1951

Some factors affecting the emulsifying properties of hen's egg

Robert Brownell Chapin
Iowa State College

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UMI®
SOME FACTORS AFFECTING THE EMULSIFYING
PROPERTIES OF HEN'S EGG

by

Robert Brownell Chapin

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Food Technology-Poultry Husbandry

Approved:

Signature was redacted for privacy.

__________________________
Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College

1951
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<th>Page</th>
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I. INTRODUCTION

The highly competitive nature of modern industry requires efficient and economic by-product utilization. The meat packing industry, of all the food industries, has probably made the most important progress in this direction. The by-products of this field include pharmaceuticals, hides, liver extracts, tankage, bone meal, buttons, etc. The poultry products industry, however, has lagged far behind in the utilization of its waste materials. The principal by-products now marketed are technical albumen, meat scraps, feathers, inedible fat, and "tanner's yolk". These products have a limited usefulness. "Tanner's yolk" is used at present in the leather industry as an emulsifier in the fat-liquoring or oiling process. It is prepared from inedible eggs by commercial hatcheries and egg drying and freezing plants. The use of tanner's egg has recently declined, however, because the product exhibits a wide variation in emulsifying ability and has poor keeping qualities.

Economic utilization of a waste material most often depends upon the successful isolation from that material of a product with some unique and valuable property. The emulsifying ability of the whole egg is one such property. The use of this action in the fat-liquoring of leather was mentioned above. The use of this action in agricultural sprays, cosmetics, medical emulsions, food emulsions, etc., could probably be developed. This potential usefulness of the emulsifying power of whole egg establishes the need for an investigation of this property.
The object of this investigation is to isolate, concentrate, and standardize the emulsifying components of whole egg. The approach to the problem may be divided into two phases: first, a gross fractionation of whole egg in order to isolate the main emulsifying component or components of the egg; and, second, a study of the effect of the important manufacturing processes on the emulsifying ability of the product.

An important phase of this study is the development of a test which will critically measure emulsifying powers. Such a test should measure one or more of the characteristics of a typical emulsion, e.g., a mayonnaise-type emulsion. It may be assumed that products which perform well under the conditions of such a test would probably perform well when used under other conditions.
II. REVIEW OF LITERATURE

A. Composition of the Egg

Critical evaluation of the existing information on the physical and chemical composition of whole egg is necessary for the development from egg of a product possessing specific properties. An examination of the composition of yolk and albumen must also be undertaken, since most of existing egg fractionation procedures are concerned with only one of these parts.

1. Whole egg and shell egg

Three of the many investigations on the physical and chemical composition of the whole egg are summarized in Table 1. Whole egg is composed chiefly of proteins and lipids. Carbohydrate and inorganic materials are present in only small amounts. Since albumen makes up about 30% of whole egg solids, an examination of the properties of the white of the egg as well as the yolk is important to this study.

2. Egg white

The white (albumen) of hen's egg has received considerable attention relative to its chemical and physical composition and biological properties. The albumen solids are composed chiefly of a mixture of proteins,
Table 1. The Constituents of Shell Egg

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Cruikshank (1940)</th>
<th>Mitchell (1932)</th>
<th>Romanoff and (1949)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shell</td>
<td>11</td>
<td>11.36</td>
<td>12.3</td>
</tr>
<tr>
<td>White</td>
<td>56</td>
<td>57.27</td>
<td>55.8</td>
</tr>
<tr>
<td>Yolk</td>
<td>31</td>
<td>30.64</td>
<td>31.9</td>
</tr>
</tbody>
</table>

Whole egg

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Cruikshank (1940)</th>
<th>Mitchell (1932)</th>
<th>Romanoff and (1949)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>73.7</td>
<td>74.1</td>
<td>73.6</td>
</tr>
<tr>
<td>Solids</td>
<td>25.3</td>
<td>25.9</td>
<td>26.4</td>
</tr>
<tr>
<td>Organic</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Protein</td>
<td>13.4</td>
<td>--</td>
<td>12.8</td>
</tr>
<tr>
<td>Fat</td>
<td>10.5</td>
<td>11.4</td>
<td>11.8</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>--</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Inorganic</td>
<td>1.0</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>White solids</td>
<td>7.9</td>
<td>7.9</td>
<td>--</td>
</tr>
<tr>
<td>Yolk solids</td>
<td>18.4</td>
<td>18.0</td>
<td>--</td>
</tr>
</tbody>
</table>

Table 2. The Chemical Constituents of Egg White

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Cruikshank (1940)</th>
<th>Mitchell (1932)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>87.95</td>
<td>87.77</td>
</tr>
<tr>
<td>Solids</td>
<td>12.15</td>
<td>12.23</td>
</tr>
<tr>
<td>Fat</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>Protein</td>
<td>10.0</td>
<td>--</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>0.38</td>
<td>0.50</td>
</tr>
<tr>
<td>Ash</td>
<td>0.32</td>
<td>0.32</td>
</tr>
</tbody>
</table>
Small amounts of carbohydrate and minerals and traces of fat are also present. Cruikshank (1940) and Mitchell (1932) reported the analysis of albumen. Their figures are summarized in Table 2.

The generally recognized proteins in egg white are ovalbumin, ovomucoid, ovomucin, somalbumin, ovoglobulin, avidin, and lysozyme. Table 3 summarizes the results of a number of investigations on the protein composition of albumen.

The remaining white constituents are vitamins, minerals, and enzymes. These materials, because of their low concentration in egg white, are probably relatively unimportant in its emulsifying action.

3. Egg yolk

The proximate analysis of egg yolk has been reported by Mitchell (1932) and Cruikshank (1940) (Table 4). Burmester (1940) in his careful experiments showed that the moisture content of the yolk depended partly on the age of the egg when analyzed since water passes into the yolk from the white until equilibrium is reached.

Extensive investigations of the proteins of egg yolk have been infrequent because the separation and purification of these proteins is complicated by the presence of lipid substances. Thus far five proteins have been isolated from yolk: vitellin, vitellenin, livetin, phosvitin, and vitellomucoid. There is evidence that livetin, a water soluble protein, is composed of more than one constituent. Table 5 summarizes certain data on these proteins. From this table it appears that egg
Table 3. Protein Composition of Egg White

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Bain and Deutsch (1947)</th>
<th>Forsythe (1949)</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per cent</td>
<td>Per cent</td>
<td></td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>60.5*</td>
<td>64.9*</td>
<td>**</td>
</tr>
<tr>
<td>Ovomucoid</td>
<td>--</td>
<td>9.2*</td>
<td>11.4*** (Lineweaver and Murray, 1947)</td>
</tr>
<tr>
<td>Globulin</td>
<td>15.0*</td>
<td>9.2*</td>
<td></td>
</tr>
<tr>
<td>Conalbumin</td>
<td>22.5*</td>
<td>13.8*</td>
<td></td>
</tr>
<tr>
<td>Ovomucin</td>
<td>--</td>
<td>1.1***</td>
<td>7.0 (Romanoff and Romanoff, 1945)</td>
</tr>
<tr>
<td>Lysosome</td>
<td>--</td>
<td>3.4*</td>
<td>2.5** (Alderton, Ward, and Fevold, 1945)</td>
</tr>
<tr>
<td>Avidin</td>
<td>--</td>
<td>--</td>
<td>0.06** (Alderton, Lewis, and Fevold, 1945)</td>
</tr>
</tbody>
</table>

*Electrophoretic analysis
**Bioassay
***Isolated

Table 4. Chemical Constituents of Egg Yolk

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Mitchell (1932)</th>
<th>Cruikshank (1940)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per cent</td>
<td>Per cent</td>
</tr>
<tr>
<td>Water</td>
<td>46.47</td>
<td>49.0</td>
</tr>
<tr>
<td>Solids</td>
<td>51.53</td>
<td>51.0</td>
</tr>
<tr>
<td>Fat</td>
<td>32.54</td>
<td>31.6</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>0.17</td>
<td>--</td>
</tr>
<tr>
<td>Protein</td>
<td>--</td>
<td>16.7</td>
</tr>
<tr>
<td>Ash</td>
<td>1.71</td>
<td>1.5</td>
</tr>
<tr>
<td>Constituent</td>
<td>Classification</td>
<td>Composition of fraction</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>1. Lipoproteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Lipovitellin</td>
<td>Lipoprotein</td>
<td>Protein + 18% phospholipid.</td>
</tr>
<tr>
<td>b. Lipovitellin</td>
<td>Lipoprotein</td>
<td>Protein + 18.8% phospholipid.</td>
</tr>
<tr>
<td>c. Lipovitellin</td>
<td>Lipoprotein</td>
<td>Protein + 16.8% phospholipid.</td>
</tr>
<tr>
<td>d. Lipovitellenin</td>
<td>Lipoprotein</td>
<td>Protein + 36-41% phospholipid.</td>
</tr>
<tr>
<td>2. Proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O insol.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Vitellin</td>
<td>Phosphoprotein</td>
<td>Protein + 1.0% phosphorus.</td>
</tr>
<tr>
<td>b. Vitellenin</td>
<td>Phosphoprotein</td>
<td>Protein + 0.25-0.3% phosphorus.</td>
</tr>
<tr>
<td>H₂O sol.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Livetin</td>
<td>Pseudoglobulin</td>
<td>Protein + 0.1% phosphorus.</td>
</tr>
<tr>
<td>b. Livetin</td>
<td>Pseudoglobulin</td>
<td>Three components.</td>
</tr>
<tr>
<td>e. Livetin</td>
<td>Pseudoglobulin</td>
<td>Less phosphorus than vitellin.</td>
</tr>
<tr>
<td>f. Phosvitin</td>
<td>Phosphoprotein</td>
<td>Protein + varying phosphorus.</td>
</tr>
<tr>
<td>g. Vitellomucoid</td>
<td>Glycoprotein</td>
<td>Protein + 10.1% glucosamine. No phosphorus.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
yolk contains three principal types of proteins: first, water insoluble proteins, vitellin and vitellenin (phosphoproteins conjugated with phospholipids, principally lecithin and cephalin); second, water soluble phosphoproteins, livetin and phosvitin (phosvitin contains a much higher percentage of phosphorus than livetin); and third, the mucoprotein, vitellomucoid.

Egg yolk contains a number of types of lipid substances among which are glycerides, phospholipids, sphingomyelin, and cholesterol. Data reported by Oxley (1945), Riemenschneider, Ellis, and Titus (1938), Cruikshank (1940), and Romanoff and Romanoff (1949) are compiled in Table 6.

B. Preparation of Egg Fractions

Very little work has been reported on the fractionation of whole egg; therefore, a critical examination of the methods used to fractionate albumen and yolk must be made to provide a basis for the isolation of the emulsifying components of mixed white and yolk.

1. Egg white

Forsythe (1949) has reviewed the many investigations concerning the preparation and properties of egg white proteins. The original basic fractionation schemes made use of fractional ammonium sulphate precipitation and various protein precipitants such as alcohol, acetone, and acids. Later, electrophoresis was employed both for qualitative and
Table 6. Composition of Egg Yolk Lipids

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Amount in yolk solids (Per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipids</strong></td>
<td></td>
</tr>
<tr>
<td>Glycerides</td>
<td>40.3</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>21.3</td>
</tr>
<tr>
<td>Lecithin</td>
<td>15.1</td>
</tr>
<tr>
<td>Cephalin</td>
<td>5.4</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>0.5</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>3.6</td>
</tr>
<tr>
<td>Cerebrosides</td>
<td>traces</td>
</tr>
<tr>
<td><strong>Fatty acids</strong></td>
<td></td>
</tr>
<tr>
<td>Saturated</td>
<td>30.0</td>
</tr>
<tr>
<td>Unsaturated</td>
<td>68.6</td>
</tr>
</tbody>
</table>

Compiled from the data of Okey (1945), Riemenschneider, Ellis, and Titus (1938), Cruikshank (1940), and Romanoff and Romanoff (1949).
quantitative analysis of albumen. The ethanol fractionation methods used with blood plasma proteins during World War II were adapted by Forsythe (1949) to egg white proteins. All of these fractionation systems were designed to isolate more or less pure constituents from the white. They are not directly applicable to the fractionation of whole egg because the general physico-chemical nature of mixed albumen and yolk differs from that of albumen alone.

2. Egg yolk

The fractionation of egg yolk is important because it forms the foundation from which a fractionation of whole egg may be developed. The presence of the same fats in yolk and whole egg and the gross similarity of yolk and white proteins makes possible the adaptation of yolk fractionation methods to the whole egg.

The fractionation of yolk has not been extensively studied. Osborn and Campbell (1906) performed the first key experiments on the isolation and characterization of lipoprotein from egg yolk. Strained yolks were added into saturated sodium chloride solution. The solution was extracted with ether and then dialyzed to precipitate the proteins. The precipitate was further extracted with ether and then redissolved in salt solution and redialyzed. The final precipitate was washed with alcohol and ether and dried. Several lipoprotein precipitates containing from 10% to 31% lecithin were obtained by fractional dialysis. The higher lecithin containing fractions were more soluble. The authors suggested
that the name "vitellin" be restricted to the protein part only and lipovitellin to the lipoprotein complex. Calvery and White (1932) modified this procedure somewhat by extracting a ten per cent sodium chloride solution of yolk with ether until the solvent was colorless. The salt solution was diluted with 20 volumes of water and centrifuged. Vitellin was prepared from the precipitated lipoprotein by repeated extraction of the precipitate with 95% alcohol.

Chargaff (1942) prepared egg yolk lipoprotein in the usual manner employing dialysis and lyophilization; however, he used acetone for the final extraction of the powder. He suggested that lipids other than lecithin were conjugated with the vitellin.

A new method for the preparation of yolk lipoproteins was introduced by Alderton and Fovold (1945). Yolk was diluted with two volumes of water and centrifuged with a force of 14,300 times gravity. The authors lyophilized the precipitate and extracted the dry powder thoroughly with ether. The values for phosphatide content of the protein were a little lower than those previously reported for similar preparations. With an extension of these methods Fovold and Lausten (1948) isolated another lipoprotein. In this case the diluted yolk supernatant was extracted with ether. A precipitate formed which was then separated and purified by extraction and reprecipitation from salt solution. The new protein contained up to 41% phospholipid (compared with 17% to 18% for previous preparations). The phosphorus content of this protein was less than one third of the phosphorus content of
vitellin. They named the new protein vitellinenin and the complex lipovitellinenin.

