FRESH AND FROZEN EGG YOLK PROTEIN FRACTIONS: EMULSION STABILIZING POWER, VISCOSITY, AND ELECTROPHORETIC PATTERNS

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ABSTRACT

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by Elizabeth Miller Davey

The primary objective of this investigation was to identify the emulsifying properties of three crude egg yolk protein fractions: lipovitellin, lipovitellenin, and livetin. Of secondary importance, was a study of the effects of freezing and thawing upon the emulsifying properties of egg yolk and all combinations of the three fractions.

Test emulsions were prepared by emulsifying oil in water using combinations of the three fractions in amounts proportional to those normally found in the egg yolk, as well as using native yolk. Emulsion drainage read at thirty-minute intervals for two hours provided the test of emulsion stability. Similar emulsions, prepared with frozen egg yolk and frozen fractions were evaluated in the same manner. The viscosity and electrophoretic mobility of both fresh and frozen egg yolk and

fractions were measured by standard procedures, and all data reported are the average of six replications.

A comparison of the emulsion stabilizing powers of native yolk and recombined fractions showed that, although total drainage was small in both cases, emulsions prepared with native yolk were more stable than those prepared with the recombined fractions after thirty minutes. Emulsions prepared with fresh yolk and recombined fresh fractions drained less than those prepared with frozen yolk and recombined frozen fractions, but this difference was significant only for the sixty minute reading.

Lipovitellin, lipovitellenin, and livetin each functioned to reduce emulsion drainage significantly, with lipovitellenin providing the best overall emulsion stabilization. Highly significant interactions, which occurred when any two of the three fractions were present, indicated that while combinations of two fractions did promote greater stability than that of either fraction alone, the stability of the emulsions was less than would be expected from independent action of the fractions *per se*. Examination of the rates of drainage suggested that lipovitellenin effectively reduced initial drainage, but increased subsequent drainage. Livetin and lipovitellin decreased initial drainage somewhat, and interacted with lipovitellenin to reduce subsequent drainage. The best emulsion stability

was observed when all three fractions were present producing an effect which equaled independent action of the fractions *per se*.

Determinations of relative viscosity, before and after lipovitellin, lipovitellenin, livetin, and native yolk were frozen and thawed, showed that freezing and thawing greatly increased the mean relative viscosity of lipovitellin, lipovitellenin, and native yolk, while the relative viscosity of livetin was essentially unchanged. The change in the mean relative viscosity of frozen lipovitellin indicated that protein denaturation had occurred, but paper electrophoresis was unable to detect any change in the mobility of the frozen lipovitellin protein. Lipovitellenin, however, produced electrophoretic patterns which indicated that a majority of its proteins were changed by freezing and thawing in such a manner that they became non-mobile after freezing.

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INTRODUCTION

The protein and lipoprotein complexes which are present in the egg yolk are responsible for its action as an excellent emulsifier in many food emulsions (Snell et al., 1935). Vincent et al. (1966) found that the livetin and lipoprotein fractions of the egg yolk contribute to its low surface energy which in turn is necessary for emulsion formation but may not influence the emulsion stability.

The use of frozen egg yolk increases efficiency in commercial emulsion production. However, the utility of freezing pure egg yolk is limited by the irreversible reaction which egg yolk undergoes as a result of freezing and thawing, resulting in a product of greatly increased viscosity which is difficult to combine with other ingredients. Although methods and additives reduce this reaction, its mechanism is unknown, and use of treated yolks is limited to products in which the treatment itself does not have deleterious effect.

There is no agreement on the influence of freezing and thawing egg yolk to be used in an emulsifying capacity. Miller and Winter (1951) found that frozen

yolk was a more efficient emulsifier in mayonnaise than fresh yolk, while Kilgore (1935) suggested that more frozen yolk than fresh yolk was required to produce a mayonnaise of acceptable viscosity. No recent studies of the emulsifying properties of frozen egg yolk have been conducted.

Although the pure proteins and lipoproteins of the egg yolk have not been characterized, the fractions which are easily separated by physical means deserve further study. Studies of egg yolk protein fractions have neither dealt with the functional properties of the fractions nor the effect of freezing upon these fractions and their properties. Knowledge of the functions of specific egg yolk fractions could provide a means of improving the quality of egg yolk products.

The present investigation was primarily concerned with determining the emulsifying properties of egg yolk and three crude egg yolk protein fractions: lipovitellin, lipovitellenin, and livetin. A secondary objective was to study the effects of freezing and thawing upon the emulsifying properties of the egg yolk and the three yolk fractions. Measurements of viscosity and electrophoretic mobility of the native yolk and fractions before and after freezing were conducted to determine the effect of the gelation reaction upon these variables.

REVIEW OF LITERATURE

The egg yolk was the subject of extensive research before study of the egg white was attempted. Much of this early work, however, was invalidated or supplemented by later studies employing modern equipment and techniques. As research continued the egg yolk was found to be more complex than formerly indicated.

This review summarizes literature on egg yolk proteins--their composition and emulsifying properties,--and the effects of freezing egg yolk. Objective tests which measure viscosity and electrophoretic protein mobility are also discussed.

Composition of Egg Yolk

Egg yolk is composed primarily of fat, protein and moisture (Table 1). Minor components include carbohydrate and ash (Ziemba, 1955; Sweetman and McKellar, 1959; Watt and Merrill, 1963). The ash of the egg yolk is composed of phosphorus, calcium, magnesium, chloride, potassium, sodium, sulfur, and iron (Romanoff and Romanoff, 1949).

TABLE 1. Approximate composition of egg yolk

Component	Percentage
Water	49.4 - 51.1
Lipid	30.6 - 31.9
Protein	16.0 - 16.7
Carbohydrate	0.2
Ash	1.0 - 1.7

Examination of the egg yolk reveals a unique physical structure. It is composed of alternating layers of white and yellow yolk globules (Table 2), white yolk constituting only three to four per cent of the total yolk material. Since separation of yellow and white yolk is extremely difficult, it is seldom attempted in egg yolk studies.

TABLE 2. Composition and properties of white and yellow yolk (Romanoff and Romanoff, 1949)

	Component			Granule Diameter
	Water	Lipid	Protein	
	%	%	%	mm
Yellow Yolk	45.4	36.4	15.0	0.015 - 0.025
White Yolk	86.0	3.5	4.6	0.004 - 0.075

Water

The amount of water in the egg yolk is influenced after laying by two factors. Water is lost through the shell of the egg. Passage of water from the white to the yolk occurs as the white becomes thinner due to loss of carbon dioxide (Triebold and Aurand, 1963).

Water exists in food products in three forms: free, bound, and mechanically occluded. All three forms occur in egg yolk. Romanoff and Romanoff (1949) stated that one-quarter of the water in the egg yolk exists in the bound form and does not freeze at -35°C. Lea and Hawke (1952) indicated that water is a structural component of the egg yolk.

Carbohydrate

Levene and Mori (1929) studied the carbohydrate of the egg yolk. They found 34.8 per cent of the sugar present as glucose. Levene and Rothen (1929), in a study of egg yolk polysaccharides, found four trisaccharides each with an approximate molecular weight of 500 on the basis of diffusion coefficients. They hypothesized a structure consisting of one molecule of glucosamine and two molecules of mannose, which could form four different combinations. These trisaccharides are associated with yolk proteins in an undisclosed manner.

Lipoprotein and protein

The protein, lipid and phosphorus of the egg yolk occur in complexes which presently defy elucidation, although theories of molecular orientation are prevalent. Much of the protein of the egg yolk occurs as lipoprotein. Lipoproteins are water soluble proteins conjugated with lecithin, cholesterol, cephalin, neutral lipid, and other similar compounds. The proteolipids are distinguished from the lipoproteins by the solubility of the former in organic solvents instead of water (White et al., 1959). Omission of the lipo- prefix from the name of a lipoprotein indicates that the lipid has been removed.

Early studies.--In 1842, Dumas and Cahours discovered a protein in egg yolk which they named vitellin, and for which they proposed a simple formula. Osborne and Campbell (1900) precipitated egg yolk protein with brine and extracted it with ether. Their nucleovitellin (now known as lipovitellin) consisted of protein, lecithin, and phosphoric acid. Osborne and Jones (1909) determined the proportion of amino acids in vitellin.

Plimmer (1908) presented the name livetin for a new egg yolk protein discovered in the solution after vitellin was removed. The slow precipitation of livetin in a solution of ammonium sulfate, magnesium chloride, and sodium chloride indicated to Kay and Marshall (1928)

that more than one protein might be present. Jukes and Kay (1932) published the quantities of basic amino acids present in livetin and vitellin. Jukes (1933) determined the quantities of additional amino acids and found carbohydrate associated with the proteins, with livetin being composed of four per cent and vitellin two per cent carbohydrate.

Fevold and Lausten (1946) isolated lipovitellenin by extracting the supernatant from the lipovitellin precipitation with ether. Centrifugation yielded three layers: ether-lipid, aqueous livetin, and a suspension of the new protein.

Phosvitin was discovered to be a high phosphorus (ten per cent) protein contaminating previous lipovitellin preparations (Mecham and Olcott, 1948). Further studies showed a ratio of one phosphate to two amino acid residues and indicated that nearly all the hydroxyamino residues of phosvitin are phosphorlyated (Mecham and Olcott, 1949).

Recent studies.--In recent studies using modern analytical techniques, each of the previously determined proteins has been found to contain more than one protein moiety. The molecular orientation of these moieties has also been studied.

1. Lipovitellin.--When egg yolk is centrifuged at high speed, approximately forty per cent of the yolk

protein sediments (Schmidt et al., 1956). This precipitate includes all of the protein phosphorus and eighty-seven per cent of the calcium and iron of the yolk, but less than thirty per cent of the yolk's total phospholipid. Lipovitellin and phosvitin are held in this high density fraction by ionic or secondary forces (Burley and Cook, 1961). Chargaff (1942a) recognized vitellin (or lecitho-vitellin) as the lipoprotein, lipovitellin. In his study of the nature of the lipid combination in this complex, he found that approximately nineteen per cent of the phosphatides were firmly bound to the protein, but could be removed by alcohol extraction and were essentially the same as the "free" lecithin and cephalin removed by ether extraction.

The hydrolysis of lecitho-vitellin (lipovitellin) by pepsin and trypsin kinase indicated that the protein portion of the molecule contained two widely separated or dissimilar phosphoric complexes, one of which was resistant to enzymic attack (Blackwood and Wishart, 1934). Electrophoretic and chromatographic studies show that lipovitellin consists of two lipoprotein moieties--α- and β-lipovitellin (Joubert and Cook, 1958a; Bernardi and Cook, 1960a, 1960b, 1960c). Both lipovitellins have the same protein lipid ratio, which is approximately four to one, but protein phosphorus is more abundant in α-lipovitellin.

The presence of two N-terminal amino acids in β-vitellin indicated the presence of more than one polypeptide chain. In aqueous buffer and urea solutions, the lipovitellins each dissociate into two subunits involving only a negative entrophy change which could be due either to changes in structure or to changes in hydration. The bonds between protein and phosphate groups are not broken during this dissociation (Sugano, 1959; Burley, 1962; Burley and Cook, 1962a, 1962b). A flexibility of structure allows the lipovitellins to absorb chloroform and β-lipovitellin in this medium gels at pH 11, perhaps because of an alteration in the position of its phospholipids. Radomski and Cook (1964) stated that the heterogeneity of α-lipovitellin arises from the hybridization of several monomers. A monomer-dimer system has been found operative in β-lipovitellin dissociation with subunits of MW = 2.27×10^5 (Cook and Wallace, 1965).

2. Phosvitin.--Although the actual structure of phosvitin is unknown, Wallace et al. (1966) indicated that it is complexed with the lipovitellins in the granules and that they may be sub-fractions of a similar protein with differing levels of phosphorylation. Phosvitin is precipitated by proper concentrations of magnesium chloride. It forms soluble complexes before precipitation and thus, may act as a bridge in the granule structure (Joubert and Cook, 1958b).

3. Lipovitellenin.--When the low density fraction obtained from the centrifugation of egg yolk is extracted with ether to remove "free" lipid, a lipoprotein with approximately forty per cent bound lipid is precipitated. The lipid removed by extraction is not really a free form, but acts to stabilize the lipoprotein (Turner and Cook, 1958; McIndoe, 1959b). The presence of more than two N-terminal amino acids in the protein moiety (Smith and Turner, 1958) and the variable lipid content of the low density fraction indicate the presence of several chains or separate protein molecules held by lipid (Martin et al., 1959). Saari et al. (1964a) showed that two low-density lipoproteins were present in their ultracentrifugal flotation patterns of the fraction previously known as lipovitellenin. Both fractions contained carbohydrate, but their resolution by paper electrophoresis was not possible.

4. α-, β- and γ-livetins.--Shepard and Hottle (1949) obtained three electrophoretic peaks when they examined livetin and designated them as α, β, and γ-livetins. Although the molecular weight of β-livetin is similar to ovalbumin, the two proteins differ in respect to composition and mobility. The immunological identities of the livetins are related to those of serum globulin of the hen (Mok and Common, 1964) but, the solubility of α-livetin is not identical to that of serum albumin. Martin

et al. (1957) found that much of the γ-livetin is precipitated with lipovitellins. Low lipid and phosphorus content differentiate γ-livetin from the lipovitellins, although its separation is difficult (Martin and Cook, 1958).

Research concerning the livetins is incomplete at the present time for starch gel electrophoresis resolves sixteen zones, four of which can be identified with β-livetin from paper electrophoresis (Hui and Common, 1966). The remaining twelve zones have not been identified.

Lipid

Early studies of the egg yolk lipid often refer to free lipid and its removal. Weinman (1956) centrifuged egg yolk under conditions in which chylomicron lipid would float to the surface. Since little lipid came to the top, he concluded that nearly all lipid of the egg yolk is bound to protein. Numerous studies support his conclusions (Vandegaer et al., 1956; Evans and Bandemer, 1957; Sugano and Watanabe, 1961).

The lipid of the egg yolk occurs as neutral lipid associated with protein and in the phosphatide portions of the lipoproteins. Fisher and Hill (1964) found that lipid held by electrostatic and hydrophobic bonds is easily removed. They postulated that the remaining fatty acids are bound to protein in association with organic phosphate.

Cook and Martin (1962) found two types of complexes in the egg yolk--the high protein lipoprotein of α- and β-lipovitellin consisting of a protein matrix with lipid interaction and the low protein lipoprotein of lipovitellenin, which follows a micellar model consisting of a lipid sphere surrounded by a film of protein. Martin et al. (1959) discovered that ether extraction of lipovitellenin removes lipid essential to the maintenance of original stability and solubility, and they concluded that the amount of protein-free micellar lipid in lipovitellenin must be small. The lipid of lipovitellenin is held in two manners such that approximately forty per cent of the lipid remains after ether extraction (Turner and Cook, 1958; Evans and Bandemer, 1957).

Because of their abundance in egg yolk, phosphatides and lipids must be taken into account in the consideration of the egg yolk's functional properties. It is interesting to note that even without complete structural elucidation, the egg yolk was the most common source of both lipoprotein and phosphatides until soybean technology made them uneconomical.

Phospholipids.--Phospholipids or phosphatides which occur in nature are glycerol derivities frequently containing a nitrogenous base in the α-position, with fatty acids in the α'- and β-positions (White et al., 1959).

Interrelationships of the phospholipids and lipoproteins may account for the structural complexities of the egg yolk constituents. Witcoff (1951) described a variety of possible linkages and the resulting compounds.

Studies have been conducted to determine the nature of the phospholipids which equal approximately twenty per cent of the weight of egg yolk and are associated with the lipoproteins. Schmidt et al. (1956) found that more than seventy per cent of the phospholipids remained in the high density fraction when egg yolk was centrifuged. The research of Smith and Turner (1958) indicated that the differences in solubility of the components of the high density fraction could be due to the amount and nature of lipid association.

Chargaff (1942a) found that the phospholipids of lipovitellin had a lower Iodine Value than those of the entire yolk. A differing rate of radioactive phosphorus incorporation in the egg yolk led Chargaff (1942b) to an explanation of the metabolic link between phospholipids and phosphoproteins. He hypothesized that protein phosphorylation at serine hydroxyls is followed by esterification with diglyceride. Peptide linkages are then broken, freeing serine phosphatides which can undergo decarboxylation to cephalin, and other compounds.

In 1954, Hanahan stated that in egg yolk lecithin, the α'-ester is unsaturated (mainly linoleic acid) and

that the β-ester is always stearic acid. Rhodes and Lea (1956) found egg lecithin more complicated. They found that not more than one-third of the lecithin could have a simple di-C_{18} structure, the remaining saturated acid being palmitic. They also concluded that phosphatidyl ethanolamine has the same type of fatty acid distribution as lecithin. Hanahan's positions of unsaturation were confirmed by Rhodes and Lea (1958) but challenged by Privett et al. (1962) who found, using thin-layer chromatography, that most but not all lecithin of the egg yolk is in the α'-saturated β-unsaturated form. Chromatographic separation of the phospholipids of egg yolk on alumina and separation on silicic acid gave the composition listed in Table 3.

TABLE 3. Composition of egg yolk phospholipids (Rhodes and Lea, 1957)

Component	Percentage
Phosphatidyl choline	73.0
Lysophosphatidyl choline	5.8
Sphingomyelin	2.5
Phosphatidylethanolamine	15.0
Lysophosphatidylethanolamine	2.1
Inositol phospholipid	0.6

Sterol.--Sterols are crystalline, neutral, unsaponifiable alcohols of high melting points (Mattil et al., 1964). They are associated with lipid either in the free form or as esters of fatty acids. Saari et al. (1964a) found that the lipid of the low density lipoprotein (lipovitellenin) contains approximately three per cent sterol. They precipitated the yolk sterol by digitonin, and the infrared spectra of this steroid material were found identical to the infrared spectrum of pure cholesterol.

Effect of hen's diet on egg yolk lipid.--Diet affects the fats and phospholipids of egg yolk. Ostrander et al. (1960) found that feeding hens corn oil increased the unsaturated fatty acid content of the yolk, while the feeding of beef tallow had the opposite effect. The influence of dietary fat is perhaps the cause of conflicting data on yolk lipid composition.

Embryological studies

By tracing the development of the egg yolk, its components may be related to the components of hen serum. Since the egg yolk provides the nourishment for the growing chick embryo, study of yolk protein utilization may give information about the protein composition and structure.

Calvary (1929) found that in the developing egg amino nitrogen decreases while non-amino nitrogen increases and that the amino acid content of the egg undergoes change (Calvary, 1930; Calvary and White, 1931-32). McCully et al. (1959) found by zone electrophoresis two lipoproteins which were similar in hen sera and egg yolk. McIndoe (1959a) precipitated a lipophosphoprotein from laying hen plasma which he felt was responsible for the transport of plasma lipid and almost half of the plasma phosphoprotein to the yolk, as related compounds were isolated from the egg yolk (McIndoe, 1959b).

Schjeide and Uhrist (1960) indicated that the yolk proteins are synthesized in the liver of the hen, transferred by blood plasma to the ovary and deposited as granules. Lipovitellin (MW ≃ 200,000) appears to be one-half the size of its serum precursor.

To determine if some yolk components were used by chick embryos in preference to others during incubation, egg yolks were removed and replaced with yolk components. The percentage survival for each component was published (Walker, 1967). The floating fraction was found to be a potent nutrient as were the granules. Supernatant plasma was not nutritionally useful. When the granules and floating fraction were combined, life support was equal to that of whole yolk.

Freezing of Egg Yolk

When the temperature of egg yolk is reduced below -6°C, an irreversible change takes place which results in a stiff paste-like putty when the egg yolk is thawed (Moran, 1925). The causes of this reaction are unknown in spite of extensive study. Various explanatory theories have been proposed and methods have been devised to partially prevent the reaction from occurring.

Gelation

The irreversible change which egg yolk undergoes upon freezing is termed gelation. It is often classified as a form of denaturation. Scheraga (1961) defined denaturation as the "process in which a protein or polypeptide is transformed from an ordered to a disordered state without the rupture of covalent bonds." Gortner (1949) defined denaturation as "any non-proteolytic modification of the unique structure of native proteins giving rise to definite changes in chemical, physical or biological properties" and gave the following properties indicative of denaturation:

1. Decreased solubility.
2. Increased digestibility by proteolytic enzymes.
3. Exposure of oxidizing and reducing groups, notably the sulfhydryl (-SH) groups.
4. Loss of enzymatic properties if the protein is an enzyme.

