of the chicken averages about seven percent (on a dry-matter basis) and that of the hog is about 22 percent, whereas beef muscle contains 19-20 percent (14). These differences are due mainly, but not entirely, to the differences in amount of neutral fat and its location in the tissues. The Psoas major muscle having a large amount of extracellular "marbling" fat (66) influences this variation.

The analysis of variance of percent neutral lipid is shown in Table 4. The differences in concentrations of neutral lipid between tissues and replications were highly significant ($P<0.01$). In Table 6, the neutral lipid concentrations in beef muscle and tissue powders are compared. The means ranged from 12.35 to 15.41 percent. According to Duncan's multiple range test, no significant differences were indicated between the means of muscle and kidney or between the means of liver and heart ($P<0.05$). The concentration of neutral lipid fluctuated from one tissue to another. Muscle and kidney gave a higher percentage of neutral lipid than either liver or heart, because of the presence of a larger amount of natural (neutral) fats located in fat cells in the interstitial

TABLE 6

PERCENT NEUTRAL LIPID OF FREEZE-DRIED BEEF MUSCLE, LIVER, KIDNEY AND HEART POWDER 1/

Replication	Muscle	Liver	Kidney	Heart
I	14.57	14.71	16.37	13.52
	16.89	14.47	15.12	14.19
	16.11	14.20	16.68	12.52
	16.20	12.34	14.71	13.30
II	14.22	11.53	14.35	11.43
	16.09	11.12	15.55	10.89
	14.07	11.24	15.10	12.33
	15.03	13.30	15.37	10.64
Mean 2/	15.40[a]	12.87[b]	15.41[a]	12.35[b]
Standard Error	1.06	1.50	0.79	1.29

1/ Percent neutral lipid was calculated on a dry-matter basis.

2/ Mean of 8 observations.

2/ Means within row followed by the same letter are not significantly different at the 0.05 level of probability.

tissue of muscle and kidney. Bloor (11) asserted that most of the natural fats stored by liver are unsaturated, which was found to be an important factor in emulsion capacity and stability by Saffle (49).

In Table 7, the concentrations of polar lipid are presented and the mean of each sample was compared with others. No significant differences were found between polar lipid content of muscle, kidney and heart ($P<0.05$). Liver tissue had the highest concentration of polar lipid (9.69 percent), followed by kidney (7.24 percent). Duncan's multiple range test indicated that there were no significant differences among the means of liver and kidney, although liver possessed the numerically highest amount of polar lipid. It is not valid to compare the concentrations of polar lipid found in this study with those reported in the literature because polar lipid was not determined directly; it was calculated as the difference between the means of percent total lipid and the means of percent neutral lipids.

Ash

The analysis of variance of ash content of freeze-dried beef muscle and meat by-products is presented in Table 8. Both the mean of each tissue and replication was significantly different ($P<0.01$). Table 9 shows the percent ash content of the samples. Liver tissue had the highest ash concentration. The differences among means of muscle, kidney and heart were not significant ($P<0.05$). Levin (35) reported the mineral content of meat protein concentrates to be 14.8 percent on a dry-matter basis, a value somewhat higher than those reported in this study.

TABLE 7

PERCENT POLAR LIPID OF FREEZE-DRIED BEEF MUSCLE, LIVER, KIDNEY AND HEART POWDER 1/ 2/

Replication	Muscle	Liver	Kidney	Heart
I	6.87	12.22	10.75	4.76
II	3.46	7.16	3.73	3.84
Mean 3/	5.16^{a}	9.69^{b}	7.24^{ab}	4.30^{a}
Standard Error	4.21	3.01	4.78	1.88

1/ Percent polar lipid was estimated as the difference between percent total lipid and percent neutral lipid.

2/ Percent polar lipid was calculated on a dry-matter basis.

3/ Mean of 8 observations.

3/ Means within row followed by the same letter are not significantly different at the 0.05 level of probability.

TABLE 8

ANALYSES OF VARIANCE OF ASH CONTENT OF FREEZE DRIED BEEF MUSCLE, LIVER, KIDNEY AND HEART POWDER

Source	D.F.	Mean Squares
		Ash
Tissues	3	37.30**
Replications	1	5.85**
Residual Error	27	3.07

** $P<0.01$.

TABLE 9

PERCENT ASH OF FREEZE-DRIED BEEF MUSCLE, LIVER, KIDNEY AND HEART POWDER 1/

Replication	Muscle	Liver	Kidney	Heart
I	9.02	11.06	12.32	14.00
	8.07	10.84	12.58	13.56
	4.76	14.53	9.56	7.87
	4.65	13.50	9.75	6.69
II	10.50	15.47	8.17	8.88
	12.49	14.60	8.39	8.23
	12.86	15.96	7.79	9.98
	11.17	14.58	8.34	9.03
Mean 2/	9.20[a]	13.82[b]	9.61[a]	9.78[a]
Standard Error	3.20	1.91	1.88	2.65

1/ Percent ash was calculated on a dry-matter basis.

2/ Mean of 8 observations.

2/ Means within row followed by the same letter are not significantly different at the 0.05 level of probability.

II. FUNCTIONAL CHARACTERISTICS OF SAMPLES

Emulsion Capacity

The analysis of variance of emulsifying capacity and stability of beef muscle and tissue powders appears in Table 10. The emulsifying capacities of tissues were significantly different at the 0.01 level of probability. The differences between replications were not significantly different at the same level of probability.

The emulsifying capacity of the samples is compared in Table 11. According to Duncan's multiple range test, the means of muscle, liver and kidney were not significantly different ($P>0.05$), but numerically liver had the highest emulsion capacity. The emulsion capacity of heart was significantly lower than that of the other tissues.

Acton and Saffle (4) reported the emulsion capacity of various sausage meats (cow meat and beef heart) to be 53.2 and 49.6, respectively. There was no indication as to whether these values were on a dry or on a fresh-weight basis.

Emulsion Stability

The analysis of variance of emulsion stability of beef muscle and tissue powders is shown in Table 10. The differences between tissues were highly significantly different at the 0.05 level of probability. Replications also were significantly different at the same probability level.

The emulsion stability of beef muscle and tissue powders appears in Table 12. Duncan's multiple range test indicated significant

TABLE 10

ANALYSES OF VARIANCE OF EMULSION CAPACITY AND STABILITY OF FREEZE-DRIED BEEF MUSCLE, LIVER, KIDNEY AND HEART POWDER

Source	D.F.	Mean Squares	
		Emulsion Capacity	Emulsion Stability
Tissues	3	268.08**	3381.60**
Replications	1	35.91	129.97**
Residual Error	27	22.74	21.20

** $P<0.01$

TABLE 11

EMULSION CAPACITY OF FREEZE-DRIED BEEF MUSCLE, LIVER, KIDNEY AND HEART POWDER 1/ 2/

Replication	Muscle	Liver	Kidney	Heart
I	86.25	93.30	97.72	74.01
	94.50	99.00	88.35	81.13
	93.22	98.00	85.37	81.88
	86.71	99.51	99.28	73.25
II	93.87	95.46	98.17	75.36
	86.27	91.01	99.04	88.21
	89.63	94.85	92.73	90.80
	95.83	90.71	89.68	93.79
Mean 3/	90.79[a]	95.23[a]	93.79[a]	80.30[b]
Standard Error	4.03	3.43	5.49	7.92

1/ Emulsion capacity was expressed as ml of oil emulsified by 100 mg freeze-dried tissue powder.

2/ Emulsion capacity was calculated on a dry-matter basis.

3/ Mean of 8 observations.

3/ Means within row followed by the same letter are not significantly different at the 0.05 level of probability.

TABLE 12

EMULSION STABILITY OF FREEZE-DRIED BEEF MUSCLE, LIVER, KIDNEY AND HEART POWDER 1/

Replication	Muscle	Liver	Kidney	Heart
I	30.05	68.35	59.86	8.65
	33.71	49.42	54.04	7.75
	29.16	54.84	45.51	11.55
	25.51	57.34	44.07	8.86
II	29.68	55.53	45.30	10.12
	23.46	58.75	47.38	8.24
	24.79	48.17	38.61	11.94
	24.78	47.32	43.48	6.63
Mean 2/	27.64[a]	54.96[b]	47.28[c]	9.21[d]
Standard Error	3.53	6.91	6.66	1.85

1/ Emulsion stability was calculated on a dry-matter basis.

2/ Mean of 8 observations.

2/ Means within row followed by the same letter are not significantly different at the 0.05 level of probability.

differences among the means of all tissues. Findings with respect to emulsion stability were similar to those with respect to emulsion capacity, in that liver ranked high in both. Liver had an emulsion stability with 54.96 percent, followed by kidney (47.28 percent) and muscle (27.64 percent), while heart seemed to be less stable (9.21 percent).

III. CORRELATION BETWEEN VARIABLES AND EMULSION CAPACITY AND STABILITY

Emulsion Capacity and Stability as Related to Total Protein Content

All correlations presented are gross correlations. It would have been statistically more appropriate to consider correlations calculated on a within-tissue or within-replication basis in which tissue or replication effects proved to be significant. However, since the numbers of observations were small and to attain consistency with respect to all correlations presented, irrespective of significance of tissue or replication effects, only gross correlations were calculated.

