SYNTHETIC MEMBRANE FORMATION AS APPLIED TO A SIMULATED EGG YOLK

By

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ABSTRACT

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Contemporary emphasis on cholesterol as an undesirable dietary ingredient for some individuals has nurtured the commercial development of fabricated liquid egg substitutions. Utilization as conventionally cooked eggs is primarily restricted to the "scrambled" style. The objective of this study is to replace the natural egg yolk with a functional, low cholesterol simulated yolk, which resembles the appearance and functionality of natural egg.

Sodium alginate can be reacted with polyvalent ions such as calcium to produce food matrix systems, and modified to simulate various food textures. When yolk-resembling emulsion, containing calcium salt is inserted into an algin solution of approximately equal density for a short period of time, film formation giving artificial yolk is obtained. This encapsulation resembles the appearance of the yolk vitelline membrane.

An acceptable emulsion was formulated using fresh liquid egg white, vegetable oils, calcium salts, flavoring, coloring, emulsifier (lecithin), and a protein stabilizer

(whey protein concentrate).

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INTRODUCTION

There has been a nutritional appeal for low cholesterol and saturated lipid diets to reduce atherosclerotic diseases, generating the production of low cholesterol food substitutes. As a major source of dietary cholesterol, the egg yolk has been widely condemned, thus reducing egg consumption for persons desiring to restrict dietary cholesterol.

Numerous substitute egg products have been fabricated, but few as functional, whole, liquid centered, multiple-use, egg yolk replacements.

This study addresses the feasibility of developing an artificial egg yolk by encapsulation of an oil-liquid emulsion in a membrane-like film. A two phase project was developed. The first, was to establish a stable emulsion, possessing yolk-like appearance and functionality. The second, and most important, was to encapsulate the emulsion, producing the appearance of a freshly separated, intact egg yolk.

The primary object of this study, therefore, was to evaluate the practicality of a simulated whole yolk, and to select ingredients appropriate for compounding such a product.

LITERATURE REVIEW

Controversy exists over the status of cholesterol and saturated lipids as undesirable ingredients in the diet. McGill and Molt (1976), summarize the widely accepted belief that high dietary cholesterol is a major determinant of high serum cholesterol in the U. S. population and the belief that mild dietary manipulations, limiting eggs, animal fats, and dairy products, can reduce cholesterol intake significantly.

Low Cholesterol Egg Products

The emphasis on cholesterol and saturated fats as undesirable dietary ingredients has nurtured the development of numerous substitute egg products. Because the lipid fraction of the egg is concentrated in the yolk, major emphasis centers on reducing, substituting or modifying the yolk.

Both United States and foreign patent literature contain numerous examples of low cholesterol egg products. However, a review of U.S. patents will suffice since few, if any, foreign patents contain novel ideas. Most patents deal with either whole egg or egg yolk replacements for use in baking or for "scrambled style" cooked eggs.

Gorman (1965) was the first to recognize the need for dietary considerations in an egg formulation. His product utilized dried egg albumin, agglomerated nonfat milk solids, grain or root flours and polyunsaturated oils. On mixing with water a scrambled-type product was obtained.

Although Jones' (1969) major purpose was to find a functionally and bacteriologically superior low-fat product, he recognized cholesterol reduction as an advantage in egg formulations. He reduced the yolk fraction of his product with milk solids, vegetable proteins, a hydrophobic thixotrophic material - such as carboxymethyl cellulose - and increased egg white content. Melnick (1971) described a solvent extraction method designed to reduce cholesterol and unsaturated fats in yolks, while Levin (1971) proposed a lipid solvent - extraction method to be used on whole raw eggs.

An artificial egg yolk formulation which is claimed to be indistinguishable in taste from actual egg yolk was developed by Perret (1974). He incorporated amino acids and an inorganic sulfide into an albumin and oil emulsion, adding emulsifying and stabilizing ingredients to achieve a slightly alkaline pH. This product was usable as a replacement in recipes or as scrambled eggs. Strong (1974) describes a scrambled-style product produced from liquid egg white, corn oil, nonfat dry milk solids, preservatives and coloring agents. The mixture was pasteurized and, emulsified to attain stability. To simulate whole egg appearance,

β-carotene and xanthophyll were added.

In 1974, Seeley updated a patent of Ziegler, Seeley and Holland, (1971) which used a mixture of nonfat milk solids, potato flour, and water, with carboxymethyl cellulose and citric acid added to whole eggs, to yield a stable egg product. Seeley reduced yolk content by adding non egg ingredients to a level that doesn't greatly impair texture or flavor, yet results in a low calorie, low cholesterol product.

When Strong and Redfern (1975), incorporated xanthan gum alone or in conjunction with guar gum or carboxymethyl cellulose to an egg white, oil, milk solid and lecithin emulsion, they obtained a low cholesterol egg product with improved texture and stability. Colors and flavors were added as desired. Glaser and Ingersen (1975), developed a method for stabilizing a liquid scrambled - egg product. Since most low cholesterol egg products are sold in the frozen state, their aim was to produce a stable liquid formulation. They succeeded by combining egg white, polyunsaturated vegetable oils and a fatty acid lactylate alkali metal salt at about 0.05 - 1.0%, with other additives and emulsifiers.

Nath and Newbold (1976) employed ultracentrifugation of egg yolks to separate three yolk fractions, one of which contained a proportionately high concentration of cholesterol. The other fractions were used as yolk replacements in fabricated foods.

A process for producing both artificial egg white and yolk which can be used to regulate lipid and cholesterol levels was described in a patent by Johnson (1972). The white was made primarily of water, oil, and gelling agents, such as low methoxy pectin and dicalcium phosphate. The yolk was comprised of water, proteins, coloring, flavoring, and binding agents similar to those incorporated into the white. Moulding gives convenient egg forms such as hard boiled, deviled, poached, or fried. These can be consumed either cold or warmed.

Perhaps the closest system to a natural appearing egg was invented by Glasser and Matos (1976). A yolk like emulsion was layered into a form and then covered with reconstituted powdered egg white. This is sealed and frozen, to be cooked later without prior thawing. Major ingredients in the yolk include dried egg white, an unsaturated oil, water and added proteins. Flavor and physical properties were adjusted by using sodium hexametaphosphate, sodium caseinate, and starch. These ingredients were stabilized by combining hydrophilic and hydrophobic emulsifiers to simulate a lecithin-cholesterol balance. Also, gums such as guar gum, gum arabic or a cellulosic derivative impart a proper yolk-like glossy sheen. Besides a sunnyside-up formed egg, the yolk emulsion gives satisfactory results in formulations. Water content can be adjusted to impart the desired runny or dry consistency.