5. Modification of the specific immunological properties.
6. Decreased diffusion coefficient and increased intrinsic viscosity of the protein.

Factors influencing gelation

The freezing point of egg yolk is 0.65°C but no gelation occurs until the temperature falls below -6°C and super-cooling results in no change in the yolk's fluidity (Moran, 1925). Lopez et al. (1954) found no change in pH between fresh and frozen egg yolks; however, adjusting the pH to 3.2 before freezing resulted in a highly viscous thawed mass. The damage is greatly reduced by rapid freezing and thawing (Lea and Hawke, 1952), but not by rapid freezing or thawing alone (Lopez et al., 1954).

Thomas and Bailey (1933) stated that the maximum gelation of egg yolk is achieved in sixty to one hundred and twenty days of storage at -21 to -18°C. Freezing has no effect on protein, amino nitrogen, or reducing sugar content of the egg yolk (Pearce and Lavers, 1949; Evans and Davidson, 1953).

Theories of gelation

The formation of ice crystals when egg yolks are frozen seems to be a requisite for gelation (Fennema and Powrie, 1964). Moran (1925) ascribed gelation to the solution of lecithovitellin by the concentrated salt

solution present in frozen yolks and its precipitation on thawing. Urbain and Miller (1930) also indicated that gelation is due to the dehydration and coagulation of a lecithoprotein. They found however that sucrose which inhibits gelation of egg yolk had no effect on lecithin alone. Thomas and Bailey (1933) found that lecithovitellin is insoluble in sugar solution and that the addition of commercial lecithin to egg yolk prior to freezing has no effect on gelation.

A protein or protein complex is thought to be responsible for gelation (Vandegaer et al., 1956; Lopez et al., 1955). Some damage may be due to the crushing or spearing action of ice crystal growth, but lipid-protein complexes rearrange and aggregate during freezing (Lovelock, 1957). In protein separations, dialysis and dilution cause protein precipitation by reducing salt concentration. A reduction in temperature causes precipitation at higher salt concentrations. A change in pH occurs if buffering salts crystallize and this pH change may account for protein denaturation, however lipovitellin is damaged more slowly at -20°C than at -3°C suggesting that it is not sensative to suspension in a solution of high ionic strength (McNally, 1959).

Lea and Hawke (1952) stated that a lowering of pH alters lipovitellin and Burley and Cook (1962a) showed that α- and β-lipovitellins dissociate with changes in hydration

or structure at specific pH values. Possibly sulfhydryl-disulfide interactions during dissociation and association of β-lipovitellin affect its structure (Burley and Cook, 1962b). Fevold and Lausten (1946) indicated that lipovitellenin was involved in gelation, and electrophoretic changes are found in the lipovitellenin from frozen egg yolks (Powrie et al., 1963). Saari et al. (1964b) isolated the low-density lipoprotein (lipovitellenin) from frozen yolk plasma. They found that freezing and thawing decreased the solubility of some plasma lipoproteins in sodium chloride solution. The insoluble fraction had a high free fatty acid content. At pH 4.0 their lipoprotein solution formed a gel after freezing and thawing and it failed to migrate in paper electrophoresis.

Prevention of gelation

It is desirable to inhibit gelation because thawed egg yolk is difficult to combine with other ingredients (Jordan et al., 1952). Gelation may be partially inhibited by mechanical and chemical treatments which are used commercially. The use of additives usually results in an egg product of limited utility. Most research involving the functional properties of frozen egg yolk has dealt with treated yolks. Methods to inhibit gelation, including explanations of the mechanisms thought to make the methods successful, follow.

Salt.--The addition of sodium chloride (1-5%) to egg yolks increases their viscosity before freezing, but causes a depression of the freezing point and reduces the viscosity after thawing (Jordan and Whitlock, 1955; Marion, 1958; Powrie et al., 1963). This treatment limits the yolks to use in products where the flavor of the added salt is desirable (Marion, 1958). Sodium chloride also inhibits gelation in low-density lipoprotein solutions (Saari et al., 1964b). The mechanism of its action may be solely the reduction in freezing point, or it may inhibit aggregation by stabilizing charged groups on the egg yolk proteins (Powrie et al., 1963).

Sugars.--A variety of sugars prevent gelation. The mechanism of this inhibition is unknown, but may be related to the chelation of iron or copper in the egg yolk which subsequently could reduce the cross-bonding of protein structures (Meyer and Woodburn, 1965). Sugars which are effective include: arabinose, galactose, glucose, fructose, sucrose, maltose, and raffinose (Urbain and Miller, 1930; Thomas and Bailey, 1933; Marion, 1958; Powrie et al., 1963). Marion (1958) observed that 9.1 per cent sucrose was necessary to produce a gelation inhibiting effect equal to that observed when only 4.76 per cent sodium chloride was used, although on a molar

basis, sucrose was more effective than sodium chloride (Powrie et al., 1963).

Water.--Lopez et al. (1954) stated that dilution or concentration of egg yolk does not affect gelation. However, Marion (1958) reported that addition of more than four per cent water did inhibit gelation somewhat. Meyer and Woodburn (1965) substantiated this report.

Homogenization.--Homogenization of egg yolk in a milk homogenizer minimizes gelation (Tongur and Ragosin, 1944). Using homogenization pressures of 0, 1000, 2000, and 3000 pounds per square inch, Marion (1958) found that the viscosity of frozen-defrosted egg yolk decreased to the same degree using the three higher pressures. When no pressure was applied, gelation was not inhibited. He also found that repeated homogenization was only slightly effective in further reducing gelation.

Other methods of reducing gelation.--When the yolks of fresh shell eggs are inoculated with Bacillus cerus and incubated at 37°C, a hardening of the yolk takes place (Colmer, 1948). The explanation given is that a lecithinase produced by the bacteria breaks down a lecithoprotein, and with the loss of lecithin's binding action the fat and protein change from their dispersed state. However, Crotoxin (rattlesnake venum lecithinase A)

prevents or reverses gelation (Feeney and Hill, 1960). Lopez et al. (1954) found the following enzymes effective in reducing gelation: papain, pepsin, trypsin, and rhozyme. Cystiene addition to egg yolks before freezing results in a slightly greater inhibition of gelation than does water (Meyer and Woodburn, 1965).

Emulsifying Properties of Egg Yolk

Ziemba (1955) stated that "the primary use of egg yolks is for emulsification." Inconsistencies exist in the literature in regard to the component responsible for egg yolk's emulsifying properties; indeed "there exists no single coherent theory of emulsion formation and stability" (Becher, 1965).

The emulsion system (Sutheim, 1947)

"Emulsions are intimate mixtures of two immiscible liquids, one of them being dispersed in the other in the form of droplets" (Sutheim, 1947). Each of the liquids is a one-phase system but a two-phase system exists after emulsification. The discontinuous or internal phase is the one which exists as separate globules in the emulsion. The continuous or external phase is the liquid enclosing the droplets. The two liquids are referred to as oil and water, dispite the fact that one or both liquids may contain other material. There are two types of emulsions,

oil-in-water and water-in-oil, with the former type being most prevalent in food emulsions. The external phase of an emulsion determines its properties. The emulsion can be diluted only by the external phase. Coloring can be achieved by using a dye soluble in the external phase. The emulsion type can seldom be determined by visual examination, but it may be determined by the behavior of emulsions toward different thinners and dyes as well as tests of conductivity.

Theories of emulsification

Many theories have been advanced to explain emulsification (Clayton, 1928). Bancroft (1913, 1915) recognized the importance of the interfacial film between the two liquids to be emulsified. Recognizing different surface tensions on the two sides of the film, Bancroft and Tucker (1927) theorized that by reducing tension on the water side of the interface, the film would curve to enclose oil droplets and prevent coalescence. Mechanical energy affects the size of the encased droplets, and coalescence is minimized as droplet size is decreased (Clayton and Morse, 1939).

Emulsifying agents have two functions; they aid in forming the emulsion and in stabilizing it. The emulsifier lowers the interfacial tension with greatest reduction on the continuous side of the film (Lowe, 1955).

Charged molecules covering the film act to keep interfacial tension to a minimum. An emulsifier acts as the separating phase and its absorption at the interface is vital (Becher, 1965). Harkins and Zollman (1926) showed that a decrease in interfacial tension eases emulsion formation, but surface active agents may not stabilize an emulsion (King, 1941; Yeadon et al., 1958).

Egg yolk as an emulsifier

Egg yolk promotes oil-in-water emulsions, however, the minimum amount of egg yolk to stabilize a given interfacial area between water and oil is not known (Clayton, 1932). Many of egg yolk's components are emulsifiers, namely, proteins, phospholipids, and cholesterol. Becher (1965) stated that in 50:50 oil-in-water emulsions inversion to the water-in-oil type will occur when the lecithin/cholesterol ratio falls below 8:1. Cholesterol promotes water-in-oil emulsions and mayonnaise is weakened when forty-four times as much cholesterol as is normally present is added (Snell et al., 1935). Lecithin was long thought to be the constituent of egg yolk responsible for its ability to emulsify oil in water. Snell et al. (1935) added lecithin to mayonnaise and found that a product of poor stability resulted. Yeadon et al. (1958) found that 1.2 per cent purified egg lecithin did not promote emulsion stability unless fatty acids and cephalins were added. It

was concluded that a lecitho-protein of the egg yolk is the emulsifying agent (Snell et al., 1935).

Vincent et al. (1966) studied the surface energy of yolk, plasma, and dispersions of yolk fractions. They found that the plasma surface-active agents effectively reduced surface energy. They indicated that the livetin fractions contribute to the low surface energy and that further reduction was due to the phospholipids released by the low-density lipoprotein for interfacial absorption. Jordan et al. (1960) found that the stability of oil-in-water emulsions was not affected by using egg yolks from hens fed diets containing different fats, even though the Iodine Values of the yolk lipids differed significantly.

The effects of freezing on egg yolk's emulsifying properties

In 1935, Kilgore stated that more frozen (salted or sugared) egg yolks were necessary to produce mayonnaise similar in consistency to that produced from fresh yolk. LeClerc et al. (1940) indicated that the functional properties of egg yolks were altered by freezing. Miller and Winter (1951) found that the viscosity of mayonnaise was increased when frozen yolks were used, and they concluded that frozen yolk could be reduced from 13.5 per cent to 8.6 per cent of the formula with no effect on the viscosity or stability of the mayonnaise. A comparison of oil separation from mayonnaises made from fresh, frozen,

freeze-dried, and spray-dried egg yolks revealed decreased mayonnaise stability when any of the processed eggs were used (Rolfes et al., 1955). The contradictory reports in the literature may be due to differences in formulae, methods and materials (Lowe, 1955).

Tests of emulsion stability

Simple systems are used to test the stability imparted by an emulsifying agent. Mayonnaise is frequently used to test the stabilizing power of egg yolk (Snell et al., 1935; Miller and Winter, 1951; Rolfes et al., 1955). Stability is determined by recording whether or not the mayonnaise emulsion breaks during storage or mechanical shaking.

Drainage from simple oil and water emulsions with a high proportion of water may be measured to indicate the stability imparted by an emulsifier. The greater the drainage, the less stable is the emulsion system. These emulsions have been used to quantify differences between emulsifying agents (Harkins and Zollman, 1926; Yeadon et al., 1958; Jordan et al., 1962).

Objective Tests

Objective tests are those tests which depend on some measure other than the human senses and include chemical, histological, and physical tests (Griswold, 1962).

They give quantitative measurements and are reproducible, with their accuracy being a function of the tools and methods employed. In the study of egg yolk proteins, electrophoretic mobility and viscosity determinations are conducted to measure changes in the proteins characteristic of denaturation.

Paper electrophoresis

Paper electrophoresis has been used extensively in the study of egg yolk proteins, both to explore their composition and to monitor separation techniques. Changes in the electrophoretic patterns of egg yolk proteins which have been frozen are apparent. Differing conditions which influence ionic migration have led to variation in the electrophoretic patterns of egg yolk proteins, dependent upon the laboratories from which the patterns were produced.

General theory of paper electrophoresis (Block et al., 1955).--Paper electrophoresis or ionophoresis is a useful tool in the study of protein mixtures. The basic principle of electrophoresis is that charged particles in solution migrate in an electrical field. When particles possess either quantitatively or electrically different charges, they migrate different distances on paper strips. Convection is prevented by draining the paper strips to

produce optimum resolution. Factors which govern ionic migration include:

1. Those characteristics related to the ion itself, namely, its charge (sign and magnitude), size, shape, tendency to dissociate, and amphoteric behavior, if any.

2. Those factors related to the environment in which the ion is being studied, such as the electrolyte concentration, ionic strength, dielectric properties, chemical properties, pH, temperature, viscosity, and the presence of non-polar molecules which may influence viscosity or dielectric properties of the electrolyte or which may interact to form charged complexes.
3. The character of the applied field, its intensity, purity (presence of alternating current components), and the distribution along the migration path (Block et al., 1955).

Electrolytic dissociation and reactions of acids or bases are the processes commonly encountered to charge particles for electrophoresis. Uncharged molecules or amphoteric materials can show apparent mobility as a result of endosmosis, a phenomenon based upon the electrokinetics of liquid-solid interfaces. A viscous medium provides a retarding force opposite to the movement of the particle in a constant field.

Many types of apparatus are used for paper electrophoresis, ranging from simple to highly complex. All embody the same basic principles; the sample is applied to a buffer impregnated filter paper strip and a current is passed through the strip, allowing the charged ions of the sample to migrate in the electrical field. The protein is then denatured, usually by drying. Control of drying

temperature, current, and evaporation from the strips are important factors in the selection of apparatus. Common buffer solutions are used as the electrolyte and the choice of solution depends upon the sample to be tested. A solution with a pH value higher than the isoelectric point of the protein in the sample prevents the adsorption of positively charged ions on the filter paper.

Irreversible adsorption of sample components on the paper, especially prevalent when mobilities are low, causes "tails" which remain superimposed on the other components and make quantification impossible. On the other hand, reversible adsorption yields comet-shaped migration bands which do not interfere with resolution.

Since paper electrophoresis is an analytical technique, methods of staining have been devised for the quantitative determination of proteins, lipids, and glycoproteins. The dyestuff must be bound stoichiometrically and permit easy elution unless the strips are to be analyzed by direct scanning. Protein dyes which give good results include bromphenol blue, Azocarmine B, and Amidoschwartz 10B.

Stained strips are analyzed by direct densitometry or by cutting the strips into the visible zones, eluting the dye and analyzing the solution colormetrically. The amount of dye bound is largely a measure of the basic groups present and cannot be correlated with other

properties. Different affinities for dye may also influence results. Unless complete resolution is obtained, cutting the strips into bands is a highly subjective procedure.

Direct scanning techniques are rapid and convenient, but direct scanners should be calibrated so that numerical values will correspond with values obtained by elution techniques, so that comparison between laboratories is possible. Scanning may involve errors. Lack of homogeneity of the paper may result in large errors where contrasts between background and dye are small.

Due to the great number of variables involved in paper electrophoresis, comparison of quantitative results can only be made if experimental techniques are precisely the same.

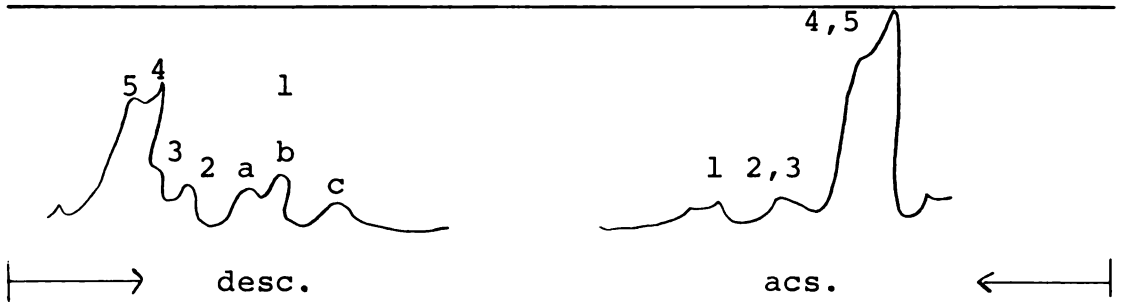
Electrophoretic mobility of egg yolk proteins.--In 1957, Evans and Bandemer studied egg yolk and egg yolk protein preparations by paper electrophoresis, using diethyl barbiturate buffer (pH 8.6, $\mu = 0.05$). The migration distances of seven bands were determined. Using elution techniques, they reported the protein composition of egg yolk (Table 4). Evans et al. (1958) noted that the yolk proteins of eggs from individual hens varied greatly in speed of migration.

TABLE 4. Protein composition of egg yolk determined by paper electrophoresis (Evans and Bandemer, 1957)

Protein	Fraction	Total Protein %
A	Ovalbumin	1.7
B	Livetin	5.5
C	Livetin	3.1
D	Lipovitellenin	41.7
E	Lipovitellin (Non-Mobile)	34.5
F	Lipoprotein	11.9
G		1.6

Using carbonate buffer (pH 9.8, μ = 0.10) to increase the resolution of low-mobility components, Saito et al. (1965) were able to separate ten proteins and lipoproteins from the egg yolk by electrophoresis. Electrophoretic study of the egg yolk's high density fraction using veronal buffer (pH 9.0, μ = 0.3) yields mobility patterns shown in Figure 1 (Bernardi and Cook, 1960a). Differences in mobility in the ascending and descending limbs result from the multiplicity of components widely different in charge density.

Sugano (1959) found that the mobility of lipovitellin depends upon the strength of the buffer solution. Meyer and Woodburn (1965) indicated that ions are important in lipovitellin migration.



$cm^2/sec/V \times 10^5$

1 a	-10 to -12	
b	-8.5	phosvitin
c	-7.5	
2	-5.4	α - livetin
3	-3.7	β - livetin
4	-3.1	α - lipovitellin
		β - lipovitellin
5	-2.4	γ - livetin

Figure 1. Typical electrophoretic mobility patterns of egg yolk's high density fraction (Bernardi and Cook, 1960a)

Livetin shows three electrophoretic peaks named α-, β-, and γ-livetin in terms of descending mobility (Shepard and Hottle, 1949; Martin and Cook, 1958). The three peaks overlap α- and β-lipovitellin in many studies (Sugano, 1958). Martin et al. (1957) found γ-livetin similar in mobility to the lipoproteins. Phosvitin precedes the livetins in migration (Sugano, 1957, 1958).

Electrophoretic patterns of frozen egg yolk.--Solubility studies indicated that lipovitellenin is altered during freezing (Fevold and Lausten, 1946). Powrie et al. (1963) electrophoretically isolated two major lipoprotein

complexes from egg yolk. Lipovitellin did not migrate from the point of application before or after freezing. Unfrozen lipovitellenin migrated 28 mm. After freezing and thawing, only a slow-migrating fraction (14 mm) was found. Optical density values showed that the slow-migrating lipoprotein was not lipovitellenin which was presumably altered by freezing and thawing and became non-mobile. In the fresh sample, the slow-migrating protein was evidently carried along to 28 mm with the lipo-vitellenin. Under the same conditions, Meyer and Woodburn (1965) found that the mobile protein fraction decreased with an increase in the non-mobile protein fraction when the egg yolk was frozen and thawed. The addition of sodium chloride or cysteine hydrochloride to the yolks before freezing reduced this difference.

Measurement of viscosity of egg yolk

Viscosity is defined as a liquid's resistance to flow and is caused by intermolecular attraction. Absolute viscosity is expressed by the work necessary to maintain a flow rate and is measured by the poise unit which equals the force of one dyne per second per square centimeter. Relative viscosity compares flow rate with that of a reference liquid, usually water.

Quantitative determinations of viscosity provide an index of denaturation (Gortner, 1949). Measurement of

viscosity before and after freezing and thawing can determine the amount of egg yolk gelation, as the phenomenon is accompanied by an obvious increase in viscosity.

Various tools have been employed to measure egg yolk viscosity. Viscosity is dependent upon the temperature and moisture content of the sample (Payawal et al., 1946).

Some of the simplest methods employed in the determination of viscosity are based upon the time necessary for the egg product to flow through a certain distance in an Ostwald or a Mohr pipet with the tip removed (Barmore, 1934; Jordan and Whitlock, 1955). This method is highly dependent upon an accurate timing devise and the dexterity of the investigator. The small sample requirement is an advantage and the method may be quite successful if the sample is not too viscous.