A water soluble protein in yolk was first examined by Flimmer in 1908. He prepared lipovitellin by extraction of salted yolk solution and dilution of the protein solution. He then acidified the lipovitellin mother liquor with acetic acid and finally boiled the solution. The precipitated proteinaceous material was named livetin. Flimmer found that livetin contained 0.11% phosphorus (as P₂O₅) and vitellin 1.1% phosphorus. Livetin was not coagulated by ether at 0° C. (32° F.) and differed from albumin in that respect.

Instead of boiling the lipoprotein supernatant to precipitate the livetin, Kay and Marshall (1928) half-saturated the supernatant with ammonium sulphate. These investigators found that it was possible to precipitate the protein from water solution by the addition of alcohol, acetone, trichloroacetic acid, and other of the usual protein precipitants. On the basis of electrophoretic analysis Shepard and Hottle (1949) concluded that livetin contained three components, one of which they were able to concentrate by ethanol precipitation.

In 1936 Once prepared a mucoprotein by precipitating livetin from the ether-extracted, water-soluble portion of yolk. He recovered the protein from the supernatant by alcohol precipitation. The "vitellomucoid" contained 10.1% glucosamine and comprised 0.1% of the yolk (wet basis).

Mecham and Clcott (1949) prepared a new dilute salt-soluble phosphoprotein from yolk by precipitation with MgSO₄, ether extraction of
the redissolved precipitate (to precipitate out the lipoproteins), and purification by reprecipitation with MgSO₄ and dialysis. The "phosvitin" contained 10% phosphorus as P₂O₅ and comprised 6.5% to 7.0% of the yolk protein.

Chargaff, Ziff, and Rittenberg (1942) prepared lecithin by adding salted and diluted egg yolk into an alcohol-ether solvent (one to one). The supernatant egg lipid-solvent solution was concentrated and poured into cold acetone. The precipitated phospholipids were dissolved in petroleum ether and reprecipitated with acetone.

3. Solubility of egg components

In order to effectively adapt the fractionation procedures outlined above to whole egg, a knowledge of the solubility of the fractions is a prime concern. Osborne and Campbell (1900) observed that the minimum salt concentration necessary to dissolve the egg yolk lipoprotein complex varied with the particular preparation. An explanation of these observations was afforded by Fevold and Lausten (1948) when they isolated lipovitellinin. This protein was soluble in lower concentrations of salt than lipovitellin. When prepared by alcohol extraction, vitellin was insoluble in salt solution. Alderton and Fevold (1945) reported, however, that vitellin is soluble in dilute alkali and is reprecipitated upon acidification. However, it has not been prepared free from lecithin and yet soluble in neutral solvents.
Livetin is a water soluble protein described by Kay and Marshall (1928) as a pseudoglobulin. It is insoluble in half-saturated ammonium sulphate. These workers observed that livetin was still soluble after prolonged dialysis and at very low salt concentrations. It was insoluble in saturated sodium chloride solution and was precipitated by boiling. The vitellomucoid of Oace (1935) was soluble in 30% alcohol and half-saturated ammonium sulphate but insoluble in 70% alcohol. It was not coagulated by boiling. Phosvitin, according to Mehan and Oloott (1949), was insoluble in water but soluble in dilute salt. The lecithin portion of the lipoprotein complex could not be removed with ether but could be removed with alcohol in the experiments of Osborns and Campbell (1900).

The solubilities of the various white proteins has been reviewed by Forsythe (1949).

C. The Emulsifying Properties of Liquid Whole Egg and Whole Egg Fractions

According to Berkman and Eglaff (1941) the efficiency of an emulsifier is often closely correlated with its ability to lower the interfacial tension between the dispersed phase and the dispersion medium. This lowering of interfacial tension is a result of adsorption of the emulsifying agent in the boundary of oil and water. According to these authors, the viscosity of the system plays no role in emulsion formation; however, a high viscosity in certain cases may hinder the coalescence of oil particles thus lending stability to the emulsion. An emulsifier to be
effective must be soluble and present in the external phase according to one definition; however, many emulsifiers exist as colloidal or even microscopic particles. Berkman and Egloff further stated that the importance of the ability of an emulsifier to form a hydrate has been greatly overestimated. The stability of an emulsion is highly influenced both by the lessening of the tendency of the oil drops to coalesce (lowering of surface tension) and the cohesiveness of the emulsifying film. King and Mukherjee (1940) emphasized the importance in emulsification of the ability of the emulsifier to form a strong solvated membrane at the oil/water interface. They felt that the viscosity of the system is important to emulsion stability.

It would appear that the desirable conditions for efficient emulsification and stabilization of the emulsion are the lowering of interfacial tension, the formation of a strong solvated membrane at the interface, and the presence of a high superficial viscosity in the system. The solubility of the emulsifier is also important not only from the standpoint of the amount of emulsifier available but also from the standpoint of the size and configuration of the emulsifying particle.

1. The influence of physical and chemical factors

So very little work has been reported on the emulsifying ability of whole egg and whole egg constituents, that recourse must be made to an examination of the properties of eggs important in the emulsifying action
outlined above in order to determine which physical or chemical treatments have a major influence upon this ability. By far the greatest amount of research has been reported on the influence of various physical and chemical treatments on the solubility of whole egg and its parts. It might be assumed that the factors which influence solubility are important to the other major emulsifying properties and ultimately to the emulsifying ability itself.

a. Influence of various physical and chemical treatments on solubility. Change in solubility is one of the major manifestations of protein denaturation and has long been used as a measure of alteration in the native protein. Wu (1927) defined denaturation as a "change in the natural protein whereby it becomes insoluble in solvents in which it was previously soluble." Neurath, et al. (1944) define denaturation as "any non-proteolytic modification of the unique structure of a native protein, giving rise to definite changes in chemical, physical, or biological properties." The important factors influencing denaturation, according to Neurath, et al., are heat, pressure, freezing, irradiation, sound waves, surface forces, pH, organic solvents, organic solutes, and enzymes. The factors likely to be encountered during the production of an emulsifier from whole egg are heat, freezing, surface forces, and the effects of organic solvents. An additional factor important in spray dried egg is the chemical change occurring during storage. The discussion of solubility below will include a discussion of viscosity changes and appearance of coagulation, since these phenomena are directly related to solubility.
(1) The effect of freezing on the solubility of egg proteins.
Moran (1925) noted that freezing and frozen storage had little effect on the egg white; however, he observed that freezing disrupted the microscopic structure of the yolk. The yolk retained its fluidity if not frozen below -5° C. (21.2° F.). At lower freezing temperatures a "pastiness" developed in the yolk when thawed. When frozen and thawed very rapidly, no thickening resulted. Moran suggested that the change in viscosity was caused by a precipitation of lipoproteins during slow freezing. Hardy (1923) postulated that between -0.7° C. and -6.0° C. (30.2° F. and 21.2° F.) much unfrozen material remained in the yolk because of local salt concentrations; however, below -6.0° C. (21.1° F.) these areas froze leading to severe chemical dissociation. Fovold and Lausten (1946) stated that lipovitellin may be partly responsible for the thickening of yolk during freezing and thawing, since the freezing process apparently left this lipoprotein less soluble. Chargaff (1942) also observed that preparations of lipoprotein, when dried from the frozen state, were only partially soluble in ten per cent sodium chloride. In experiments on whole egg, Thomas and Bailey (1933) concluded that the amount of gelation of thawed whole egg was predominantly affected by the mechanical treatment before freezing. The addition of sodium chloride, sucrose, or dextrose retarded the effect of freezing. Homogenization was observed to be the most effective means of preservation. Urbain and Miller (1930) concluded that dextrose and levulose were more effective than sucrose in preventing whole egg magma from thickening during freezing and thawing.
The effect of heat and storage of spray dried powders on the solubility of egg yolk, egg albumen, and whole egg. The effect of heat and spray drying will be discussed together because spray drying includes, besides atomization, the application of heat. The effect of the development of surface (such as in atomization) on egg albumen will be discussed later.

Romanoff (1943) reported that the coagulation point of albumin was 61° C. (142° F.). Payawal, Lowe, and Stewart (1946) found that as the temperature of egg white was increased, its viscosity did not change up to a temperature of 62.5° C. (144° F.) and when raised above that temperature, the viscosity was irregular. Yolk exhibited a gradually increasing rate of thickening until coagulation took place at 70° C. (158° F.). The viscosity of whole egg increased slowly up to 66° C. (151° F.). From 66° C. to 70° C. (151° F. to 158° F.), a drop occurred indicating fractional precipitation of some component or components. A rapid increase in viscosity was noted above a temperature of 70° C. (158° F.). Whole egg was heated at a temperature of 58° C. (136° F.) for 6½ minutes without loss of solubility. Mecham and Olcott (1947) heated dried egg albumen in boiling inert hydrocarbons for eighteen hours. The solubility of the heated powder decreased as the temperature of heating increased. After being held for eighteen hours at a temperature of 100° C. (212° F.) the egg white powder was 52% soluble in water. The greater heat stability of dry protein over native protein is, of course, well recognized. Anson and Mirsky (1931) observed that no change in the viscosity of acidified
or diluted ovalbumin occurred during heating up to a temperature of 100° C. (212° F.) for four minutes. Bancroft and Hetsler (1931) noted that dextrose in sufficient concentration could retard the denaturation of egg white.

These observations become important when applied to processes to which eggs might be subjected such as pasteurization and spray drying. Payzval, Lowe, and Stewart (1943) applied their data on the effect of heat on the denaturation of liquid egg pulp to the pasteurization of eggs. Brooks and Hawthorne (1943) found that the addition of 10% sucrose, lactose, or dextrin prior to drying had no appreciable effect on the initial solubility of the powders. Stewart, Best, and Lowe (1943) and Bates-Smith and Hawthorne (1945) observed that acidification of liquid egg before spray drying increased the initial solubility of the dried product to a small extent over that of the unacidified egg.

The production of a spray dried egg powder with good solubility is, of course, important; however, the retention of this solubility during storage is equally important. This retention of solubility is also a problem since loss of the solubility of spray dried egg powders may be rapid if the conditions of production and storage are not carefully chosen. Hawthorne (1943) found that low moisture powders (less than 2.5% moisture) retained their solubility during storage better than powders containing higher amounts of water. White and Thistle (1943) also observed the higher stability of low moisture spray dried egg and warned that a spray dried egg must be rapidly cooled after drying in order to prevent a rapid loss of solubility. Brooks and Hawthorne (1943)
produced a relatively stable powder by the addition of 10% sucrose, lactose, or dextrin to the egg before spray drying. The solubility of the sugared egg was about twice that of the unsugared egg after storage for 30 days. Stewart, Best, and Lowe (1943) produced a whole egg powder with good solubility characteristics by fermenting the egg pulp before drying. They also found that low moisture (less than 3.0%) powders were more stable. In accelerated storage studies at 50°C (122°F) one spray dried powder containing 1.4% moisture had a solubility of 57% after twelve days while a 4.0% moisture powder was soluble to an extent of only 37%. Adjustment of the pH to 6.5 prior to drying greatly enhanced shelf life. Bate-Smith and Hawthorne (1945) observed that the addition of all reducing sugars to fermented egg increased the rate of loss of solubility and accelerated the rate of increase of florescence (rates similar to those encountered with unfermented egg). Shelf life of spray dried whole egg was improved by the addition of 10% to 15% sucrose or lactose to unfermented egg before drying. Greene, Conrad, Olsen and Wagoner (1948) acidified the whole egg to a pH of 5.5 with hydrochloric acid, spray dried the egg using a redrier to bring the moisture content down to 2%, immediately cooled the powder, and packed the powder under gas (using a mixture of 20% carbon dioxide and 80% nitrogen). They claimed a useful storage life for their powder of six months at 100°F.

(3) The effect of surface formation on the solubility of ovalbumin. The rate and amount of surface formation is a factor in the
denaturation of many proteins. Since a large surface is formed rapidly during spray drying, it might be suspected that surface denaturation may have an influence upon the solubility of spray dried, protein containing materials such as whole egg. Most of the work on the surface phenomena of egg proteins has been performed on purified ovalbumin. Bull (1933) and Bull and Neurath (1937) found that the rate of denaturation of ovalbumin varied directly with the rate of surface formation and with the concentration of the protein solution up to 2% (under the conditions of their experiments). The rate of denaturation of ovalbumin would probably be altered in the presence of other proteins and suspended fat, thus the importance of this factor cannot be judged solely from these investigations.

(4) The effect of organic solvents on the solubility of egg proteins. Wu (1927) studied the coagulation of egg white by alcohol and concluded that it was essentially the same process as heat denaturation. Booth (1930) in a related study found that as the concentration of alcohol increased in a hemoglobin solution, the rate of denaturation increased; and as the temperature of the solution increased, the rate of solubility loss increased very markedly. The reaction thus has a high temperature coefficient. Ferry, Cohn, and Newman (1936) during their studies on solubility of the protein employed a temperature of -5°C, (25°F.) in order to prevent denaturation of ovalbumin in 25% ethanol. They observed that the protein was completely denatured in 25% ethanol at room temperature. Ether extraction of the lipoprotein complex of egg yolk renders the lipoprotein partially insoluble according to Bate-
Smith (1935). He stated that if 20% to 30% of sucrose is added prior to extraction of egg yolk lipoprotein, the protein/fat acid ratio may be raised to 5.1 to 1 without loss of solubility or even to 7.5 to 1 with very little loss. This sensitivity of most proteins to denaturation by organic solvents can probably be controlled not only by temperature and the presence of other substances but also by the moisture content.

b. The emulsifying ability of whole egg constituents. Egg yolk has long been used for its emulsifying ability particularly in the food industry as an emulsifier in salad dressings and mayonnaise; however, little work has been reported on the emulsifying efficiency of egg white and of yolk components. Clark and Mann (1922) employed a benzene/water system to measure the emulsifying ability of albumen. The albumen when compared with sucrose, starch, dextrin, and gum arabic was an excellent emulsifier. In concentrated solutions the white was found to lower surface tension to a greater degree than any of the other agents. Wilson (1927) tested the emulsifying ability of white, yolk, whole egg, and thickened whole egg (whole egg that had been allowed to stand for three weeks or more) in fat-liquor emulsions. The emulsions were formed by emulsifying neat's foot oil in a mixture of egg and water. The egg materials were compared on a wet basis. This method, of course, took no account of the solids content of the egg materials. The pH of all emulsions was about 7.6. The most stable emulsions were made from the thickened product (this product had the same moisture content as fresh whole egg). Egg white and whole egg were the next best emulsifiers under these conditions. Yolk made very poor emulsions when compared with the other
emulsifiers. Wilson used a shaking procedure to make up the emulsions, so the foaming power of white and to a lesser extent whole egg might have been a factor in the stability of emulsions made from them. Corran and Lewis (1924) compared the emulsifying ability of lecithin and cholesterol. Cholesterol tended to form water-in-oil emulsions and lecithin, oil-in-water emulsions. When lecithin and cephalin were used as a mixed emulsifier, the resulting emulsion was less stable than when either one was used alone.