The coefficients of correlation of emulsion capacity and stability with total protein, salt-soluble protein, total lipid, neutral lipid, polar lipid and ash are presented in the Appendix. The relationships of emulsion capacity and stability with other variables are shown graphically in Figures 4 through 15, where the means of muscle and tissue powder in two replcates and the grand means were plotted. Although the graphs indicated an interaction between tissues and replications, it was not

determined in analysis of variance because of the small number of observations. Furthermore, since this was a preliminary study, no such interaction was anticipated, nor planned for the experimental design. If further studies are conducted in this area, this should be investigated.

Relationship of emulsion capacity with total protein of freeze-dried beef muscle and tissue powders appears in Figure 4. The emulsifying capacity was inversely and apparently curvilinearly related ($r = -.63$). This negative relation was in agreement with the results reported by Ivey et al. (29). The presence of a negative correlation between emulsion capacity and total protein of fish muscle was found also by Childs (18).

Figure 5 shows the relationship of emulsion stability with total proteins of muscle and tissue powders. A negative correlation also was observed here ($r = -.78$). Because of this negative and obviously curvilinear relationship of emulsion stability with concentration of total protein, emulsion stability decreased when the concentration of total proteins increased.

Emulsion Capacity and Stability as Related to Salt-Soluble Protein Content

Figure 6 indicates a positive relationship between emulsion capacity and salt-soluble protein concentration, although it was not a strong linear relationship ($r = 0.56$). Results of Carpenter and Saffle (15) revealed that the emulsifying capacity was nearly linear with salt-soluble protein when its concentration was held constant. Satterlee

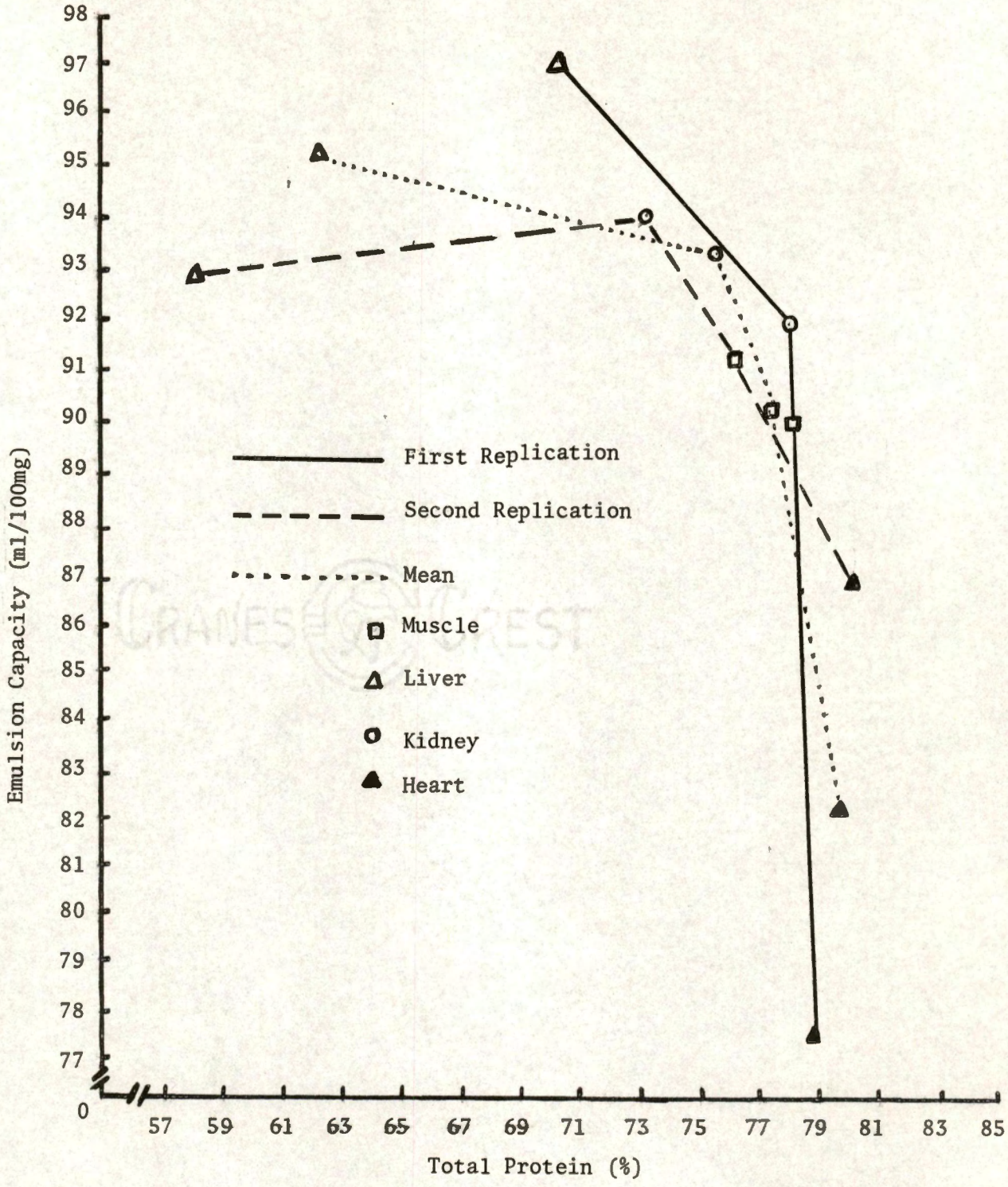

Figure 4. Relationship between emulsion capacity and total protein of freeze-dried beef muscle, liver, kidney and heart powder.

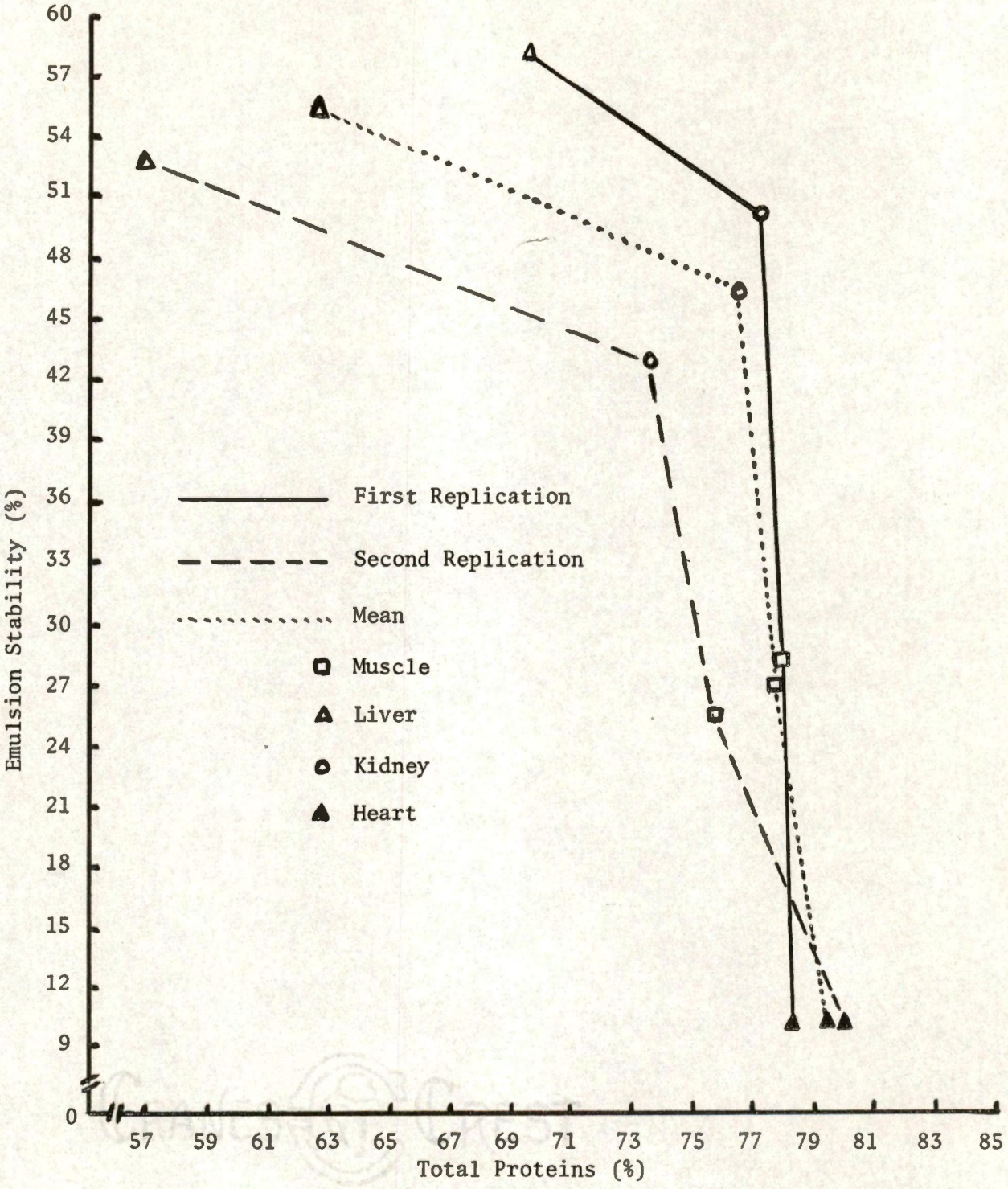

Figure 5. Relationship between emulsion stability and total protein of freeze-dried beef muscle, liver, kidney and heart powder.