The capillary viscometer (Payawal et al., 1946) or Bingham plastometer (Bateman and Sharp, 1928) measures the pressure required to pass a sample through a length of uniform bore capillary tubing. By this method, Payawal et al. (1946) found the viscosity of egg yolk (49-49.5 per cent water) to be 800 centipoises at 25°C.

After gelation has taken place, the increased viscosity of the sample necessitates the use of some other instrument. The Gardner-Parks mobilometer measures the time necessary for a weighted piston to fall a pre-determined

distance into the sample (Pearce and Lavers, 1949). Forsythe et al. (1953) found this instrument satisfactory for measuring the viscosity of frozen whole egg fortified with yolk. In a study of the viscosity of frozen whole egg magma, Thomas and Bailey (1933) found each successive plunge of the mobilometer piston to be more rapid than the previous plunge.

Jordan and Whitlock (1955) used the MacMichael viscometer to measure the viscosity of egg yolk. This instrument measures the angel of torque of a disk suspended by a wire into the sample which is rotated at a constant speed. The Stormer viscometer measures the rotation rate of a cylinder moving through the sample impelled by a uniform force. Miller and Winter (1950) used this instrument to study the viscosity of frozen pasteurized whole eggs.

The Brookfield viscometer successfully measures the relative viscosity of egg yolk which has undergone gelation (Jordan and Whitlock, 1955; Lopez et al., 1954; Marion, 1958). A spindle of chosen size is rotated through the sample at a predetermined number of revolutions per minute and the force exerted against rotation by the sample is read from the instrument and converted to centipoise units by means of a table, dependent upon rotation rate and spindle size. Meyer and Woodburn (1965) reported a mean relative viscosity of 27.4 poises for fresh yolk at 24°C

and a mean relative viscosity of 907.9 poises for thawed yolk frozen at -25°C for 20 to 24 hours. Powrie et al. (1963) reported a mean relative viscosity of 24 poises for fresh yolk at 25°C and of 628 poises for thawed yolk which had been frozen at -14°C for 150 minutes. Fresh yolk viscosity is influenced by the temperature and moisture content of the sample, whereas thawed yolk viscosity is dependent upon the additional factors of rate, time, and temperature of freezing and the rate and temperature of thawing (Marion, 1958; Powrie et al., 1963).

EXPERIMENTAL PROCEDURE

Design of Experiment

This experiment was designed to study the emulsion stabilizing ability of native egg yolk and three crude egg yolk fractions: lipovitellin, lipovitellenin, and livetin. Oil-in-water emulsions, stabilized by the yolk fractions, were prepared according to a 2^4 factorial design. The four factors were the three fractions and the storage treatment. The two levels of the three yolk fractions were their absence or presence in the emulsion mixture. The two levels of storage were designated as fresh and frozen. Thus, there were sixteen treatment combinations for each replication of the design. Emulsions prepared with fresh and frozen native yolk served as a control for comparison with emulsions containing recombined lipovitellin, lipovitellenin, and livetin.

To determine the effects of freezing on the physical properties of egg yolk and the three yolk fractions, samples of both fresh and frozen native yolk and fractions were analyzed for moisture, protein, pH, and viscosity. Studies of electrophoretic behavior were conducted on

native yolk and the three yolk fractions both before and after they were frozen. The entire design was replicated six times.

Procurement of Eggs

The requirement for fresh egg yolk and the amount of time required to complete this study obviated the method of mixing and storing egg yolk sufficient for all replications. Therefore, eggs were obtained from a controlled group of sixty-five S.C. White Leghorn hens (Foreman Strain) for each replication.

Hens were approximately eighteen months of age, housed in experimental floor pens and fed an all-mash laying ration designated as LB-63, the formula of which is included in the Appendix.

Eggs were gathered two times each day and held at room temperature over night prior to delivery for research. All eggs were laid the day before they were taken to the laboratory where they were held at 4-5°C prior to use within twelve hours. Eggs were sorted and only grade A Large eggs were used. All eggs were dry cleaned by sanding before packaging.

Preparation of Egg Yolk

Following removal from refrigeration, the eggs were broken and separated by hand. Each yolk was carefully

rinsed in distilled water, which was deionized by means of an Illco-Way Research Model deionizer (product impurities < 0.5 ppm.). The yolks were rolled on four layers of cheesecloth to remove adhering white, and the chalazae were removed by hand. The vitelline membrane was punctured and the yolk contents allowed to drain through two layers of cheesecloth into a tared 2000 ml beaker. The yolks from four dozen eggs were mixed by stirring three minutes with a rubber spatula, afterwhich 135 ± 0.01 g of the native yolk was weighed on a Torbal torsion balance (Model PL-800, 800 g-capacity) into a labeled one-half pint polyethylene freezer container. The native yolk was stored covered at 4-5°C until divided and analyzed.

Separation of Egg Yolk Fractions

A modification of the technique employed by Evans and Bandemer (1957) was used to separate three crude egg yolk protein and lipoprotein fractions which were designated as lipovitellin, lipovitellenin, and livetin. Walker (1967), who used a similar method of separation, listed the components of the three fractions. Thus, according to Walker's classification, the crude lipovitellin used in this experiment actually consisted of α-lipovitellin, β-lipovitellin, phosvitin, and low-density lipoprotein. The crude lipovitellenin was fairly pure and the crude

livetin consisted of α-, β-, and γ-livetins in addition to small amounts of unrecovered lipovitellenin.

Lipovitellin

A Toledo Balance (Model 4030, 5 kg-capacity) was used to weigh the mixed egg yolks to the nearest 1.0 g. Deionized water in an amount equal to twice the weight of the egg yolk was added, and the diluted egg yolk was mixed three minutes with a rubber spatula, before being placed into eight 50 ml Teflon centrifuge tubes and centrifuged in a Servall angle centrifuge, Type SS-1, equipped with a refrigeration unit which maintained a temperature of 0 ± 2°C in the interior chamber during centrifugation. The centrifuge timer was set for thirty minutes and the speed was brought to 10,000 rpm as quickly as possible (approximately three minutes) without exceeding a 5 amp reading on the ammeter. The 10,000 rpm speed was maintained until the centrifuge automatically stopped at the end of the preset time. Since four centrifuge runs were necessary for each replication, the Saran-covered diluted egg yolk mixture was held at 4-5°C between runs, and stirred for thirty seconds with a rubber spatula before additional tubes were filled.

The supernatant solutions from all eight tubes were combined in a 1000 ml beaker before any further separation steps were begun. The creamy, white precipitate in

each centrifuge tube was rinsed with approximately 5 ml of deionized water and the rinsings were discarded before the precipitate was removed from the tubes with a metal spatula. All precipitates were mixed in a tared one-half pint polyethylene freezer container before weighing to the nearest 0.01 g. The container was tightly covered, labeled, and stored at 4-5°C until division, analyses, and objective testing. This creamy, white precipitate was designated as lipovitellin.

Lipovitellenin

The supernatant solution obtained from the lipovitellin preparation was used to prepare the crude lipovitellenin. The combined supernatant solution from each set of eight centrifuge tubes was placed in 36 inches of seamless, cellulose dialyzer tubing (1 1/8 inches diameter, inflated). Dialysis was carried out against deionized water at 4-5°C until a negative test for chloride was obtained. Qualitative determination of chloride was made according to the method outlined by Caldwell and King (1961). Three drops of concentrated HNO_3 were added to 5 ml of the dialysis water. One drop of approximately 0.1 N $AgNO_3$ was added. The presence of a white precipitate was indicative of chloride presence and dialysis was continued until a clear solution was obtained on $AgNO_3$ addition.

The contents of all dialysis tubes were mixed in a 2000 ml beaker and placed on a Cenco Magnetic Stirrer. Using a 1 3/4 inch Teflon-coated stirring bar, the mixture was stirred at medium speed throughout the pH adjustment. A Beckman Zeromatic pH Meter equipped with a thermocompensator and standardized with a pH 7 buffer solution was used to measure the pH of the solution while 0.169 N NaOH (pH 12.0) was added gradually from a 25 ml buret supported over the center of the solution. Sufficient NaOH (approximately 10 ml) was added until the solution reached pH 6.2.

The solution was placed in 50 ml Teflon centrifuge tubes which were balanced and placed in a Servall angle centrifuge at 0 ± 2°C. The timer was set for ten minutes and the speed increased to 10,000 rpm as rapidly as possible without exceeding a 5 amp reading on the ammeter (approximately three minutes). Following centrifugation, a sticky, yellow, low-density fraction was suspended in the tubes above a clear solution. The contents of the tubes were placed in funnels (4 inch diameter) fitted with Whatman No. 4 filter paper (24.0 cm diameter) which were set into 500 ml Erlenmeyer flasks. The top of each funnel was covered with Saran and filtration was allowed to continue overnight at 4-5°C. The sticky yellow low-density fraction which collected on the filter paper was designated as lipovitellenin. It was carefully removed from the filter

paper, placed in a labeled one pint polyethylene freezer container and weighed to the nearest 0.01 g. The lipovitellenin was stored at 4-5°C until division, analyses, and objective testing were conducted.

Livetin

The filtrate remaining in the Erlenmeyer flasks after lipovitellenin removal was designated as livetin. It was mixed in a tared and labeled one quart polyethylene freezer container and weighed to the nearest 0.01 g on a Torbal torsion balance. Until division, analyses, and objective testing were conducted, the livetin was stored at 4-5°C.

Freezing of Egg Yolk and Egg Yolk Fractions

The experimental material for each replication was divided into two parts. One-half of the native yolk and each of the three fractions were weighed to the nearest 0.01 g on a Torbal torsion balance into labeled polyethylene freezer containers, using one-half pint containers for the native yolk, lipovitellin, and lipovitellenin and a one pint container for the livetin. The half-portions of the native yolk and the three fractions were frozen and held at -23°C for one week, after which they were removed from the freezer and thawed six hours at room temperature.

Chemical analyses and objective testing were conducted on the frozen samples following the same procedures used on fresh samples with one exception. No determination of total protein was made on the frozen samples, since total protein has been shown to remain constant when egg yolk is frozen (Evans and Davidson, 1953). Changes in moisture content, however, made necessary recalculation of the percentage protein contained in the frozen samples.

Chemical Evaluation

Fresh egg yolk and yolk fractions were analyzed for total protein and total moisture content to determine the proportions to be used in emulsion preparation. Frozen egg yolk and yolk fractions were analyzed for total moisture content. Percentage protein in the frozen egg yolk and yolk fractions was recalculated using revised sample sizes due to changes in moisture content.

Moisture

Total moisture was determined by the AOAC vacuum oven method 16.3 (a) (1955). Sample weights of native yolk, lipovitellin, lipovitellenin, and livetin were approximately 2 g and drying was carried out at 90°C with 25-30 in. vacuum for six hours in a Hotpack vacuum oven, Model 633. An average of two determinations for each sample was recorded.

Protein

Total reduced nitrogen was determined by the Kjeldahl method as modified by Gunning and Arnold (Triebold and Aurand, 1963). The oxidation catalyst used consisted of an 8:1 mixture of K_2SO_4 and $CuSO_4$. Sample sizes were determined so that the titration step would require 20-40 ml of 0.1 N HCl. The approximate weights used were as follows: lipovitellin, 1 g, lipovitellenin, 3 g, livetin, 30 g, and native yolk, 1 g. Wet oxidation of the samples was accomplished by a three hour digestion period. Ammonia was distilled into 150 ml 2% boric acid and the solution in the receiving flasks was titrated with standard 0.1 N HCl using a mixed indicator consisting of 0.083 g methylene blue and 0.125 g methyl red per 100 ml ethanol. The average percentage of protein in each sample was calculated using the general conversion factor, 6.25. An average of two determinations for each sample was recorded.

Objective Measurements

Objective tests were performed on fresh and frozen native yolk and yolk fractions to determine differences in relative viscosity, pH, emulsion stabilizing power, and electrophoretic behavior of egg yolk proteins.

Emulsion stability

Emulsions stabilized by native yolk and yolk fractions were studied to determine the stability each fraction and all combinations of yolk fractions imparted to an oil-in-water emulsion system and to determine the effects of freezing on the emulsion stabilizing properties of the fractions.

Emulsion formula.--The emulsion formula selected to test the emulsion stability imparted by all possible combinations of the egg yolk fractions was based on the formula used by Jordan et al. (1962), which consisted of 15 g egg yolk, 15 g corn oil, and 85 g deionized water. It was necessary to modify the formula for each emulsion mixture to maintain desired protein ratios and a constant moisture content.

An emulsion containing 15 g egg yolk contains 2.45 g protein, using 16.4 as the average percentage protein in egg yolk (Sweetman and MacKellar, 1959). The average amount of moisture contained in 15 g egg yolk is 7 g, based on 49.4 per cent moisture in egg yolk (Sweetman and MacKellar, 1959). Adding this amount of water to the 85 g recommended by the basic formula resulted in a 92 g moisture content for the emulsion mixture. The amount of moisture contained in the yolk, yolk fraction or combination of fractions used in each emulsion mixture was

subtracted from 92 g to determine the amount of water to be used in each preparation.

Two emulsions were prepared for each replication using fresh and frozen native yolk as the emulsion stabilizing agent. Protein and moisture contents of the native yolk were used to determine the amount used to control the protein content of each emulsion to 2.45 g. These emulsions were prepared for comparison to emulsions containing proportional amounts of the three yolk fractions. Table 5 lists the weights of ingredients used in the native yolk emulsions.

TABLE 5. Formulae used in preparation of native yolk emulsions[a]

Storage Treatment	Ingredients		
	Native Yolk	Deionized Water	Corn Oil
	g	ml	g
Fresh	14.90 - 15.25	85	15
Frozen	14.96 - 15.04	85	15

[a]The amount of native yolk was corrected for variance in moisture and protein content between replications.

Lipovitellin, lipovitellenin, and livetin each contribute to the total protein of the egg yolk. For each replication, the proportional amounts of protein contributed by each fraction were calculated. Using the results

of the analyses for total protein contained in each fraction, the amount of each fraction to be used in the emulsion mixture was determined.

Preparation of sixteen yolk fraction emulsions was required to complete each replication of the 2^4 factorial design. Table 6 lists the weights of the ingredients used in the yolk fraction emulsions.

A sufficient quantity of a commercial brand[1] of corn oil for all replications was procured at the beginning of the investigation and stored at 4-5°C until before a set of emulsions was prepared, when it was removed from refrigeration and thoroughly mixed. A Torbal torsion balance was used to weigh 15 ± 0.01 g oil into each of nine 25 ml pyrex test tubes. The tubes were tightly covered with Parafilm until the oil was used.

Preparation of emulsions.--The nine emulsions composing the fresh or frozen portion of each replication were prepared as one set. The same order of emulsion preparation was followed throughout the investigation. Also maintained throughout the experiment was the time elapsing from the day the eggs were laid until the day the emulsions were prepared. This time was seven days for the

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[1]Miesel Brand, Stabilized Pure Corn Oil distributed by Geo. Miesel & Son, Detroit, Michigan.

TABLE 6. Formulae used in preparation of yolk fraction emulsions[a]

Emulsion Type[b]		Ingredients				
		Yolk Fractions[b]			Deionized Water	Corn Oil
		A	B	C		
		g	g	g	ml	g
Fresh	O	-	-	-	92	15
	A	4.27 - 4.96	-	-	90 - 89	15
	B	-	11.02 - 12.80	-	86 - 84	15
	C	-	-	29.63 - 34.79	63 - 58	15
	AB	4.27 - 4.96	11.02 - 12.80	-	83 - 82	15
	BC	-	11.02 - 12.80	29.63 - 34.79	56 - 51	15
	AC	4.27 - 4.96	-	29.63 - 34.79	60 - 55	15
	ABC	4.27 - 4.96	11.02 - 12.80	29.63 - 34.79	54 - 48	15
Frozen	O	-	-	-	92	15
	A	4.25 - 4.95	-	-	90 - 89	15
	B	-	11.08 - 12.82	-	86 - 84	15
	C	-	-	28.40 - 34.78	64 - 59	15
	AB	4.25 - 4.95	11.08 - 12.82	-	83 - 82	15
	BC	-	11.08 - 12.82	28.40 - 34.78	57 - 50	15
	AC	4.25 - 4.95	-	28.40 - 34.78	61 - 55	15
	ABC	4.25 - 4.95	11.08 - 12.82	28.40 - 34.78	55 - 48	15

[a]The amount of each yolk fraction and deionized water was corrected for variance in moisture and protein content between replications.

[b]Fractions are labeled as follows: O = no fractions present, A = lipovitellin, B = lipovitellenin, and C = livetin. Emulsion type refers to both storage treatment and stabilizer composition.

fresh yolk and fresh yolk fraction emulsions and fourteen days for the frozen yolk and frozen yolk fraction emulsions.

The predetermined amounts of the yolk and yolk fractions for each set of emulsions were weighed to the nearest 0.01 g into tared one-half pint glass jars which were then covered until mixing was started. Weighed corn oil at room temperature was added to the yolk or yolk fractions and the oil tube was allowed to drain in a vertical position for thirty seconds, before addition of the required amount of deionized water, at room temperature, from a 100 ml graduate cylinder.

The blade of an Osterizer blender (Model 452) was attached and the jar containing the emulsion ingredients was inverted on the blender assembly. The blender was connected to a Fisher Scientific Powerstat (Type 3PN116) to control blender speed, and to a GraLab timer (Model 171) to control blending time. Blending was begun and continued for one minute with the powerstat set at 55. The powerstat was then adjusted to 110 and the emulsion homogenized for five minutes. If after one minute at the higher speed, particles still adhered to the container, timing was stopped and the particles were scraped from the sides and bottom of the container with a metal spatula. Mixing was then resumed for the remainder of the five minutes.

Measurement of emulsion stability.--Immediately after the preparation of each emulsion, two 15 ml graduated centrifuge tubes were filled with the emulsion mixture, covered with Parafilm to prevent surface drying, and placed in a vertical position in a test tube rack. Emulsion stability was determined by measuring the amount of separation of the oil and water phases at four time intervals: thirty, sixty, ninety, and one hundred and twenty minutes after the emulsions were poured. The amount of drainage to the nearest 0.05 ml was recorded. The average drainage of the two tubes for each of the four times was calculated and used in statistical analysis.

pH of emulsion mixtures, native yolk, and yolk fractions

The pH of the emulsion mixture remaining in each container after the tubes had been filled was determined using a Beckman Zeromatic pH meter. The pH value was recorded to the nearest 0.1 pH unit when the meter needle maintained its position on the scale. The pH values of the yolk and the three yolk fractions, before and after frozen storage, were determined in the same manner.

Viscosity of native yolk and yolk fractions

Measurements of relative viscosity were made on samples of native yolk and the three yolk fractions, before and after frozen storage using a Brookfield Syncho-lectric

Viscometer, Model RVT. Lipovitellin, lipovitellenin, and native yolk samples were placed in 50 ml tapered pyrex centrifuge tubes, to a depth one-half inch from the top. The tubes were supported in a vertical position by a 150 ml beaker and spindle 7 was lowered to its indentation mark into the center of the sample. Care was taken to avoid the entrapment of air bubbles. It was necessary to adjust the rotating speed of the viscometer from sample to sample depending upon the composition of the sample and its storage treatment. A table of rotation speeds for each variable and for each replication is included in the Appendix. Three readings were recorded for each sample; the first reading was taken after one minute of rotation, and additional readings were taken at thirty second intervals. Livetin samples were placed in 600 ml beakers to a depth of 8.0 cm. Spindle 1 and a constant speed of 100 rpm were used for both fresh and frozen samples. After lowering the spindle carefully into the liquid until its indentation mark was reached, three readings were recorded following the procedure previously described.

Since the speed and spindle sizes varied between samples, values were converted to poises to facilitate comparison. The three values for each sample were averaged.

Paper electrophoresis

Samples of lipovitellin, lipovitellenin, and native yolk were prepared for electrophoresis by dispersing 1 g of the yolk or yolk fraction in 1 g deionized water. The yolk or fraction and water were weighed into a 50 ml beaker and hand stirred with a wooden applicator stick until the mixture was homogeneous. Livetin was sufficiently dilute to be used as obtained. Samples were placed in 10 x 75 mm tubes, covered with Parafilm, and allowed to reach room temperature before application to filter paper (Schleicher and Schuell, 2043-A mgl, 3.0 x 30.6 cm) strips.