Snell, Olsen, and Kremers (1935) investigated the emulsifying ability of egg yolk fractions. To prepare the emulsifier, egg yolk was salted and extracted several times with a hexane-acetone mixture. The final extractions were performed with hexane alone. The dry preparation was a light yellow powder, which they termed the "lecitho-protein" fraction. It contained 20.2% lecithin (on a salt free basis). The viscosity (measured by means of a Gardner mobilometer) of mayonnaises made from this material was used as a criterion of emulsifying efficiency. The authors also examined several other egg fractions for their emulsifying ability. The results are reproduced in Table 7. It is evident from this table that the "lecitho-protein" was an efficient emulsifier when compared with salted yolk. It is also evident that the addition of cephalin and lecithin greatly lowered the efficiency of "lecitho-protein". The "egg oil" of lot three was prepared by filtering off the liquid portion of the solvent free lipids extracted from the egg yolk. It thus probably contained a small amount of dissolved solid fats and phospholipids. The
Table 7. Emulsifying Powers of Egg Fractions
(Snell, Olsen, and Kremers, 1935)

<table>
<thead>
<tr>
<th>Lot</th>
<th>Emulsifier</th>
<th>Added ingredients</th>
<th>Consistency (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Salted yolk</td>
<td>None</td>
<td>15</td>
</tr>
<tr>
<td>2.</td>
<td>Reconstituted yolk</td>
<td>None</td>
<td>114</td>
</tr>
<tr>
<td>3.</td>
<td>Lecitho-protein and egg oil</td>
<td>None</td>
<td>900+</td>
</tr>
<tr>
<td>4.</td>
<td>Lecitho-protein and cottonseed oil</td>
<td>None</td>
<td>900+</td>
</tr>
<tr>
<td>5.</td>
<td>Same as 4</td>
<td>1.2 gms. cephalin</td>
<td>295</td>
</tr>
<tr>
<td>6.</td>
<td>&quot;</td>
<td>6.0 gms. lecithin</td>
<td>20</td>
</tr>
<tr>
<td>7.</td>
<td>&quot;</td>
<td>2.0 gms. cholesterol</td>
<td>900+</td>
</tr>
<tr>
<td>8.</td>
<td>&quot;</td>
<td>5.0 gms. fat</td>
<td>900+</td>
</tr>
<tr>
<td>9.</td>
<td>&quot;</td>
<td>5.0 gms. pigment</td>
<td>900+</td>
</tr>
</tbody>
</table>
"fat" of lot eight is the solid fat not including phospholipids, cholesterol, or pigments. The "lecitho-protein" preparation contained more lecithin than the lipoprotein preparations reported by other investigators (Osborne and Campbell, 1900; Calvery and White, 1932; Chargaff, 1942; Alderton and Fevold, 1945). It is probable that some residual "free lecithin" and perhaps some non-phospholipid oil were not extracted by their hexane-acetone extraction. Besides the "lecitho-protein" of egg yolk, there were also present the water soluble proteins, livetin, phosvitin, and vitellomucoid since no water extraction had been performed. In addition at least one other phospholipid besides lecithin was probably present. Their "lecitho-protein" thus was probably a mixture of lipoproteins, water soluble proteins, "free phospholipids, and egg oils. Since in previous experiments Snell, Olsen, and Kremers showed that the egg oil, egg pigments, and cholesterol had almost no emulsifying ability and since lecithin and cephalin were shown in the later experiments to actually interfere with the emulsification, the important emulsifying principles in their preparation were probably the proteins and lipoproteins.

Bennion, Hawthorne, and Bate-Smith (1942) noted a similar antagonism between the whipping properties of egg oil containing phospholipids and of the remaining portion of spray dried whole egg. They extracted some of the fat from spray dried whole egg and obtained a preparation exhibiting improved whipping properties. They concluded that spray drying changed
the lipids in some detrimental manner. Since both whipping properties and emulsifying properties relate in part to the formation of films a close relationship of these effects might be expected. Brooks and Hawthorne (1943) found that the addition of regular spray-dried whole egg to fresh egg reduced the latter's whipping properties more than the addition of an equivalent amount of water. According to the authors this indicated that spray drying released some foam-inhibiting factor in whole egg.

It appears that the principal emulsifying components of egg yolk are among the proteins and lipoproteins. Cholesterol and the phospholipids seem to interfere with the emulsification even though they possess some emulsifying ability of their own. Egg white proteins also possess emulsifying ability, thus for the purposes of this investigation the non-fat portion of whole egg would seem to be the most important of the whole egg components.

2. Methods for making oil-in-water emulsions

There are two common methods for making oil-in-water emulsions. The first method, similar to that employed by Corran and Lewis (1924), involves a simple shaking motion to incorporate all ingredients simultaneously and is best suited to the preparation of thin, unstable emulsions. In the second method the emulsion is formed by adding the oil to a mixture of the salt, sugar, spices, vinegar, and emulsifier. Kilgore (1935) outlined a basic procedure:
1. Egg yolk, sugar, salt, spices, and a small amount of vinegar are mixed together thoroughly.

2. Oil is added intermittently and slowly until the emulsion begins to form; the rate of addition is then increased.

3. Half of the oil is added then more vinegar then the rest of the oil followed by the rest of the vinegar.

The physical condition of the starting material, according to Kilgore, controls the quality of the final product. There must be sufficient moisture present to incorporate the emulsifier easily; however, too much water will dilute it excessively. Clayton (1935) explained that intermittent mixing is necessary to allow time for adsorption of the emulsifying agent at the interface. Lowe (1947) stated that as temperature rises, emulsification is generally more effective. The optimum temperature is limited, of course, by the coagulation point of the emulsifier. It is evident that the efficiency of emulsification is dependent in part upon the details of procedure; thus, a test of emulsifying ability would have to be designed to suit the needs of the particular experiment. The procedure in order to provide a valid comparison between emulsifiers would have to be carefully standardized.

3. Tests of efficiency of emulsification

The efficiency of emulsification may be defined in at least three ways, the degree of dispersion of oil (measured by the oil/water interface area or by the size distribution of the oil particles), the resistance of
the emulsion to the coalescence of oil drops, and the resistance of the emulsion to creaming (the appearance of a water layer). Which definition of efficiency is chosen depends upon the use to which the emulsion is to be put.

Tests of the efficiency of emulsification fall into two general categories, tests on the initial state of the emulsion (viscosity, oil/water interface area, size distribution of oil particles, etc.) and tests of the changes with time of emulsion characteristics (stability).

Kilgore (1933) diluted a sample of mayonnaise with an equal weight of water, shook the diluted emulsion, poured it into graduated cylinders, and allowed it to stand twenty-four hours. He used the amount of creaming for a measure of stability. Finkle, Draper, and Hildebrand (1923) suggested a direct microscopic count of oil particle sizes. Stamm (1925) diluted stable emulsions to hasten separation. The light scattering properties of the separating emulsion were then recorded photographically. Using this information and the height of the sedimenting phase, he derived a mathematical scheme for determining the size distribution of the oil particles. Gardner and Van Heukeroth (1927) developed a viscometer (called a "mobilometer") that has been widely used in the paint industry. It was employed by Snell, Olsen, and Kremers (1935) to measure the viscosity of their test emulsions. In operation of the "mobilometer" a plunger of known weight was made to fall through a vertical tube filled with the material to be measured. The time required for the plunger to
travel a given distance was taken as a measure of the viscosity. Gray and Southwick (1929) devised minor improvements providing greater operating ease and greater sensitivity.

Kraemer and Stamm (1934) determined the size distribution of the oil particles by a mathematical calculation derived from observation of the rate of change of the apparent density of the upper part of a standing emulsion. Their device measured this value continuously without disturbing the emulsion.

Of these methods for measuring emulsion efficiency, the most rapid and least tedious is the determination of initial viscosity. This measure presumably is correlated with the degree of dispersion of oil. Of the methods used to determine the distribution of oil drop sizes, the direct microscopic count would be the most accurate and the method of Kraemer and Stamm probably the fastest. Kilgore’s method would seem to be simple but like the method of Stamm measures stability under artificial conditions.

Bennett (1947) suggested two methods for obtaining a measure of stability with time. He suggested that the emulsion be centrifuged and the time required for separation of the phases be used as a measure of the stability. Bennett also designed a device consisting of a glass cylinder with a bulbous extension on one end. The bulb was fitted with a stop cock. The apparatus was filled with the test emulsion and stored at a definite temperature. After a time an aliquot was removed from the bottom and analyzed for the amount of moisture. This gave a measure of
the stability. The method of Kraemer and Stamm (1924) described above could be adapted directly to an empirical measure of rate of creaming with thimer emulsions. The various size distribution measurements and the determination of viscosity could of course also be used at intervals of time for stability measurements. It is suspected, however, that the appearance of water layers on the bottom of the emulsion, the appearance of oil layers on the top, and the usual unstable condition of an aged emulsion would render such measurements difficult.

D. Solvent Extraction

Solvent extraction has not been widely applied to inedible eggs because the value of the products has not been great enough to sustain the expense of the operation. It has, however, been applied to soybeans and is now a common industrial operation.

In the application of the solvent extraction process to whole egg, the solvent must be selected with several considerations in mind including price, efficiency, effect on egg constituents, health hazards, convenience in handling, and availability. Some of the solvents generally considered are listed in Table 8 along with a few of their properties and their current quantity prices. The use of some of the higher priced solvents would not be prohibitive if they could be recovered economically. Trichloroethylene, chloroform, and carbon tetrachloride are essentially non-flammable, while ethyl ether and acetone possess a dangerous flammability. The other solvents must be handled with great care. Ayers and Dooley (1948) studied
<table>
<thead>
<tr>
<th>Solvent</th>
<th>Lbs./gal.(^a)</th>
<th>Boiling point</th>
<th>Flammability (flash pt.)</th>
<th>Toxicity(^b)</th>
<th>Current price(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>6.5</td>
<td>69.0(^\circ) C.</td>
<td>Mildly</td>
<td></td>
<td>$ .17/gal.</td>
</tr>
<tr>
<td>Iso-propyl ether</td>
<td>6.1</td>
<td>82-85(^\circ) C.</td>
<td></td>
<td></td>
<td>$ .31/gal.</td>
</tr>
<tr>
<td>Methyl alcohol</td>
<td>6.6</td>
<td>66.0(^\circ) C.</td>
<td>Highly</td>
<td></td>
<td>$ .49/gal.</td>
</tr>
<tr>
<td>Carbon tetra-chloride</td>
<td>15.3</td>
<td>74.8(^\circ) C.</td>
<td>Non-flam.</td>
<td>Mildly</td>
<td>$1.06/gal.</td>
</tr>
<tr>
<td>Acetone</td>
<td>6.6</td>
<td>55-56(^\circ) C.</td>
<td>-17(^\circ) C.</td>
<td>Very mildly</td>
<td>$ .58/gal.</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>12.2</td>
<td>96.7(^\circ) C.</td>
<td>Non-flam.</td>
<td>Toxic</td>
<td>$1.22/gal.</td>
</tr>
<tr>
<td>Chloroform</td>
<td>12.5</td>
<td>61.3(^\circ) C.</td>
<td>Not easily flam.</td>
<td>Toxic</td>
<td>$2.12/gal.</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>7.5</td>
<td>74-77(^\circ) C.</td>
<td>-2(^\circ) C.</td>
<td>Non-toxic</td>
<td>$1.35/gal.</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>6.6</td>
<td>73.5(^\circ) C.</td>
<td>412(^\circ) C.</td>
<td>Very mildly</td>
<td>$1.25/gal.</td>
</tr>
</tbody>
</table>

\(^a\)Calculated from Lange’s Handbook of Chemistry (1946).

\(^b\)Lehmann and Flury (1943).

the efficiency of various petroleum hydrocarbons in the extraction of oil from cottonseed. They found that the methyl pentanes were the most efficient followed by normal paraffins, highly branched isohexanes, cyclo- paraffins, and aromatics in that order. Sweeney and Arnold (1949) reported a low solvent loss and high operating efficiency in a pilot plant study of the use of trichloroethylene for the extraction of soybeans.

Organic solvents in general are known to be toxic in varying degrees. Lahmann and Flury (1943) reported that a concentration of 190 parts per million by volume of trichloroethylene in the air is safe, but that toxic effects from higher concentrations seemed to be cumulative. Clinton (1948) pointed out that trichloroethylene was particularly dangerous because it can be breathed without discomfort. Wilson (1949) stated that the chlorinated hydrocarbons are generally toxic, sufficient exposure to them resulting in such conditions as jaundice, diarrhea, nausea, headache, and cirrhosis of the liver.

E. Industrial Applications of the Emulsifying Ability of Whole Egg

Egg by-products have been commonly used as emulsifiers in the fat- liquoring (oiling) operation in leather manufacture. According to USDA Circular 583 (1941) "Tanner's yolk", as the product is called, is made from inedible whole eggs, yolks, or mixtures of these. Sources of tanner's egg include rejects from dealers and cold storage, infertile eggs from hatcheries, inedibles from egg breaking operations, etc. Usually the
...would be produced from fresh whole eggs. It is probable that an additional estimate that would find use in the breeding of these eggs would have to be produced from other sources. The egg of the Phaeton produces for some mysterious reason very large amounts of mucus, serous, and albuminous, secreted directly from the egg. A similar power of eggs to make the need for one to see the world is eliminated when the egg is taken...
Whole egg fractions were prepared from incubated 16 day infertile eggs from the College Poultry Farm and from similar eggs obtained from a local hatchery. The examination of the effect of processing variables on the emulsifying ability of the whole egg emulsion was performed on fresh whole eggs. The fresh eggs, except in certain cases noted later, were obtained from the College Poultry Farm.

1. Preparation of yolk, white, and fresh whole egg samples.

Three day eggs were secured from the College Poultry Farm, stored at 4°C (39.2°F) until broken, and separated on a standard commercial egg breaker (braking tray and separator). The whites were separated into two equal portions, the yolk was immediately homogenized. Whole egg samples were prepared by mixing with a rotary hand beater until macroscopically homogenous. Whole egg samples were used (the maximum storage period was six hours).

Eggs from the College Poultry Farm were set at 37.7°C (99.9°F).