Although each patent portrays some characteristic feature(s), actual commercial egg replacements exhibit marked similarities. Common items for baking formulations include soy or wheat flour or starch, lecithin, egg white or vegetable protein, and artificial color. Varying with intended use, other ingredients are gums, lactose, preservatives, glycerin, and spice or flavor enhancers. Examples of commercial products available include: Monark Egg Corporation (Mogold-CEB and Mogold-Tex#10R), Stauffer Corporation (Triet 2C and Triet TMERB), and Precision Foods Company (Artiyolk).

Claiming 96% cholesterol removal, the Fanning Chemical Company and Viobin Corporation produce defatted egg powders to be utilized either in scrambled egg mixes or in cooking preparations. These are essentially fat-free egg preparations. Similarly, scrambled breakfast egg products contain many common ingredients. Most are comprised of 80% liquid egg white, a protein source such as milk solids, soy isolate or egg white solids, and vegetable oils. As of July, 1976, the following companies offered such products: Draper Products (Egg Delight), Fleishmann (Egg Beaters), Avoset Food Corporation (Second Nature), General Mills (Scramblins) and Miles Laboratories (Scramblers). All are cholesterol free except Egg Delight, which is advertised as "low cholesterol". And all are frozen products except Second Nature, which is a refrigerated product. Second Nature can be used as baking replacement for whole eggs and is

fortified with vitamins and minerals.

Emulsion Stability

According to Glaser and Ingerson (1975), a major disadvantage of most egg substitute products is their poor physical stability; partly due to the inability of egg white to combine with oil to form a stable emulsion. This characteristic necessitates the distribution of most simulated egg product in frozen form.

In a system where numerous components are incorporated, as an egg emulsion, the emulsion becomes quite complex. It is much more complicated than the simple oil micelle, where according to Walnack, Barrington, and Faller (1960), emulsifier is adsorbed to the oil droplet decreasing interfacial tension and giving each droplet a similar net electrostatic charge, thus repelling one another. Although much is known concerning the behavior and properties of emulsion systems containing only two or three components under given conditions (i.e. mixing, temperature, concentration), most food systems are multicomponent. Increasing complexity decreases predictability of emulsion characteristics. In the past researchers adopted an Edisonian approach (Wolnick et al., 1960), to obtain satisfactory, stable emulsions. Trial and error selection of emulsifiers for most food products is based on experience and pilot plant tests (Krog and Lauridsen, 1976). Because this approach is time consuming and expensive, an ideal approach is to use model systems for

objective measurements of functional properties (Kinsella, 1976). But the number and complexity of food systems make it difficult to validly extrapolate data from model systems to specific food application. The trial and error approach can be altered somewhat, as discussed by Smith et al. (1977), by using computer plotting and response methodology, but ingredients and processing variables still must be limited. Krog and Lauridsen (1976) states that most food emulsions are not governed by simple emulsion or emulsifier evaluations such as the common hydrophile-lypophile balance (HLB) system due to narrow classification and the lack of accounting for complex formations from such components as protein and starch. Simulated egg yolk formulations fit these characteristics.

A brief look at factors and ingredients that might influence egg emulsions is necessary to obtain a feel for their tendency toward instability. The type and amount of lipid and emulsifier in a food emulsion significantly affect emulsion stability. Their influence, however, can be minimized and predicted by knowing what type of emulsifier or oil is needed for desired conditions or effects. Oil-emulsifier matches are made much easier by following recommendations presented in the emulsifier literature and selecting those which work best in water-oil ($^{W}/O$) or oil-water ($^{O}/W$) systems. Proper hydrophile-lypophile balance, pairing emulsifiers and oil; and such characteristics as phase inversion temperature (PIT) and desired texture or

viscosity also influence ingredient selection.

In general ions (particularly cations), destabilize emulsions, with polyvalent ions showing much greater destabilizing effects than monovalent ions (Friberg, 1976). However, Princen (1972) notes that, occasionally, stability may be increased by addition of electrolytes. For example, anionic emulsifiers in contact with cations in O/W emulsions may form soaps which then form stable W/O emulsions.

Petrowski (1976) observes that salts may have markedly variable affects on proteins in emulsions. They influence ion binding, ionic strength, and alter solvent properties. The salting-in or salting-out characteristics affect emulsion stability.

Petrowski (1976) states that heat effects on an emulsion are basically twofold. Upon manufacture, heat is advantageous for both sterilization and emulsification, but promotes destabilization once the emulsion is formed.

Krog and Lauridsen (1976) describe three main functions of food emulsifiers:

1) The reduction of surface tension at oil interfaces, induces and stabilizes emulsification, resulting in phase equilibrium.

2) Interaction with starch and protein components in foods modify texture and rheological properties.

3) The crystallization of fats and oils is modified.

They note also that the method of emulsifier incorporation significantly influences optimum results in many

types of food products.

Petrowski (1976) points out the importance of droplet size as a major consideration in emulsion stability. The larger the droplet size the greater the attractive forces and the less stable the emulsion. Emulsions destabilize in the order of drop aggregation or floculation, creaming (density differences), and coalescence. When mixing, it must be determined whether or not particle size reduction for stability is more important than possible adverse shearing or denaturation.

Occasionally more than one necessary agent used in food preparation will show antagonistic emulsifying properties, with competing O/W and W/O tendencies and thus create an overall instability.

Perhaps the greatest area of interest for emulsion technology in relationship to egg emulsions is that of the role of proteins. These play a significant part in the characteristics and functionality of an emulsified food system. Kinsella (1976) gives a good summary of the functionality and variables involved in emulsions and particularly those involving proteins.

In solution proteins adsorb and absorb other food ingredients, including volatile components, flavors, lipids and water. They influence viscosity, film formation, adhesion, and fiber formations. Emulsion characteristics and binding properties of proteins respond according to conditions of temperature, pH, ionic strength, chemical or

enzymatic modification, presence and type of carbohydrates and lipids, mechanical agitation and protein particle size, and concentration. Ionic strength and pH influence both surface properties and surface area of protein molecules, regulating counter ions, conformation and degree of hydration.

Additional factors important to the water-binding capacity of proteins in solutions vary with protein source and composition, previous processing and the nature of polysaccharides present.

Functionally, proteins no longer in their native environment, vary according to source, method of isolation, precipitation, concentration, modification (enzymatic or chemical) dehydration, and associated environmental conditions (temperature, pH, ionic strength). Where several discrete proteins are involved (which usually is the case) each exhibits characteristic properties, thus system functionality and reproducability depend on strictly controlled "make" conditions.