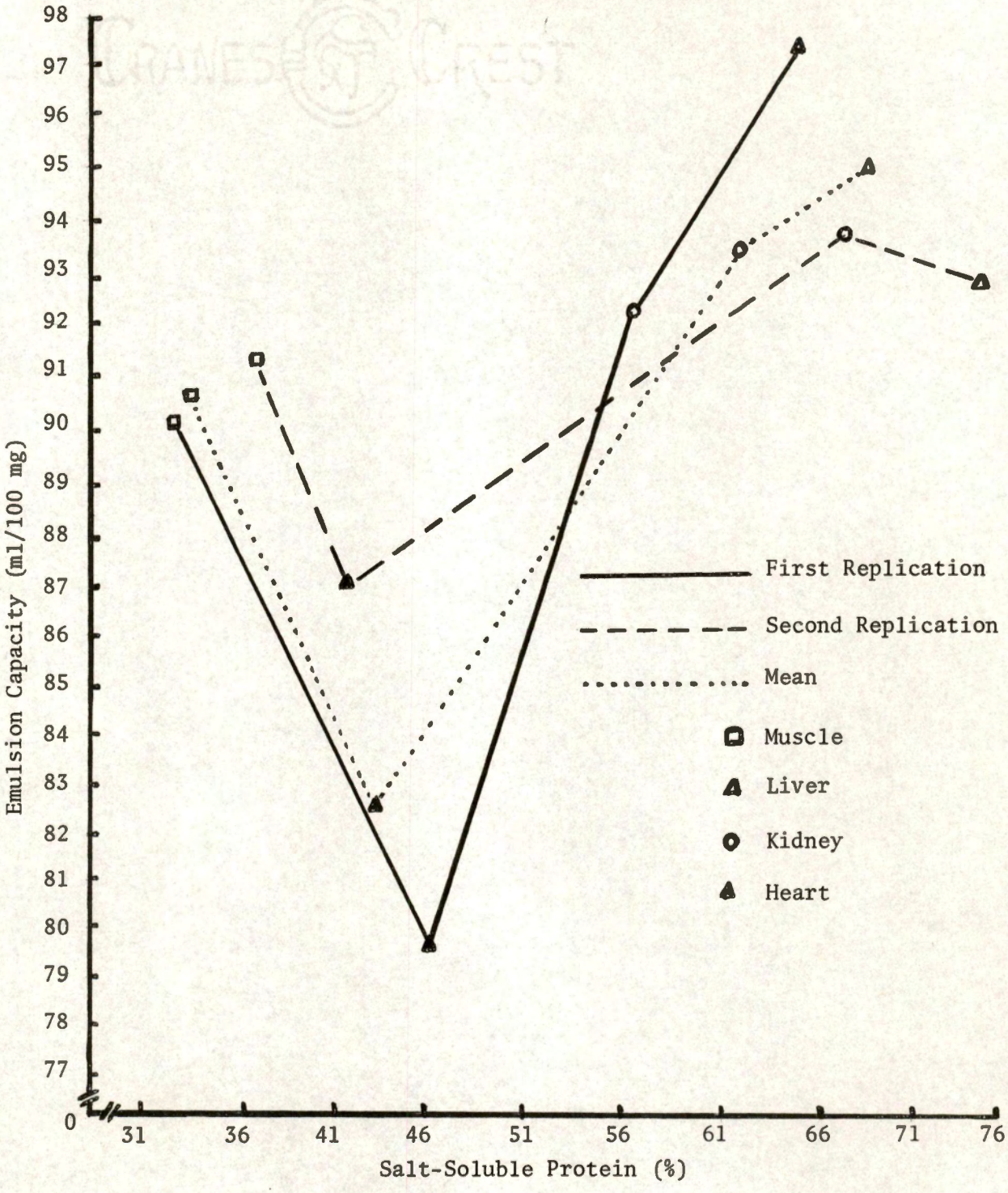

Figure 6. Relationship between emulsion capacity and salt-soluble protein of freeze-drief beef muscle, liver, kidney and heart powder.

et al. (51), however, reported a coefficient of correlation of 0.982 between ml of oil emulsified and amount of salt-soluble protein. There may be several plausible explanations for these variations in correlation coefficients. The denaturation of proteins which might occur during the freeze-drying process may have a great influence on emulsion capacity. Parkes (44) reported that the emulsion capacity of proteins became nonexistent at higher temperatures, although it was not affected at lower temperatures.

The ability of salt-soluble proteins to emulsify fat depends on the shape and net charges of protein molecules. Since the shape and net charges of salt-soluble proteins may vary in individual tissues (64), a variation in emulsifying capacity and, consequently, in correlation is expected.

A positive but stronger correlation ($r = 0.75$) than that with emulsion capacity was observed between emulsion stability and salt-soluble protein content (Figure 7).

Emulsion Capacity and Stability as Related to Total Lipid Content

Figure 8 shows the relationship of emulsion capacity with total lipid of tissue powders. A strong relationship with a coefficient of correlation of 0.99 indicated that total lipid content was an important factor in emulsion capacity. Saffle (49) reported a similar correlation coefficient ($r = 0.995$) between the amount of fat emulsified and emulsion capacity.

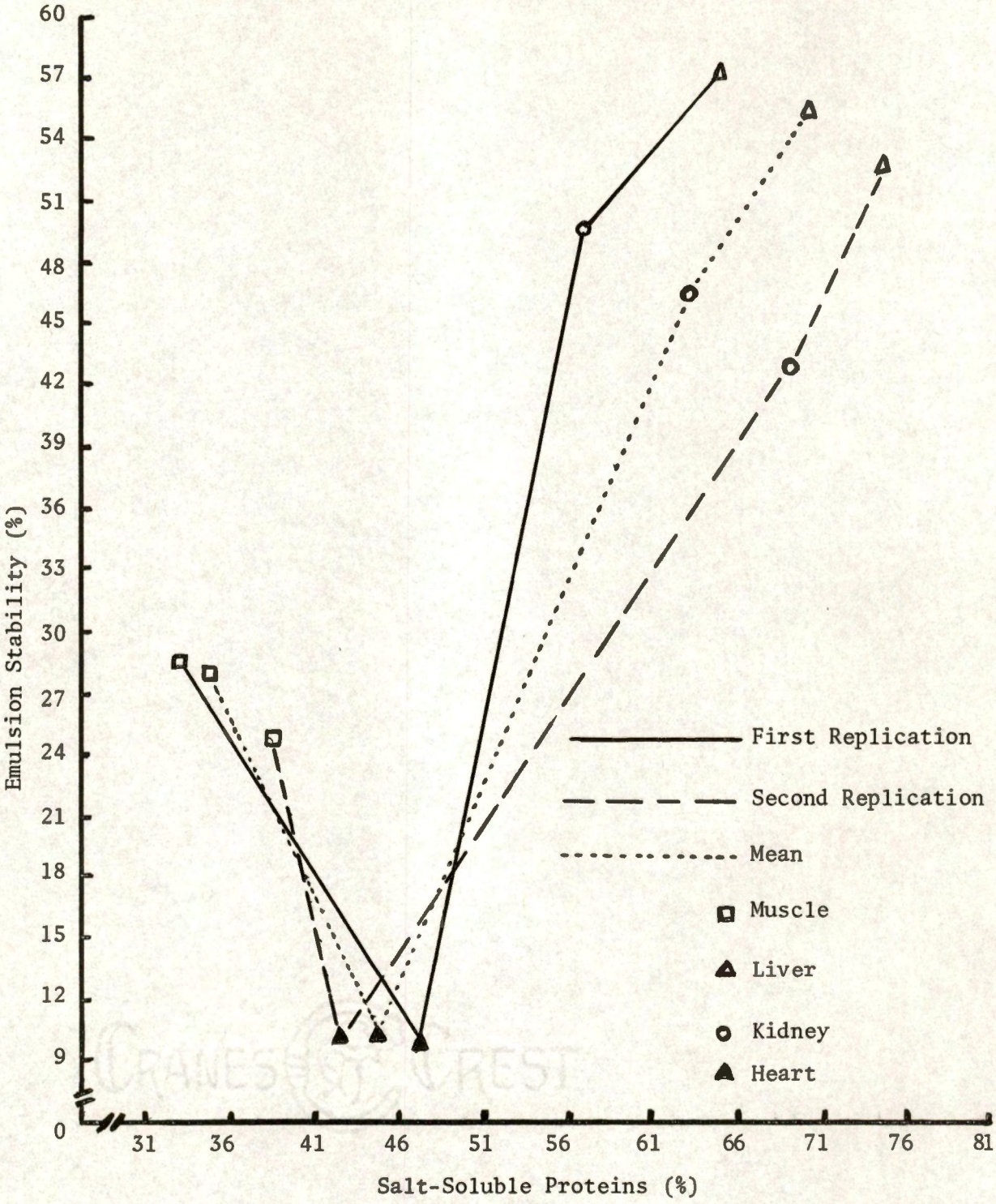

Figure 7. Relationship between emulsion stability and salt-soluble protein of freeze-dried beef muscle, liver, kidney and heart powder.