2. Preparation of whole egg from incubator infertiles.
in a regular forced draft electric incubator. At the end of eighteen days, the eggs were candled and the fertiles were removed. The infertiles were then broken, mixed, and stored in the same manner as the fresh whole eggs. The shells were scraped out with a spatula to remove all adhering white and yolk material. A second lot of eighteen day infertile eggs was purchased from a local hatchery and broken in the same manner.

B. Processing of Samples

The processing methods used for the various preparations are outlined in the appropriate sections in the Results and Discussion. The general methods are explained below.

1. Centrifugation

The centrifuging process was carried out with a motor-driven Sharples Supercentrifuge at 14,500 times gravity (26,000 revolutions per minute). A continuous-feed bowl of about 400 ml. capacity was used. The feed nozzle had an orifice of 2.2 mm. in diameter.

2. Spray drying

The spray drier was previously built in this laboratory by Mr. D.H. Bergquist. The construction of this drier is shown by Figure 1. Two two-fluid spray nozzles were used (air was the second fluid), one an internal mixing nozzle with an orifice 1.6 mm. in diameter, and the second an external mixing nozzle with an orifice 0.9 mm. in diameter. The powder
Fig. 1 Schematic Diagram of Laboratory Spray Drier
was collected by shutting down the operation and removing the spray dried material from the bag and drying chamber. One spray drying run required fifteen to twenty minutes and produced from 150 to 200 grams of whole egg powder.

3. Lyophilisation

Lyophilisation was performed on an apparatus previously built in this laboratory by Dr. Ralph W. Kline (Hanson, 1945). The material to be dried was frozen in flasks immersed in the condenser bath maintained at $-35^\circ$ C. to $-45^\circ$ C. ($-31^\circ$ F. to $-49^\circ$ F.). The drying was performed at a vacuum of one to four mm. absolute pressure. A ten to twenty hour drying period was sufficient to reduce the moisture content below 10%.

4. Extraction

The solvents used were ethyl ether, ethyl alcohol, trichloroethylene, and water. The extraction procedure was the same for both liquids and solids. The ether extractions were carried out at $0^\circ$ C. ($32^\circ$ F.) in six liter flasks. The extraction was considered complete if the solvent was colorless after four hours contact with the egg product. The solvent was removed by decanting. During decantation the temperature of the material did not rise appreciably above the extraction temperature. After the solvent had been decanted, the remaining ether was removed from liquids by vacuum distillation. The remaining solvent was removed from extracted powders by filtration followed by air drying at room temperature. The water extractions were carried out in a similar manner.
Trichloroethylene extractions were performed in a glass Soxhlet extractor of 1.2 liters capacity (Figure 2). The temperature of the oil bath was controlled at 120° C. (248° F.) by a thermoregulator. For extractions made at 71° C. to 77° C. (160° F. to 171° F.), the regular side arm of the extractor conducted the solvent vapors to the condenser. For lower temperature extractions (18° C. to 21° C. or 68° F. to 70° F.), a special boiling flask was used. This flask had a long detachable side arm which conveyed the vapors to the top of the condenser. The liquified solvent travelled the full length of the condenser before entering the extraction flask. Solvent temperatures as low as 18° C. (65° F.) at the point of extraction were attainable using this method.

The powder to be extracted was placed in a filter bag made of ordinary muslin sheeting. The bag was supported in a framework of copper tubing so that the top edge of the filter was higher than the siphon level. The extraction cycle varied from twenty-five to fifty minutes. The temperature of both the condensed solvent and the extracted powder was measured by means of a thermocouple-potentiometer setup.

5. Concentration and vacuum drying

A large water bath was constructed to hold three vacuum desiccators. The temperature of the water was controlled at 27° C. ± 1° C. (80.6° F. ± 1.8° F.) by a thermoregulator. The material to be concentrated was weighed into the desiccators. A vacuum of two mm. of mercury was then drawn on the system and maintained until the desired concentration had been achieved. A freezing trap was employed to condense the vaporizing moisture. The
Fig. 2  Soxhlet Extraction Flask (1.2 liters cap.) with special side arm for Extractions at 18°C to 21°C (65°F to 70°F)
approximate solids content was determined by periodically weighing the desiccators and contents. Concentration of liquid whole egg from 25% solids to 40% solids required about seven hours with the size sample used (300 to 400 ml.). Concentration to dryness (about 10% moisture) required about twenty hours. With laboratory prepared samples no off odors developed in the egg during concentration; however, since the commercial samples of liquid whole egg were of low quality when collected (at the spray drying plant), it was necessary to add salt prior to concentration to inhibit further microbial action.

6. Heating of powders

Extracted whole egg powders were placed in 20 x 175 mm. test tubes and heated at 71° C. to 77° C. (160° F. to 171° F.) in a hot air oven. Internal temperatures were measured by means of a thermocouple-potentiometer setup. The heating time was measured from the moment the powder reached a temperature of 66° C. (150° F.). This generally required about fifteen minutes. At the end of the heating period, the powders were emptied onto a sheet of paper where they were allowed to cool in air.

7. Homogenization

Homogenization was performed on a hand homogenizer (Fisher Scientific Company) by forcing the liquid between two flat plates held together by a spring. Homogenizing pressures up to 600 p.s.i. were developed.
8. **Freezing and frozen storage**

Each sample to be frozen was placed in an enameled \#2 tin can. The tops of the cans were covered with wrapping paper and the paper was secured with tape. The cans were then placed in a single layer in the freezing compartment of a deep freeze unit maintained at \( -17^\circ \text{C.} \) \((1^\circ \text{F.})\). The cans were so spaced that they did not touch each other. All cans were as far from the freezer walls as possible. After the appropriate storage time, the contents were thawed overnight in a forced current of \( 4^\circ \text{C.} \) \((39^\circ \text{F.})\) air.

**G. Chemical Analyses**

1. **Fat analysis**

Fat analysis was carried out by the solvent extraction method. Prior to use, extraction flasks were heated in a \( 110^\circ \text{C.} \) \((230^\circ \text{F.})\) oven for twenty minutes, cooled in a desiccator, and weighed. Dry samples of powder were weighed in cones of filter paper, placed in Whatman 22 x 80 mm, double thickness extraction thimbles, and extracted in a Goldfisch-type extractor. The extracting solvent was a mixture of five parts of ethyl alcohol to one part of ethyl ether. The extraction was continued for approximately sixteen hours. At the end of this period the solvent was evaporated slowly from the extraction flasks, which were then placed in a \( 110^\circ \text{C.} \) \((230^\circ \text{F.})\) oven for one hour. The flasks were cooled in a desiccator and weighed. Liquid samples were dried for three hours at \( 95^\circ \text{C.} \) \((203^\circ \text{F.})\) under a vacuum of 30 inches (gauge) prior to extraction.
2. Moisture analysis

The samples were weighed into moisture tins and then dried in a vacuum oven for three hours at 95°C. (203°F.) and under a vacuum of 30 inches (gauge). They were cooled in a desiccator and reweighed.

D. Emulsifying Ability Test

A mayonnaise-type emulsion was used to test the emulsifying ability of the egg preparations. The ability of a material to emulsify oil in water may be expressed by the rate of increase of the emulsion viscosity with the increase in concentration of the emulsifier in the emulsion. To provide a measure of this rate, five test emulsions, each with a different amount of emulsifier present, were made from each egg preparation. The viscosities of these five emulsions were then plotted against the concentration of the emulsifier and from this plot an emulsifying index was determined. The test procedure described below was used for the major portion of this study: namely, the examination of the effect of processing variables on the emulsifying ability of the isolated emulsifier (Section D, Results and Discussion). This test was a refinement of the earlier test used to examine the emulsifying abilities of the isolated egg fractions (Sections B and C, Results and Discussion).

1. Preparation of the emulsifier

The various egg preparations contained different amounts of non-fat-solids, fat, and water. The preparation of these materials for the emulsi-
measured out to make up the following mixture:

defer applicable quantities of the other components were
determined, e.g. model (b) shown diagrammed, according to the same concentration to make the glass best compatible with and tranformed to a

a

The preparation was made as described below:

the EE extract was dissolved in the same solvent of the preparation for preparation of the mixture. When the procedure all the preparations were based under the same conditions. The dehydrated procedure for the preparation of the 31° + 0.20 + N to 1. When all the EE preparations were made up to the same composition before the test, the same composition was used for the test.

soil and the non-15/0.5 (0.5 to 1) Bodman orthophosphate and sucrose were added to the water/15/0.5 (0.5 to 1) What was added to make the water/15/0.5 (0.5 to 1) made up to the same composition with respect to these ingredients. Corn oil was added to make the 15/0.5 (0.5 to 1) made up to the same composition with respect to these ingredients.
The sucrose and NaCl were dissolved in the water, and a quantity of this solution sufficient to make a thick paste was mixed into the egg fraction by hand. This paste was then mixed on the machine for four minutes at the lowest speed. At the end of this time the remaining aqueous solution was added. The sides of the bowl were then scraped down and the mixing was continued for three minutes. The preparation was stored at 1°C. (34°F.) until used. Just prior to testing, the proper amount of fat (corn oil) was added from a buret and mixed in at the second speed for 15 seconds.

b. Wet preparations. A sufficient quantity of the liquid egg fraction was weighed into a beaker. Appropriate quantities of the other emulsifier components were measured out to make up the ratios given above. The three ingredients were added to the preparation and stirred in until the emulsifier was homogeneous. The material was then stored at 1°C. (34°F.) until tested. Just before testing the proper amount of oil was measured out from a buret and mixed in at second speed for 15 seconds.

2. Procedure for preparation of the emulsion

The method for making the emulsion is a modification of the method of Kilgore (1935) for making mayonnaise. In general the emulsion was formed by mixing oil into the emulsifier in fourteen additions with a rest period between each addition. Between certain of the oil additions, a portion of a water solution of NaCl, 50% acetic acid, and sucrose, was added. (Originally this acid was added when the emulsifier was first
prepared; however, it was noted that the high acid conditions caused the emulsifier to thicken and in some cases precipitate overnight. The acid was thus added during the mixing of oil to avoid local excesses in concentration.) The ingredients were incorporated on a Hobart "KitchenAid, model 4" mixer. The formulae for the emulsions are given in Table 9.

The detailed procedure for mixing in the materials is outlined in Table 10. Five emulsions were made from each emulsifier. The concentrations were selected (Table 9) on the basis of the expected emulsifying ability of the preparation.

From Table 10, it may be seen that the usual rest periods were fifteen seconds; however, during the scraping down operations, the period was one minute. The last additions of oil and water solution were mixed in for thirty instead of fifteen seconds.

The procedure described above was used to test the emulsifying ability of all spray dried preparations; however, some of the tests were conducted on the effect of freezing, homogenization, and vacuum concentration on the emulsifying ability of whole egg. Since the water/non-fat-solids ratio of whole egg is about 6.0 to 1, an alteration was made in the procedure so that the emulsifying ability of the whole egg preparations could be tested. The water/non-fat-solids ratio used in preparing the emulsifier was raised to 6.2 to 1. The NaCl/non-fat-solids ratio was raised to 0.43 to 1 and the sucrose/non-fat-solids ratio was raised to 0.62 to 1. The fat/non-fat-solids ratio remained at 1.2 to 1. Only one
Table 9. Formulae for the Test Emulsions

<table>
<thead>
<tr>
<th>NFS emulsifier weight (%)</th>
<th>50% acetic acid (ml.)*</th>
<th>Oilb of NaCl, Sucrose, and Acetic Acid (ml.)c</th>
<th>Water solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>16.3</td>
<td>0.3</td>
<td>330</td>
</tr>
<tr>
<td>1.4</td>
<td>32.6</td>
<td>0.7</td>
<td>324</td>
</tr>
<tr>
<td>2.1</td>
<td>48.9</td>
<td>1.0</td>
<td>319</td>
</tr>
<tr>
<td>2.3</td>
<td>65.2</td>
<td>1.4</td>
<td>312</td>
</tr>
<tr>
<td>3.5</td>
<td>61.5</td>
<td>1.7</td>
<td>306</td>
</tr>
<tr>
<td>4.2</td>
<td>97.6</td>
<td>2.1</td>
<td>299</td>
</tr>
<tr>
<td>4.9</td>
<td>114.2</td>
<td>2.4</td>
<td>293</td>
</tr>
<tr>
<td>5.6</td>
<td>130.5</td>
<td>2.8</td>
<td>287</td>
</tr>
</tbody>
</table>

*a Added during the third oil addition.

*b Added in fourteen additions (Table 18, Appendix).

*c Added in four additions (Table 19, Appendix). The solution was made up to the following ratios:

\[
\frac{NaCl}{Water} = 0.07 \\
\frac{Sucrose}{Water} = 0.10 \\
\frac{Acetic acid}{Water} = 0.04
\]

These are the same ingredient water ratios used for preparing the emulsifier (previously expressed as ingredient non-fat-solids ratios).
Table 10. Procedure for Making Emulsion

<table>
<thead>
<tr>
<th>Time</th>
<th>Operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:00</td>
<td>Emulsifier prepared.</td>
</tr>
<tr>
<td>0:15</td>
<td>Mix oil addition 1.*(speed #2).</td>
</tr>
<tr>
<td>0:30</td>
<td>Rest.</td>
</tr>
<tr>
<td>0:45</td>
<td>Mix oil addition 2. (speed #2).</td>
</tr>
<tr>
<td>1:00</td>
<td>Rest.</td>
</tr>
<tr>
<td>1:15</td>
<td>Mix oil addition 3. (speed #2). Add acetic acid (column #3, Table 10).</td>
</tr>
<tr>
<td>1:30</td>
<td>Rest.</td>
</tr>
<tr>
<td>1:45</td>
<td>Mix oil addition 4. (speed #2).</td>
</tr>
<tr>
<td>2:00</td>
<td>Rest. Scrap down bowl and mixing blade.</td>
</tr>
<tr>
<td>3:00</td>
<td>Mix solution addition 1.** (speed #1).</td>
</tr>
<tr>
<td>3:15</td>
<td>Rest.</td>
</tr>
<tr>
<td>3:30 to 3:45</td>
<td>Mix oil additions 5 to 10 with 15 second rest periods between additions. (speed #1).</td>
</tr>
<tr>
<td>4:00</td>
<td>Rest.</td>
</tr>
<tr>
<td>4:15</td>
<td>Mix solution addition 2. (speed #1).</td>
</tr>
<tr>
<td>4:30</td>
<td>Rest.</td>
</tr>
<tr>
<td>5:00 to 5:15</td>
<td>Mix oil additions 11 and 12 with 15 second rest periods between additions. (speed #1).</td>
</tr>
<tr>
<td>5:45</td>
<td>Rest.</td>
</tr>
<tr>
<td>6:00</td>
<td>Rest.</td>
</tr>
<tr>
<td>6:15</td>
<td>Mix solution addition 3. (speed #1).</td>
</tr>
<tr>
<td>6:30</td>
<td>Rest.</td>
</tr>
<tr>
<td>6:45</td>
<td>Mix oil addition 13. (speed #1).</td>
</tr>
<tr>
<td>7:00</td>
<td>Rest.</td>
</tr>
<tr>
<td>7:15</td>
<td>Mix oil addition 14. (speed #1).</td>
</tr>
<tr>
<td>8:00</td>
<td>Rest.</td>
</tr>
<tr>
<td>8:15</td>
<td>Mix solution addition 4. (speed #1).</td>
</tr>
<tr>
<td>8:30</td>
<td>Rest.</td>
</tr>
<tr>
<td>8:45</td>
<td>Mix oil addition 15. (speed #1).</td>
</tr>
<tr>
<td>9:00</td>
<td>Rest.</td>
</tr>
<tr>
<td>9:15</td>
<td>Mix oil addition 16. (speed #1).</td>
</tr>
<tr>
<td>10:30</td>
<td>Rest. Scrap down bowl and mixing blade.</td>
</tr>
<tr>
<td>11:00</td>
<td>Mix solution addition 4.</td>
</tr>
</tbody>
</table>

*See Table 18, Appendix, for the volumes of the fourteen additions. The total volume of oil is given in Table 9.