Most proteins appear to retain optimum emulsifying properties at the pH where they are most soluble. Generally globular proteins in their native conformation, where polar amino acids are exposed to the aqueous phase, favor solubility and thus this conformation is usually preferred for emulsification applications.

Rand (1976) discusses the affects of oils on proteins. In direct contact with lipids, proteins, that in water

exhibit an optimum free energy with their hydrophilic groups inside in the tertiary conformation, may turn inside out or become denatured and exhibit entirely different functional properties. These interactions may develop tenaceous lipid protein bonds, stabilized by ionic, polar, hydrogen, and hydrophobic interactions.

Friberg (1976) states that polymers such as proteins, when used as emulsifiers, reach saturation adsorption at extremely low concentration in solution. And, when emulsions flocculate, proteins stabilize the anisotropic skins which develop between the droplets.

In summary Petrowski (1976) implies, when emulsifiers are used with such compounds as proteins, that no unambiguous generalization can be made regarding the effect of emulsifier concentration on critical micelle concentration and its resultant effect on emulsion stability.

Protein Crosslinking And Film Formation

As previously described, several attempts have been made to develop a substitute which would appear to have an intact yolk, but none with the versatile appearance or function of fresh egg. To accomplish this, one might encapsulate the emulsion in a transparent film or membrane. Although numerous compounds have been used to develop synthetic membrane or film structures, few have even remote feasibility as food ingredients.

Perhaps the most desirable method of obtaining an encapsulated emulsion would be to cross-link emulsion

surface oriented proteins. An idea of the multitude of crosslinker functional groups and resulting compounds usable in protein reactions can be obtained from Table I (Uy and Wold, 1977).

Several possible reactive systems, whether currently food related or not, will be discussed. A major condition for most crosslinkers described is their ability to react in aqueous environment at ambient temperatures and under moderate pH conditions. Most agents find some utilization in such industries as animal feeds, pharmaceuticals, textiles, and foods.

Sulfur Compounds

Needles and Whitfield (1969) describe a method used for coatings and sizings for paper and fabrics in which a collagen-protein is bound together in the presence of water soluble per-sulfate. The crosslinking reaction can be carried out at room temperature with the addition of a reducing agent. Shapiro and Gazit (1977) noted that at neutral pH and physiological temperatures bisulfite will catalyze the transamination of cytosine (nucleic acids) and amines. Both α and ε aminos of L-lysine are reactive.

In practical application, Jensen (1959) described the crosslinking of a monomolecular layer of fibrinogen to yield a semi-permeable protein membrane for use in model cell membrane studies. A thin layer of fibrinogen in saline is carefully floated on an aqueous surface. The addition of

Table I. Typical Bifunctional Protein Crosslinking Reagents (Uy and Wold).

Functional Groups (x,y) in Bifunctional Reagents (x-R-x, x-R-y)

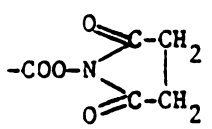
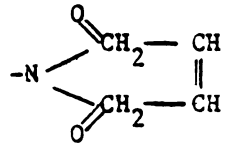
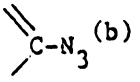
	Functional Group	Reacts with	Examples[a]
1.	Aromatic 	Lys, (Tyr, Cys)	A. p,p'-difluoro-m,m'-dinitrodiphenylsulfone (1); 1,5-difluoro-2,4-dinitrobenzene (2). B. 1-Fluoro-2-nitro-4-Azidobenzene (3).
2.	$-SO_2Cl$	Lys	A. Phenol-2,4-disulfonyl chloride; α-naphthol-2,4-disulfonyl chloride (4).
3.	$-COOC_6H_4NO_2$	Lys	A. Adipate bis-(p-nitrophenyl ester (5); carbonyl bis(methionine p-nitrophenyl ester (6).
4.	$-CON_3$	Lys	A. Tartaryl diazide; Tartryl bis-(glycylazide) (7).
5.		Lys	A. Succinate bis-(hydroxysuccinimide ester) (8). B. N-(Azidonitrophenyl)γ-aminobutyrate hydroxysuccinimide ester (3).
6.	$-COCH_2$ Br(I)	Cys, Met, His, Lys	A. 1,3-dibromoacetone (9) B. p-azidophenacyl bromide (10) See also 7-B and 10-B.
7.	$-CO-CHN_2$	Asp, Glu (Cys)	A. 1,1-bis-(diazo acetyl)-2-phenylethane (11). B. 1-diazoacetyl-1-bromo-2-phenylethane (11).
8.	Aromatic 	Tyr, His	A. Bis diazo benzidine (12).
9.	-CHO	Lys (Cys,His,Tyr)	A. Glutaraldehyde (13).
10.		Lys	A. Polymethylene (n=3-12) di-imidates (14); Di-

Table I - Cont.

			methylsuberimidate (15). B. Ethyl (chloroacetimidate (16).
11.	-N = C = O	Lys	A. Hexamethylene diisocyanate (17). B. Toluene 2-isocyanate, 4-isothiocyanate (18).
12.	-N = C = S	Lys	B. See above (11B).
13.		Cys (Lys)	A. Bis(maleidomethyl)ether (19); N,N'-phenylenedimaleimides (20). B. (See Trommer et al., this volume).
14.	Aromatic  [b]	Nonspecific	A. N,N'-Bis(p-Azido-o-nitrophenyl)1,3 diamino-2-propanol (21). B. See above (1B, 6B).

[a] The examples have been selected to illustrate homofunctional reagents (A) and heterofunctional reagents (B) with recent references either to reagent preparations or applications. For a more complete survey of reagents and applications see Uy and Wold (1976).

[b] Photoactivated: $-N_3 \xrightarrow{h\nu} -\ddot{N}$ nitrene is the reactive species.

References to Table 3:

1. Macleod and Hill (1970)
2. Grow and Fried (1975)
3. Yagub and Guire (1974)
4. Herzig et al. (1964)
5. Brandenburg (1972)
6. Busse and Carpenter (1974)
7. Lutter et al. (1974) See also Wetz et al. (1974)
8. Lindsay (1971)
9. Husain and Lowe (1970)
10. Hixson and Hixson (1975)
11. Husain et al. (1971)
12. Silman et al. (1966)
13. Josephs et al. (1973)
14. Hucho et al. (1975)
15. Tinberg et al. (1976) See also Wang and Richards (1975)
16. Olomucki and Diopoh (1972)
17. Snyder et al. (1974)
18. Schick and Singer (1961)
19. Freeberg and Hardman (1971)
20. Chang and Flaks (1972)
21. Guire (1976)

cysteine to the water yielded a two dimensional crosslinking of the proteins to form a stable film.