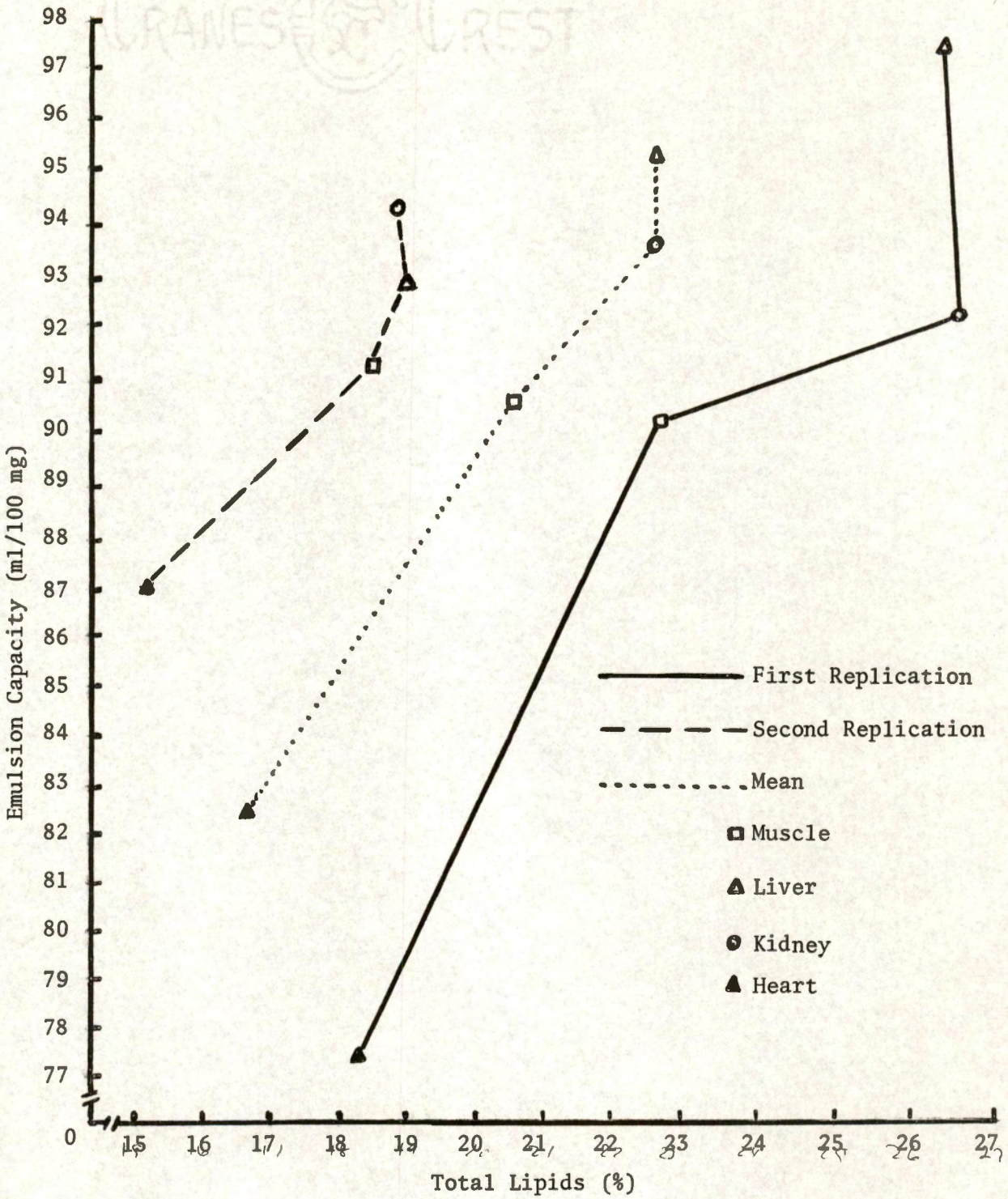

Figure 8. Relationship between emulsion capacity and total lipid of freeze-dried beef muscle, liver, kidney and heart powder.

Figure 9 also indicates the existence of a strong linear correlation between the emulsion stability and total lipid (r = 0.97).

Emulsion Capacity and Stability as Related to Neutral Lipids Content

The relationship of emulsion capacity with neutral lipid was weak (r = 0.55), as compared to that with total lipid (Figure 10). Figure 11 indicates that the concentration of neutral lipid had little effect on emulsion stability (r = 0.32).

The literature survey yielded no report of a correlation of neutral lipid with either emulsion capacity or stability for comparison with results of the present study. But, it is quite natural that neutral lipids (triglycerides), having no asymetric electronic charge, which are considered to be nonpolar lipids, could not have had high affinity with proteins (21) and, hence, would not show strong relationship with emulsion capacity and stability.

Emulsion Capacity and Stability as Related to Polar Lipid Content

The concentration of polar lipid appears to be quite important because of its high relationship with emulsion capacity and stability. Figures 12 and 13 indicate a strong linear relationship of polar lipid content with emulsion capacity and stability (r = 0.77 and r = 0.91, respectively). Carpenter and Saffle (16) pointed out the possible effects of polar lipid content on emulsion capacity, along with the saponification of free fatty acids.

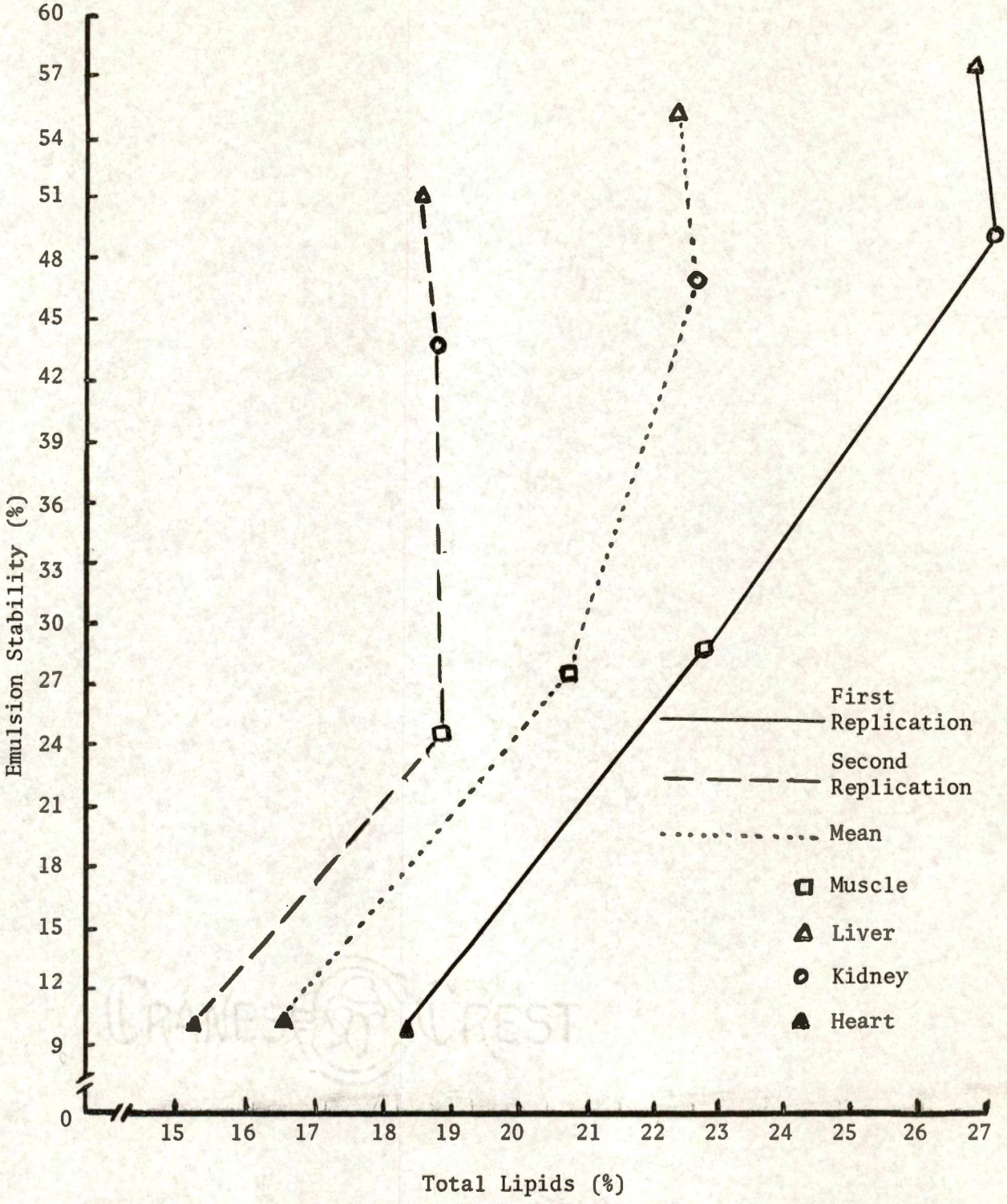

Figure 9. Relationship between emulsion stability and total lipid of freeze-dried beef muscle, liver, kidney and heart powder.