**See Table 19, Appendix, for the volumes of the four additions. The total volume of this solution is given in Table 9.
that the number of drops per 60 second interval was increased from each test specimen. Each mount was counted once. If the assumed

maximal number of 100 to 200 drops was exceeded, the number of drops per a 100

min test specimen was then counted, until that test specimen was completed with 100 of

a maximal number of drops per 60 second interval. The number of all drops per a

maximal number of 0.04 min test specimen was increased until a gel support of a test-

piece was obtained beyond the 0.04 min test specimen, then the sample was taken out to com-

plete this period. Further, when the gel support became clear, the sample was taken out of

the cover slip of the gel plate used for the homograft mounting cell was then

covered. Either 1% or 0.05% above the stage jutt revealed of the mount 0.1 min

The two horizontal supports of the gel were ground down so that the

surface of the gel to cover the stage of a specimen-prepared hemocytometer.

The examination to cover the stage of a specimen prepa-red hemocytometer

this period just enough material was revealed from beneath the surface of

gel acetate to be observed then the gel acetate for ten minutes. At the end of

clear inspection (in order to establish the relation between the difference between the
drop size and dosage). The samples from the examination were made, three of

these observations were made during the inspection.

a. Only those observations were made on the completed test specimen.

b. Observations were made on the complete test specimen.

three criteria may be found in Section A, Results and Discussion.

this examination is the completion of the test specimen of these

the test specimen were done to the first drop determine, and the second drop determine.

For this procedure are given in Tables 20 and 21, Appendix.
average diameter of the oil drops.

b. **Emulsion viscosity.** A Gardner mobilometer (Simer and Amend) was used to measure the viscosity. A stainless steel cylinder 235 mm. high and 39 mm. in diameter was secured in a vertical position. The material to be measured was placed inside the cylinder. A weight pan to which was added 20 grams of weight was attached to the top of a plunger (the plunger, weight pan, and weight weighed 54 grams). The plunger, fitted at the bottom and with a disc perforated with 51 small holes 1.8 mm. in diameter, was allowed to fall through the test emulsion. The time required for the plunger disc to travel four inches from a point eight inches above the bottom of the cylinder was taken as a measure of the viscosity. In all cases only one reading was taken.

c. **Emulsion stability.** These observations were made during the investigation of the emulsifying ability of egg fractions (Section A, Results and Discussion) in order to establish the relationship between stability and viscosity. After the sample had been secured for the viscosity measurement, the remaining emulsion was poured into a \( \frac{1}{2} \) pint mason jar, which was sealed and stored at 21° C. \( \pm \) 2° C. (70° F. \( \pm \) 4° F.). The time of the first appearance of water was used as a measurement of the stability of the emulsion (the appearance of oil was much slower and even less definite than the appearance of water).
The effect of increased concentration of the emulsifier on the stability of the emulsion was increased.

I. Effect of Increased Concentration of the Emulsifier on the Emulsion

As the concentration of the emulsifier in the emulsion was increased,
Fig. 3 Effect of Concentration of Emulsifier on the Viscosity of the Test Emulsions (Spray Dried Whole Egg).
Fig. 4 Graphical Procedure for Obtaining Emulsifying Index.
concentration" is a characteristic of the emulsifying ability of the various egg fractions, and thus can be used to measure this ability.

It appears that the critical concentration can be satisfactorily represented by a measurement on the X axis of the point at which the plot of viscosity versus non-fat-solids curves most sharply; that is, the point at which the rate of change of the tangent to the curve is greatest. The portion of the curve immediately adjacent to this point of maximum curvature closely approximates an equilateral hyperbola having the X and Y axes as asymptotes. The point of maximum curvature of an equilateral hyperbola with these asymptotes is represented by the tangent to the curve drawn at an angle of 45°. Thus a 45° tangent drawn to the plots of viscosity versus non-fat-solids provides an approximate measure of the point of maximum curvature and thus of the critical concentration.

Figure 4 illustrates a comparison of two materials of different emulsifying ability. Curve A evidently represents a preparation which has an emulsifying ability greater than that of the preparation represented by Curve B. Tangents to the curves were drawn at an angle of 45° and vertical lines were drawn through the point of tangency to the X axis. The critical concentration of preparation A is measured as 1.97% non-fat-solids and that of preparation B, 3.43% non-fat-solids. To convert these figures to emulsifying index, the following formula was used:

\[ EI = 10(10-C). \]

Where: \( EI \) = Emulsifying index,
\( C \) = Critical concentration.

Thus \( EI \) equals 80.3 for emulsifier A and 65.7 for emulsifier B. It was
noted that the EI determined in this manner afforded in almost all cases conclusions similar to those resulting from visual examination of the curves.

It is apparent that there is need for refinement of the test to allow a more precise measurement of the critical concentration. Since the viscosity of the emulsion increases so rapidly after a particular concentration is reached, it is possible that the use of a greater weight on the weight pan of the mobilometer might reduce the range at which this concentration becomes evident and thus reduce the error of its estimation. It might also be possible to adjust the shape of the curve by judicious alteration of the procedure for making the emulsions. It was noted that the curve could be altered by changes in the rates of oil and water addition and by changing the initial ratios of ingredients.

2. Reproducibility

In order to evaluate differences in EI such as those determined above, the reproducibility of the testing procedure was investigated by conducting repeated tests on a homogeneous lot of fresh egg yolk. A second series of tests was performed to measure the error introduced by reconstitution of dried preparations. A third series of tests on spray dried whole egg were done over a period of 70 days in order to detect any changes in the test results that might have occurred during that time. These measurements were used for the purpose of comparing differences in results throughout this study except in the study of the effect of freezing on
the emulsifying ability of whole egg. The freezing experiments, since
the effect of each treatment was determined by three replications, were
treated in a statistical manner.

a. Reproducibility of the emulsifying test. The reproducibility
tests were performed on a large amount of fresh egg yolks prepared as
described. A total of twenty tests were performed in a randomized order
at four egg yolk concentrations. The yolk was stored at 1°C, (34°F.)
during the two overnight periods until the tests were completed. The
shaded area between the lines of Figure 5 includes the extremes of all
data. This area represents the reproducibility of the test itself and
may be used to make a visual comparison between two lines representing
the emulsifying ability of two emulsifiers. The extremes of emulsifying
index are 60.8 and 58.1 the difference between them being 2.7. It would
thus appear that two emulsifiers whose emulsifying indices differ more
than 3.0 (when tested under these conditions) probably differ in their
emulsifying ability.

b. Reproducibility of reconstitution of dried powders. Five samples
of spray dried whole egg were reconstituted (according to Section D-l-a,
Experimental Procedure) within two days of each other and tested for
emulsifying ability. Four concentrations were employed for each sample.
The results are illustrated in Figure 6. Visually the results were
similar to those of Figure 5. The emulsifying index interval was 1.3
(66.2 - 64.9) compared with 2.7 for test reproducibility, thus the
standardization of the reconstitution procedure was considered adequate.
Fig. 5    Reproducibility - Emulsifying Test
Fig. 6 Reproducibility—Reconstitution of Spray Dried Whole Egg Powder.
c. Reproducibility of the test over a period of time. Spray dried whole egg was kept in a $1^\circ$ C. ($34^\circ$ F.) refrigerator when not used so as to minimize storage effects. Tests of the emulsifying ability were performed at intervals of time over a period of 70 days. A total of 25 tests was performed at five concentrations. The results are illustrated in Figure 7. The emulsifying index interval was 1.5 ($67.7 - 66.1$) compared with 2.7 for the test reproducibility; thus time was not considered to be an important factor in reproducibility of results.

Why the limits of emulsifying index for the operator reproducibility were greater than the limits for the reproducibility of reconstitution (error here consisted of operator plus reconstitution error) and were greater than the limits for the tests performed over a period of time (error consists of operator plus reconstitution plus time error) is not clear. It is possible that some of the more unusual values were encountered during the operator tests. In view of this possibility the conclusion that a difference in the emulsifying indices of 5.0 probably represents an actual difference in emulsifying ability of any two preparations would seem to be a logical one.

3. Choice of a criterion for determination of degree of emulsification in test emulsions

Throughout these tests, viscosity of the test emulsion has been used as a criterion of degree of emulsification, and conclusions have been drawn on the differences in the viscosity of these emulsions. Viscosity is only one of the possible criteria that might have been used, others being stabil-
Fig. 7 Reproducibility—Reconstitution of Spray Dried Whole Egg over Period of 70 Days.
If I were to paint a picture of the world, I'd make sure that every drop counted and that every stroke was deliberate and precise. But in reality, we often struggle to achieve such perfection. The same applies to our daily lives. We aim for excellence, but sometimes our efforts fall short. Nevertheless, we strive to improve, learning from our mistakes and striving for excellence in every aspect of our lives.

The key is to keep moving forward, even when progress is slow. Each step, no matter how small, is a step in the right direction. And just as in painting, it's the details that make the difference. So let's keep working on ourselves, pushing ourselves to be better every day. After all, life is a work in progress, and we are all artists in our own lives.
Fig. 8 Relationship between Viscosity and the Number of Oil Drops per Microscope Field.
Fig. 9 Relationship between Stability and Number of Oil Drops per Microscope Field.
was considered into two parts. Half was removed to the 6.6% or 1 minute end

This translated to 710°C or 770°C.

*Subject

drawn from each and the same manner

type of tests, it was desirable to determine whether or not comparable

performant procedure.

section (6.6 to 1) (see section on Ex-

erty that were made up to a lower ratio (6.6 to 1) (1).

other tests were performed on samples

rather on natural shape of the given test,

in kase of the effect of freezing and frozen storage on the enamel.

<table>
<thead>
<tr>
<th>High water/non-ratio.</th>
<th>Low water/non-ratio.</th>
</tr>
</thead>
</table>

*6. Plateau formation between the low water/non-ratio. Formations and

Enamel.

Examine of the enamel

type of enamel used at the time the plaster was separated into two parts. The roughness of the surface of the plaster was determined by a micrometer.

A test was made up to a lower ratio (6.6 to 1) (1) (see section on Ex-

section (6.6 to 1) (1) (see section on Ex-

experiments on enamel. The enamel was then tested for the enamel.

step formation in the enamel.

experiments on enamel. The enamel was then tested for the enamel.

then separated into two parts. The roughness of the surface of the plaster was determined by a micrometer.

A test was made up to a lower ratio (6.6 to 1) (1) (see section on Ex-

section (6.6 to 1) (1) (see section on Ex-

experiments on enamel. The enamel was then tested for the enamel.

step formation in the enamel.
half to the 3:1 to 1 ratio. These preparations were then tested with their respective formulae. The results are shown in Figure 10. The emulsifying indices for the 6:2 to 1 ratio formulae were 90.8, 85.2, and less than 75.0; and the emulsifying indices for the 3:1 to 1 ratio formulae were 87.4, 74.8, and less than 65.0. These data indicate that the results are similar only in that an increase in the measurement of emulsifying ability by one test is accompanied by an increase in the measurement by the other. The emulsifying indices cannot be directly compared but conclusions drawn from these indices can.

5. Operation of the mobilometer

Early in this study the viscosity measurements were calculated as an average of five readings from five successive plunges of the disc. It was noted, however, that shorter times were obtained with each successive plunge (Table 11). It was thus concluded that the treatment broke down the emulsions and only one reading was taken as a measure of the viscosity.

E. Emulsifying Ability of Egg Fractions

The primary purpose of this portion of the study was to isolate and test the emulsifying components of whole egg. The emulsifying abilities of egg albumen and egg yolk fractions were examined to further separate the emulsifying principles. Egg yolk fractionation was also undertaken with a view toward the possible application of this study to fresh eggs. The original low water/non-fat-solids ratio formulae were used to measure emulsifying ability in this section.

1. The emulsifying ability of whole egg fractions

The most extensive fractionation procedure was carried out on spray
Fig. 10 The Relation between Low and High Water/non-fat-solids Ratio Formulas.
<table>
<thead>
<tr>
<th>Material</th>
<th>Emulsion viscosity (Seconds)</th>
<th>Material</th>
<th>Emulsion viscosity (Seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh egg yolk</td>
<td>139.0</td>
<td>Fresh whole egg</td>
<td>94.0</td>
</tr>
<tr>
<td></td>
<td>131.5</td>
<td></td>
<td>76.1</td>
</tr>
<tr>
<td></td>
<td>122.0</td>
<td></td>
<td>76.3</td>
</tr>
<tr>
<td></td>
<td>125.7</td>
<td></td>
<td>65.0</td>
</tr>
<tr>
<td></td>
<td>119.0</td>
<td></td>
<td>58.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>56.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>51.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>43.5</td>
</tr>
</tbody>
</table>
dried whole egg prepared in this laboratory. This concentration step was necessary to allow the preparations to be tested with the existing emulsion formulae. The fractionation procedure was duplicated, the first fractionation being carried out on eighteen day infertile eggs incubated at the College Poultry Farm and the second fractionation being carried out on a lot of infertile, incubated eggs obtained from a local hatchery. The fractionation procedure is outlined in Figure 11.

A third preparation was undertaken to examine the emulsifying ability of a fraction analogous to the lipovitellin fraction of Alderton and Fevold (1945) as compared with a fraction probably containing the lipovitellinenin of Fevold and Lausten (1946) and other of the more soluble whole egg constituents. Eighteen day infertile eggs were prepared as described and subjected to supercentrifugation at 14,300 times gravity. The fractionation procedure is outlined by Figure 12.