Ruminant Feeds

Crosslinking systems are used to protect proteins in feeds from microbial utilization in the near neutral environment of the rumen; but are available to the animal under the acid condition of the abomasum and intestines. Table II lists examples of a broad range of compounds utilized in patents by Miller, 1972; Miller, 1973; Wildi and Miller, 1973; and Scott and Hills, 1975. Most reactions can be carried out at neutral pH and room temperatures.

Polyelectrolytes

Sternberg and Hershberger (1974) reviewed the use of polyelectrolytes to precipitate protein. These include amylopectin sulfate, dextran sulfate, carboxymethyl cellulose, anionic hydrocolloids and heteropolyacids. As an example, polyacrylic acid precipitates many proteins between pH 3 and 6; above and below this point they are released. The polyacrylates can be recovered by precipitation with a suitable polyvalent metal salt such as calcium chloride after protein removal. Then the calcium is removed with acid. This principle was used by Sternberg (1975), to precipitate and recover cheese whey proteins. Suitable polyacids are listed in Table III; additional information on these and other precipitants are discussed by Sternberg, Chiang, and Eberts (1976).

Table II. Examples of Ruminant Feed Crosslinking Compounds

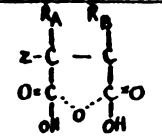
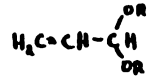
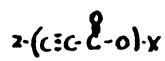
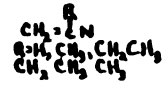
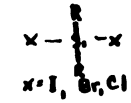
Patent and Number	Class of Compounds	Examples of Chemicals	Reactive Group	Specific Conditions of Reaction	Known Amino Reactions
Miller 3,685,998	Polymerized unsaturated carboxylic acid or anhydrides	Polymaleic anhydride homopolymer, ethylenemaleic anhydride copolymer		Non aqueous solvents	
Miller 3,711,290	Acrolein Acetal	Acrolein, dimethyl acetal			Terminal α amino groups, ε amino of lysine, amide of asn. and gln, guanidyl arg.
Wildi and Miller 3,718,478	Acetylenic monoesters, and acetylenic diesters	Ethylene glycol, bispropiolate, n-decyl propiolate		Basic pH	
Miller 3,720,765	Organic acid anhydrides	Maleic anhydride, pthalic anhydride			Terminal α amino, ε amino lysine, amide asn. and gln., guanidyl arg
Miller 3,726,971	Alkene Nitrile	Acrylonitrile, Methyl acrylonitrile		Basic pH	
Miller 3,726,972	Halosilanes	Trimethyl chlorsilane, phenyl trichlosilane		Nonpolar ionic solvents	Terminal α amino, ε amino lysine, amide asn. & gln., guanidyl arg.
Scott and Hills 3,925,560	Aldehyde	Formaldehyde, glyoxal, glutaraldehyde		Droplets of lipid protein mixture	

Table III. Compounds Used for Whey Precipitation (Sternberg, 1975).

Compound, General Title	Trade Name	Manufacturer
Polyacrylics	Carbopol 934, 940, 941, 960, 961. Goodrite K-702, K-714	B.F. Goodrich
Ethylene-maleic anhydride copolymers	EMA 11, 21, 22, 31, 61	Monsanto Chemical Company
Methylvinylether-maleic anhydride	Gantrez AN 119, AN 139, AN 169	General Aniline and Film Corporation

A look at commercial literature yields an understanding of the way these polymerized crosslinkers perform. Gantrez (GAF Corporation, 1965), for example, coacervates with proteinaceous material at low pH in aqueous media but redissolves when the pH is raised. Crosslink formation is via primary valence bonds with polyfunctional compounds.

Wool and Fiber Reactions

Another area of continued research on crosslinkers is being conducted by the wool and fiber industries to modify texture, strength and shrink properties of these products. Several reviews (Ziegler et al., 1975; Friedman, 1977; and Tillin et al., 1977) discuss chemicals, reactions, and effects, based on wool crosslinking studies.

Some compounds which have been grafted successfully to wool fibers are acrylic acid and other acrylate compounds (Pavlath, 1974) as well as Zinc acetate, which reacts with

both amino and carboxyl groups (Koenig et al., 1974).

Enzyme Immobilization and Glutaraldehyde

Perhaps the greatest input into protein crosslinking and chemical modification emanates from studies on enzyme immobilization. Olson and Stanley (1974) review some common crosslinkers including glutaraldehyde, tannic acid and formaldehyde. Numerous chemicals and processes have been developed with varying degrees of success. Broun (1976) also discusses chemical crosslinking of proteins and enzymes; and again glutaraldehyde is a major crosslinker discussed due to its satisfactory and reproducible results. Glutaraldehyde is a water soluble dialdehyde. Its reaction with proteins (Olson and Stanley, 1974) in solution, progress with time; insolubilization is most rapid for most proteins at their isoelectric point (Jensen, Tomimatsu, and Olson, 1971). Essentially reactivity is with the ε amino group of lysine. Effective reactions occur with either high concentrations of a lysine-rich protein or lower lysine proteins in combination with high lysyl residue proteins such as bovine serum albumin (Broun, 1976).

Reactions can be controlled by attaching proteins either to insoluble supports (Wirth and Tixier, 1974) or on a solid, internally insolubilized compound (Jansen et al., 1971). Atallah and Hultin (1977) crosslinked glucose oxidase and catalase with glutaraldehyde in the presence of BSA to obtain a bifunctional enzyme system with increased

thermal stability.

Korn, Keairheller, and Filachione (1972) discussed the glutaraldehyde/protein response. In reaction (at acid or neutral pH) glutaraldehyde covalently attaches primarily with lysine or hydroxylysine at ratios of about four moles of glutaraldehyde to one of lysine. The reaction is complicated, yielding at least three protein bonded products besides glutaraldehyde polymers. They used u.v. absorption spectra studies and observed several possible polymer structures. Also, there is a direct correlation between the amount of unreacted glutaraldehyde and the amount combined with proteins. Under alkaline conditions, polymerization is different and protein response less effective.

Habeeb and Hiromota (1968) noted that in addition to lysine and terminal amino group responses with glutaraldehyde the sulfhydryl group of cysteine, the phenolic ring of tyrosine, and the imidazol ring of histidine also may be partly reactive.