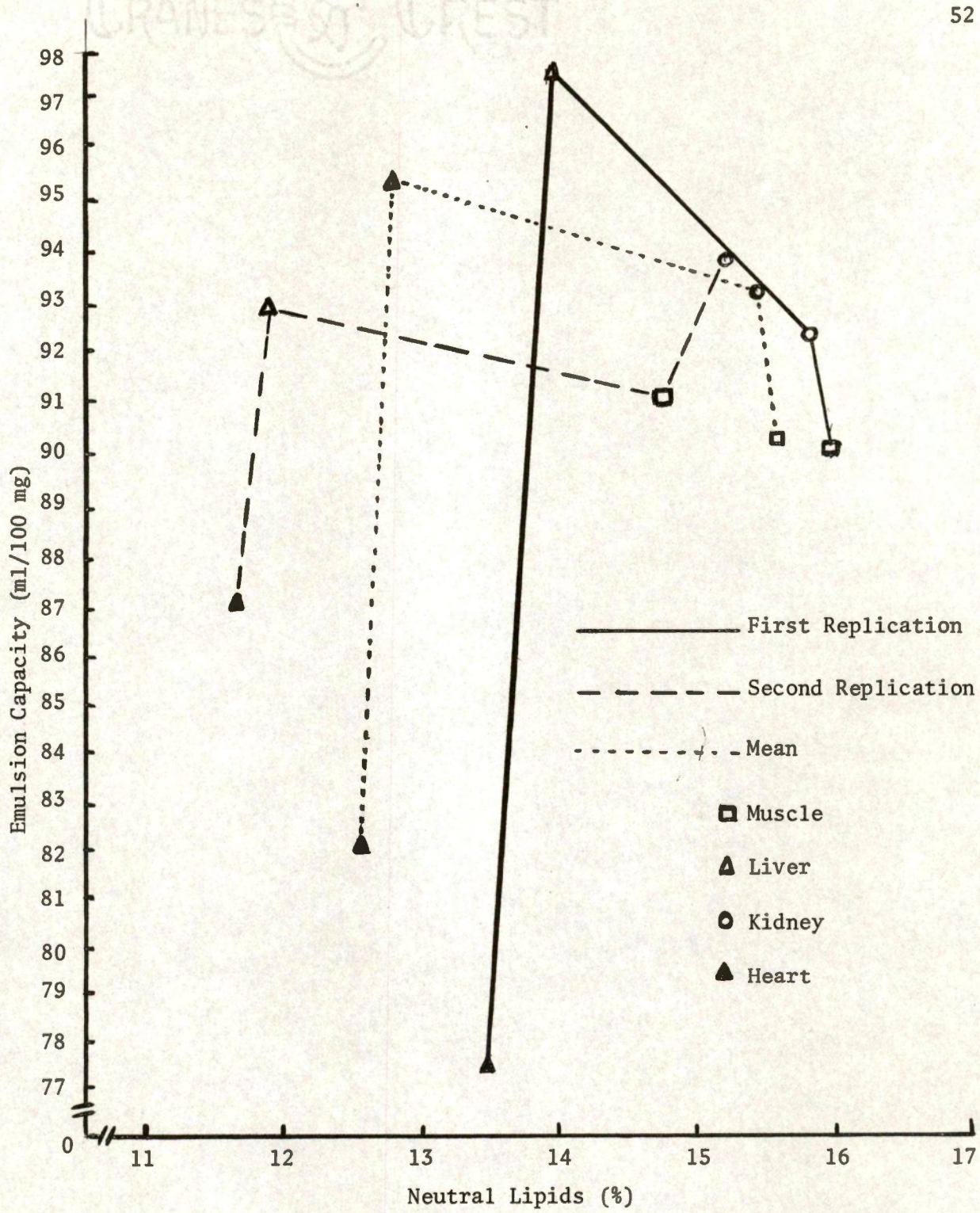

Figure 10. Relationship between emulsion capacity and neutral lipid of freeze-dried beef muscle, liver, kidney and heart powder.

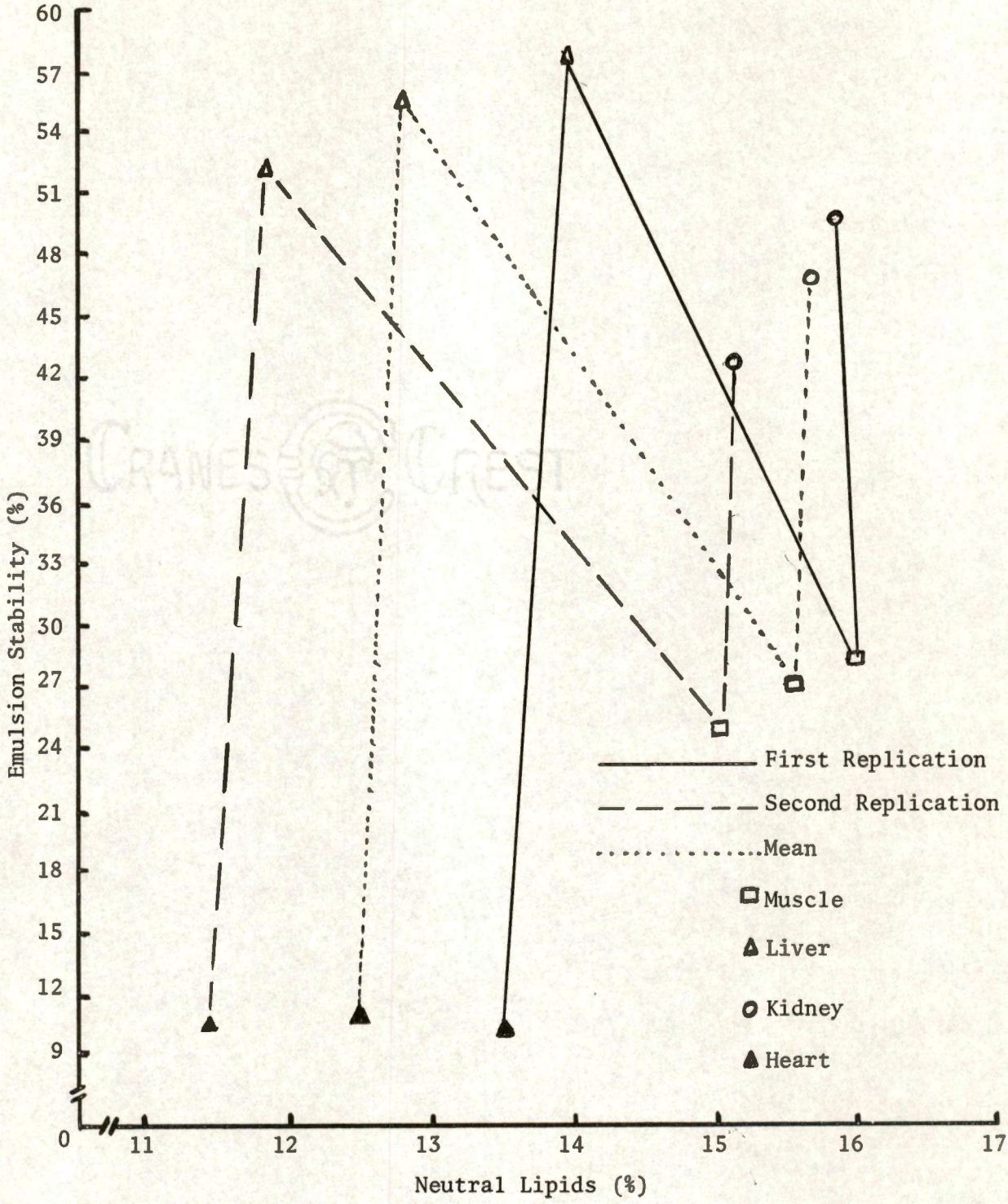

Figure 11. Relationship between emulsion stability and neutral lipid of freeze-dried beef muscle, liver, kidney and heart powder.

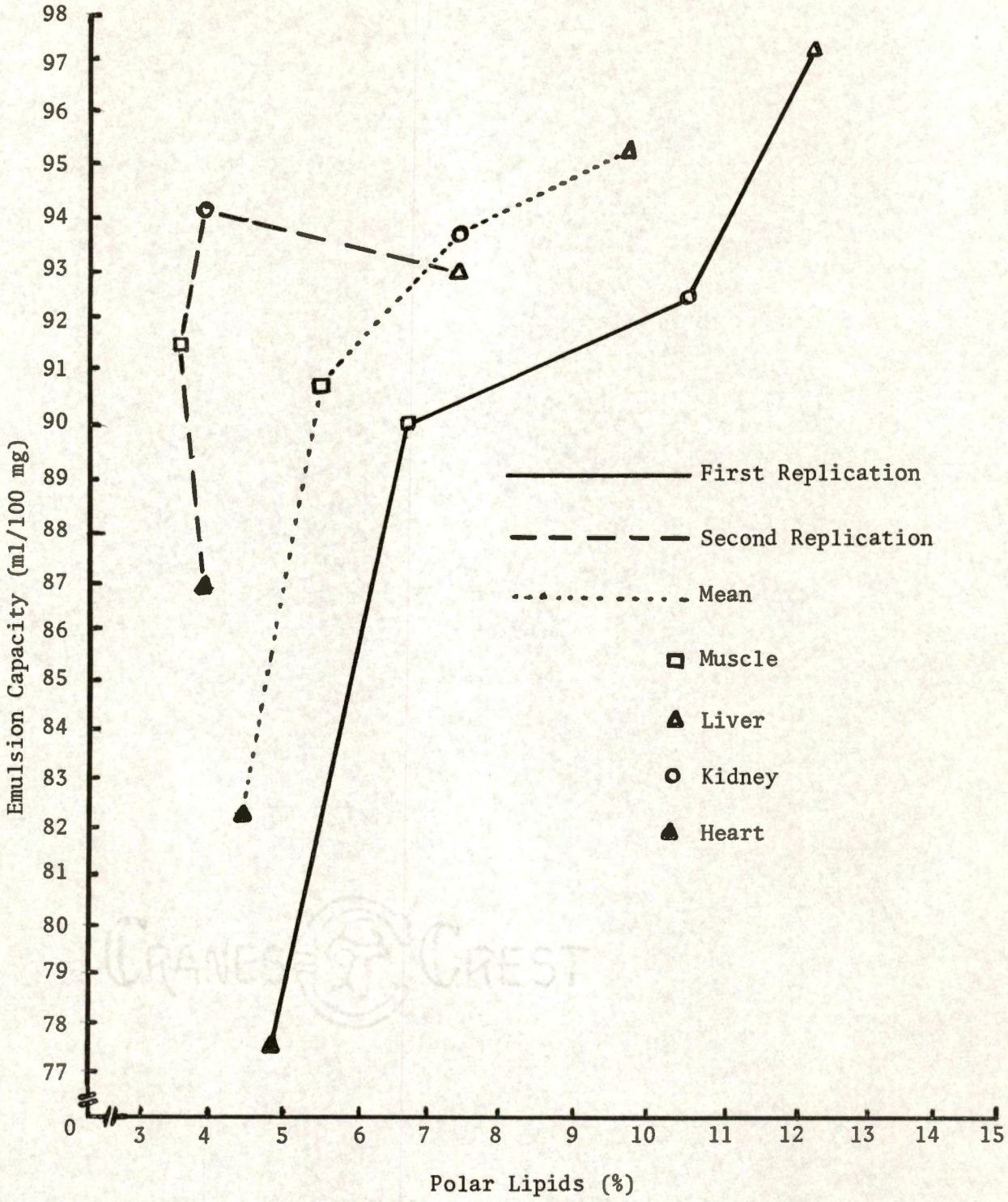

Figure 12. Relationship between emulsion capacity and polar lipid of freeze-dried beef muscle, liver, kidney and heart powder.