The emulsifying indices of these preparations are given in Tables 12a and 12b. The emulsifying abilities are illustrated in Figures 13 and 14. It is evident from an examination of Figure 13 and Table 12a that the most efficient emulsifiers prepared from spray dried whole egg were the ether insoluble portions (prep. 2a and 2b). The ether soluble lipids (prep. 4a) possessed little or no emulsifying ability. In the first of the fractionation experiments, further fractionation of the ether insoluble spray dried whole egg powder with water failed to further concentrate the emulsifying principles, since both the residue (prep. 3a) and filtrate (prep. 5a) were inferior to the unextracted powder (prep. 2a). In the second experiment, however, the residue after extraction of the
Fig. 11 The Fractionation of Spray Dried Whole Egg.

a. SEE FIG. 13
b. PRINCIPALLY PROTEINS (BY ANALOGY FROM THE WORK OF CHARGAFF ON YOLK, 1942).
c. PRINCIPALLY LIPOPROTEINS (BY ANALOGY FROM CHARGAFF, 1942).
d. PRINCIPALLY PHOSPHOLIPIDS (BY ANALOGY FROM CHARGAFF, ET. AL, 1942).
DILUTED 05:1 WITH WATER. SUPERCENTRIFUGED

RESIDUE

WATER INSOLUBLE PORTION

(PREP. 7)

SUPERNATANT

SPRAY DRIED

DRIED WATER SOL. PORTION

EXTRACTED WITH ETHER AT 0°C (32°F)

ETHER INSOLUBLE PORTION

(PREP. 9)

48c.

WHOLE EGG

a. SEE FIG. 14

b. PRINCIPALLY LIPOVITELLIN PLUS SOME FAT (BY ANALOGY FROM ALDERTON AND FEVOLD, 1945).

c. PRINCIPALLY WATER SOLUBLE PROTEINS, LIPOVITELLENIN, AND SOME FAT (BY ANALOGY FROM FEVOLD AND LAUSTEN, 1946).

d. PRINCIPALLY WATER SOLUBLE PROTEINS PLUS LIPOVITELLENIN (BY ANALOGY FROM FEVOLD AND LAUSTEN, 1946).

Fig. 12 The Fractionation of Liquid Whole Egg.
Table 12a. Emulsifying Indices of Egg Fractions

<table>
<thead>
<tr>
<th>Prep. no.</th>
<th>Means of preparation</th>
<th>Emulsifying index a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Whole egg, spray dried</td>
<td>42.5</td>
<td>53.5</td>
</tr>
<tr>
<td>2.</td>
<td>Ether ins. portion of #1 (&quot;proteins&quot; of whole egg)</td>
<td>71.3</td>
<td>63.7</td>
</tr>
<tr>
<td>3.</td>
<td>Water ins. portion of #2 (&quot;lipoprotein&quot; plus some water soluble &quot;proteins&quot;)</td>
<td>54.3</td>
<td>39.9</td>
</tr>
<tr>
<td>4.</td>
<td>Ether sol. portion of #1 (egg lipids)</td>
<td>&lt;32.0</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Water sol. portion of #2 (mostly &quot;livetin&quot; plus some albumen)</td>
<td>&lt;32.0</td>
<td>&lt;36.0</td>
</tr>
<tr>
<td>6.</td>
<td>Acetone ins. portion of #4 (&quot;phospholipids&quot;)</td>
<td>37.5</td>
<td></td>
</tr>
</tbody>
</table>

*First experiment.  
*Second experiment.

Table 12b. Emulsifying Indices of Egg Fractions

<table>
<thead>
<tr>
<th>Prep. no.</th>
<th>Means of preparation</th>
<th>Emulsifying index a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.</td>
<td>Supercant. res. of whole egg</td>
<td>30.4</td>
<td>23.7</td>
</tr>
<tr>
<td>8.</td>
<td>Supernatant from cent. of whole egg (spray dried). (&quot;lipoprotein&quot; plus water sol. &quot;proteins&quot; plus some fat)</td>
<td>51.3</td>
<td>&lt;36.0</td>
</tr>
<tr>
<td>9.</td>
<td>Ether ins. portion of #6 (#6 minus ether sol. fat)</td>
<td>67.7</td>
<td></td>
</tr>
</tbody>
</table>

*First experiment.  
*Second experiment.
Fig. 13 Emulsifying Ability of Whole Egg Fractions.
Fig. 14 Emulsifying Ability of Whole Egg Fractions Obtained by Supercentrifugation.
The experimental studies of the effects of temperature and 
E.E. voltages on the physical properties of the water samples 
showed that the water samples contained a significant amount 
of dissolved oxygen. When the water samples were exposed to 
E.E. voltages, the amount of dissolved oxygen decreased 
markedly. However, when the water samples were subjected to 
E.E. voltages at temperatures above 50°C, there was a slight 
increase in the amount of dissolved oxygen. This effect was 
more pronounced in water samples that had been heated to a high 
temperature before exposure to the E.E. voltage. The results 
of these experiments suggest that E.E. voltages can affect the 
properties of water, particularly in the presence of high 
temperatures.
thus difficult to recover these proteins from this material. A second approach was therefore undertaken. Yolk was spray dried and extracted with water as described. The supernatant was spray dried. This preparation would presumably be similar to the "livetin" preparations of Plimmer (1908) and Kay and Marshall (1939). In addition, egg white was lyophilized and tested for emulsifying ability. The fractionation procedures are outlined in Figures 15a and 15b. The emulsifying abilities of these fractions are illustrated in Figure 16 and the emulsifying indices are given in Table 12a.

Egg white as well as yolk protein exhibited considerable emulsifying ability; thus the separation of these egg parts would not be advisable purely from an emulsification standpoint. The isolated water-ether insoluble portion of the liquid yolk (the "lipovitellin" preparation) exhibited a greater emulsifying ability than that of the natural yolk. These observations suggest that the important emulsifying fractions of whole egg are the albumen and the "lipoprotein" portions. The emulsions formed with albumen, however, were atypical appearing under the microscope as lakes and pools of oil and water intermingled with large numbers of air bubbles.

The water soluble-ether insoluble portion of yolk (the "livetin" preparation) possessed poor emulsifying properties by itself; however, when added to the water insoluble part (the yolk "lipoproteins") in natural proportions, the emulsifying index of the resulting emulsifier was 90.1 (compared with 90.5 for the water insoluble portion alone) (Figure 17). This suggests that although not possessing a marked inherent emulsifying ability,
EGG YOLK (4% SALT) (PREP. 10)

EXTRACTED WITH ETHER AT 0°C (32°F).

RESIDUE

EXTRACT

ETHER INSOLUBLE PORTION

DILUTED WITH 20 VOL. OF WATER

CENTRIFUGED

ETHER SOLUBLE PORTION (DISCARDED)

SUPERNATANT

RESIDUE

WATER INSOLUBLE PORTION (PREP. II)

WATER SOLUBLE PORTION (DISCARDED)

a. SEE FIG. 16

b. PRINCIPALLY LIPOPROTEINS (CHARGAFF, 1942).

Fig. 15a Fractionation of Yolk.
a. SEE FIG. 16
b. PRINCIPALLY PROTEINS AND LIPOPROTEINS (CHARGAFF, 1942).
c. PRINCIPALLY LIVETIN (KAY AND MARSHALL, 1928).

Fig. 15b Fractionation of Yolk.
Fig. 16 Emulsifying Ability of Yolk Preparations and Albumen.
### Table 12c. Emulsifying Indices of Egg Fractions

<table>
<thead>
<tr>
<th>Prep. no.</th>
<th>Means of preparation</th>
<th>Emulsifying index</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Egg yolk</td>
<td>66.8</td>
</tr>
<tr>
<td>11</td>
<td>Ether and water ins. portion of #10 (&quot;lipoprotein&quot;)</td>
<td>90.5</td>
</tr>
<tr>
<td>12</td>
<td>Spray dried yolk</td>
<td>91.0</td>
</tr>
<tr>
<td>13</td>
<td>Ether ins. portion of #12 (&quot;proteins&quot; of yolk)</td>
<td>87.9</td>
</tr>
<tr>
<td>14</td>
<td>Water sol. portion of #13° (&quot;livetin&quot;)</td>
<td>&lt;75.6</td>
</tr>
<tr>
<td>15</td>
<td>Albumen (lyophilized)</td>
<td>92.7</td>
</tr>
<tr>
<td>16</td>
<td>#15 and #14 added in natural proportions (&quot;lipoprotein&quot; plus &quot;livetin&quot;)</td>
<td>90.1</td>
</tr>
<tr>
<td>17</td>
<td>#15, #14, and #6 added in natural proportions (&quot;lipoprotein&quot;, &quot;livetin&quot;, and &quot;phospholipids&quot;)</td>
<td>86.3</td>
</tr>
</tbody>
</table>

*a* First experiment. 

*b* Second experiment. 

*Only one concentration was tested corresponding to this emulsifying index.*
Fig. 17 Effect of Addition of Phospholipids on the Emulsifying Ability of "Protein" Portions of Yolk.
the water soluble portion exhibits an emulsifying ability approximately equal to that of the water insoluble portion when added to that material in natural proportions. The removal of this portion of the yolk would serve no purpose even though the material has little ability to emulsify by itself.

The addition of yolk phospholipids to the ether insoluble portion of egg yolk in natural proportions produced an emulsifier with an emulsifying index of 86.3 (compared with 50.1 for the ether insoluble portion alone) (Figure 17). It would appear that the phospholipids reduced the emulsifying ability of the latter material.

The most important emulsifying principles of whole egg evidently are included in the ether insoluble portion. Both egg albumen and the ether and water insoluble portion of yolk ("lipoprotein" preparations) contribute to this action. The water soluble non-lipid materials have little emulsifying ability but aid in emulsification when added to the water insoluble non-lipids. Egg oil has almost no ability to emulsify the corn oil. The phospholipids, while they have slight emulsifying ability, apparently decrease the emulsifying efficiency of the ether insoluble emulsifiers.

C. The Selection of a Process for the Production of an Emulsifier from Whole Egg

Selection of a process for the production of a satisfactory emulsifier (in this case the ether insoluble portion of whole egg) from whole egg is governed in part by the emulsifying efficiency of the product, the cost of production, and the degree of separation of the emulsifying materials from
the non-emulsifying materials afforded by the process. From the stand-
point of storage life and ease of handling, a dried product is desirable.
The processes studied were supercentrifugation, (with subsequent drying
and extraction), extraction of liquid egg and subsequent spray drying, and
spray drying and subsequent extraction. The original low water/non-fat-
solids ratio formulae were used to test the emulsifying ability of the
preparations in this section.

1. Supercentrifugation of liquid whole egg

This process was discussed in relation to the examination of the emulsi-
fying ability of the "lipovitellin" of Alderton and Fevold (1945) (see
Figures 12 and 14 and Table 12b). Divergent results were obtained in
duplicate experiments. In the first experiment the residue from super-
centrifugation had an EI of 80.4 and the spray dried supernatant had an EI
of 51.3. In the second experiment the residue had an EI of 93.7 and the super-
натant had an EI of less than 36.0. Approximately 30% of the total ether
insoluble solids of whole egg were obtained in the residue in either case. It
may be concluded that while the emulsifying ability of the residue obtained
from the supercentrifugation process is satisfactory, the yield is too low
to permit economic operation. On this basis, supercentrifugation was re-
jected as a possible method for preparing an emulsifier from whole egg.

2. Extraction of liquid whole egg

Three liquid-liquid extractions of whole egg were made. In the first,
5% sugar was added prior to extraction; in the second, 3% salt was added;
and in the third, nothing was added before extraction. All three preparations were spray dried following the extraction process. The emulsifying abilities of these preparations are illustrated in Figure 18. The emulsifying indices are shown in Table 13a. All of these preparations exhibited a high emulsifying ability. In all cases the spray dried materials were better emulsifiers than the liquid. The high level of emulsifying ability of these products suggests the feasibility of the use of a liquid-liquid extraction process followed by spray drying. The addition of salt or sugar had almost no effect and their presence was considered of little importance.

3. Extraction of spray dried whole egg

Again three preparations were studied, spray dried whole egg, egg to which 2% salt had been added, and egg to which 6% sugar had been added prior to spray drying. Each spray dried material was then extracted with ether. The emulsifying abilities of these preparations are illustrated in Figure 19. Their emulsifying indices are given in Table 13b. Again the dried and extracted materials exhibited a good emulsifying ability. In each case the extraction process (at 0°C) enhanced the emulsifying ability of the egg fractions. The addition of salt or sugar did not affect the emulsifying ability to an appreciable extent and their presence was considered to be of little importance.

The emulsifying indices of the extracted and spray dried materials were similar to those of the spray dried and extracted egg fractions. Both processes effectively separated almost all of the emulsifying portions of
Fig. 18 Emulsifying Ability of Ether Extracted (Concentrated) and Spray Dried Whole Egg
Table 13a. Emulsifying Indices of Extracted Whole Egg Products

<table>
<thead>
<tr>
<th>Prep. no.</th>
<th>Method of preparation</th>
<th>Emulsifying index</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Whole egg extracted with ether (liquid)</td>
<td>38.5</td>
</tr>
<tr>
<td>21</td>
<td>#20 spray dried</td>
<td>91.2</td>
</tr>
<tr>
<td>22</td>
<td>Whole egg salted (5%) and extracted with ether</td>
<td>78.2</td>
</tr>
<tr>
<td>23</td>
<td>#22 spray dried</td>
<td>82.7</td>
</tr>
<tr>
<td>24</td>
<td>Whole egg sugared (5%) and ext. with ether</td>
<td>84.5</td>
</tr>
<tr>
<td>25</td>
<td>#24 spray dried</td>
<td>89.8</td>
</tr>
</tbody>
</table>

*It was considered in Section A that any two emulsifiers whose emulsifying indices were different by more than 3.0 units probably possessed different emulsifying abilities.