Working with papain, Ottesen and Svensson (1971) noticed that mild increases in temperature, pH, and glutaraldehyde concentration increased the tendency to form insoluble derivatives. Simplistically, the glutaraldehyde reaction sequence may follow the scheme:

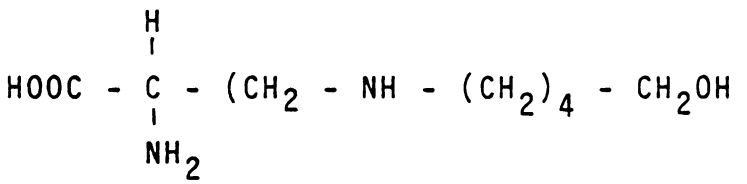
$$\text{Protein} - NH_2 + OHC\,(CH_2)_3\,CHO \rightleftharpoons$$

$$\text{Protein} - NH - CHOH\,(CH_2)_3 - CHO \rightleftharpoons - H_2O$$

$$\text{Protein} - N = CH\,(CH_2)_3\,CHO \longrightarrow$$

$$\text{Protein} - NHCH_2\,(CH_2)_3\,CH_2OH$$

Although it's possible for more than one molecule of glutaraldehyde to react with each amino group, for steric reasons it appears that only one usually does. The proposed lysine derivative obtained upon hydrolysis is:



Tannins

With greater application to food, but with less defined structure and reactivity are the tannins. Strumeyer and Malin (1975) presented an elementary discussion on the nature and properties of tannins. These complex phenolic compounds are divided into hydrolyzable and condensed tannins. The hydrolyzable are readily cleaved by enzymes and relatively dilute acids into simple sugars such as glucose and a phenolcarboxylic acid such as gallic acid. Condensed tannins are comprised primarily of polyphenolics which resist enzyme and mild acid degradation.

Although very common in nature, the elucidation of tannin reactivity and chemistry seems relatively incomplete. Loomis and Battaile (1966) review the interaction of tannins with proteins. From tanning experiments, it becomes apparent that only the peptide bonds are required for formation of tannin-protein complexes, through hydrogen bonding. The hydrolyzable tannins, especially, exert pH-reducing effects, producing hydrogen bonds between the un-ionized carboxyl

groups of the tannins and protein hydroxyl groups.

Oliver and Boyd (1972) list three types of bonding mechanisms which occur between tannins and other polymers:

1) Hydrogen bonds between tannin phenolic groups and receptor groups (-NH, CO-, or OH) on proteins or other polymers.

2) Ionic interactions between anionic groups of the tannins (ionized phenolic or carboxylic groups) and the cationic groups on protein (ε amino of lysine).

3) Covalent bonds between quinones (form part of tannin structure or are produced by oxidation) and reactive groups of the reacting polymer. These bonds are especially important to final reaction stability. Loomis and Battaile (1966) identify possible protein covalent bond sites as free amino groups, sulfhydryls, and the imino group of proline.

Phosphates and Starch Crosslinkers

In addition to proteins, crosslinking has been studied in starches, using both phosphates and other reactive chemicals. Ellinger (1972) sites work of researchers who used phosphates to bind proteins. Ortho and meta-phosphates were bound to hydroxyl groups of amino acids. Others have used phosphates as protein precipitants, and with acidification under proper conditions this can form an edible coating (Ellinger, 1972; and Gordon, 1945).

Phosphates have been used for protein stabilization and viscosity control for milk, eggs, and gelatin, and for precipitation of gelatin and blood plasma. An example is the use of sodium hexametaphosphate or polyphosphoric acid to prepare industrial gelatin films (Stauffer Chemical Company, 1975).

Low levels of phosphates tend to promote protein emulsification and stabilization, whereas higher levels coagulate proteins (F.M.C. Corporation). This characteristic is utilized in manufacture of firm puddings and in whey protein recovery.

Much of the phosphate reaction technology is derived from the starch industry, where two types of reactions can be forced to occur. This first is phosphate esterification to a single starch molecule and second by phosphate crosslinks between starch molecules. Different reactive compounds promote different responses.

Other starch reactive molecules have possible use due to similarity between starch and protein binding sites, i.e. hydroxyls. Epichlorohydrin in the presence of NaOH and mild heat over a period of time crosslinks dextran molecules by bonding hydroxyl groups with a glyceryl bridge (Floden, 1962).

In most starch reactions, crosslinks with phosphorus oxychloride, epichlorohydrin, or sodium trimethaphosphate, can be initiated with sodium chloride, with reactions occuring at room temperature in solution (Radley, 1968).

Polysaccharide Films

Another possible method of encapsulating an emulsion is to coat it with a polysaccharide gum. The gums which work best are alginates and pectates, in the presence of a polyvalent ion such as calcium. Alginates are the most versatile and useful, and as a result of their unique properties numerous recent artificial food products have been developed. Rees (1972) and Wylie (1973) describe the alginic acid and pectic acid similarities that make them different from other polysaccharides, and readily reactive with polyvalent ions. The similarity is based on their uronic acid units and regular 1:4 glycosidic linkages.

Altinates are composed almost entirely of L-guluronic (G) and D-mannuronic (M) residues. Most alginate is produced as sodium alginates. In contrast, Rees (1972) classifies pectins as D-galacturonic acid units exhibiting weaker and less predictable reactivity with polyvalent cations than those of alginates. Weaker pectin-ion bonds are due to varying degrees of methyl esterification and foreign residues in the basic backbone chain, such as L-rhamnose. Frequently, side chains of neutral sugar residues and other imbellishments limit reactivity.

Algin structures (McDowel, 1975) are generally unbranched block polymers, (short polysaccharide units), divided into categories of M blocks, G blocks, and MG blocks, each 20 to 50 residues long. Blocks are alternately or randomly distributed. For film and gel forming properties

the most important variable is the percent of G blocks, which range from 0 to 60%, depending mainly on plant species and to some extent on part of plant from which derived or stage of growth. Available calcium ions bind preferentially with the G blocks by cooperative binding, possibly due to the fact that G blocks are stiffer than M blocks which, in turn, are stiffer than MG blocks. Presumably the G block reaction with each other and with calcium is energetically more favorable than with the other two blocks (Rees, 1972).

The algins exhibit linear extended structures, like corrugated ribbons (rather than carrageenan-like spiral structures). They aggregate to form interstices, coordinating calcium ions in a favorable way without altering the crystal conformation of polyuronic acid (particularly the poly-guluronic acid fraction). Calcium concentrations in excess of the amount necessary to combine with G blocks will react with other parts of the alginate molecule (McDowell, 1975). Where this occurs gel strength increases over a period of hours, indicating a slow rearrangement. Excess calcium and acid causes chain rearrangement and syneresis.

Calcium ions fit into folds of the uronic acid and are coordinated with oxygen atoms in neighboring chains, stabilizing the entire structure which firmly holds the calcium ions. In a partly reacted calcium gel, alginates which are still sodium ionized tend to repel each other, but calcium

serves to stabilize the entire aggregation.