Filter paper electrophoresis was conducted using a ridgepole electrophoresis cell, Spinco Model R. Veronal buffer (15.40 g sodium barbital and 2.76 g barbital per liter), pH 8.6, ionic strength 0.075 was used. The filter paper strips were wet with buffer solution and the cell was allowed to equilibrate thirty minutes before a micropipette and sample applicator were used to apply 0.006 ml of sample to each strip. For each sample duplicate strips were run. A constant current of five milliamperes was applied for eighteen hours at room temperature after which the strips were dried for fifteen minutes at 120°C.

To facilitate analysis of protein mobility, the denatured protein on the strips was stained. Following a six minute pre-rinse in methanol, the strips were immersed in alcoholic bromphenol blue solution (1 g dye per liter

of methanol) for thirty minutes. The strips were rinsed three times for fifteen minutes each in five per cent acetic acid before being blotted on filter paper and dried fifteen minutes at 120°C. Color was fixed by holding the strips in NH_4OH vapors for fifteen minutes.

In order to determine the electrophoretic patterns, the stained strips were analyzed using a Spinco Model RB Analatrol equipped with two 500 mμ filters and a B-5 cam, using a 1.0 mm slit width. Percentages of mobile and non-mobile fractions were calculated following visual examination of the electrophoretic patterns. Migration distance was measured in millimeters from the point of application to the most distant portion of the curve.

Analyses of Data

Two computer programs designed to perform statistical analysis using the CDC-3600 Computer at Michigan State University were used to calculate the desired statistical tests on the objective data. The Rand Routine and AOV Routine were used to calculate analysis of variance and standard deviations of the means. Significant differences detected by analyses of variance were further examined by graphing of interaction means and use of Studentized range tests (Duncan, 1957).

RESULTS AND DISCUSSION

The present investigation was primarily concerned with identifying the emulsifying properties of egg yolk and three crude egg yolk fractions: lipovitellin, lipovitellenin, and livetin. A second objective was to study the effects of freezing and thawing upon the emulsifying properties of egg yolk and all combinations of the three fractions.

Test emulsions were prepared by emulsifying oil in water using combinations of the three fractions in amounts proportional to those normally found in the egg yolk as well as using native yolk. Emulsion stability was determined for all emulsions by recording drainage at thirty-minute intervals for two hours. Similar emulsions, prepared with frozen egg yolk and egg yolk fractions, were evaluated in the same way. The viscosity and the electrophoretic mobility of egg yolk and the three fractions were measured by standard procedures before and after the samples had been frozen and thawed to determine the effects of gelation upon these parameters.

Chemical Analyses

Chemical determination of moisture and protein content of the native yolk and the three fractions--lipovitellin, lipovitellenin, and livetin--were conducted for three reasons. First, the determinations were necessary in order to correctly balance the emulsion formulae. Second, since a different lot of eggs from the same flock of hens was used in each replication, these determinations were conducted to detect differences in egg yolk composition between replications, and finally, these determinations were conducted on both fresh and frozen materials to determine whether changes in moisture or protein content accompanied the gelation reaction. Replicate averages for moisture and protein content and pH determinations of both fresh and frozen native yolk and fractions appear in the Appendix.

Moisture

The results of the analysis of variance for moisture is included in Table 7. As expected, lipovitellin, lipovitellenin, livetin, and native yolk were different from one another in respect to moisture content at the 0.1% level of probability (Table 8). There was no significant difference in moisture attributed to replications. Lipovitellin, lipovitellenin, and livetin all exhibted higher moisture contents than native yolk.

TABLE 7. Analyses of variance for differences in protein and moisture content among native yolk, lipovitellin, lipovitellenin, and livetin with two storage treatments--fresh and frozen

Source of Variance	Degrees of Freedom	Mean Squares	
		Moisture	Protein
Total	47		
Replications	5	1.71	1.14
Storage Treatment	1	0.08	0.01
Fractions	3	5907.11***	1618.60***
ST x F	3	0.35	0.03
Error	35	3.02	0.91

***Significant at the 0.1% level of probability.

TABLE 8. Treatment means, standard deviations, and significant differences for moisture and protein content of lipovitellin (A), lipovitellenin (B), livetin (C), and native yolk (Y) with two storage treatments

Factor	Storage Treatment	Treatment means/standard deviations[a]				Significant Differences at 0.1% level[b]
		A	B	C	Y	
Moisture	Fresh	56.47 ± 0.98	59.26 ± 2.65	97.91 ± 0.75	48.07 ± 0.59	C>B>A>Y
	Frozen	55.98 ± 1.93	59.46 ± 3.04	97.89 ± 0.80	48.15 ± 0.63	C>B>A>Y
Protein	Fresh	27.61 ± 1.80	7.39 ± 0.56	0.91 ± 0.14	16.31 ± 0.13	A>Y>B>C
	Frozen	27.78 ± 1.90	7.37 ± 0.53	0.91 ± 0.15	16.30 ± 0.09	A>Y>B>C

[a]Treatment means expressed as per cent ± standard deviation. Abbreviations given in title.

[b]Duncan, 1957.

This was due to the dilution involved in the separation procedure. The moisture of these fractions is certainly higher under these experimental conditions than it is when the fractions are found in their combined state as native yolk.

There were no significant differences in moisture content between the fresh and frozen fractions and between fresh and frozen samples of native yolk. This indicates that freezing and thawing did not change the binding of water in such a way that more or less of the water was removed by the moisture determination employed.

Protein

The analysis of variance for protein (Table 7) indicated a very highly significant difference in protein content among the three fractions and native yolk. Mean protein content and standard deviations are included in Table 8.

The lipovitellin fraction contained the highest percentage of protein on a wet basis and accounted for 52.36 per cent of the egg yolk's total protein. Lipovitellenin contributed 35.70 per cent of the yolk's total protein and the livetin fraction being extremely dilute, represented 11.09 per cent of the yolk's total protein.

The protein contents of the fractions and native yolk did not differ significantly among replications and

were not significantly greater after freezing and thawing. These findings are in agreement with those of Evans and Davidson (1953) who found no difference in protein content between fresh and stored shell eggs which had been layed at the same time by the same hens.

pH

The mean pH and standard deviations for the three fractions and native yolk for both storage treatments are shown in Table 9. Freezing did not appear to change the

TABLE 9. Means and standard deviations for pH determinations of fresh and frozen samples of native yolk, lipovitellin, lipovitellenin, and livetin

Storage Treatment	Fraction	Mean/standard deviation
Fresh	Native yolk	5.9 ± 0.08
	Lipovitellin	5.9 ± 0.16
	Lipovitellenin	6.3 ± 0.22
	Livetin	6.0 ± 0.23
Frozen	Native yolk	5.8 ± 0.10
	Lipovitellin	5.8 ± 0.08
	Lipovitellenin	6.4 ± 0.22
	Livetin	6.1 ± 0.25

pH of the native yolk, lipovitellin, lipovitellenin, or livetin. This is in agreement with the work of Lopez et al. (1954) who found that freezing did not alter the pH of egg yolk. The pH of lipovitellin was the same as that of the native yolk, whereas the pH values of lipovitellenin and livetin were more neutral due to the adjustment of pH necessary to effect their separation.

Emulsion Stability

The drainage data from the emulsion studies were analyzed to determine which factors influenced the stability of emulsions prepared with native yolk, or all combinations of lipovitellin, lipovitellenin, and livetin. The effect of storage treatment was determined by comparing drainage data from emulsions prepared with fresh and frozen egg yolk or fractions.

Recombined fractions vs. native yolk

The results of the analyses of variance for determining differences in emulsion stabilizing power between fresh and frozen native yolk and recombined fresh and frozen lipovitellin, lipovitellenin, and livetin appear in Table 10.

No significant differences in drainage between native yolk and recombined fraction emulsions or between the two storage treatments occurred at the thirty minute

TABLE 10. Analyses of variance for differences in emulsion stabilizing power between fresh and frozen native yolk and fresh and frozen recombined lipovitellin, lipovitellenin, and livetin

Drainage Time	Source of Variance	Degrees of Freedom	Mean Squares
30 minutes	Total	23	
	Replications	5	0.01
	Storage Treatment	1	0.02
	Fractions	1	0.01
	ST x F	1	0.02
	Error	15	0.01
60 minutes	Total	23	
	Replications	5	0.03
	Storage Treatment	1	0.12*
	Fractions	1	0.17***
	ST x F	1	0.07
	Error	15	0.02
90 minutes	Total	23	
	Replications	5	0.03
	Storage Treatment	1	0.12
	Fractions	1	0.45***
	ST x F	1	0.04
	Error	15	0.04
120 minutes	Total	23	
	Replications	5	0.04
	Storage Treatment	1	0.13
	Fractions	1	0.83***
	ST x F	1	0.02
	Error	15	0.06

*Significant at the 5% level of probability.

**Significant at the 1% level of probability.

***Significant at the 0.1% level of probability.

reading. However, after sixty, ninety, and one hundred and twenty minutes, emulsions prepared with native yolk had significantly less drainage than those prepared with the recombined lipovitellin, lipovitellenin, and livetin. The emulsions prepared with fresh yolk and recombined fractions drained less than those prepared with frozen yolk and frozen recombined fractions, but this difference was significant only for the sixty minute reading.

The mean drainage for these two emulsion formulae is plotted against time in Figure 2. It should be noted that although the two emulsion formulae differed significantly in stability, total drainage was small in both cases (Table 11). These findings indicate that the separation techniques may have changed the fractions in such manner that the emulsions prepared with recombined fractions decreased in stability to a greater extent as time increased than did those emulsions prepared with native yolk, with both emulsion formulae imparting equal stability at thirty minutes. Freezing influenced both emulsion formulae to the same extent, significantly increasing drainage at sixty minutes in both cases.

The differing emulsion stability of the two formulae may also be explained by the differences in pH of the emulsion mixtures. Replicate averages, means, and standard deviations for pH determinations of the emulsion formulae appear in the Appendix. The mean pH of emulsions

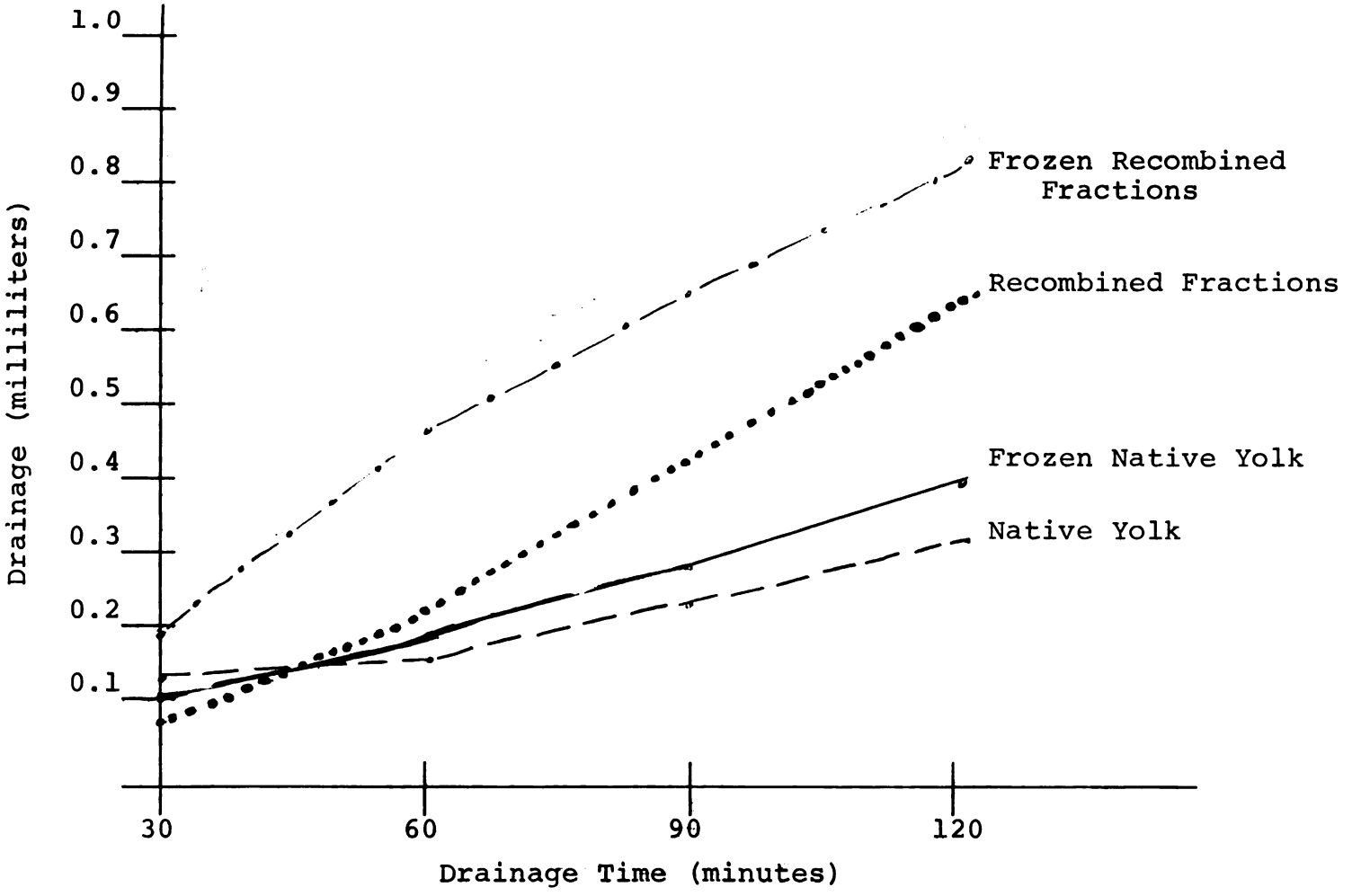


Figure 2. Amount of drainage plotted against drainage time for emulsions stabilized by fresh and frozen native yolk and fresh and frozen recombined fractions

TABLE 11. Treatment means and standard deviations for drainage at four time intervals from emulsions prepared with fresh and frozen native yolk (Y) and fresh and frozen recombined lipovitellin, lipovitellenin, and livetin (ABC)

Storage Treatment	Emulsions Stabilized by:	Drainage (ml) at four time intervals (min) 30	60	90	120
Fresh	ABC	0.08 ± 0.05	0.21 ± 0.06	0.41 ± 0.18	0.62 ± 0.26
	Y	0.11 ± 0.04	0.14 ± 0.04	0.22 ± 0.03	0.31 ± 0.04
Frozen	ABC	0.19 ± 0.13	0.45 ± 0.27	0.63 ± 0.32	0.83 ± 0.38
	Y	0.10 ± 0.00	0.18 ± 0.06	0.28 ± 0.09	0.39 ± 0.13

prepared with native yolk was 5.7 in comparison to a pH of 5.9 for emulsions prepared with the recombined fractions. More research is necessary to determine the effect of pH on the stability of emulsions prepared with egg yolk and egg yolk fractions.

Replicate averages for drainage from emulsions prepared with fresh and frozen native yolk and the recombined fractions appear in the Appendix.

Combinations of lipovitellin, lipovitellenin, and livetin

The results of the analysis of variance for determination of the effects of the presence of lipovitellin, lipovitellenin, and livetin as well as the effects of storage treatment, replication, and drainage time on the stability of oil-in-water emulsions appear in Table 12. A table of the means used in the interpretation of the analysis appears in the Appendix.

Effects of lipovitellin, lipovitellenin, and livetin on emulsion drainage.--The presence of lipovitellin, lipovitellenin, or livetin in the emulsion formula reduced emulsion drainage significantly, at the 0.1% level of probability. The effects of the protein fractions *per se* are depicted in Figure 3. Although all three fractions were effective emulsion stabilizers, lipovitellenin reduced drainage by 7.1 ml and thus, provided the best overall

TABLE 12. Analysis of variance for determination of the effects of fractions, storage treatment, replications, and drainage time on the stability of emulsions prepared with all possible combinations of lipovitellin, lipovitellenin, and livetin

Source of Variance	D.F.	Mean Squares
Total	383	
Replications	5	0.85
Storage Treatment	1	2.39
Drainage Time	3	24.24***
A[a]	1	328.39***
B	1	4815.67***
C	1	136.83***
A x B	1	69.91***
B x C	1	27.14***
A x C	1	24.24***
A x B x C	1	0.56
A x ST	1	2.54
B x ST	1	1.22
C x ST	1	0.98
A x B x ST	1	4.31
B x C x ST	1	2.78
A x C x ST	1	0.01
A x B x C x ST	1	0.21
ST x DT	3	0.01
A x DT	3	0.03
B x DT	3	0.30
C x DT	3	0.02
A x B x DT	3	3.94***
B x C x DT	3	2.02***
A x C x DT	3	0.07*
A x B x C x DT	3	0.22***
A x ST x DT	3	0.03
B x ST x DT	3	0.04
C x ST x DT	3	0.04
A x B x ST x DT	3	0.03
B x C x ST x DT	3	0.01
A x C x ST x DT	3	0.00
A x B x C x ST x DT	3	0.06
Remaining Error	240	0.03

*Significant at the 5% level of probability.

***Significant at the 0.1% level of probability.

[a]A denotes lipovitellin.

B denotes lipovitellenin.

C denotes livetin.

ST denotes storage treatment.

DT denotes drainage time.

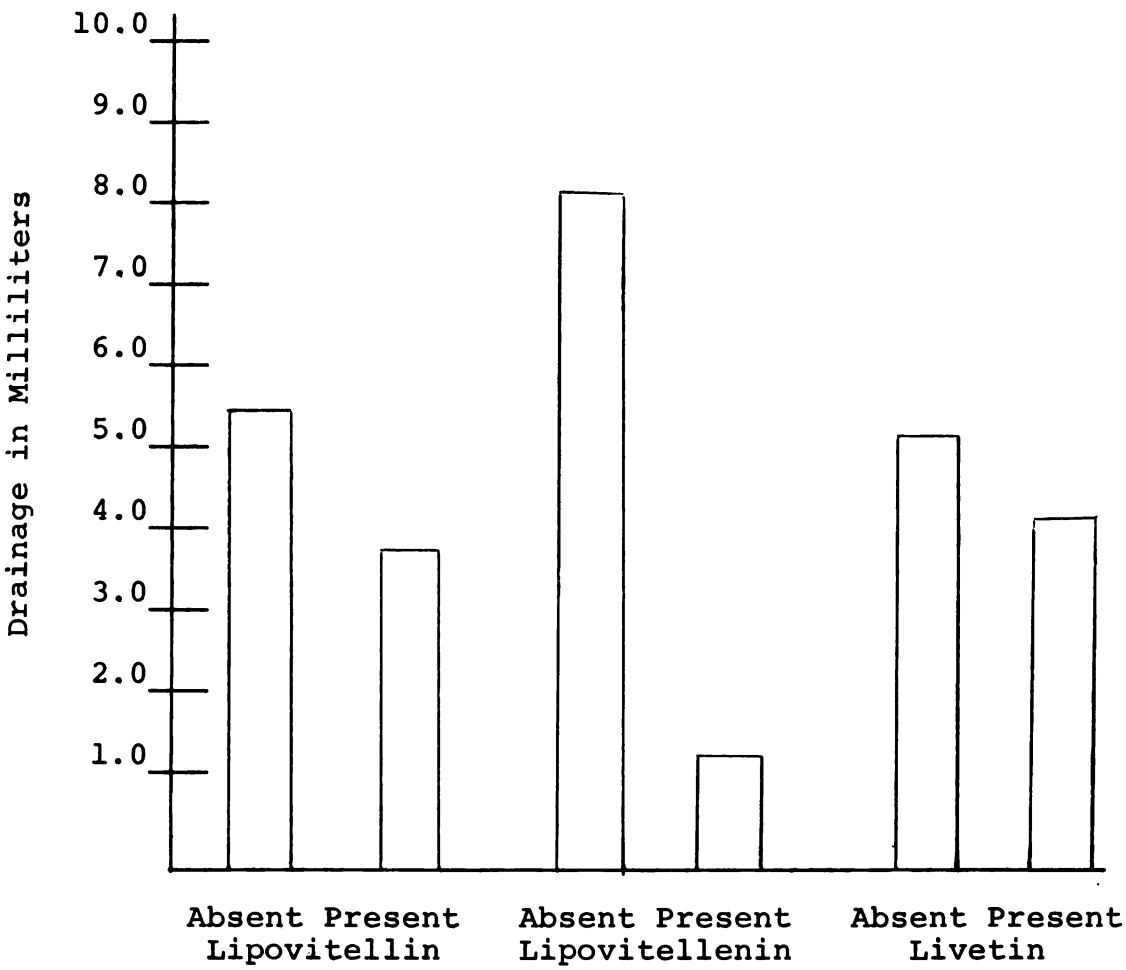


Figure 3. Bar-graph of emulsion drainage depicting the main effects of lipovitellin, lipovitellenin, and livetin in emulsion stabilization

stabilizing effect of the three. Lipovitellin reduced drainage by 1.3 ml, and livetin reduced drainage by 1.1 ml in effects which were similar.