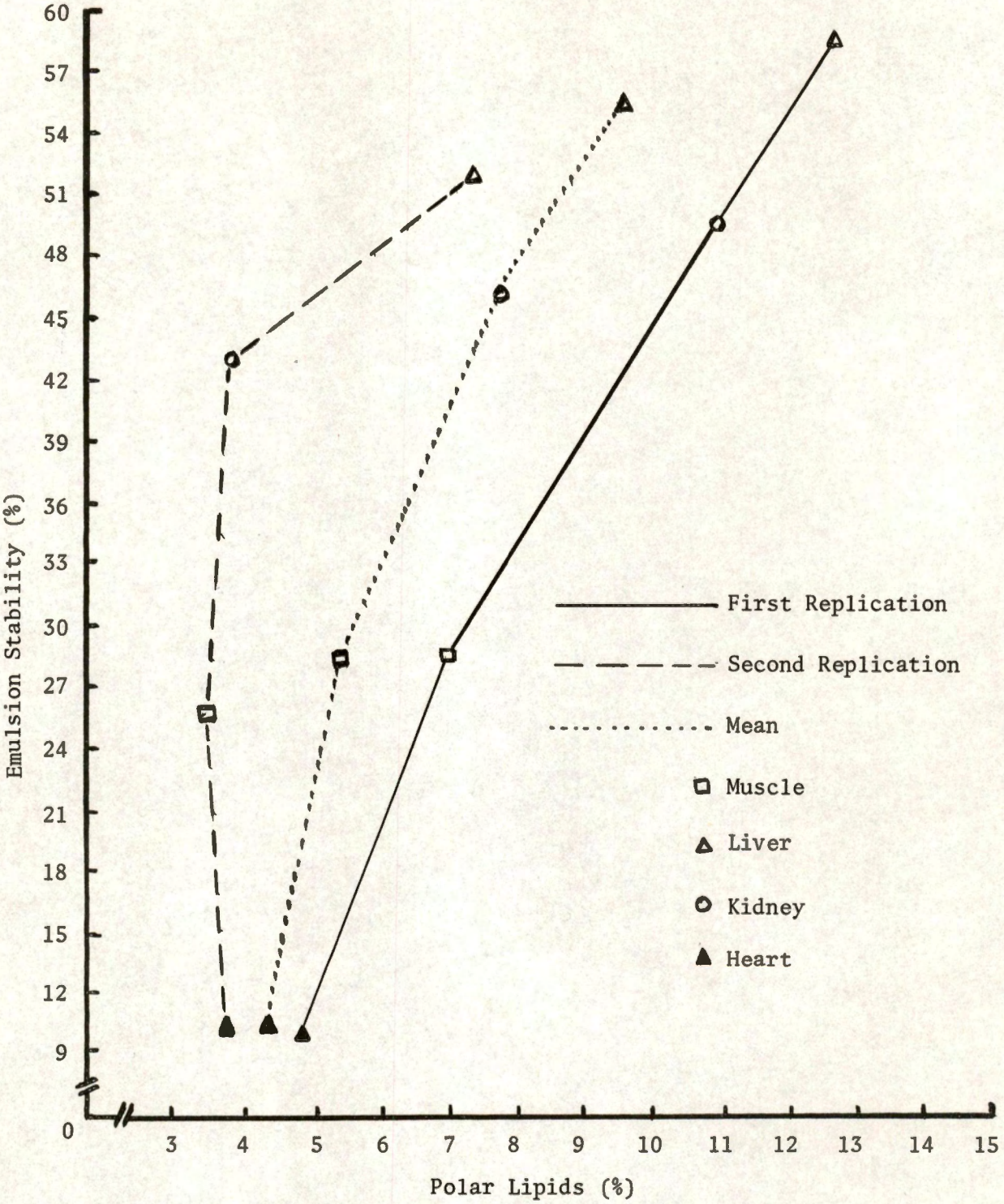

Figure 13. Relationship between emulsion stability and polar lipid of freeze-dried beef muscle, liver, kidney and heart powder.

Emulsion Capacity and Stability as Related to Ash Content

Figures 14 and 15 indicate only a slight relationship of ash content of beef muscle and tissue powders with emulsion capacity and stability ($r = 0.39$ and $r = 0.59$, respectively).

In this study it has been established that the amount of total and/or salt-soluble protein alone cannot be the sole factor determining emulsifying capacity and stability of muscle or meat by-product tissues. Lipids, specifically total lipids and polar lipids, emerged as being among the most important emulsifying agents in emulsifications. These results clearly justify continued research on the effects of phospholipids, sterols, fatty acids, bile acids especially in liver and molecular weights of various proteins on emulsion capacity and stability.

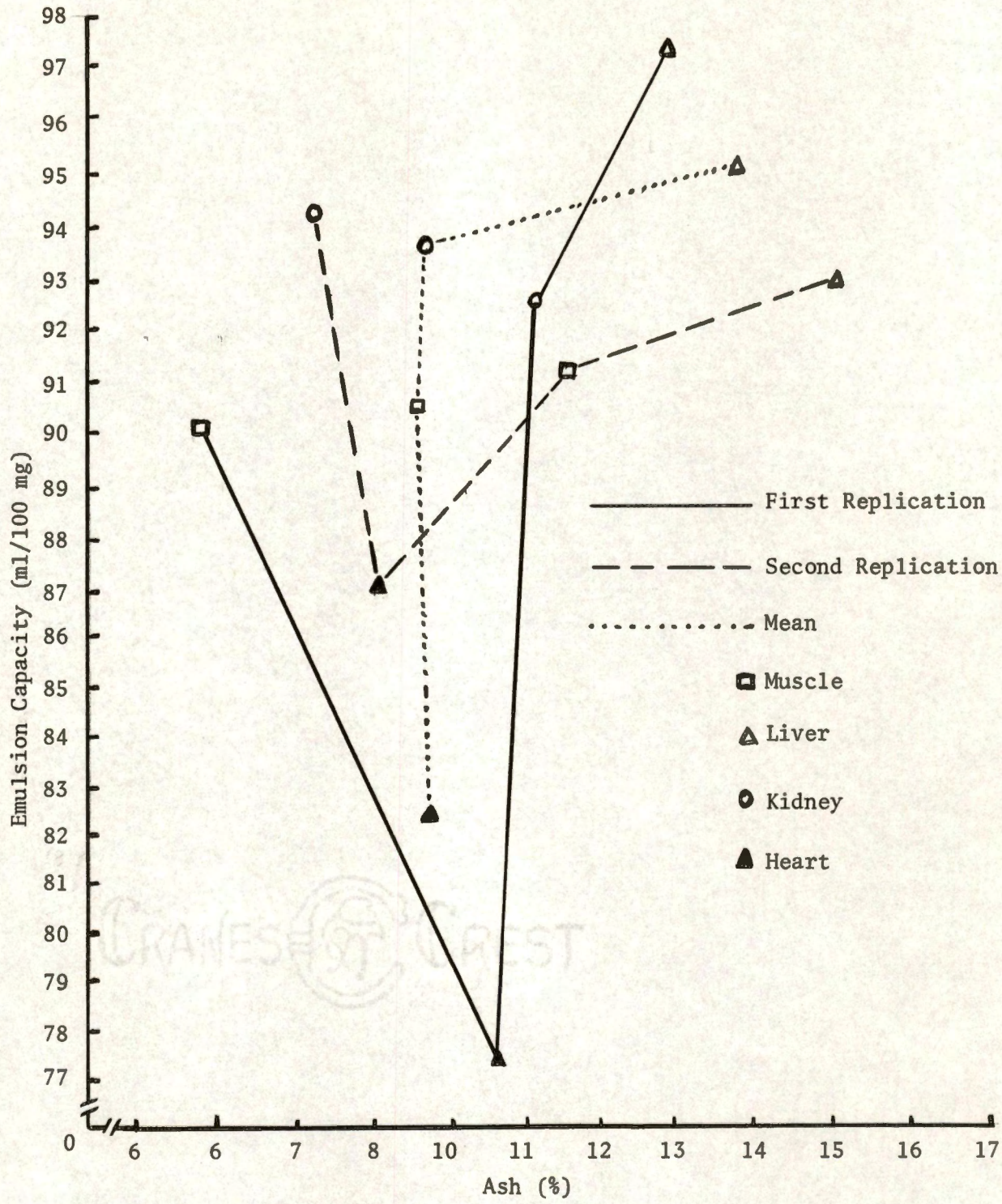

Figure 14. Relationship between emulsion capacity and ash of freeze-dried beef muscle, liver, kidney and heart powder.