Most protein reactivity with algins is electrostatic (McDowell, 1975), however reactions may occur if proteins have a positive charge (are at a pH below their isoelectric point), since the alginate molecule in solution reacts as an anion (Alginate Industries Limited, 1975).

A great deal of technical information is furnished by the Kelco Co. Inc. (ref.). The following discussion summarizes much of this information.

Gels with a large proportion of polyguluronic residues form rigid, brittle gels that tend to undergo syneresis. Polymannuronic gels form elastic gels with less tendency toward syneresis. The general reaction mechanism is:

$$2\text{Na alginate} + Ca^{++} \longrightarrow \text{Ca alginate} + 2\text{Na} +$$

Gel reactivity can be controlled by several methods: Calcium salt selection to vary solubility for desired temperature or pH; sequestrant utilization to adjust calcium solubility and thus gellation time and final texture; acid incorporation to control reaction rate and setting time; and other ingredients addition to algin, such as sugar to produce softer gels.

Usually texture and setting time are controlled by calcium availability and algin composition. Greater metal ion concentration reduces setting time and sequestrants increase it. Normally, algin gels are compatible with

starches, sugars, water-soluble gums and proteins.

Other algin variations are ramified in particular characteristics; high molecular weight and greater concentration produce stronger gels. Highly refined algins yield crisper gels and greater clarity. Finer mesh algin powders produce smoother gels especially in quick set application.

Due to commercial availability, cost and acceptability for food use, calcium salts are the most practical and common gelling agents. Calcium forms the strongest and most stable gels at 30 - 40% of the stoichiometric cross-link value of 7.2 g/100 g of algin. Greater concentrations increase syneresis by precipitating calcium alginate.

Salts that perform best include calcium chloride, calcium acetate and calcium lactate. Calcium chloride is optimal but imparts a bitter taste unless the product is rinsed to remove free ions. Calcium acetate may impart an acetic acid taste; while a greater concentration of salt is required to provide the desired amount of calcium. Calcium gluconate is usable but has a low percentage of calcium per molecular weight. Other calcium salts such as sulfate, citrate, tartrate, carbonate, and phosphates have poor solubility and except under modified conditions such as high acidity the calcium is unavailable for reaction.

Applications of Algin in Foods

Besides its' use as a thickener, the gelling ability of algin has led to numerous applications in food coatings, as

texture modifiers or in synthetic food systems. Glicksman (1975) describes a U.S. system for producing artificial caviar. Sodium alginate containing color, flavor, salt and texture modifying ingredients are extruded as droplets into a calcium salt bath. The caviar appearance and texture is created by the instantaneous formation of an insoluble calcium alginate membrane around the droplet. Texture is varied by type and concentration of alginate, calcium salt, droplet size and soaking time. Although this type of product has not been commercialized in the U.S., the Soviets have pursued it to production. Slonimsky et al. (1973) describes a somewhat similar method utilizing a gelatin protein solution containing pectinate or alginate. Droplets are formed in an oil bath, fixed in a bivalent metal salt solution, then treated in a vegetable tannin solution to impart desirable caviar similarities.

Luh, Flink and Karel (1976, 1977) reviewed and described fabrication of simulated foods when calcium ions are reacted with alginate or pectates. In most of these applications the food matrix is obtained by introducing polysaccharide gel structure into an aqueous solution of calcium ions. Due to calcium ion migration into the food system, uniform texture is obtained throughout the structure rather than as an encapsulated product. Luh et al. (1976) developed a simulated fruit gel acceptable for freeze drying. They developed a weak algin-gelatin gel which allowed for solidification and shaping before the algin was crosslinked.

Final gel structure was obtained by varying the concentration of algin, sucrose, and calcium in the gelling bath. To demonstrate that various modifications can be made to impart desired textural differences to fabricated food matrices, Luh et al. (1977) studied the rheological properties of algin gels as influenced by various ingredient modification and gelling conditions.

Other methods of producing alginate products have been introduced. Szcesniak (1968) patented a process for interacting an alginic acid solution with solutions containing an alkaline earth metal salt. First, an insoluble film was formed around the algin, then a calcium solution was adjusted to obtain a uniform cellular structure throughout to simulate a particular fruit or vegetable. Unilever (1972) developed a process that simulates fruit pieces by quickly and simultaneously mixing combinations of an alginate or pectate solution, fruit pulp, a suitable insoluble calcium salt such as dicalcium hydrophosphate with an edible acid. Wood (1974) describes an artificial fruit of heterogeneous eating texture in which particles of a gelling agent like agar, carrageenan, gelatin or starch are dispersed in an alginate or pectinate solution. The solution around the particles is then gelled by introducing calcium ions at a predetermined ratio.

Unilever (1974) described a method for mixing an alginate or pectate solution containing fruit pulp or puree with a calcium ion insufficient to cause gelling on rapid

mixing, but capable of gelling under shear-free conditions.

A method for preserving fresh foods (meats in particular) was patented by Earle (1968). An aqueous algin dispersion containing at least one mono or disaccharide was subjected to an aqueous gelling solution, containing an effective amount of water-soluble calcium for sufficient time to bond the coating to the food product without imparting bitter taste. For specific meat preservation properties, Earle and McKee (1976) patented a method whereby aqueous algin is applied to the meat followed by a suitable gelling solution to form a continuous film.

Using Flavor-Tex ® (malt dextrin and sodium alginate), Williams, Oblinger and West (1978) and Lazarus et al. (1977) demonstrated the control of shrinkage and microbiol contamination of meat products with an algin film. The film is formed over the meat, dipping first in Flavor-Tex and then in a calcium chloride-carboxymethyl cellulose solution.

Earle (1975) also applied the coating principle to vegetables and in particular onions. A cold water-insoluble, amylaceous material was applied directly to raw onion which was then dipped in an algin solution. The product is then dipped in a calcium solution to complete film formation. Another method of coating (Unilever, 1971a) for frozen food products was obtained by emersing in a solution of partially reacted calcium or aluminum alginate (which may also contain xanthan gum) followed by drying in a stream of cold air to accelerate hardening.

By adding calcium or aluminum salt directly to fruit puree or other product to be encapsulated, Unilever (1971b) obtained liquid centered droplets. The product was brought into contact with a stream of alginate or pectate solution as free falling droplets. Excess pectate or alginate was gelled by recontact with calcium or aluminum solution. Algin can also be incorporated into spun protein fibers and the fibers insolubilized by reaction with polyvalent ions (Glicksman, 1975).