Very highly significant first-order interactions occurred when any two of the three fractions were combined. These interactions showed that when two fractions were combined, their emulsion stabilizing powers were not independent.

Table 13 indicates the responses of lipovitellenin and lipovitellin to one another. The presence of both lipovitellin and lipovitellenin in the emulsion formula reduced emulsion stability by 0.3 ml, in comparison to the effects of both fractions alone.

TABLE 13. Interaction responses of lipovitellin and lipovitellenin to one another

Lipovitellin	Lipovitellenin Absent	Lipovitellenin Present	Response to Presence of Lipovitellenin
	ml	ml	ml
Absent	8.1	1.5	-6.6
Present	6.8	0.5	-6.3
Response to Presence of Lipovitellin	-1.3	-1.0	Interaction -0.3

Table 14 indicates the responses of lipovitellenin and livetin in respect to the presence of one another. Lipovitellenin and livetin were also antagonistic toward one another in emulsion stabilization and the magnitude of this antagonism was -1.0 ml.

The responses of lipovitellin and livetin to one another in emulsion formulae appear in Table 15. In this case, the antagonistic interaction effect was also -1.0 ml.

TABLE 14. Interaction responses of lipovitellenin and livetin to one another

Livetin	Lipovitellenin Absent	Lipovitellenin Present	Response to Presence of Lipovitellenin
	ml	ml	ml
Absent	9.0	1.4	-7.6
Present	7.3	0.7	-6.6
Response to Presence of Livetin	-1.7	-0.7	Interaction -1.0

TABLE 15. Interaction responses of lipovitellin and livetin to one another

Livetin	Lipovitellin Absent	Lipovitellin Present	Response to Presence of Lipovitellin
	ml	ml	ml
Absent	6.3	4.0	-2.3
Present	4.6	3.3	-1.3
Response to Presence of Livetin	-1.7	-0.7	Interaction -1.0

These antagonistic interactions which occurred when two fractions were present in the emulsion mixture suggested that a major decrease in stability might be observed when all three fractions were present in the emulsion system. This was not the case. The lack of any

significant interaction when all fractions were present in the emulsions indicated that all of the fractions in this case reduced drainage in the manner to be expected from the consideration of fractions per se. This may be partially explained by a consideration of the mean drainage from the eight emulsion formulae (Table 16). Combinations of any two fractions promoted greater stability than either of the fractions alone, although the very highly significant first-order interactions indicated that the stability of these emulsions was not as great as would be expected from independent action of the fractions per se. When all fractions were recombined, stability was the best, being equal to the combined independent action of the fractions.

Becher (1965) stated that in oil-in-water emulsions, inversion to water-in-oil will occur if the lecithin/cholesterol ratio falls below 8:1. Lipovitellenin contains approximately thirty-eight per cent lecithin and six percent cholesterol for a lecithin/cholesterol ratio of 6.33:1, and yet this fraction was the most effective of the three fractions in promoting emulsion stability. Since most of the cholesterol of the egg yolk is obtained from the lipovitellenin fraction, further imbalance of the lecithin/cholesterol ratio when another fraction is added is unlikely.

TABLE 16. Mean drainage and standard deviations for drainage from emulsions prepared from fresh and frozen lipovitellin (A), lipovitellenin (B), and livetin (C) and read at four time intervals

Storage Treatment	Egg Yolk Fractions[a]	Mean/Standard Deviation for Drainage at: 30'	60'	90'	120'
		ml	ml	ml	ml
Fresh	0	10.8 ± 0.44	10.9 ± 0.44	11.0 ± 0.44	11.0 ± 0.46
	A	6.6 ± 0.72	7.2 ± 0.59	7.5 ± 0.54	7.7 ± 0.52
	B	0.7 ± 0.44	1.7 ± 0.98	2.3 ± 0.87	2.7 ± 0.91
	C	7.7 ± 0.36	8.3 ± 0.34	8.5 ± 0.32	8.7 ± 0.29
	AB	0.1 ± 0.04	0.3 ± 0.16	0.7 ± 0.26	1.0 ± 0.28
	BC	0.2 ± 0.10	0.7 ± 0.26	1.1 ± 0.41	1.5 ± 0.51
	AC	4.7 ± 1.25	5.9 ± 1.05	6.3 ± 1.00	6.6 ± 0.85
	ABC	0.1 ± 0.05	0.2 ± 0.06	0.4 ± 0.18	0.6 ± 0.26
Frozen	0	10.1 ± 0.54	10.3 ± 0.53	10.3 ± 0.50	10.4 ± 0.52
	A	6.7 ± 0.40	7.3 ± 0.21	7.7 ± 0.36	8.1 ± 0.53
	B	0.9 ± 0.33	2.1 ± 0.55	2.8 ± 0.59	3.3 ± 0.55
	C	7.6 ± 0.78	8.3 ± 0.58	8.6 ± 0.51	8.7 ± 0.42
	AB	0.3 ± 0.19	0.6 ± 0.33	1.0 ± 0.51	1.2 ± 0.59
	BC	0.4 ± 0.09	0.9 ± 0.18	1.3 ± 0.27	1.7 ± 0.35
	AC	5.6 ± 0.84	6.5 ± 0.68	6.9 ± 0.63	7.2 ± 0.57
	ABC	0.2 ± 0.13	0.5 ± 0.27	0.6 ± 0.32	0.8 ± 0.38

[a]Letters denote the fractions used in the oil-in-water emulsions.

0 = no fractions.

Harkins and Zollman (1926) stated that oil-in-water emulsions form readily if interfacial tension is below ten dynes. The research of Vincent et al. (1966) indicated that lipovitellin has a high interfacial tension and livetin a low interfacial tension. Yet the stability of emulsions prepared with lipovitellin and livetin were similar, with lipovitellin being slightly more effective in promoting emulsion stability. Solutes which lower interfacial tension are efficient emulsifiers, but low interfacial tension apparently does not influence emulsion stability.

The findings of this study, indicate that the mechanism of emulsion stabilization by egg yolk is complex, and do not contradict the statement by Yeadon et al. (1958) that "those phosphatide preparations possessing good emulsifying characteristics are presumably mixtures or complexes of the phosphatides with other substances."

Drainage between storage treatments.--No significant difference in drainage was detected between emulsions prepared with fresh yolk fractions and emulsions prepared with fractions which had been frozen. In this experiment, freezing was not detrimental to the emulsion stabilizing power of any fraction or fraction combination, however, when the mean drainage from the fresh emulsions was compared to that of the frozen emulsions, (Table 16) there

was a trend toward greater drainage when frozen fractions were used. This trend may become a significant decrease in stability in emulsions where only a small proportion of egg yolk is employed as the stabilizing agent. Miller and Winter (1951) stated that the amount of frozen egg yolk in mayonnaise could be reduced from 13.5 per cent to 8.6 per cent of the formula with no effect on stability. The trend for frozen yolk emulsions to be less stable than fresh yolk emulsions, in the present study, does not lend support to their suggestion. It appears that as Kilgore (1935) suggested, more frozen than fresh yolk would produce mayonnaises of equal stability, although equal quantities of fresh and frozen yolk could produce products with acceptable stability. The viscosity of the emulsions in this study were not examined, and while no great differences in viscosity were noted by this investigator, frozen yolk may significantly effect the viscosity of the emulsion system of mayonnaise.

Drainage variance among time intervals.--The time at which drainage from the emulsions was read produced a very highly significant main effect on the magnitude of the drainage (Table 12). The mean drainage curve for all emulsions (Figure 4) showed that drainage from the emulsions was greatest in the first thirty minutes, after which there was a leveling off with a significant and

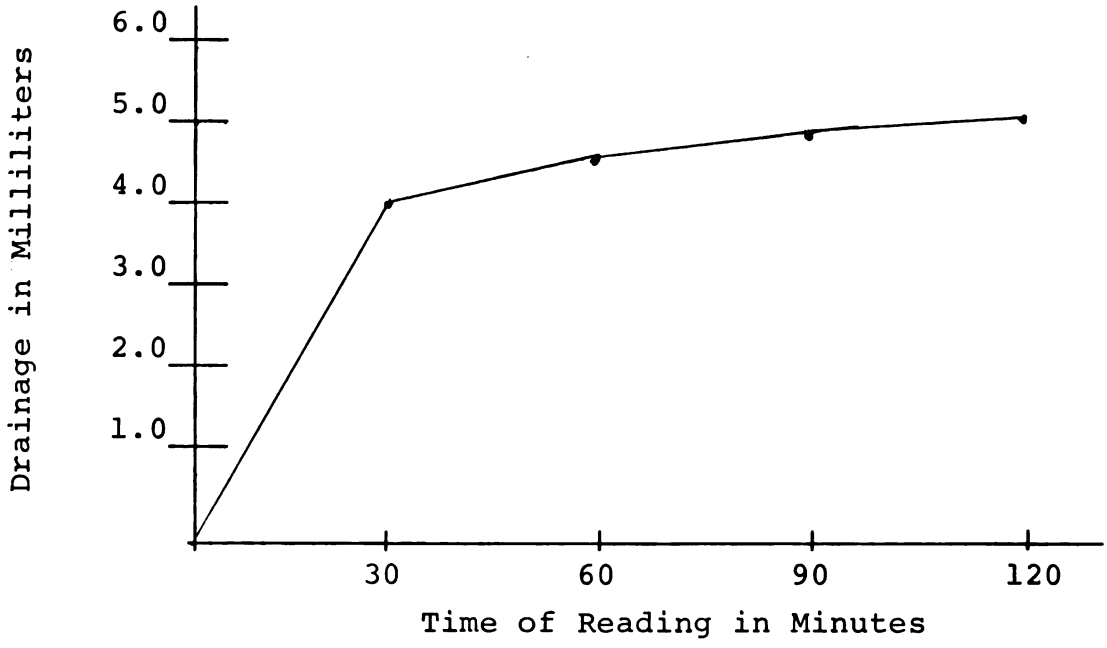


Figure 4. Mean drainage from all emulsions plotted against the times at which the readings were taken

nearly linear increase in drainage from thirty to one hundred and twenty minutes.

The use of frozen fractions in the emulsions had no effect on the rate of drainage from the emulsions, as indicated by the low mean square for the interaction of storage treatment with drainage time (Table 12). Since storage treatment had no significant effect, drainage data from both fresh and frozen fraction emulsions were averaged in the explanation of significant interactions of drainage and fraction combinations.

Interaction of drainage time and fraction combinations.--The lack of significant interactions of drainage time with each of the three fractions indicated

that the magnitude of drainage for these fractions was the same between thirty and one hundred and twenty minutes, although as previously stated, the presence of any fraction significantly reduced drainage.

The magnitude of significance in the second-order interactions of drainage time with combinations of any two of the three fractions and an examination of the first-order interaction mean squares for drainage time and fractions revealed that the presence of lipovitellenin was an important, though not exclusive, factor in the second-order interactions. The mean square for the interaction of drainage time and lipovitellenin, while not significant at the 5% level of probability, was greater than the mean squares of corresponding interactions of drainage time with lipovitellin or livetin. The second-order interaction of drainage time with livetin and lipovitellin was significant at the 5% level of probability, whereas corresponding second-order interactions which included lipovitellenin were significant at the 0.1% level of probability.

The interaction of drainage time with lipovitellin and lipovitellenin is depicted in Figure 5. It is apparent that the initial drainage--from zero to thirty minutes--was greatest when the lipovitellenin fraction was not present.

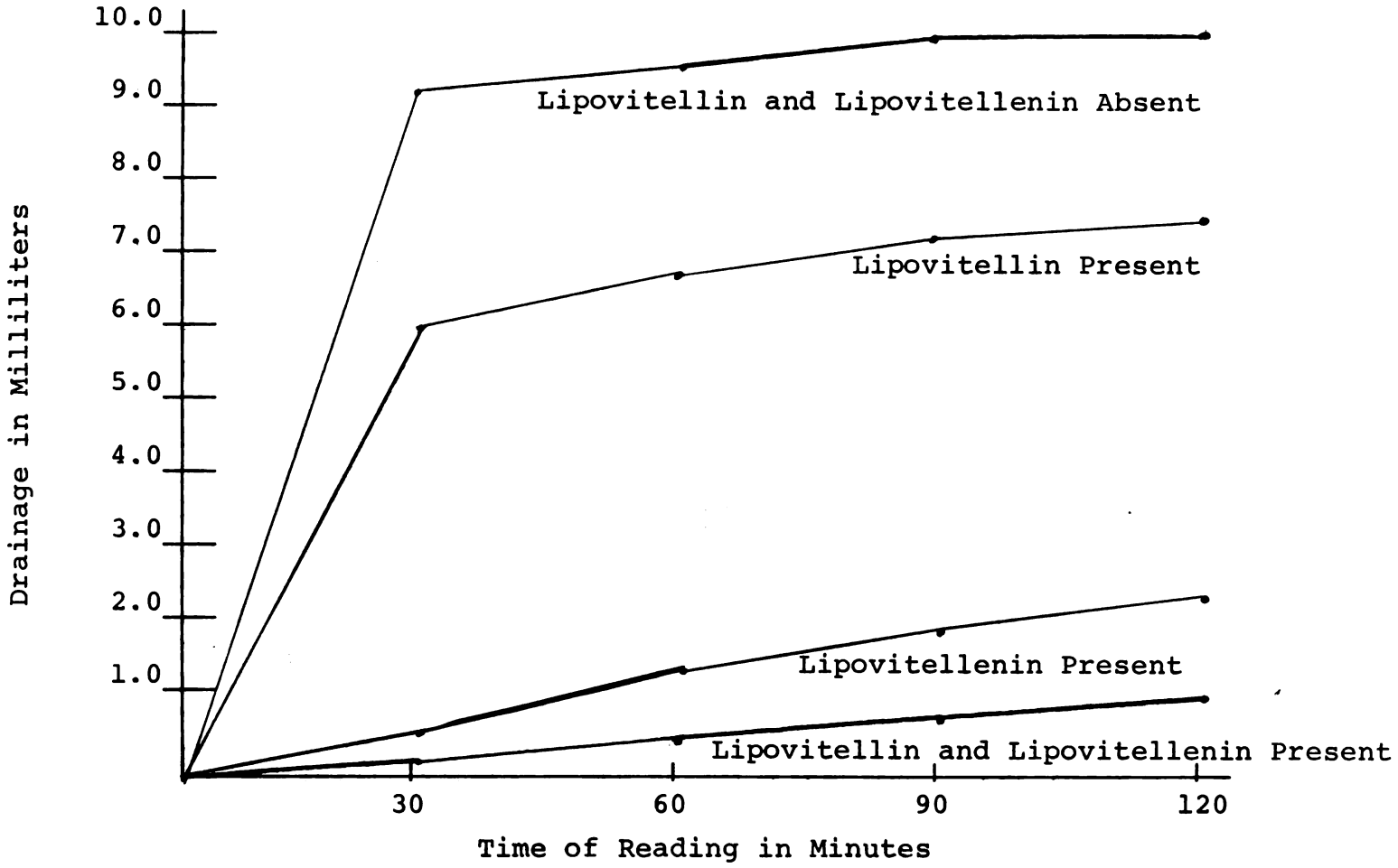


Figure 5. Interaction of drainage time with lipovitellin and lipovitellenin

Figure 6 depicts the interaction of drainage time with lipovitellenin and livetin. Again, the presence of lipovitellenin greatly reduced the initial drainage, while increasing the rate of drainage from thirty to one hundred and twenty minutes.

The interaction of drainage time, lipovitellin, and livetin, significant at the 5% level of probability, is depicted in Figure 7. Lipovitellin and livetin both reduced initial drainage, although not to the extent of the lipovitellenin reduction (Figures 5 and 6). The rate of drainage from thirty to one hundred and twenty minutes was essentially the same for these emulsion formulae, indicating that neither lipovitellin, livetin, nor their combination was very effective in reducing the rate of drainage from thirty to one hundred and twenty minutes.

A comparison of Figures 5, 6, and 7 indicates that the presence of lipovitellenin reduced the drainage rate from zero to thirty minutes, but increased the rate of drainage from thirty to one hundred and twenty minutes. Lipovitellin interacted with lipovitellenin (Figure 5) to reduce the rate of drainage from thirty to one hundred and twenty minutes, while it did not greatly reduce this rate when present alone. Livetin interacted with lipovitellenin in a similar manner (Figure 7), but did not reduce drainage in the presence of lipovitellenin