Table 13b. Emulsifying Indices of Extracted Whole Egg Products

<table>
<thead>
<tr>
<th>Prep. no.</th>
<th>Method of preparation</th>
<th>Emulsifying index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Whole egg, spray dried</td>
<td>55.1</td>
</tr>
<tr>
<td>2</td>
<td>#1 extracted with ether</td>
<td>55.7</td>
</tr>
<tr>
<td>16</td>
<td>Whole egg salted (2%) and spray dried</td>
<td>54.2</td>
</tr>
<tr>
<td>17</td>
<td>#16 extracted with ether</td>
<td>55.7</td>
</tr>
<tr>
<td>18</td>
<td>Whole egg sugared (5%) and spray dried</td>
<td>52.3</td>
</tr>
<tr>
<td>19</td>
<td>#18 extracted with ether</td>
<td>93.9</td>
</tr>
</tbody>
</table>

*It was considered in Section A that any two emulsifiers whose emulsifying indices were different by more than 3.0 units probably possessed different emulsifying abilities.
Fig. 19  Emulsifying Ability of Spray Dried Whole Egg (Unextracted and Ether Extracted)
the egg, thus the selection of the process would depend on other factors. From a technological standpoint, extraction of a dry powder is simpler and less costly than a liquid-liquid extraction. It was concluded that the process to be studied would include the spray drying and organic solvent extraction of whole egg.

D. Study of the Effect of Processing Treatments on the Emulsifying Ability of Whole Egg

Based on these studies the commercial production of an emulsifier from whole egg would probably include breaking, freezing and thawing, spray drying, and solvent extraction. In addition, homogenization might be employed prior to the freezing step. These processes (with the exception of breaking) were studied with respect to their effect upon the emulsifying ability of whole egg. The effects of concentration and vacuum drying of liquid whole egg were also examined. The influence of heat on extracted powder and the influence of the addition of extracted egg oil back to the powder were studied in an effort to partially explain the effect of extraction on the emulsifying ability of spray dried whole egg.

1. The effect of freezing and frozen storage on the emulsifying ability of whole egg

Commercial whole egg (mixed white and yolk) is generally available as a frozen product. Thomas and Bailey (1923) studied the effect of freezing on the gelation of whole egg magma and observed that the viscosity of the
frozen and thawed material increased with an increase in the time of frozen storage, reaching a maximum in about 30 to 60 days. They also noted that the addition of salt and sugar prior to freezing retarded this effect and that homogenization largely prevented it.

An investigation into the effect of freezing on fresh whole eggs was carried out to determine if these viscosity changes affected the emulsifying properties. Additional treatments studied were the addition of salt before freezing, and homogenization before freezing. Three portions of a homogeneous lot of whole egg magma were frozen and stored for periods of approximately 2, 4, 8, 16, 32, and 64 days. The first portion was not treated before freezing; the second portion was salted to 5% before freezing; and the third portion was homogenized before freezing. Each portion was run in triplicate.

A second lot of whole egg magma was treated in a similar but much attenuated experiment. One portion of the egg was homogenized, frozen, stored fifteen days, thawed, and tested (performed in triplicate). The second portion (control) from the same lot was also frozen, stored, thawed, and tested. The high water/non-fat-solids ratio formulae were used to test these samples; thus the emulsifying indices of these materials cannot be compared with the emulsifying indices of emulsifiers prepared with the low water/non-fat-solids ratio (as in parts 3, 4, and 5, this section and in Sections B and C). The results of these tests are given in Table 14 and Figures 20 and 21.

Freezing and frozen storage had only a slight effect on the emulsifying ability of whole egg. A small increase, most evident after 16 to 32
Table 14. Effect of Freezing and Frozen Storage on the Emulsifying Ability of Whole Egg

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Emulsifying Index (Average of Three Replications)</th>
<th>Days in frozen storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control-frozen</td>
<td></td>
<td>82.6</td>
</tr>
<tr>
<td>Test A*</td>
<td></td>
<td>82.3</td>
</tr>
<tr>
<td>Test B</td>
<td></td>
<td>80.9</td>
</tr>
<tr>
<td>5% NaCl-frozen</td>
<td></td>
<td>75.0</td>
</tr>
<tr>
<td>Test A*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test B</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*All test A's are from the same homogeneous lot of whole egg magma and can be compared directly.*
Fig. 20 Effect of Homogenization on the Emulsifying Ability of Whole Egg
days, was brought about. Although this increase is significant at the
5% level (see analysis of variance, Table 22, Appendix), it is so small
as to be commercially insignificant. The addition of salt prior to
freezing had no influence on this effect. Homogenization initially reduced
the emulsifying ability of the whole egg (Figure 20); however, this ability
was recovered during frozen storage (Table 14, Figure 21). Apparently
freezing is not detrimental to the emulsifying ability of whole egg, thus
it is unnecessary to homogenize or to add salt to whole egg before freeze-
ing (as is often done) to preserve its properties.

The greatest difference in the means of the emulsifying indices among
the treatments (control, Test A, Table 14) was 2.1. Since this figure is
influenced by treatment errors as well as operator error, it lends credence
to the use of the figure 3.0 (proposed in Section A, Results and Discussion)
as a basis of comparison of any two emulsifying indices where the treatment
error is not considered along with the operator error (as in Sections B,
C, and D, excepting this portion of Section D).

2. Concentration, vacuum drying, and spray drying

Portions of each of three lots of whole egg were concentrated under
vacuum (see Experimental Procedure) to four different total solids concen-
trations. The first lot was prepared from Grade B eggs obtained from a
local supermarket. The second lot was prepared from fresh eggs obtained
from the College Poultry Farm. The third lot, from which duplicate prepar-
ations were made, was obtained from a commercial spray drying plant. The
liquid whole egg from this plant was secured just before it was pumped to the drying chamber. At the same time a sample of the spray dried product was collected. The commercial liquid lot was of low quality when collected from the plant and it was found necessary to add salt before concentrating to retard further microbial action. The high water/non-fat-solids ratio formulas were used to test the emulsifying ability of these preparations, thus the emulsifying indices of these materials cannot be compared with the emulsifying indices of emulsifiers prepared with the low water/non-fat-solids ratios (as in parts 3, 4, and 5, this section and Sections B and C).

The effect of concentration (between temperatures of 26° C. and 28° C., 75° F. and 82° F.) on the emulsifying ability of whole egg is shown in Table 15. In each case, any egg material concentrated beyond about 40% solids (including dryness) exhibited an emulsifying ability inferior to the unconcentrated controls. This suggests that removal of moisture (or drying) per se reduces the emulsifying ability of whole egg.

The effect of spray drying on the emulsifying ability of whole egg would be dependent upon a combination of factors including drying, heat, and atomization. The effect of drying was investigated above; the effect of heat on extracted whole egg powder will be explained below; and information relative to the effect of atomization in a laboratory drier probably cannot be applied to commercial driers because of differences in design and operating conditions. However, during these studies many preparations of laboratory spray dried whole egg were made. The emulsifying ability of some of these is shown by Figure 22. These preparations
Table 15. The Effect of Vacuum Concentration on the Emulsifying Ability of Whole Egg

<table>
<thead>
<tr>
<th></th>
<th>Test A</th>
<th></th>
<th>Test B</th>
<th></th>
<th>Test C</th>
<th></th>
<th>Test D</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Solids %</td>
<td>E.I.</td>
<td>Solids %</td>
<td>E.I.</td>
<td>Solids %</td>
<td>E.I.</td>
<td>Solids %</td>
<td>E.I.</td>
<td>Solids %</td>
</tr>
<tr>
<td>-------</td>
<td>--------</td>
<td>-------</td>
<td>--------</td>
<td>-------</td>
<td>--------</td>
<td>-------</td>
<td>--------</td>
<td>-------</td>
</tr>
<tr>
<td>25.0*</td>
<td>78.0</td>
<td>25.0*</td>
<td>80.3</td>
<td>26.6*</td>
<td>75.1</td>
<td>24.6*</td>
<td>85.8</td>
<td></td>
</tr>
<tr>
<td>33.2</td>
<td>&lt;75.0</td>
<td>80.3</td>
<td>55.1</td>
<td>78.6</td>
<td>47.1</td>
<td>&lt;75.0</td>
<td>50.1</td>
<td>&lt;75.0</td>
</tr>
<tr>
<td>66.6</td>
<td>&lt;75.0</td>
<td>64.2</td>
<td>&lt;75.0</td>
<td>64.9</td>
<td>&lt;75.0</td>
<td>64.6</td>
<td>75.1</td>
<td></td>
</tr>
<tr>
<td>96.1</td>
<td>&lt;75.0</td>
<td>95.7</td>
<td>&lt;75.0</td>
<td>94.7</td>
<td>&lt;75.0</td>
<td>94.6</td>
<td>75.1</td>
<td></td>
</tr>
<tr>
<td>Spr. Dry</td>
<td></td>
<td>Spr. Dry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>94.2</td>
<td>83.2</td>
<td>94.2</td>
<td>83.2</td>
<td>82.4</td>
<td>82.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Fresh whole egg, unconcentrated.
Fig. 22  Emulsifying Ability of Four Samples of Laboratory Spray Dried Whole Egg
Wax paper was used in the tests to regulate the flow of the extractant (see Figure 22 and Table 16) and to determine the number of times the sample powder was extracted. Toluene was used as the solvent and acetonitrile was used as the extraction solvent. The tests were performed in an extraction apparatus and an extraction of 10% water content or water soluble extractant was performed.

1. Introduction to 16, 17, 18.

2. Extraction and 16.

3. The further extraction with acetonitrile

because of the extraction of the process.

The initial cost of the solvent was higher, the quantity was commended

solution containing the extractant or solvent. They reported that although

point, and low heat of evaporation.

The non-extractable solid fraction of the

extractant were measured for extractable or where the EE powder because

2. Extraction

* of the untested compounds.

extract the homogenous EtOAc-EE extract from the unreacted EtOAc.

The ee value in Table 26 (tests a and b) were the same source and of similar.

were made from EE's from the same source and of similar.

568.
Fig. 23 Effect of Extraction with Trichloroethylene at 18°C to 21°C (65°F to 70°F) on the Emulsifying Ability of Spray Dried Whole Egg
Fig. 24 Effect of Extraction with Trichloroethylene at 18°C to 21°C (65°F to 70°F) upon the Emulsifying Ability of Spray Dried Whole Egg
<table>
<thead>
<tr>
<th>temp. °F</th>
<th>wt% powder</th>
<th>wt% powder</th>
<th>wt% powder</th>
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</tbody>
</table>

*NOTE: The data presented above is for the gas-phase decomposition of the compound at various temperatures. The weight percent of powder in each test is shown.*

**Table 16. Effect of Extraction with Tetrachloroethylene at 170 °C to 210 °C (66 °F to 70 °F).**

*68d*
dried whole egg. The third lot was a powder that had been acidified with hydrochloric acid to a pH of 5.5 before spray drying. Sufficient sodium bicarbonate had been added to bring the pH to between 6.5 to 7.0 upon reconstitution of the powder. Duplicate runs were performed on one of the regular spray dried lots and on the acidified lot.

An increase in emulsifying ability of the whole egg powders was noted after the first or second extraction (Figures 23 and 24 and Table 16). This increase usually became greater with successive extractions. The acidified powder seemed to be more resistant to this effect than the unacidified powder. It is evident from these data that the EI, varied directly with the number of extractions (excluding the first) and inversely with the amount of fat present. The highest EI, attained by the extraction procedure was 83.3 (Table 16) representing an increase of 16.9 over the control (EI, of control was 66.4). Noticeable variation in the emulsifying ability of the preparations existed between duplicate extractions of the same material. These variations could be attributed in part to variation in the fat content of the powders, variations in the rate of extraction, and variations in solvent temperature (dependent upon the temperature of the cooling water and the rate of boiling). Regardless of these variations, however, extraction of spray dried whole egg powder at a temperature not expected to greatly influence the physical state of the proteins produced in all cases except one an increase in the emulsifying ability of the whole egg powder. (The one exception is illustrated by Figure 24, 17 extractions).
b. **Extraction at 71° C. to 77° C.** Two lots of commercial spray-dried powder were extracted at 71° C. to 77° C. (160° F. to 171° F.) a varying number of times (Figure 25 and Table 17). One lot was a regular spray-dried whole egg on which a duplicate run was made. The second lot was the acidified powder described above.

The results of the high temperature extractions were somewhat more definite. The effect of extraction upon the emulsifying ability of the powders seemed to vary directly with the number of extractions, and also, therefore, with time (Figure 25, Table 17). There was no evidence that a large number of extractions at high temperatures injured the emulsifying abilities even though the powders which had been extracted ten (EI, equals 80.0) and 24 times (EI, equals 86.0) (Figure 25) were noticeably browner than powders produced by fewer extractions (e.g., 3 extractions, EI, equals 69.3). Thus solvent extraction at temperatures expected to have considerable adverse effect on the physical state of the proteins resulted in an appreciable increase in the emulsifying ability of the powders.

c. **Alcohol extractions.** It was observed that trichloroethylene was unable to extract the powders below a fat content of about ten per cent. Since commercially it would be desirable to remove this fat, a further study was undertaken to determine the effect of extracting the remaining lipid material. Experience indicated that one of the few solvents that would remove most of the lipid from the lipoproteins was ethyl alcohol. The possibility that a mixed solvent of trichloroethylene and alcohol
Fig. 25 Effect of Extraction with Trichloroethylene at 71° C. to 77° C. (160° F. to 171° F.) on the Emulsifying Ability of Spray Dried Whole Egg
Table 17. Effect of Extraction with Trichloroethylene at 71° C. to 77° C. (160° F. to 171° F.) on the Emulsifying Ability of Whole Egg Powder

| No. of extractions | Test A* | | Test B* | | Test C** | |
|--------------------|--------|----------------|--------|----------------|--------|
|                    | % fat in powder | EI. | % fat in powder | EI. | % Fat in powder | EI. |
| 0                  | 49.9   | 63.4 | 49.9   | 65.7 | 49.5   | 59.8 |
| 1                  | 31.9   | 78.1 | 23.5   | 56.0 | 27.0   | 59.8 |
| 5                  | 11.3   | 83.0 | 17.0   | 71.7 | 14.2   | 69.3 |
| 6                  |        |      | 10.3   | 78.5 | 11.5   | 76.6 |
| 10                 | 10.7   | 98.7 | 9.3    | 88.6 | 10.5   | 80.8 |
| 24                 |        |      | 10.9   | 98.0 |        |      |

*Produced from the same regular spray dried whole egg.

**Produced from acidified egg (internally neutralized with NaHCO₃).
It was noted from the above experiments that the extraction continued

whereas the effervescent powder, when prepared at the lower temperature

of 110° C. to 170° C. (77° C. to 70° C.) seemed to produce powders

out at the higher temperature (77° C. to 70° C.) of the extraction at

Effect of heat on extracted powder

The heat required for the preparation of these proportions is sufficient in Figure 2.

The extracted powder contained only 1.8% to 2.2% and kept well.