Other patents, particularly those from Japan, demonstrated the continued application of alginate as food coatings. In most instances, a solid food was dipped into a solution containing alginic acid or sodium alginate followed by reaction in a polyvalent metal salt solution. Variations were based on solutions applied before the algin treatment or on flavors or other compounds incorporated with the algin before salt treatment, and finally in method of drying.

EXPERIMENTAL

Exploratory Methods and Materials

As a research initiation exploratory trials were performed with ingredients commonly used in egg substitute formulations to determine coagulation characteristics; and film-forming potential with common protein crosslinkers and egg white precipitants.

Corn oil was mixed with liquid egg white, at 32% of the final weight in a Waring blender or homogenized three times at approximately 1000 psi. Five ml aliquots were coagulated in pyrex test tubes in boiling water at 15 second intervals from 15 sec. to 210 sec. A corn oil-lecithin mixture was mixed with egg white in 5% increments from 0 - 35% and homogenized twice at 1000 psi. These preparations were stored at 4 C for several days to detect phase separation, then acidified to approximately 6.0 and restored for further observation.

Based on a fresh yolk average composition of 50% water, 16% protein, 1.0% salts and 33.0% lipids (Powrie, 1972), a model system was formulated containing 100 g liquid egg white, 18.8 g whey protein concentrate (Stauffer Chemical Co., ENRPRO 50), 1.2 g calcium phosphate, 40.6 g corn oil and 17 g lecithin. After mixing for 30 seconds at high

speed in a Waring blender, the emulsion was held at 4 C for four days, then adjusted to pH 6.3-6.4 (approximate pH of natural yolk) with 7% acetic acid. Following twelve additional days of cold storage, coagulation was observed in test tubes as previously described for heating periods of up to four min.

Additional emulsions were made under similar conditions modifying the percentage of various ingredients. Milk-derived proteose peptone (Cante and Moreno, 1975) was also incorporated up to 0.5% as an emulsifier. Gelatin was incorporated up to 6% as a protein additive and potential crosslinking enhancer.

A meat-ball press with 3.5 cm internal diameter and a volume capacity of 22.5 ml was adapted to form a coagulated emulsion of yolk shape and approximate size (Fig. 1). The hole on one side of the apparatus was sealed with a flat head screw (to the inside) and a nut. The opposite hole was used for inserting emulsion. The middle of the sphere was sealed with plastic tape before emulsion addition and the top was sealed with either tape or a piece of rubber held in place with a clamp.

Heating was performed at various time intervals in boiling water and the contents inspected for general appearance and degree of coagulation. Crosslinking solutions tried included tannic acid (0.05-10%) and glutaraldehyde (0.05-25%). Reactions were observed with egg white or model emulsion using 5-10 ml samples in 20-50 ml of reactive

Figure 1. Metal forms used to fabricate simulated hard boiled "yolks" for texture studies. On the left is the modified meat-ball press and to the right, the milled aluminum form. Coagulated simulated yolks from the milled from are shown whole and halved at the top. Actual similar size yolks from hard boiled eggs are shown in the lower section of the picture.

solutions in beakers. Other potential film formers tried include ethanol and albumin reactive compounds such as aluminum potassium sulfate, hydrogen peroxide, urea, zinc acetate, and succinic acid. Some combinations of the above agents were also tested. Reaction times were varied from ten minutes to several hours.

General Methods and Materials

The sources for ingredients, chemicals, and equipment used in numerous experiments throughout the project will be listed with only one procedure, usually the first. Most work was performed at ambient temperatures. However, when not in use or in preparation for use, emulsion ingredients, emulsions, and most solutions were stored at 4 C. Emulsion ingredient concentrations are based on w/w ratios. Concentrations of crosslinkers, polysaccharides, and other solutions are w/v, and were prepared with distilled water.

Oil Selection and Drop Spreading

Commercial oils were purchased and analyzed for emulsifying characteristics, adapting the simple method described by Becker (1960). Twenty five milliliters of various emulsion ingredients and mixtures, dissolved in water, were placed in disposable petri dishes. Each oil sample (0.5 ml) was pipetted onto the center of the liquid surface. Oil droplets were allowed to spread for ten minutes and approximate diameter of the oil droplet spread was measured. Oils used included Mazola (corn oil),

Pompeian (olive oil), Wesson (a soy blend with cottonseed and polyglycerides), Crisco (soy oil), Planters (peanut oil), Hollywood (safflower oil), and Hollywood Blend (soy, peanut, walnut and safflower oils). Table IV lists the liquid solutions used for oil drop spread tests to determine emulsification potential of the oils.

Solutions were mixed using a Tekmar Super Dispax agitator, model SD45K with a G-301 generator operated at approximately 60 on the speed control for one minute or until mixing appeared complete. Samples were weighed on a Mettler 800 g capacity K7t top loading balance.

To verify these screening evaluations 50 ml portions of liquid egg white were mixed with 10 ml of various oils (peanut, olive, soy, and soy blend) and mixed on the Dispax at speed 40 for 10 sec. Fifteen milliliter alliquots were removed and centrifuged for five min. at ∿500xg and recentrifuged for 15 min. at ∿1,200xg.

The effects of pH on oil drop spread was observed by adjusting 30 ml egg white samples to whole number pH values from 4 to 11 ± 0.05 with either sodium hydroxide or citric acid, and peanut oil applied as previously described.

Emulsion Stability, Texture and Taste Studies

After selection of an oil, emulsions were made to study texture, stability and taste characteristics, by changing various emulsion properties. Emulsion formulations, and method of mixing are listed in Table V.

Table IV. Solutions and Conditions Used for Oil Drop Spread Tests

Solutions and Ingredients	Ingredient Incorporation (%)				Supplier
A. Distilled water	---				---
B. Lecithin	1.0				Central Soya-Centrolex F or Centrolex R[a]
C. Liquid egg white	---				MSU-Poultry Science Dept.
D. Nonfat Dry Milk (NFDM)	17.0				Food Club Brand
E. Emulsion Mixtures:[b,c]	1	2	3	4	Seymour Foods, Topeka, Kansas
Liquid egg white	56.7	56.7	56.7	0	
Whey solids	0	0	10	0	
Dried egg white	10	0	0	16.7	
NFDM	0	10	0	0	
Water	0	0	0	50	
Each with emulsifier levels of: Lecithin	0, 0.5, 0.5+0.5 PP[d], 1.0, 3.0				

a) Varies only in granule size
b) A total of twenty mixtures, four mixture variations at five emulsifier levels each
c) Made without oil but with percentages as if 33.7% lipids were present
d) Proteose-Peptone

Table V. Emulsion Conditions and Methods of Mixing Used to Study Stability, Texture and Taste Properties