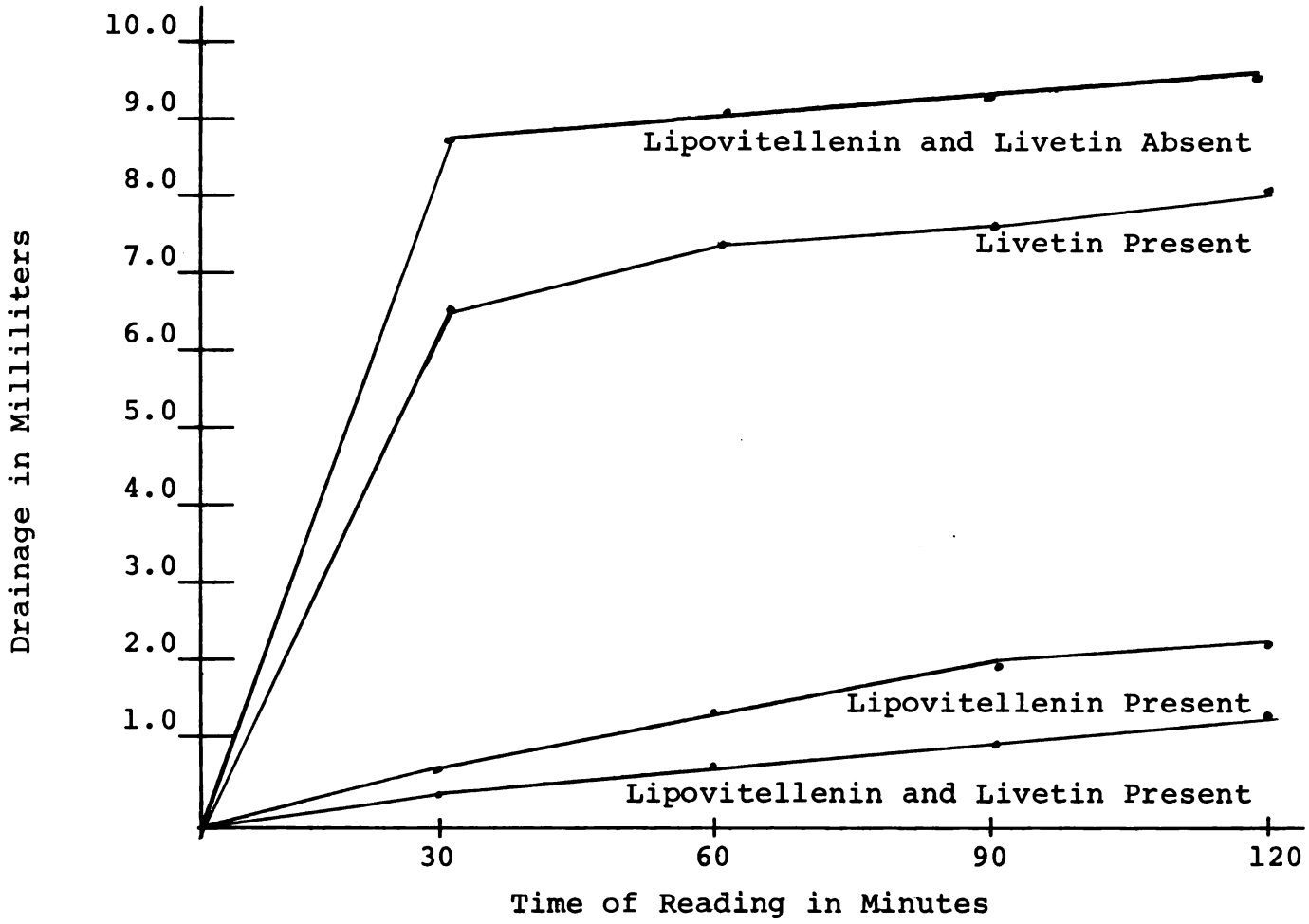


Figure 6. Interaction of drainage time with lipovitellenin and livetin

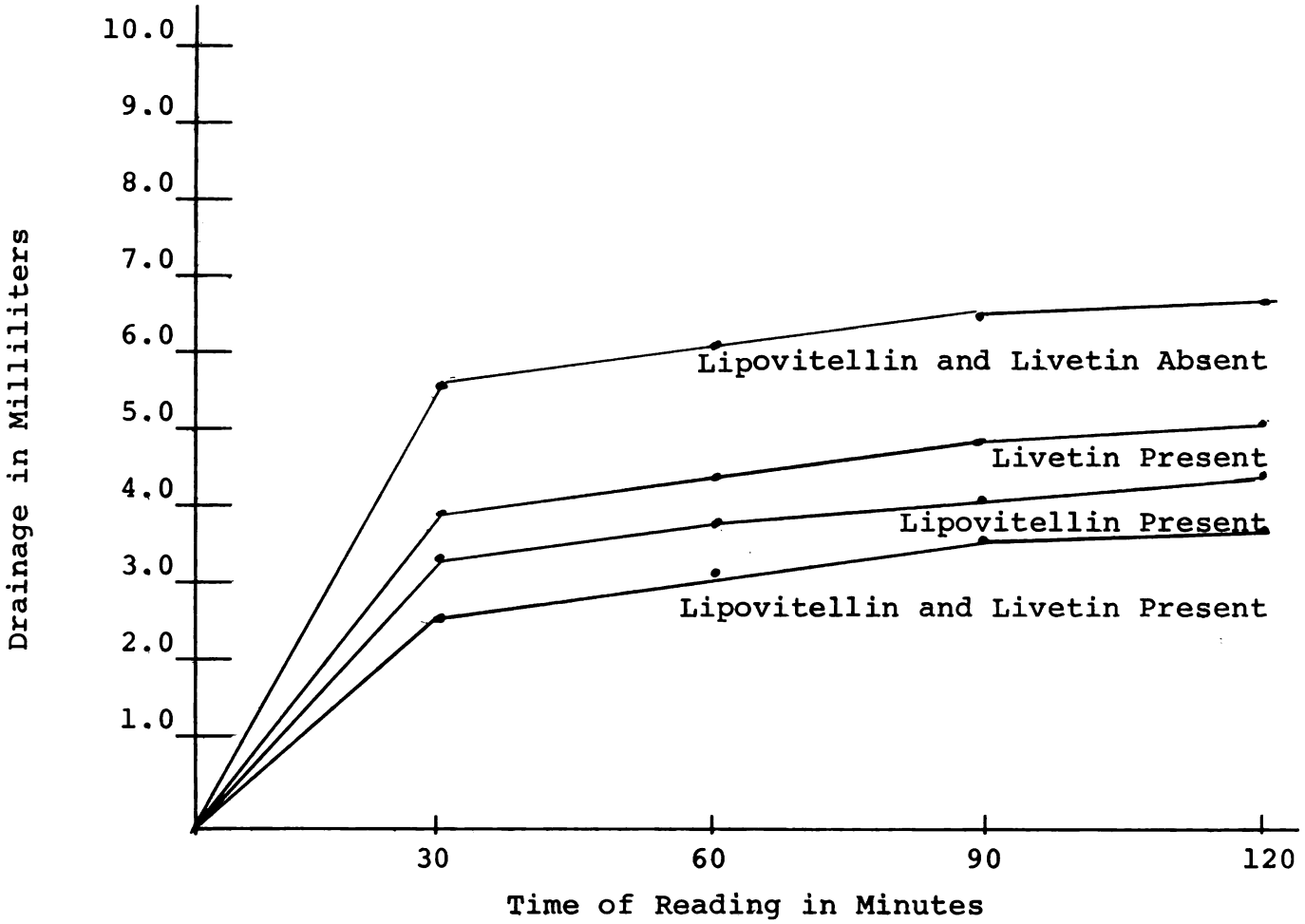


Figure 7. Interaction of drainage time with lipovitellin and livetin

as much as the lipovitellin-lipovitellenin combination. Although both livetin and lipovitellin reduced initial drainage somewhat, neither of them nor their combination effected a reduction in drainage from thirty to one hundred and twenty minutes.

The third-order interaction of drainage time with lipovitellin, lipovitellenin, and livetin is depicted in Figure 8. Drainage from each fraction combination emulsion from zero to thirty minutes and the increase in drainage from thirty to one hundred and twenty minutes is shown in Table 17. Examination of this interaction gives the best overall picture of the manner in which the three fractions function to promote emulsion stability.

The highest initial drainage occurred when none of the fractions were present, although in this case subsequent drainage was low. Thus, the maximum drainage which could occur in this emulsion system was apparently achieved in the early stages of drainage. Lipovitellenin was more effective in reducing initial drainage than lipovitellin or livetin. When more than one fraction was present, the comparison of initial drainage to subsequent drainage is found to be more than a simple effect of different rates of drainage toward a maximum. Both lipovitellin and livetin, when present with lipovitellenin, further reduced initial drainage, while counteracting lipovitellenin's effect to increase subsequent drainage. While the

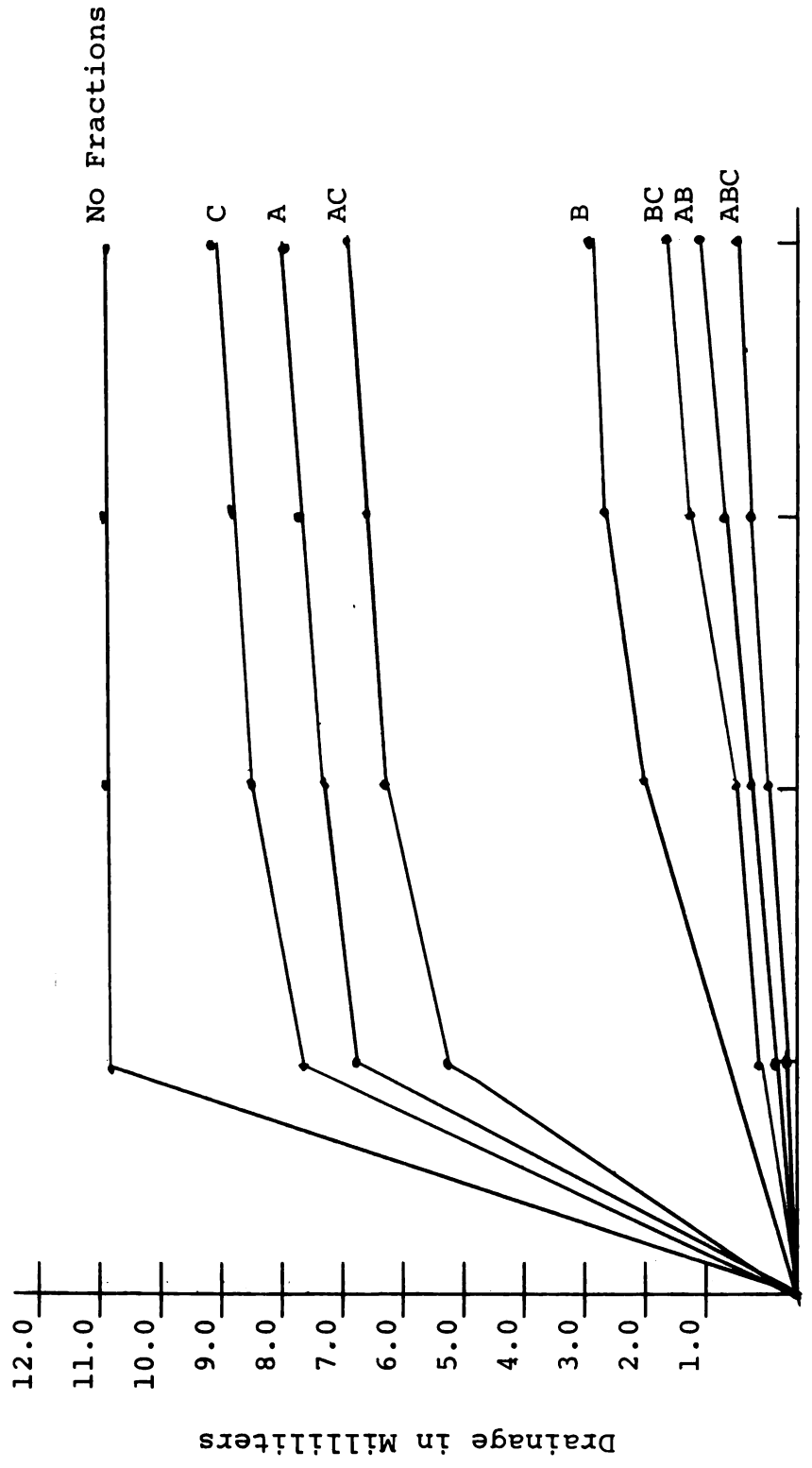


Figure 8. Interaction of drainage time with lipovitellin (A), lipovitellenin (B), and livetin (C)

TABLE 17. Drainage from zero to thirty minutes and the increase in drainage from thirty to one hundred and twenty minutes for emulsions prepared with combinations of lipovitellin, lipovitellenin, and livetin

Fractions in Emulsions[a]			Drainage from:	
Lipovitellin	Lipovitellenin	Livetin	0 to 30'	30 to 120'
			ml	ml
-	-	-	10.5	0.2
+	-	-	6.7	1.2
-	+	-	0.8	2.2
-	-	+	7.7	1.0
+	+	-	0.2	0.9
-	+	+	0.3	1.3
+	-	+	5.1	1.8
+	+	+	0.1	0.6

[a]Presence denoted by +.

Absence denoted by -.

lipovitellenin and livetin fractions together did reduce drainage from thirty to one hundred and twenty minutes, the reduction was only slightly less than that of livetin alone. In the presence of both lipovitellin and livetin, initial drainage was slightly reduced, while subsequent drainage was greater than that observed when either lipovitellin or livetin was present alone. Only when all three fractions were present was the best emulsion stability obtained. All three fractions functioned to reduce initial drainage to a minimum. The high drainage from thirty to one hundred and twenty minutes which was apparent when only lipovitellenin or the combination of lipovitellin and livetin were present was, in this case, compensated by the ability of lipovitellin-lipovitellenin and lipovitellenin-livetin combinations to reduce subsequent drainage. When all fractions were present, these complex interactions, which can be resolved only when drainage time is considered, produced effects upon total drainage which suggested independent action of the three fractions.

Relative Viscosity

Replicate averages for the relative viscosity of fresh and frozen lipovitellin, lipovitellenin, livetin and native yolk appear in the Appendix. Means and standard deviations are listed in Table 18. An analysis of variance test was not conducted since the prerequisite

TABLE 18. Means and standard deviations for the relative viscosity of both fresh and frozen lipovitellin, lipovitellenin, livetin, and native yolk

Fraction	Storage Treatment	Mean	Standard Deviation
		poises	poises
Lipovitellin	Fresh	1.64×10^6	0.84×10^6
	Frozen	14.26×10^6	27.00×10^6
Lipovitellenin	Fresh	8.14×10^5	6.38×10^5
	Frozen	44.71×10^5	45.71×10^5
Livetin	Fresh	0.16	0.03
	Frozen	0.18	0.05
Native Yolk	Fresh	1.37×10^2	0.11×10^2
	Frozen	1.62×10^6	0.25×10^6

of equal variance was obviously not fulfilled. The extremely large standard deviations for relative viscosity determinations of fresh and frozen lipovitellin and lipovitellenin indicated that these fractions differed greatly in viscosity from one replication to another. This could have been due to several factors. The spindle of the Brookfield viscometer tended to cut a path within these highly viscous samples, rather than rotate through them. Also, the small differences in moisture content between replications for these samples may have resulted in larger

changes in relative viscosity. Finally, the consistency of the lipovitellin fraction samples particularly, tended to remain somewhat lumpy, despite efforts of homogenization. An examination of the means and replicate averages for relative viscosity does, however, establish definite patterns of viscosity change.

Native yolk

Freezing and thawing increased the relative viscosity of native yolk from 1.37×10^2 to 1.62×10^6 poises. This increase, indicating that gelation had occurred, is a sign of protein denaturation as expressed by Gortner (1949). Forsythe et al. (1953) found that the thick gel-like consistency of frozen whole egg fortified with yolk had no adverse effects on its performance in sponge cakes, however, Pearce and Lavers (1949) pointed out that viscous frozen yolk is difficult to combine with other ingredients and Jordan et al. (1952) were unable to prepare satisfactory custards or plain cakes with untreated frozen egg yolk.

Lipovitellin

The relative viscosity of lipovitellin was influenced in an unpredictable manner by freezing and thawing. The relative viscosity of frozen lipovitellin for two replications was actually lower than that of the unfrozen

sample, although in all other replications, relative viscosity increased after freezing and thawing with the average increase of 1.26×10^7 poises being the greatest change associated with any fraction. Powrie et al. (1963) examined the relative viscosity of a lipovitellin solution containing $CaCl_2$. They found that the relative viscosity of the solution increased after freezing and thawing, indicating that structural changes of the lipovitellin's proteins had occurred. Lea and Hawke (1952), in their study on the effects of freezing on the solubility of lipovitellin also had a great deal of variability in their results.

Lipovitellenin

Frozen and thawed lipovitellenin had a higher relative viscosity than fresh lipovitellenin for all replications. The average increase was 3.66×10^6 poises--approximately a four-fold increase in relative viscosity. The lipovitellenin fraction contributed to the high relative viscosity of frozen native yolk as indicated by Fevold and Lausten (1946) and Feeney and Hill (1960). Saari et al. (1964b), studying the effects of freezing on a low-density lipoprotein solution (pH 4.0), indicated that freezing and thawing low-density egg yolk lipoprotein resulted in gel formation. Such a gel was noted by this

investigator, in conjunction with a definite loss of glossiness after the lipovitellenin fraction had been frozen and thawed.

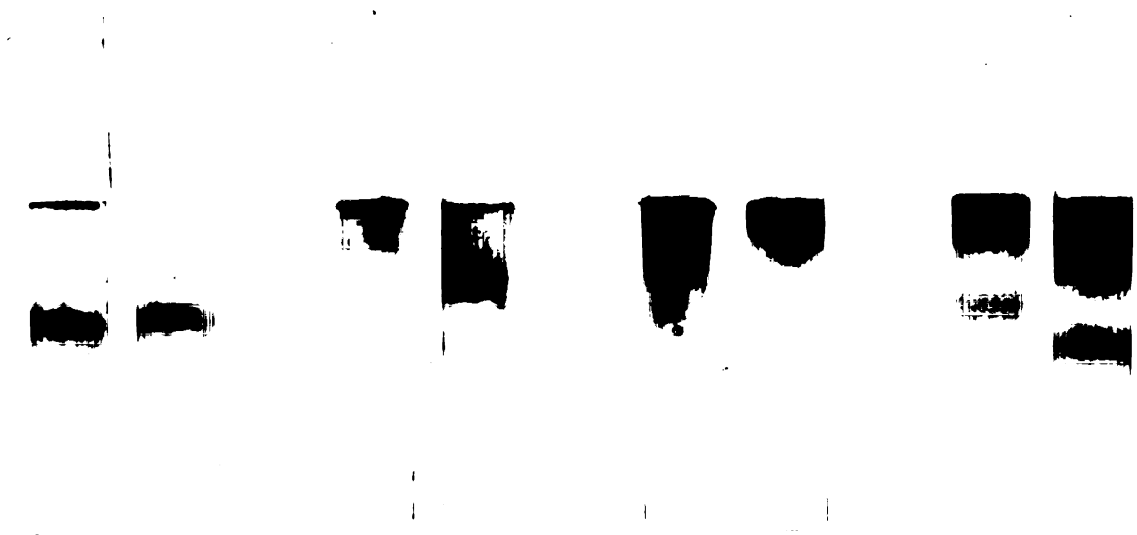
Livetin

The relative viscosity of livetin remained essentially the same after freezing and thawing. Gelation does not seem to involve livetin, perhaps because it is not a lipoprotein.

It is apparent that the increase in relative viscosity which occurs when native yolk is frozen and thawed is due to changes in both the lipovitellin and lipovitellenin fractions of egg yolk. Further studies of the effects of the gelation reaction are necessary, using highly purified and further sub-divided samples of lipovitellenin and lipovitellin.

Paper Electrophoresis

Filter paper electrophoresis was conducted to determine the effect of freezing and thawing on the proteins of lipovitellin, lipovitellenin, livetin, and native yolk. This procedure also aided in identifying the components of the three crude protein fractions. Typical electrophoretic patterns are depicted in Figure 9. Migration distances and percentages of mobile and non-mobile protein for each replication are listed in the



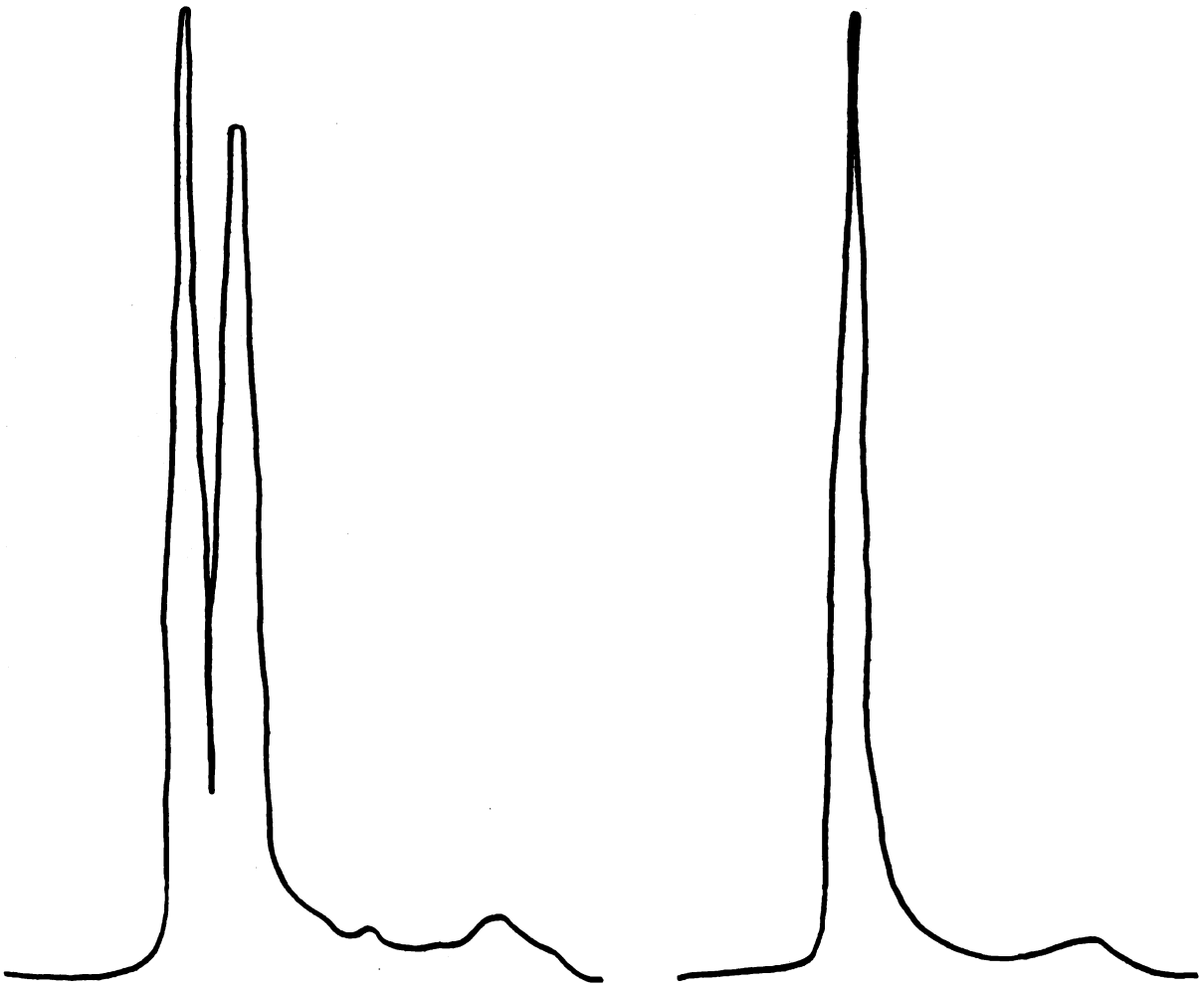
Frozen Fresh Livetin | Frozen Fresh Lipovitellenin | Frozen Fresh Lipovitellin | Frozen Fresh Native Yolk

Figure 9. Typical dyed paper strips for samples of both fresh and frozen livetin, lipovitellenin, lipovitellin, and native yolk

Appendix. Migration to the left of the origin was calculated, but since it was considered to be mainly due to spreading of the sample, it was included as part of the non-mobile protein. The migration to the left could also have been due to a very slight contamination of the samples with egg white lysozyme, which becomes colored with dye in this location, but does not contribute greatly to the area under the curve of the electrophoretic pattern. An analysis of the proportional composition of the native yolk was not attempted, as difficulty in the application of some samples led to an indeterminate sample volume.