The extracted powder was then dried in a drying oven at 110° C. to 170° C.

were then evaporated for a single extraction and then extracted at 77° C. to

water solution used for reconstruction of the preparation.oy

A mixture that appeared to be crystalline in the cake, except a small

where the intermolecular distance was barely hydrogen

after about five extractions, the powder had formed into hard nuggets,

to the theoretical proportion. The extraction was carried out at room temp-

1938). This mixture was made up

composition from the molecular structure of the molecule. A water

and water 5.0%.

would extract most of the rube, in one operation with 20% acetic acid.
Fig. 26  Effect of Ethyl Alcohol Extractions on the Trichloroethylene Extracted Powder (six extractions at 71° C. to 77° C.)


The effect of heat on the extraction of EGF oil to the emulsifier.

* Results (Table 22, appendix)

The effect of heat on the extraction of EGF oil to the emulsifier was repeated with similar results. The temperature was kept between 71°C to 74°C (160°F to 165°F) for 170 minutes. The effect of extraction at 71°C to 74°C (160°F to 165°F) for 70 minutes was observed in three hours. It is evident that the powder prepared at each temperature between the effect of heating the powder at 71°C to 74°C (160°F to 165°F) for 70 minutes had no much difference between the effect of heating three hours. However, it was observed that the EGF oil contained in the powder of about 1.0% after three hours of extraction. The extraction index was in three hours of about 58%. This was an increase of about 27% over the control of 70% for the control of the EGF powder from an E.G. of 70% for the control of the EGF powder from an E.G. of 70% for the extraction at 71°C to 74°C (160°F to 165°F) for three hours. The action of the heat increased the emulsification efficiency to the time when heated in a not very oven at this temperature for one.

66.
Fig. 27 Effect of Heat on the Emulsifying Ability of Trichloroethylene Extracted Whole Egg Powder. Heated at 71°C to 77°C. (160°F to 171°F.)
extracted on the same sample of the extractable EEF. The further test used was that when the extractable component was contained some component which was then recovered from 60°C to 60°C. The extractable powder was then recovered from 60°C to 60°C. The extractable powder was then heated at 60°C. (10°C) in under which vacuum* the oil from the recovered was then condensed and collected after four hours. The ether-di solution was then concentrated to 60°C until the extractable solution was still

66e.
Fig. 28  Effect of Egg Fat (added in Natural Proportions) on the Emulsifying Ability of Ether Extracted Whole Egg (Whole Egg "Proteins" plus "Lipoproteins")
V. CONCLUSIONS

The following conclusions are based on the assumption that the rate of change of emulsion viscosity with the change of the quantity of the emulsifier used, or the emulsifying index computed from such data, is a reliable measure of emulsifying ability. It should be emphasized that the viscosity measurements are not necessarily related only to emulsifying ability but probably also reflect the initial viscosity of the emulsifier.

1. The ether insoluble portion (the "protein" and "lipoprotein" portion) is the most important emulsifying substance of mixed albumen and yolk (whole egg). Egg albumen and the ether insoluble portion of yolk when examined separately have an emulsifying ability equal to that of the whole egg "protein" and "lipoprotein".

2. Ether soluble egg lipids contain some substance, probably phospholipid in nature, which reduces the emulsifying ability of the ether insoluble portion of the egg.

3. Homogenization and low temperature vacuum concentration reduce the emulsifying ability of whole egg.

4. Freezing has only a slight tendency to increase the emulsifying ability of whole egg. This tendency is not affected by the addition of salt prior to freezing.
5. Spray drying, extraction of spray dried whole egg with ether or trichloroethylene, and dry heat increase the emulsifying ability of the emulsifying substances in whole egg (the "proteins" and "lipoproteins").

6. The effect of solvent extraction of whole egg powder on the emulsifying ability of that powder is due in part to the removal of fat substances, probably phospholipid in nature, and in part to the effect of dry heat.
VI. SUMMARY

The abundance and relatively low economic value of waste materials in the poultry industry have established a need for by-product development. One possibility for this development lies in the isolation and standardization of the emulsifying components of whole egg wastes. These wastes accumulate from hatcheries, breaking and freezing operations, and cold storage. The emulsifying ability of eggs is well known and is used widely in the food industry. The production of a reliable emulsifier from the waste materials could probably find successful application in the non-food industries. This study was directed toward the development of a test for measuring emulsifying ability, the isolation of the emulsifying components from whole egg, and an investigation of the effects of processing treatments on the emulsifying ability of these components.

The method used to test the emulsifying ability of the egg preparations was a quantitative adaptation of commercial methods for making mayonnaise. A measure of the viscosity of the complete emulsion was used as a criterion of the degree of emulsification. An emulsifying index was determined from these viscosity measurements. This emulsifying test and the index derived from it proved to be a satisfactory measure of the emulsifying ability of the preparations.

Spray dried whole egg and fresh egg yolk fractions were prepared. The ether insoluble portion of the egg (the "lipoproteins" and "proteins")
showed the greatest emulsifying ability. Albumen and the "lipoprotein" from yolk were equal in emulsifying ability to the whole egg "proteins"; however, the albumen emulsions were atypical. The phospholipids, when added back to the whole egg emulsifier, reduced the latter's emulsifying ability.

Several processes for the production of an emulsifier were investigated. The residue from supercentrifugation was an excellent emulsifier but was obtained in a yield of only 30% of the ether insoluble solids of whole egg; thus this process was rejected as a possible commercial procedure. The emulsifying ability of the whole egg "proteins" was similar whether prepared by liquid-liquid extraction followed by spray drying or by spray drying followed by extraction of the dry powder. Since the cost of a liquid-liquid extraction exceeds that of a liquid-powder extraction, the latter method was chosen for further study.

The effect of various processes on the emulsifying ability of whole egg and whole egg powder was investigated. Homogenization reduced the emulsifying ability of liquid whole egg; however, this ability was recovered during frozen storage. Freezing and frozen storage had only a slight tendency to change the emulsifying ability of whole egg. This tendency was not influenced by the addition of salt before freezing. Vacuum concentration and drying at low temperatures markedly reduced the emulsifying ability of the egg. Spray drying appeared to increase the emulsifying ability of both extracted and unextracted whole egg.
Extraction of whole egg powder with trichloroethylene at room and elevated temperatures increased the emulsifying ability of the powder. This increase was more pronounced at the higher temperatures. Alcohol extractions of the trichloroethylene extracted powder removed most of the residual fat but had a small detrimental effect on the emulsifying ability of the powder. Heating of the trichloroethylene extracted powder increased its emulsifying ability appreciably. The addition of egg oil in natural proportions back to the extracted powder decreased the emulsifying ability of that powder. This effect and the effect of heat on the spray dried and extracted egg provided an explanation of the effect of extraction of the emulsifying ability of whole egg powder.
VII. LITERATURE CITED


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Ferry, R.M., Cohn, E.J., and Newmann, E.S. 1936. Physical chemistry of the proteins. XIII. The solvating action of sodium chloride on egg albumin in 25% ethanol at -5°. J. Am. Chem. Soc. 58:2370-2375.


U.S. Department of Agriculture. 1941. Eggs and egg products. USDA Circ. 583.


VIII. ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. George F. Stewart for his continued interest and guidance throughout this study. The writer also wishes to express his gratitude to Dr. Richard H. Forsythe for his valuable criticisms and assistance, and to the members of the Food Processing Laboratory and the Poultry Husbandry Department for their assistance and cooperation.
IX. APPENDIX
Table 18. Volumes of the Fourteen Additions of Oil Added During the Test of Emulsifying Ability (Low ratio emulsifiers) (ml.)

<table>
<thead>
<tr>
<th>Non-fat solids</th>
<th>1 *</th>
<th>2 *</th>
<th>3 *</th>
<th>4 *</th>
<th>Total (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>3, 4, 5, 6, 8, 10, 14, 20, 25, 30, 45, 55, 50, 55</td>
<td>330</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td>6, 8, 10, 12, 15, 15, 15, 20, 20, 45, 50, 45, 50</td>
<td>324</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>5, 12, 15, 15, 15, 15, 15, 15, 15, 40, 45, 45, 45</td>
<td>318</td>
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<td>2.8</td>
<td>12, 15, 20, 25, 9, 10, 15, 15, 15, 15, 40, 40, 40, 40</td>
<td>312</td>
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<tr>
<td>3.5</td>
<td>15, 20, 25, 31, 10, 10, 10, 10, 15, 35, 35, 35, 40</td>
<td>306</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>18, 24, 30, 37, 10, 10, 10, 10, 10, 35, 30, 30, 30</td>
<td>299</td>
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<td></td>
<td></td>
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<td>4.9</td>
<td>21, 28, 35, 43, 6, 10, 10, 10, 10, 25, 20, 20, 20</td>
<td>293</td>
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<td></td>
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<tr>
<td>5.6</td>
<td>24, 32, 40, 49, 7, 5, 5, 10, 10, 20, 25, 25, 25</td>
<td>287</td>
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*Points of addition of water solution of salt, sugar, and acetic acid (See Tables 9 and 19).

Table 19. Amount of Water Solution of Salt, Sugar, and Acetic Acid Added During the Test of Emulsifying Ability (Low ratio emulsifiers) (ml.)

<table>
<thead>
<tr>
<th>Non-fat solids</th>
<th>Addition 1</th>
<th>Addition 2</th>
<th>Addition 3</th>
<th>Addition 4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>24.0</td>
<td>24.0</td>
<td>24.4</td>
<td>14.6</td>
<td>87.0</td>
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<tr>
<td>1.4</td>
<td>31.0</td>
<td>21.0</td>
<td>20.4</td>
<td>14.5</td>
<td>77.9</td>
</tr>
<tr>
<td>2.1</td>
<td>17.0</td>
<td>17.0</td>
<td>17.9</td>
<td>14.4</td>
<td>66.3</td>
</tr>
<tr>
<td>2.8</td>
<td>14.0</td>
<td>14.0</td>
<td>15.5</td>
<td>14.3</td>
<td>55.8</td>
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<tr>
<td>3.5</td>
<td>10.5</td>
<td>10.5</td>
<td>10.3</td>
<td>14.3</td>
<td>45.6</td>
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<tr>
<td>4.2</td>
<td>7.0</td>
<td>7.0</td>
<td>6.9</td>
<td>14.2</td>
<td>35.1</td>
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<tr>
<td>4.9</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>14.2</td>
<td>24.7</td>
</tr>
<tr>
<td>5.6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>14.2</td>
<td>14.2</td>
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Table 20. Emulsion Formulae for the High Water/Non-fat-solids Emulsifiers

<table>
<thead>
<tr>
<th>Non-fat-solids</th>
<th>Emulsifier (gms.)</th>
<th>Acetic acid (ml.)</th>
<th>Oil (ml.)</th>
<th>Water (ml.)</th>
<th>Solution of salt, sugar, and acetic acid in water (ml.)</th>
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</thead>
<tbody>
<tr>
<td>0.5</td>
<td>18.9</td>
<td>0.5</td>
<td>364</td>
<td>50.7</td>
<td>56.9</td>
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<td>1.0</td>
<td>37.7</td>
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<td>359</td>
<td>33.0</td>
<td>42.7</td>
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<td>1.5</td>
<td>57.6</td>
<td>1.5</td>
<td>355</td>
<td>25.3</td>
<td>38.4</td>
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<td>2.0</td>
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<td>2.0</td>
<td>350</td>
<td>12.7</td>
<td>14.3</td>
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<tr>
<td>2.5</td>
<td>94.3</td>
<td>2.5</td>
<td>346</td>
<td>0.0</td>
<td>0.0</td>
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</table>

*50% acetic acid.

Table 21. Volumes of the Fourteen Additions of Oil Added During the Test of Emulsifying Ability (High Water/Non-fat-solids Emulsifiers) (ml.)

<table>
<thead>
<tr>
<th>Non-fat-solids</th>
<th>Total (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>(ml.)</td>
</tr>
<tr>
<td>0.5</td>
<td>364</td>
</tr>
<tr>
<td>1.0</td>
<td>359</td>
</tr>
<tr>
<td>1.5</td>
<td>355</td>
</tr>
<tr>
<td>2.0</td>
<td>350</td>
</tr>
<tr>
<td>2.5</td>
<td>346</td>
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</tbody>
</table>

*Points of addition of water solution of salt, sugar, and acetic acid. (See Table 20).
Table 22. Analysis of Variance, Effect of Freezing and Frozen Storage on the Emulsifying Ability of Liquid Whole Egg (Table 14)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
</tr>
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<tbody>
<tr>
<td>A. Control</td>
<td>Days in storage</td>
<td>5</td>
<td>6.96</td>
<td>1.392*</td>
</tr>
<tr>
<td></td>
<td>Individuals</td>
<td>12</td>
<td>4.08</td>
<td>0.340</td>
</tr>
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<td></td>
<td>Total</td>
<td>17</td>
<td>11.04</td>
<td>F = 4.09</td>
</tr>
<tr>
<td>B. 5% NaCl</td>
<td>Days in storage</td>
<td>6</td>
<td>3.86</td>
<td>1.493</td>
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<tr>
<td></td>
<td>Individuals</td>
<td>14</td>
<td>9.07</td>
<td>0.648</td>
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<tr>
<td></td>
<td>Total</td>
<td>20</td>
<td>17.03</td>
<td>F = 2.30</td>
</tr>
<tr>
<td>C. Homogenized</td>
<td>Days in storage</td>
<td>5</td>
<td>24.28</td>
<td>4.856**</td>
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<td>Individuals</td>
<td>12</td>
<td>4.43</td>
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<tr>
<td></td>
<td>Total</td>
<td>17</td>
<td>28.71</td>
<td>F = 13.16</td>
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</table>

Table 23. Effect of Dry Heat of the Emulsifying Ability of Extracted Powders

<table>
<thead>
<tr>
<th>Time of Heating (hours)</th>
<th>Emulsifying Index</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>74.2</td>
</tr>
<tr>
<td>1</td>
<td>81.9</td>
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<tr>
<td>3</td>
<td>83.5</td>
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<tr>
<td>6</td>
<td>83.3</td>
</tr>
</tbody>
</table>
Table 24. Effect of Egg Oil (Added in natural proportions) on the Emulsifying Ability of Trichloroethylene Extracted Whole Egg Powder

<table>
<thead>
<tr>
<th>Viscosity of emulsions made from extracted powder (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. No egg oil added</td>
</tr>
<tr>
<td>20.9</td>
</tr>
<tr>
<td>21.2</td>
</tr>
<tr>
<td>21.2</td>
</tr>
<tr>
<td>20.2</td>
</tr>
<tr>
<td>22.9</td>
</tr>
<tr>
<td>18.9</td>
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