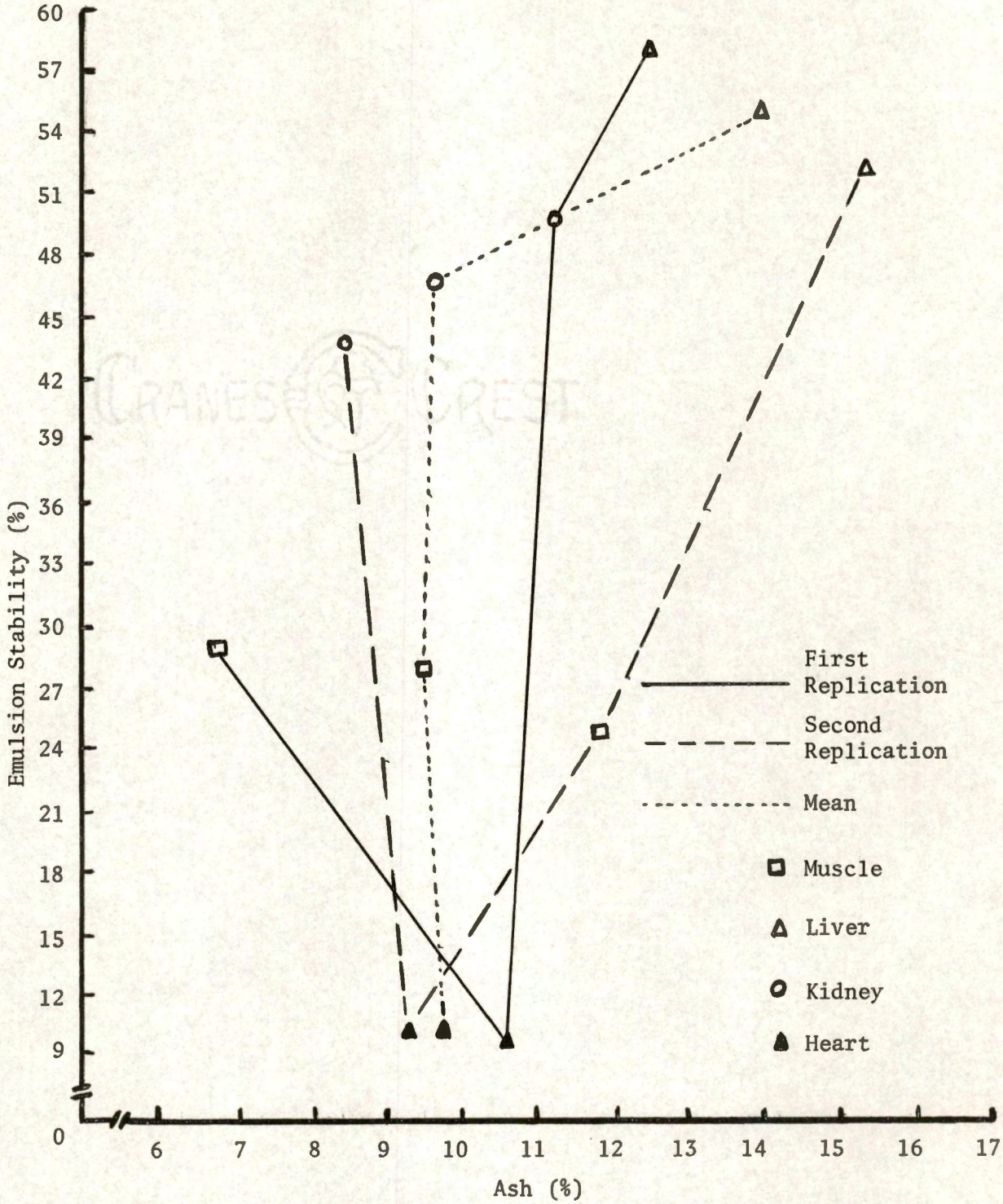

Figure 15. Relationship between emulsion stability and ash of freeze-dried beef muscle, liver, kidney and heart powder.

CHAPTER V

SUMMARY

The relationship of emulsifying capacity and emulsion stability with bovine tissue parameters (total protein, salt-soluble protein, total lipid, neutral lipid, polar lipid and ash) was studied. Beef muscle (Psoas major) and meat by-products (liver, kidney and heart) were chopped, freeze-dried, ground and screened through No. 10 and No. 20 mesh size sieves, respectively. The samples were analyzed for emulsion capacity and stability, and the tissue parameters were determined. Based on the results of this study, the following conclusions were made:

1. Of the four tissues, liver had the highest emulsion capacity, although there were no significant differences among the means of muscle, liver and kidney ($P<0.05$). Heart tissue had significantly lower emulsion capacity than the other tissue powders.

2. Liver possessed the highest emulsion stability, followed by kidney and muscle. Emulsions of heart tissue were the least stable.

3. A negative nonlinear relation was established graphically between total protein and emulsion capacity and stability ($r = -.63$ and $r = -.78$, respectively). As the concentration of protein increased, emulsion capacity and stability decreased.

4. In liver, salt-soluble protein constituted the greatest proportion of total protein, followed by heart and kidney. Regardless

of the amount of salt-soluble protein, a relatively weak relation was observed with emulsifying capacity ($r = 0.56$). However, a stronger relation was found between emulsion stability and salt-soluble protein ($r = 0.75$).

5. Total lipid content appeared to be one of the factors with most influence on emulsion capacity and stability. Strong linear correlations were observed ($r = 0.99$ and $r = 0.97$, respectively). By its biological nature, liver tissue contained the highest amount of total lipids, which was assumed to be directly responsible for its higher emulsion capacity and stability.

6. Neutral lipid content did not seem to exert any effect on either emulsion capacity or stability and exhibited weak relations with them ($r = 0.55$ and $r = 0.32$, respectively).

7. Along with total lipid content, polar lipid content also seemed to be an important factor in emulsion capacity and stability. Polar lipid content exhibited a moderate correlation with emulsion capacity ($r = 0.77$) but was highly correlated with emulsion stability ($r = 0.91$).

8. Ash content of muscle and tissue powders did not exhibit any strong correlation with either emulsion capacity or stability ($r = 0.39$ and $r = 0.59$, respectively).

LITERATURE CITED

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1. Acton, J. C., and R. L. Saffle. 1970. Stability of oil-in-water emulsions. I. Effects of surface tension, level of oil, viscosity and type of meat protein. J. Food Sci., 35:852.

2. Acton, J. C., and R. L. Saffle. 1971. Stability of oil-in-water emulsions. II. Effects of oil phase volume, stability test, viscosity, type of oil and protein additive. J. Food Sci., 36:1118.

3. Acton, J. C., and R. L. Saffle. 1972a. Film observations at an oil-water interface. J. Food Sci., 37:795.

4. Acton, J. C., and R. L. Saffle, 1972b. Emulsifying capacity of muscle protein: phase volumes at emulsion collapse. J. Food Sci., 37:904.

5. American Meat Institute Committee on Textbooks. 1953. In "Sausage and Ready-to-Serve Meats," pp. 55-56 and 78-99. University of Chicago Press, Chicago.

6. A.O.A.C. 1965. "Official Methods of Analysis," 10th ed. Association of Official Chemists, Washington, D. C.

7. Bate-Smith, E. C. 1935. The proteins of meat. J. Soc. Chem. Ind., 54:152.

8. Becher, P. 1965. "Emulsions: Theory and Practice," 2nd ed. Reinhold Publishing Corp., New York.

9. Bennett, H., J. L. Bishop, Jr., and M. F. Wulfinghoff. 1968. "Practical Emulsions," Vol. I. Chemical Publishing Company, Inc., New York.

10. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37:911.

11. Bloor, W. R., and R. H. Snider. 1930. The neutral fat of beef liver and other tissues. J. Biol. Chem., 87:399.

12. Borton, R. J., N. B. Webb, and L. J. Bratzler. 1968. Emulsifying capacities and emulsion stability of dilute slurries from various meat trimmings. Food Technol., 21:162.

13. Brennan, J. G., J. R. Butters, N. D. Cowell, and A. E. V. Lilly. 1968. "Food Engineering Operations," pp. 81-98. Elsevier, London.

14. Cantarow, A., and B. Schepartz. 1967. "Biochemistry," 4th ed. W. B. Saunders Company, Philadelphia.

15. Carpenter, J. A., and R. L. Saffle. 1964. A simple method of estimating the emulsifying capacity of various sausage meats. J. Food Sci., 29:774.

16. Carpenter, J. A., and R. L. Saffle. 1965. Some physical and chemical factors affecting the emulsifying capacity of meat protein extracts. Food Technol., 19:1567.

17. Chapman, D. 1969. Physical studies of lipid-lipid and lipid-protein interactions. Lipids, 4(4):251.

18. Childs, E. A. 1973. Functionality of fish muscle: Emulsification capacity. Tech. Paper No. 3654. Oregon State University, Agri. Experiment Station.

19. Clayton, W. 1954. "The Theory of Emulsions and Their Technical Treatment," Blakiston Co., New York.

20. Crenwelge, D. D., C. W. Dill, P. T. Tybor, and W. A. Landsmann. 1974. A comparison of the emulsification capacities of some protein concentrates. J. Food Sci., 39:175.