Native yolk

Typical electrophoretic patterns of fresh and frozen native yolk (Figure 10) indicated that the proteins of egg yolk were changed by the gelation phenomenon. Fresh native yolk contained an average of 35.0 per cent non-mobile protein and 65 per cent mobile protein, while in frozen samples, the average amount of non-mobile protein increased to an average of 51.3 per cent and mobile protein decreased to 48.7 per cent. These findings are in agreement with those of Meyer and Woodburn (1965) who indicated that freezing increased the proportion of non-mobile protein in native yolk. The large ranges in migration distance are consistant with the findings of Evans et al. (1958) that the yolk proteins of eggs from individual hens



Fresh

Mean Migration - 48.9 mm
Mobile Protein - 65.0%
Non-Mobile Protein - 35.0%

Frozen

Mean Migration - 48.3 mm
Mobile Protein - 48.7%
Non-Mobile Protein - 51.3%

Native Yolk

Figure 10. Typical paper electrophoretic patterns of fresh and frozen native yolk

vary greatly in speed of migration. A comparison of the typical stained paper strips of lipovitellin, lipovitellenin, livetin, and native yolk (Figure 9) indicated that overlap occurs when all fractions are combined as was indicated by Sugano (1958).

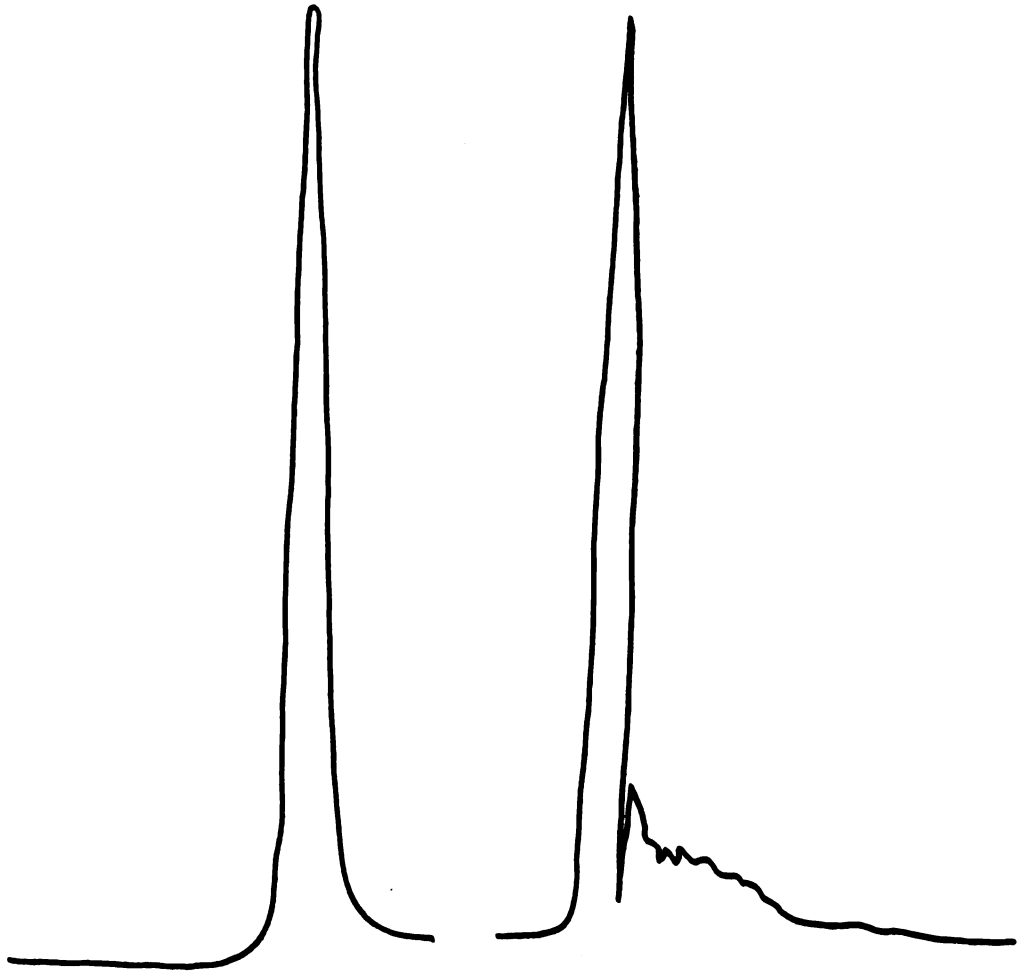
The fraction responsible for the change in protein mobility of native yolk after freezing may be found by an examination of the electrophoretic data from the fresh and frozen fractions.

Lipovitellin

The mean migration distance of lipovitellin remained essentially unchanged after freezing, and little change in the mobility was noted (Figures 9 and 11). These findings indicated that lipovitellin was not mobile either before or after freezing, under the conditions of this research. The findings of Bernardi and Cook (1960a) indicated that the slight mobility observed was due to the presence of γ-livetin, phosvitin, and the low-density lipoprotein in the crude lipovitellin fraction. These results substantiate the non-mobile nature of lipovitellin found by Powrie et al. (1963).

Lipoyitellenin

Freezing caused a dramatic change in the electrophoretic patterns of lipovitellenin (Figure 12). The



Fresh

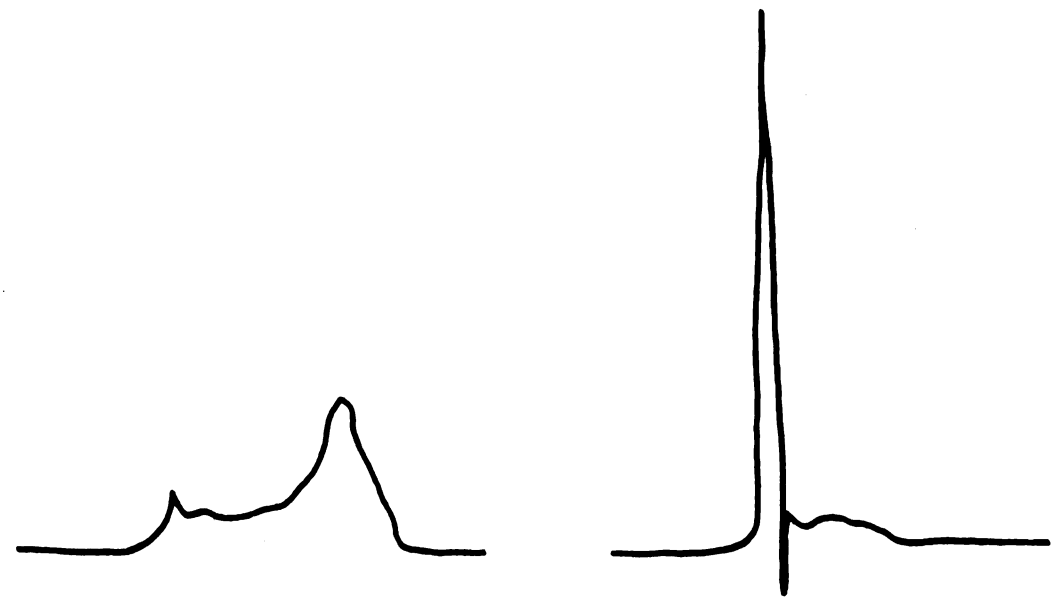
Mean Migration - 44.3 mm
Mobile Protein - 28.7%
Non-Mobile Protein - 71.3%

Frozen

Mean Migration - 46.8 mm
Mobile Protein - 26.7%
Non-Mobile Protein - 73.3%

Lipovitellin

Figure 11. Typical paper electrophoretic patterns of fresh and frozen lipovitellin



Fresh

Mean Migration - 37.6 mm
Mobile Protein - 89.3%
Non-Mobile Protein - 10.7%

Frozen

Mean Migration - 24.3 mm
Mobile Protein - 32.6%
Non-Mobile Protein - 67.4%

Lipovitellenin

Figure 12. Typical paper electrophoretic patterns of fresh and frozen lipovitellenin

majority of protein in this fraction was mobile when fresh samples were analyzed, but after freezing, the majority of the protein was non-mobile. Examination of typical stained paper strips for fresh and frozen lipovitellenin samples (Figure 9) revealed the presence of a slow-migrating protein after freezing, in results similar to those of Powrie et al. (1963). These researchers showed that this slow-migrating protein was not lipovitellenin, which became non-mobile after freezing. They indicated that in fresh samples the slow-migrating fraction, which is unidentified at present, was carried a greater distance by the more mobile lipovitellenin.

Livetin

Typical electrophoretic patterns of livetin (Figure 13) indicated the presence of α- and β-livetins in this fraction. The increase in non-mobile protein after freezing was probably due to contamination with lipovitellenin in the separation technique. Migration distances remained essentially the same for fresh and frozen samples.

Fresh

Mean Migration - 54.8 mm
Mobile Protein - 95.9%
Non-Mobile Protein - 4.1%

Frozen

Mean Migration - 55.8 mm
Mobile Protein - 87.6%
Non-Mobile Protein - 12.4%

Livetin

Figure 13. Typical paper electrophoretic patterns of fresh and frozen livetin

SUMMARY AND CONCLUSIONS

This investigation was primarily concerned with identifying the emulsifying properties of three crude egg yolk fractions: lipovitellin, lipovitellenin, and livetin. A second objective was to study the effects of freezing and thawing upon the emulsifying properties of egg yolk and all combinations of the three fractions.

Test emulsions were prepared by emulsifying oil in water using combinations of the three fractions in amounts proportional to those normally found in the egg yolk, as well as using native yolk. Emulsion stability was determined for all emulsions by recording drainage at thirty-minute intervals for two hours. Similar emulsions, prepared with frozen egg yolk and egg yolk fractions were evaluated in the same manner. The viscosity and electrophoretic mobility of egg yolk and the three fractions were measured by standard procedures before and after the samples had been frozen and thawed, to determine the effects of gelation upon these parameters. All data reported are the average of six replications.

Lipovitellin, lipovitellenin, livetin and native yolk differed from one another in respect to moisture and

protein content (0.1% level of probability). Freezing and thawing did not significantly influence these determinations.

A comparison of the emulsion stabilizing powers of native yolk and recombined fractions revealed no significant difference in emulsion drainage between the two formulae or the fresh and frozen storage treatments after thirty minutes of drainage. However, after sixty, ninety, and one hundred and twenty minutes, emulsions prepared with native yolk were more stable than those prepared with the recombined fractions (0.1% level of probability). Emulsions prepared with fresh yolk and recombined fresh fractions drained less than those prepared with frozen yolk and frozen recombined fractions, but this difference was only significant (5% level of probability) for the sixty minute reading. Although the two emulsion formulae differed in stability, total drainage was small in both cases. The difference may be explained by changes in the fractions during their separation or by differences in the pH of the emulsion mixtures.

The presence of lipovitellin, lipovitellenin, or livetin in the emulsion formula reduced emulsion drainage significantly at the 0.1% level of probability. Although all of the fractions were effective emulsion stabilizers, lipovitellenin provided the best overall stabilizing effect of the three.

Very highly significant interactions occurred when any two of the three fractions were combined, indicating that emulsion stabilizing powers were not independent. Close examination of these interactions revealed that, while combinations of any two fractions promoted greater stability than either of the fractions alone, the stability of the emulsions was less than would be expected from independent action of the fractions *per se*.

Freezing and thawing was not detrimental to the emulsion stabilizing power of any of the fractions or fraction combinations, although there was a trend toward higher drainage when frozen fractions were used, which might become significant in an emulsion system of different proportions.

Drainage increased throughout the two hour testing time. Emulsion mixtures without any stabilizing agent drained a large amount initially, apparently reaching the drainage maximum early in the drainage period. The presence of lipovitellenin reduced initial drainage, but increased the rate of drainage from thirty to one hundred and twenty minutes. Livetin and lipovitellin reduced initial drainage somewhat and interacted with lipovitellenin to reduce subsequent drainage, although the latter effect was not apparent when lipovitellin and livetin were present alone or combined with one another. The best emulsion

stability was observed when all three fractions were recombined.

Determinations of relative viscosity, before and after lipovitellin, lipovitellenin, livetin, and native yolk were frozen, revealed that freezing and thawing greatly increased the viscosity of native yolk. The increased relative viscosity of lipovitellenin after freezing contributed to the thickness of the frozen yolk. The lipovitellin fraction had the greatest mean relative viscosity increase after freezing, but it was quite variable and in two replications the relative viscosity of lipovitellin was decreased by freezing and thawing.

Paper electrophoresis was unable to detect any change in the mobility of the proteins of the lipovitellin fraction after freezing which would account for its change in relative viscosity. Lipovitellenin, however, produced electrophoretic patterns which indicated that a majority of its proteins were changed in such a manner that they became non-mobile after freezing and thawing. It was concluded that livetin did not contribute to gelation, as its relative viscosity and electrophoretic patterns remained the same after freezing and thawing.

The results of this investigation, while elucidating the emulsifying properties of three crude egg yolk fractions, indicated that research is needed in the following areas: (1) an analysis to determine the effect

of the lipid portion of lipovitellin and lipovitellenin on their emulsion stabilizing powers; (2) a study to determine the effects of various concentrations of egg yolk proteins and lipids on emulsion stability; (3) an investigation to determine the emulsifying properties of further separated and more highly purified egg yolk proteins; (4) an investigation of the emulsion stability imparted to mayonnaise by the egg yolk proteins and lipoproteins; (5) an investigation to determine the effects of varying pH on the stability of egg yolk emulsions; (6) development of a more accurate tool for determining the relative viscosity of frozen native yolk and the yolk fractions which are thickened by gelation; (7) further studies to determine the cause of the increase in relative viscosity which lipovitellin and lipovitellenin undergo after freezing and thawing; and (8) a study to examine other functional properties of crude egg yolk protein fractions, such as coagulation and foaming.

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APPENDIX

Diet of Hens Producing Eggs for Research

Tables 19 and 20 give the composition and analysis of the diet fed to the hens producing the eggs for this research.

TABLE 19. Composition of ration identified as LB-63 (Dawson, 1968)

Ingredient	Lbs. per ton
Ground yellow corn	1320.00
Soybean meal, dehulled, 50% protein	310.00
Alfalfa meal, dehyd., 17% protein	60.00
Meat and bone scraps, 50% protein	50.00
Fish meal, Vitaproil (with solubles added, 55% protein)	60.00
Dried whey	40.00
Ground limestone	100.00
Dicalcium phosphate (24% Ca, 18.5% P)	20.00
Salt, iodized	6.00
Vitamin trace mineral premix[a]	5.00 to 10.00
Choline chloride 25%	2.00
Zinc oxide (73-80% zinc)	0.25
Animal fat	25.00
Oyster shells	25.00

[a]Dawes Vitefac No. 1 (10 lbs. per ton) or Nopco M-4 (5 lbs. per ton) or equivalent product.

TABLE 20. Chemical analyses of ration identified as LB-63 (Dawson, 1968)

Component	%
Phosphorus	0.76
Calcium	2.73
Crude fiber	3.28
Ether extract	4.82
Protein	19.00
Water	7.72

TABLE 21. Percentage of total protein contributed by each of the three fractions

Rep.	Lipovitellin		Lipovitellenin		Livetin	
	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen
	%	%	%	%	%	%
1	55.43	55.52	34.94	34.93	9.63	9.55
2	52.62	53.14	32.76	32.36	14.62	14.50
3	54.65	53.58	33.14	34.14	12.21	12.28
4	51.13	51.46	36.26	35.87	12.61	12.67
5	49.74	50.74	39.90	39.31	10.37	9.95
6	49.89	50.46	37.73	37.19	12.38	12.35
Mean	52.36		35.70		11.07	

TABLE 22. Spindle size and viscometer speeds for measurement of the relative viscosity of lipovitellin, lipovitellenin, livetin, and native yolk before and after frozen storage over six replications

Replication	Storage Treatment	Spindle size/viscometer speed (rpm)			
		Lipovitellin	Lipovitellenin	Livetin	Yolk
1	Fresh	7/20	7/10	1/100	7/100
	Frozen	7/10	7/10	1/100	7/ 10
2	Fresh	7/10	7/20	1/100	7/100
	Frozen	7/ 0.5	7/10	1/100	7/ 10
3	Fresh	7/10	7/20	1/100	7/100
	Frozen	7/10	7/10	1/100	7/ 10
4	Fresh	7/10	7/20	1/100	7/100
	Frozen	7/10	7/10	1/100	7/ 10
5	Fresh	7/10	7/10	1/100	7/100
	Frozen	7/10	7/ 2.5	1/100	7/ 10
6	Fresh	7/10	7/10	1/100	7/100
	Frozen	7/ 0.5	7/10	1/100	7/ 10

TABLE 23. Replicate averages for fractions and storage treatment for chemical determination of moisture content

Rep.	Storage Treatment	Percentage moisture in: Lipovitellin	Lipovitellenin	Livetin	Native Yolk
1	Fresh	56.10	58.28	98.45	47.79
	Frozen	55.08	57.52	98.40	48.18
2	Fresh	56.38	63.82	96.75	48.14
	Frozen	54.60	64.24	96.72	47.99
3	Fresh	56.66	58.79	97.46	48.62
	Frozen	59.28	55.85	97.36	48.14
4	Fresh	56.55	59.48	97.91	48.75
	Frozen	56.33	60.67	97.84	49.07
5	Fresh	58.06	55.67	98.91	47.12
	Frozen	56.28	57.65	99.02	47.14
6	Fresh	55.40	59.52	97.98	48.10
	Frozen	53.78	60.82	98.01	48.10

TABLE 24. Replicate averages for fractions and storage treatment for chemical determination of protein content

Rep.	Storage Treatment	Percentage protein in: Lipovitellin	Lipovitellenin	Livetin	Native Yolk
1	Fresh	27.66	7.44	0.81	16.35
	Frozen	27.94	7.50	0.81	16.29
2	Fresh	30.04	6.47	1.13	16.35
	Frozen	30.60	6.44	1.13	16.38
3	Fresh	27.63	7.35	1.00	16.28
	Frozen	26.94	7.53	1.00	16.28
4	Fresh	27.13	8.06	0.91	16.07
	Frozen	27.19	7.94	0.91	16.13
5	Fresh	24.60	7.85	0.72	16.44
	Frozen	25.04	7.72	0.69	16.38
6	Fresh	28.60	7.19	0.88	16.35
	Frozen	28.97	7.10	0.88	16.32

TABLE 25. Replicate averages for fractions and storage treatment for the determination of pH

Rep.	Storage Treatment	pH for: Lipovitellin	Lipovitellenin	Livetin	Native Yolk
1	Fresh	6.1	6.1	5.9	5.9
	Frozen	5.8	6.1	5.9	5.8
2	Fresh	6.0	6.7	6.3	5.9
	Frozen	5.9	6.7	6.3	5.8
3	Fresh	5.7	6.2	5.8	5.8
	Frozen	5.8	6.5	6.3	5.8
4	Fresh	5.9	6.4	6.2	5.9
	Frozen	5.7	6.2	6.0	5.8
5	Fresh	5.8	6.2	5.8	5.9
	Frozen	5.7	6.3	5.8	5.7
6	Fresh	5.7	6.2	6.2	5.7
	Frozen	5.8	6.4	6.4	6.0

TABLE 26. Replicate averages for drainage from emulsions prepared with fresh and frozen native yolk (Y) and recombined lipovitellin, lipovitellenin, and livetin (ABC) at four time intervals

Rep.	Storage Treatment	Emulsions Stabilized by:	Drainage in milliliters at four time intervals (minutes)			
			30	60	90	120
1	Fresh	ABC	0.10	0.30	0.60	0.85
		Y	0.10	0.10	0.18	0.23
	Frozen	ABC	0.15	0.35	0.60	0.85
		Y	0.10	0.13	0.20	0.25
2	Fresh	ABC	0.10	0.15	0.20	0.30
		Y	0.10	0.15	0.20	0.30
	Frozen	ABC	0.45	1.00	1.25	1.55
		Y	0.10	0.25	0.35	0.55
3	Fresh	ABC	0.15	0.25	0.65	1.00
		Y	0.15	0.20	0.25	0.30
	Frozen	ABC	0.15	0.45	0.55	0.65
		Y	0.10	0.23	0.35	0.45
4	Fresh	ABC	0.10	0.20	0.40	0.60
		Y	0.10	0.15	0.25	0.35
	Frozen	ABC	0.10	0.30	0.40	0.60
		Y	0.10	0.20	0.35	0.50
5	Fresh	ABC	0.00	0.13	0.25	0.50
		Y	0.13	0.25	0.25	0.30
	Frozen	ABC	0.13	0.30	0.40	0.50
		Y	0.10	0.13	0.25	0.35
6	Fresh	ABC	0.05	0.20	0.35	0.45
		Y	0.05	0.10	0.20	0.30
	Frozen	ABC	0.15	0.35	0.60	0.80
		Y	0.10	0.13	0.15	0.25

TABLE 27. Replicate averages for pH, means and standard deviations for pH of emulsion formulae prepared with fresh and frozen native yolk and fresh and frozen recombined lipovitellin, lipovitellenin, and livetin

Storage Treatment	Rep.	pH of emulsion formula prepared with: Native Yolk	Mean/std. dev.	Recomb. Fractions	Mean/std. dev.
Fresh	1	5.8	5.7 ± 0.137	6.2	5.9 ± 0.250
	2	5.5		5.6	
	3	5.8		6.2	
	4	5.7		5.5	
	5	5.7		5.9	
	6	5.7		6.0	
Frozen	1	5.8	5.7 ± 0.172	6.0	5.9 ± 0.242
	2	5.8		6.2	
	3	5.5		5.5	
	4	5.5		5.9	
	5	5.9		5.9	
	6	5.8		6.1	

TABLE 28. Replicate averages for emulsion drainage at four time intervals from emulsions prepared with no stabilizer (O) and with fresh lipovitellin (A), lipovitellenin (B), and livetin (C) combinations as stabilizers

Rep.	Fraction	Average drainage in ml at:			
		30 min.	60 min.	90 min.	120 min.
1	O	10.55	10.55	10.60	10.60
	A	6.85	7.35	7.70	7.90
	B	0.65	2.40	2.50	2.95
	C	7.55	8.10	8.35	8.60
	AB	0.05	0.18	0.40	0.65
	BC	0.20	0.85	1.25	1.50
	AC	5.20	6.50	7.00	7.20
	ABC	0.10	0.30	0.60	0.85
2	O	10.65	10.85	10.95	10.95
	A	7.55	8.00	8.30	8.50
	B	0.35	1.15	1.40	1.75
	C	6.90	7.90	8.20	8.45
	AB	0.10	0.25	0.45	1.00
	BC	0.10	0.30	0.70	0.90
	AC	2.35	3.90	4.45	5.05
	ABC	0.10	0.15	0.20	0.30
3	O	11.10	11.15	11.20	11.20
	A	6.95	7.30	7.65	7.95
	B	1.50	3.30	3.90	4.30
	C	7.60	8.25	8.40	8.70
	AB	0.15	0.60	1.00	1.40
	BC	0.40	1.00	1.65	2.10
	AC	4.45	5.55	6.05	6.40
	ABC	0.15	0.25	0.65	1.00
4	O	11.50	11.60	11.65	11.70
	A	6.05	6.80	7.30	7.50
	B	0.75	1.45	2.05	2.40
	C	7.90	8.40	8.60	8.85
	AB	0.15	0.40	0.80	1.00
	BC	0.20	0.50	0.85	1.05
	AC	5.00	6.20	6.60	6.90
	ABC	0.10	0.20	0.40	0.60
5	O	10.95	11.00	11.05	11.15
	A	5.50	6.25	6.65	6.95
	B	0.30	0.55	1.75	1.95
	C	7.80	8.20	8.40	8.50
	AB	0.10	0.20	0.50	0.85
	BC	0.15	0.75	1.55	2.05
	AC	5.10	6.30	6.60	6.90
	ABC	0.00	0.13	0.25	0.50
6	O	10.10	10.35	10.40	10.40
	A	6.60	7.25	7.45	7.65
	B	0.45	1.40	2.20	2.75
	C	8.30	8.90	9.10	9.25
	AB	0.10	0.40	0.90	1.30
	BC	0.15	0.55	0.80	1.20
	AC	6.00	6.80	7.20	7.40
	ABC	0.05	0.20	0.35	0.45

TABLE 29. Replicate averages for emulsion drainage at four time intervals from emulsions prepared with no stabilizer (O) and with frozen lipovitellin (A), lipovitellenin (B), and livetin (C) combinations as stabilizers

Rep.	Fraction	Average drainage in ml at:			
		30 min.	60 min.	90 min.	120 min.
1	O	9.75	9.95	10.25	10.25
	A	6.60	7.25	7.65	7.95
	B	0.60	1.70	2.60	3.00
	C	8.10	8.50	8.70	8.85
	AB	0.25	0.60	1.05	1.25
	BC	0.30	0.75	0.95	1.30
	AC	4.70	5.70	6.25	6.65
	ABC	0.15	0.35	0.60	0.85
2	O	9.80	9.95	10.05	10.05
	A	6.45	7.15	7.45	7.80
	B	1.50	2.90	3.70	4.00
	C	7.20	8.00	8.35	8.55
	AB	0.65	1.25	1.90	2.25
	BC	0.40	0.90	1.30	1.80
	ABC	0.45	1.00	1.25	1.55
3	O	10.80	10.95	10.95	11.00
	A	7.35	7.60	8.35	9.10
	B	0.80	1.90	2.60	3.00
	C	7.35	8.15	8.55	8.65
	AB	0.20	0.50	0.75	0.95
	BC	0.40	1.05	1.45	2.05
	AC	5.60	6.40	6.85	7.15
	ABC	0.15	0.40	0.55	0.65
4	O	10.60	10.65	10.65	10.65
	A	6.25	7.05	7.30	7.60
	B	1.10	2.55	3.45	3.95
	C	6.40	7.30	7.70	8.00
	AB	0.35	0.70	1.15	1.40
	BC	0.50	1.05	1.70	2.15
	AC	5.50	6.90	6.90	7.10
	ABC	0.10	0.30	0.40	0.60
5	O	10.40	10.50	10.60	10.70
	A	7.00	7.50	7.70	8.00
	B	0.70	1.70	2.25	2.85
	C	8.45	8.80	9.00	9.00
	AB	0.15	0.50	0.65	0.90
	BC	0.40	0.85	1.35	1.80
	AC	6.95	7.45	7.85	8.05
	ABC	0.13	0.30	0.40	0.50
6	O	9.45	9.55	9.55	9.55
	A	6.70	7.35	7.65	7.90
	B	0.90	1.55	2.40	2.85
	C	8.25	8.85	9.10	9.20
	AB	0.15	0.30	0.45	0.55
	BC	0.25	0.60	1.05	1.35
	AC	5.90	6.85	7.15	7.50
	ABC	0.15	0.35	0.60	0.80

TABLE 30. Main effect and significant interaction means from the analysis of variance for determination of the effects of fractions, replication, storage treatment, and drainage time on the stability of oil-in-water emulsions

Factor and Category[a]						
Rep.	Lipovitellin	Lipovitellenin	Livetin	Storage	Time	Mean Drainage (ml)
1	0	0	0	0	0	4.52
2	0	0	0	0	0	4.42
3	0	0	0	0	0	4.77
4	0	0	0	0	0	4.59
5	0	0	0	0	0	4.57
6	0	0	0	0	0	4.55
0	0	0	0	Fresh	0	4.49
0	0	0	0	Frozen	0	4.65
0	0	0	0	0	30'	3.91
0	0	0	0	0	60'	4.47
0	0	0	0	0	90'	4.81
0	0	0	0	0	120'	5.08
0	-	0	0	0	0	5.48
0	+	0	0	0	0	3.66
0	0	-	0	0	0	8.11
0	0	+	0	0	0	1.03
0	0	0	-	0	0	5.17
0	0	0	+	0	0	3.97
0	-	-	0	0	0	9.45
0	-	+	0	0	0	1.51
0	+	-	0	0	0	6.77
0	+	+	0	0	0	0.54
0	0	-	-	0	0	8.97
0	0	-	+	0	0	7.25
0	0	+	-	0	0	1.36
0	0	+	+	0	0	0.70
0	-	0	-	0	0	6.33
0	-	0	+	0	0	4.63
0	+	0	-	0	0	4.00
0	+	0	+	0	0	3.31
0	-	-	0	0	30'	9.06
0	-	-	0	0	60'	9.43
0	-	-	0	0	90'	9.60
0	-	-	0	0	120'	9.70
0	-	+	0	0	30'	0.54
0	-	+	0	0	60'	1.32
0	-	+	0	0	90'	1.89
0	-	+	0	0	120'	2.29
0	+	-	0	0	30'	5.89
0	+	-	0	0	60'	6.72
0	+	-	0	0	90'	7.09
0	+	-	0	0	120'	7.40
0	+	+	0	0	30'	0.17
0	+	+	0	0	60'	0.41
0	+	+	0	0	90'	0.68
0	+	+	0	0	120'	0.92
0	0	-	-	0	30'	8.56
0	0	-	-	0	60'	8.91
0	0	-	-	0	90'	9.13
0	0	-	-	0	120'	9.29
0	0	-	+	0	30'	6.39
0	0	-	+	0	60'	7.24
0	0	-	+	0	90'	7.56
0	0	-	+	0	120'	7.81
0	0	+	-	0	30'	0.50
0	0	+	-	0	60'	1.18

TABLE 30. Continued.

Factor and Category[a]						
Rep.	Lipovitellin	Lipovitellenin	Livetin	Storage	Time	Mean Drainage (ml)
0	0	+	-	0	90'	1.70
0	0	+	-	0	120'	2.05
0	0	+	+	0	30'	0.21
0	0	+	+	0	60'	0.55
0	0	+	+	0	90'	0.87
0	0	+	+	0	120'	1.16
0	-	0	-	0	30'	5.64
0	-	0	-	0	60'	6.23
0	-	0	-	0	90'	6.61
0	-	0	-	0	120'	6.83
0	-	0	+	0	30'	3.97
0	-	0	+	0	60'	4.52
0	-	0	+	0	90'	4.88
0	-	0	+	0	120'	5.16
0	+	0	-	0	30'	3.43
0	+	0	-	0	60'	3.86
0	+	0	-	0	90'	4.21
0	+	0	-	0	120'	4.51
0	+	0	+	0	30'	2.63
0	+	0	+	0	60'	3.26
0	+	0	+	0	90'	3.55
0	+	0	+	0	120'	3.81
0	-	-	-	0	30'	10.47
0	-	-	-	0	60'	10.59
0	-	-	-	0	90'	10.66
0	-	-	-	0	120'	10.68
0	-	-	+	0	30'	7.65
0	-	-	+	0	60'	8.28
0	-	-	+	0	90'	8.54
0	-	-	+	0	120'	8.72
0	-	+	-	0	30'	0.80
0	-	+	-	0	60'	1.88
0	-	+	-	0	90'	2.57
0	-	+	-	0	120'	2.98
0	-	+	+	0	30'	0.29
0	-	+	+	0	60'	0.76
0	-	+	+	0	90'	1.22
0	-	+	+	0	120'	1.60
0	+	-	-	0	30'	6.65
0	+	-	-	0	60'	7.24
0	+	-	-	0	90'	7.60
0	+	-	-	0	120'	7.90
0	+	-	+	0	30'	5.12
0	+	-	+	0	60'	6.20
0	+	-	+	0	90'	6.58
0	+	-	+	0	120'	6.90
0	+	+	-	0	30'	0.20
0	+	+	-	0	60'	0.49
0	+	+	-	0	90'	0.83
0	+	+	-	0	120'	1.13
0	+	+	+	0	30'	0.14
0	+	+	+	0	60'	0.33
0	+	+	+	0	90'	0.52
0	+	+	+	0	120'	0.72

[a]The following are the factors and an identification of the catagories of each of the factors:

Rep.--replications one thru six.
Lipovitellin--absence (-) or presence (+).
Lipovitellenin--absence (-) or presence (+).
Livetin--absence (-) or presence (+).
Storage--fresh or frozen.
Time--Reading at 30, 60, 90, or 120 minutes.

0 denotes that these factors were not separated when the mean was determined.

TABLE 31. Replicate averages for relative viscosity of lipovitellin, lipovitellenin, livetin, and native yolk, before and after frozen storage

Rep.	Storage Treatment	Relative viscosity (poises) of: Lipovitellin	Lipovitellenin	Livetin	Native Yolk
1	Fresh	1,226,700.00	846,700.00	0.12	14.67
	Frozen	2,893,300.00	2,540,000.00	0.13	1,280,000.00
2	Fresh	2,906,700.00	176,700.00	0.13	13.33
	Frozen	74,533,300.00	1,006,700.00	0.14	1,653,300.00
3	Fresh	1,773,300.00	366,700.00	0.16	13.33
	Frozen	1,013,300.00	2,880,000.00	0.25	1,546,700.00
4	Fresh	920,000.00	406,700.00	0.21	12.00
	Frozen	800,000.00	2,413,300.00	0.23	1,413,300.00
5	Fresh	526,700.00	2,093,300.00	0.19	15.33
	Frozen	833,300.00	14,560,000.00	0.19	1,780,000.00
6	Fresh	2,493,300.00	993,300.00	0.18	13.33
	Frozen	5,493,300.00	3,426,700.00	0.12	2,066,700.00

TABLE 32. Calculated average migration distances, mobile and non-mobile protein fractions of fresh and frozen native yolk as separated by paper electrophoresis

Storage Treatment	Rep.[a]	Migration[b] Left	Migration[b] Right	Mobility[c] Non-Mobile	Mobility[c] Mobile
		mm	mm	%	%
Fresh	1 a	10	46	35.2	64.8
	1 b	9	43	23.9	76.1
	2 a	10	47	31.3	68.7
	2 b	10	51	25.1	74.9
	3 a	4	47	33.2	66.8
	3 b	7	56	37.4	62.6
	4 a	9	44	44.7	55.3
	4 b	11	46	23.8	76.2
	5 a	15	49	45.6	54.4
	5 b	13	53	34.8	65.2
	6 a	13	55	39.9	60.1
	6 b	14	50	44.7	55.3
Mean		10.0	48.9	35.0	65.0
Range		4-15	43-56	23.8-45.6	54.4-76.2
Frozen	1 a	10	44	38.8	61.2
	1 b	11	46	53.9	46.1
	2 a	6	54	55.8	44.2
	2 b	6	49	55.3	44.7
	3 a	8	52	55.1	44.9
	3 b	9	46	57.8	42.2
	4 a	8	54	58.3	41.7
	4 b	8	52	52.8	47.2
	5 a	11	54	58.4	41.6
	5 b	10	52	51.8	48.2
	6 a	8	38	53.1	46.9
	6 b	7	39	24.0	76.0
Mean		8.5	48.3	51.3	48.7
Range		6-11	38-54	24.0-58.4	41.6-76.0

[a]Two strips (a and b) run for each replication.

[b]Migration from origin.

[c]Non-Mobile includes any mobility to the left.

TABLE 33. Calculated average migration distances, mobile and non-mobile protein fractions of fresh and frozen lipovitellin as separated by paper electrophoresis

Storage Treatment	Rep.[a]	Migration[b] Left	Migration[b] Right	Mobility[c] Non-Mobile	Mobility[c] Mobile
		mm	mm	%	%
Fresh	1 a	9	27	67.1	32.9
	1 b	10	30	80.5	19.5
	2 a	6	51	77.3	22.7
	2 b	5	44	69.7	30.3
	3 a	7	47	57.3	42.7
	3 b	8	58	62.4	37.6
	4 a	9	41	72.7	27.3
	4 b	10	45	73.1	26.9
	5 a	7	40	79.0	21.0
	5 b	10	40	79.6	20.4
	6 a	12	55	71.2	28.8
	6 b	9	53	65.7	34.3
Mean		8.5	44.3	71.3	28.7
Range		5-12	27-58	62.4-80.5	19.5-37.6
Frozen	1 a	8	51	69.2	30.8
	1 b	11	29	83.3	16.7
	2 a	5	51	80.3	19.7
	2 b	6	53	79.3	20.7
	3 a	5	50	54.6	45.4
	3 b	4	62	51.2	48.8
	4 a	6	33	90.1	9.9
	4 b	9	37	88.0	12.0
	5 a	6	51	50.8	49.2
	5 b	4	53	52.9	47.1
	6 a	10	45	90.8	9.2
	6 b	32	46	88.9	11.1
Mean		8.8	46.8	73.3	26.7
Range		4-32	29-62	50.8-90.8	9.2-49.2

[a] Two strips (a and b) run for each replication.

[b] Migration from origin.

[c] Non-Mobile includes any mobility to the left.

TABLE 34. Calculated average migration distances, mobile and non-mobile protein fractions of fresh and frozen lipovitellenin as separated by paper electrophoresis

Storage Treatment	Rep.[a]	Migration[b] Left	Migration[b] Right	Mobility[c] Non-Mobile	Mobility[c] Mobile
		mm	mm	%	%
Fresh	1 a	5	32	14.9	85.1
	1 b	4	33	15.5	84.5
	2 a	5	35	6.0	94.0
	2 b	7	44	8.1	91.9
	3 a	5	39	6.7	93.3
	3 b	4	42	2.5	97.5
	4 a	5	49	6.5	93.5
	4 b	4	52	8.3	91.7
	5 a	13	33	20.3	79.7
	5 b	8	43	13.5	86.5
	6 a	9	23	15.4	84.6
	6 b	11	26	10.5	89.5
Mean		6.7	37.6	10.7	89.3
Range		4-13	23-52	2.5-20.3	79.7-97.5
Frozen	1 a	8	20	80.0	20.0
	1 b	7	19	70.8	29.2
	2 a	9	24	79.2	20.8
	2 b	5	23	70.4	29.6
	3 a	6	27	70.8	29.2
	3 b	6	21	71.4	28.6
	4 a	6	30	69.2	30.8
	4 b	7	26	58.8	41.2
	5 a	7	27	59.4	40.6
	5 b	7	26	54.8	45.2
	6 a	28	24	75.9	24.1
	6 b	6	25	48.5	51.5
Mean		8.5	24.3	67.4	32.6
Range		5-28	19-30	48.5-80.0	20.0-51.5

[a]Two strips (a and b) run for each replication.

[b]Migration from origin.

[c]Non-Mobile includes any mobility to the left.

TABLE 35. Calculated average migration distances, mobile and non-mobile protein fractions of fresh and frozen livetin as separated by paper electrophoresis

Storage Treatment	Rep.[a]	Migration[b] Left	Migration[b] Right	Mobility[c] Non-Mobile	Mobility[c] Mobile
		mm	mm	%	%
Fresh	1 a	4	50	5.0	95.0
	1 b	4	50	4.3	95.7
	2 a	3	56	14.3	85.7
	2 b	2	54	0.0	100.0
	3 a	2	58	0.0	100.0
	3 b	5	60	0.0	100.0
	4 a	5	47	0.0	100.0
	4 b	5	51	0.0	100.0
	5 a	4	56	0.0	100.0
	5 b	7	58	8.3	91.7
	6 a	8	61	8.7	91.3
	6 b	7	57	9.1	90.9
Mean		4.7	54.8	4.1	95.9
Range		2-8	47-61	0.0-14.3	85.7-100.0
Frozen	1 a	3	51	33.3	66.7
	1 b	6	54	13.6	86.4
	2 a	4	52	16.7	83.3
	2 b	4	56	15.4	84.6
	3 a	3	59	13.3	86.7
	3 b	4	59	7.4	92.6
	4 a	3	62	0.0	100.0
	4 b	2	64	0.0	100.0
	5 a	2	53	16.7	83.3
	5 b	4	67	12.5	87.5
	6 a	4	48	12.1	87.9
	6 b	3	45	7.5	92.5
Mean		3.5	55.8	12.4	87.6
Range		2-6	45-67	0.0-33.3	66.7-100.0

[a]Two strips (a and b) run for each replication.

[b]Migration from origin.

[c]Non-Mobile includes any mobility to the left.