21. Fullington, J. G. 1969. Lipid-protein interaction. Bakers Digest, 43(6):34.

22. Galanos, D. S., and V. M. Kapoulas. 1962. Isolation of polar lipids from triglyceride mixtures. J. Lipid Res., 3:134.

23. Hanahan, D. J., and G. A. Thompson, Jr. 1963. Complex lipids. Ann. Rev. Biochem., 32:215.

24. Hansen, L. J. 1960. Emulsion formation in finely comminuted sausage. Food Technol., 14:565.

25. Haq, A., N. B. Webb, J. K. Whitfield, A. J. Howell, and B. C. Barbour. 1973. Measurement of sausage emulsion stability by electrical resistance. J. Food Sci., 38:1224.

26. Hegarty, G. R., L. J. Bratzler, and A. M. Pearson. 1963. Studies on the emulsifying properties of some intracellular beef muscle proteins. J. Food Sci., 28:663.

27. Hornstein, I., P. F. Crowe, and M. J. Heimberg. 1961. Fatty acid composition of meat tissue lipids. J. Food Sci., 26:581.

28. Inklaar, P. A., and J. Fortuin. 1969. Determining the emulsifying and emulsion stabilizing capacity of protein meat additives. Food Technol., 23:103.

29. Ivey, F. J., N. B. Webb, and V. A. Jones. 1970. The effect of disperse phase droplet size and interfacial film thickness on emulsifying capacity and stability of meat emulsions. Food Technol., 24:1279.

30. Jirgensons, B., and M. E. Straumanis. 1954. "Colloid Chemistry," John Wiley and Sons, Inc., New York.

31. Karmas, E. 1972. "Sausage Processing," Noyes Data Corp., New Jersey.

32. Korschgen, B. M., and R. E. Baldwin. 1973. Fish protein concentrate as an emulsifier. J. Food Sci., 38:179.

33. Kuchmak, M., and L. R. Dugan, Jr. 1963. Phospholipids of pork muscle tissues. J. Am. Oil Chemist's Soc., 40:734.

34. Lea, C. H. 1962. Adipose tissue and animal lipids. In "Recent Advances in Food Science," Vol. I, eds. J. Hawthorn, and J. M. Leitch, Butterworth, London.

35. Levin, E. 1970a. MPC + edible fat. Food Technol., 24:19.

36. Levin, E. 1970b. Conversion of meat by-products into edible meat protein concentrate powder. Proceedings of the meat industry research conference, p. 29. American Meat Institute Foundation.

37. Levin, E. 1971. Private communication. VioBin Corporation, Monticello, Illinois.

38. Levin, E. 1973. Meat flour-new protein food. Canner/Packer, 138:50.

39. Lu, J., and W. E. Townsend. 1973. Feasibility of adding freeze-dried meat in the preparation of fermented dry sausage. J. Food Sci., 38:837.

40. MacKenzie, D. S. 1966. "Prepared Meat Product Manufacturing," AMI Center for Continuing Education, American Meat Institute.

41. Olson, F. C. 1970. Nutritional aspects of offal proteins. Proceedings of the meat industry research conference, p. 23. American Meat Institute Foundation.

42. Ostrander, J., and L. R. Dugan, Jr. 1961. A rapid quantitative lipid extraction method. American Meat Institute Foundation Bull. No. 50.

43. Pals, C. H. 1970. The practical aspects of blood component procurement. Proceedings of the meat industry research conference, p. 17. American Meat Institute Foundation.

44. Parkes, M. R. 1967. Retention of emulsifying capacity of salt-soluble protein during freezing, freeze-drying and other preservation techniques. M. S. Thesis. University of Georgia Library, Athens, Ga.

45. Porter, J. W. G., and B. A. Rolls. 1973. "Proteins in Human Nutrition," Academic Press, New York.

46. Price, J. F., and B. S. Schweigert. 1971. "The Science of Meat and Meat Products," 2nd ed. W. H. Freeman Co., San Francisco.

47. Saffle, R. L. 1964a. II. An objective method of determining emulsification value and color of various sausage meats. "IBM Meat Packing Symposium," pp. 67-87. White Plains, New York.

48. Saffle, R. L., and J. W. Galbreath. 1964b. Quantitative determination of salt-soluble protein in various types of meat. Food Technol., 18:119.

49. Saffle, R. L. 1968. Meat emulsions. In "Advances in Food Research," pp. 105-160. Academic Press, New York.

50. Satterlee, L. D., N. Y. Zachariah, and E. Levin. 1973a. Utilization of beef and pork skin hydrolyzates as binder/extenders in sausage emulsions. J. Food Sci., 38:268.

51. Satterlee, L. D., B. Free, and E. Levin. 1973b. Utilization of high protein tissue powders as a binder/extender in meat emulsions. J. Food Sci., 38:306.

52. Schwartz, A. M., and J. W. Perry. 1949. "Surface Active Agents." Interscience Publishers, Inc., New York.

53. Scopes, P. K. 1970. Characterization and study of sarcoplasmic proteins. In "The Physiology and Biochemistry of Muscle as a Food," eds. E. J. Briskey, R. G. Cassens, and B. B. Marsh. pp. 471. The University of Wisconsin Press, Madison, Wisconsin.

54. Steel, R. G., and J. H. Torrie. 1960. "Principles and Procedures of Statistics," pp. 107-109. McGraw-Hill Book Company, New York.

55. Sulzbacher, W. L. 1973. Meat emulsions. J. Sci. Food Agri., 24: 589.

56. Swift, C. E., C. Lockett, and A. J. Fryar. 1961. Comminuted meat emulsions: The capacity of meats for emulsifying fats. Food Technol., 15:468.

57. Swift, C. E., and W. L. Sulzbacher. 1963. Comminuted meat emulsions: Factors affecting meat proteins as emulsion stabilizers. Food Technol., 17:106.

58. Swift, C. E. 1965. The emulsifying properties of meat protein. In "Proc. Meat Ind. Research Conf." eds. (O. E. Kolari and W. J. Aunan) pp. 78-93. Am. Meat Sci. Assoc. and Am. Meat Inst. Foundation, Chicago, Illinois.

59. Taylor, J. F. 1953. The isolation of proteins. In "The Proteins," eds. (H. Neurath and K. Bailey) Vol. 1, Part A, 40. Academic Press, New York.

60. Townsend, W. E., L. P. Witnauer, J. A. Riloff, and C. E. Swift. 1968. Comminuted meat emulsions: Differential thermal analysis of fat transitions. Food Technol., 22:319.

61. Trautman, J. C. 1964. Fat-emulsifying properties of pre-rigor and post-rigor pork proteins. Food Technol., 18:1065.

62. Trautman, J. C. 1966. Role of muscle protein in processed foods. In "The Physiology and Biochemistry of Muscle as a Food," eds. E. J. Briskey, R. G. Cassens, and J. C. Trautman, pp. 403-418. The University of Wisconsin Press, Madison, Wisconsin.

63. Tsai, R. R., G. Cassens, and E. J. Briskey. 1972. The emulsifying properties of purified muscle proteins. J. Food Sci., 37:286.

64. Webb, N. B., F. J. Ivey, H. B. Craig, V. A. Jones, and R. J. Monroe. 1970. The measurement of emulsifying capacity by electrical resistance. J. Food Sci., 35:501.

65. Wierbicki, E., E. Kunkle, V. R. Cahill, and F. E. Deatherage. 1956. Post mortem changes in meat and ther possible relation to tenderness together with some comparison of meat from heifers, bulls, steers, di-ethylstilbestrol treated bulls and steers. Food Technol., 10:80.

66. Ziegler, T. 1966. "The Meat We Eat." The Interstate Printers and Publishers, Inc., Danville, Illinois.

APPENDIX

TABLE A-1

COEFFICIENTS OF CORRELATION OF EMULSION CAPACITY WITH TOTAL PROTEIN, SALT-SOLUBLE PROTEIN, TOTAL LIPID, NEUTRAL LIPID, POLAR LIPID AND ASH CONTENT OF FREEZE-DRIED BEEF TISSUES

Replication	Total Protein	Salt-Soluble Protein	Total Lipid	Neutral Lipid	Polar Lipid	Ash
I	- 0.60	0.37	0.96	0.55	0.86	0.13
II	- 0.56	0.70	0.96	0.60	0.30	0.24
Mean 1/	- 0.63	0.56	0.99	0.55	0.77	0.39

1/ Mean of 8 observations.

TABLE A-2

COEFFICIENTS OF CORRELATION OF EMULSION STABILITY WITH TOTAL PROTEIN, SALT-SOLUBLE PROTEIN, TOTAL LIPID, NEUTRAL LIPID, POLAR LIPID AND ASH CONTENT OF FREEZE-DRIED BEEF TISSUES

Replication	Total Protein	Salt-Soluble Protein	Total Lipid	Neutral Lipid	Polar Lipid	Ash
I	- 0.69	0.53	0.98	0.42	0.94	0.30
II	- 0.84	0.89	0.89	0.22	0.66	0.50
Mean 1/	- 0.78	0.75	0.97	0.32	0.91	0.59

1/ Mean of 8 observations.

VITA

The author was born in Aksaray, Turkey, on December 2, 1941. He attended The University of Ankara from 1961 to 1966, and was graduated with a Bachelor of Science degree in Medecine Veterinary. He served for six months as a veterinarian in a state institution.

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