Emulsion Formulation[a]	Method of Mixing
A. Emulsions described in Table IV, F, but with peanut oil added to 31.7% of the Emulsion.	Dispax, speed 35-40 for 15 sec.
B. Lecithin, 1%; peanut oil, 34%; egg white, 65%; pH adjustments to 4.08, 5.07, 6.95, 8.24, 8.55, 9.00, 9.66, 10.42	Dispax, speed 35, until visibly well mixed
C. Lecithin, 1%; peanut oil, 32%; whey solids, 10.75%; egg white; 56.25%; pH adjustments to 5.3, 6.15, 6.9, 7.2, 8.1, 8.95, 9.95, 11.00	Dispax, speed 30, 30 sec.; speed 70, 45 sec.
D. Lecithin, 1%; peanut oil, 32%; whey solids and egg white together, 67%; with whey at 0, 2.5, 5, 7.5, 10.0, 12.5, 15 and 20%; pH adjusted to 6.0 ± 0.1	Dispax, speed 40, 30 sec.
E. 1) Lecithin, 1%; whey solids, 10%; oil and albumin together, 89%; with oil from 0 to 60% at 5% increments; pH adjusted to 6.0 ± 0.1 2) Same as above with oil varied from only 15 to 40%	Dispax, speed 35 - 40, until visibly well mixed
F. Lecithin, 1%; oil, 32%; whey solids, 8.5%; pH adjusted egg white, 58.5%; pH adjusted to 5.7, 6.1, 6.5, 6.95, 7.25, 7.65, 7.9	Dispax, speed 35 - 40 until well mixed
G. Lecithin, 1%; oil, 32%; whey solids, 8.5%; pH adjusted egg white yielding final pH values of 6.05, 6.15, 6.25, 6.35, 6.4, 6.6, 6.7, 6.75, 6.85	Dispax, speed 35 - 40 until well mixed

Table V (cont'd.).

Emulsion Formulation[a]	Method of Mixing
H. Lecithin, 1%; oil, 32%; whey solids, 8.5%; egg white, 57.0%; calcium chloride dihydrate, 1.0%, and 0.5% of the following stabilizers: additional lecithin; carboxymethylcellulose, (CMC); 7LF and 7MF, and DD slow set pectin (Hercules, Inc.); sodium hexametaphosphate and sodium tripolyphosphate (FMC Corp.); Sodium trimetaphosphate (Stauffer Chemical Co.). All samples were pH adjusted to approximately 6.85	Dispax, speed 40, 1 min., then homogenized once at 500 psi

a) Emulsion pH's were adjusted with 10% sodium hydroxide or 10 or 20% citric acid

Emulsion characteristics were assessed by some or all of the following methods:

1) Centrifugation - model CL International table top centrifuge; 15 ml samples at 1,200xg for 15 min. Egg white separated at the bottom from the remainder of the emulsion, was observed.

2) Measurements of pH and adjustments - Chemtrix, type 60A, with a single reference glass electrode; or an Instrumentation Laboratory Inc. Model 245 pH/mv electrometer.

3) Microscopic inspection - American Optical Co. Microstar Series 10 microscope with fluorolume illuminator, lens setting at 0, light setting B and 45x eye piece. Emulsion samples were inspected for both predominant and overall droplet size ranges.

4) Visual separation - observation of samples stored overnight or for several days, to detect phase separation.

5) Texture studies - a) Large eggs were placed in cool water and brought to a boil for 18 min., quickly cooled, until cool enough to handle, shelled and the yolks removed. b) Small eggs were prepared the same way with the exception that boiling was ended at 14 min. c) Samples prepared as described in Table V, A, were coagulated for seven min. in the metal sphere described in the preliminary methods section. d) Other samples, as described in Table V, were formed for 5.5 min. in an aluminum sphere, (fabricated by the MSU machine shop) comprised of two 2.9 cm diameter, overlapping hemispheres, total capacity, 12.5 ml, and clamped together

with a three prong extension clamp. Samples were poured into a quarter inch hole at the top and sealed with a flat head screw (Fig. 1). These are comparable to small egg yolks obtained from eggs ranging in size from 17 - 21 oz. per dozen. Texture comparisons were obtained using an Allo-Kramer shear press, Model SP12 with recording attachment, a 100 lb. ring, and aluminum CS-1 standard shear compression cell. The instrument was operated with the range setting at 50 for samples described in condition A, Table V, and at range 20 for the other coagulated emulsion samples (Table V) and for egg yolks.

6) Taste - shear press samples were tasted after shearing to observe flavor and mouth feel characteristics.

7) Authentic yolks of small size and of some fabricated samples were weighed to substantiate size comparison for textural studies.

8) Viscosity - a 20 ml volumetric pipette with part of the tip removed was mounted vertically to a ring stand. To provide relative comparisons of emulsion and yolk viscosities, the pipette emptying time was recorded. Yolks were from fresh eggs (2 days old) and eggs stored 4 and 8 weeks.

Crosslinker Studies

In addition to unadjusted solutions, potential film forming crosslinkers were assessed by adjusting the simulated egg yolk emulsion to pH values of from 3 to 12 in

whole number values. Most pH adjustments were made with 10% sodium hydroxide and 10 or 20% citric acid. Thirty-five to fourty-five milliliters of crosslinker solutions were poured into 3.5 inch petri dishes. Five milliliters of the simulated emulsion, containing typically, 1% lecithin, 10% whey solids, 59% liquid egg white, and 31% oil, was layered onto the surface of the crosslinker solution.

Periodic observations were made for up to a minimum of one hour. Type of interaction and, specifically, incidence of film formation were noted. Chemicals and suppliers, solution concentration and pH ranges, and literature references, where applicable, are listed in the Appendix.

Polysaccharide Films and Emulsion Interaction

Development of polysaccharide and polyvalent cation interactions to develop optimum film formation around emulsion globules was studied by varying parameters of polysaccharides and salts; their concentrations, combinations, reaction times and effects of pH adjustment.

Several approaches were used to determine optimum film formation potential and effectiveness with algin and pectin solutions. These include:

1) Pectinate and alginate mixed with emulsion before addition to polyvalent cation salt solution.

2) Addition of salts at various concentrations and mixtures, then insertion into polysaccharide solutions.

3) pH adjustment of emulsions and of algins before

interaction.

pH and Crosslinker Mixing Effects

The influence of pH on algin reactivity was tested by fabricating emulsions with and without 0.5% sodium alginate. Portions of each were adjusted to pH 5.8 and 6.5. Emulsions with alginate were studied in pH-adjusted water; and those without algin in pH adjusted 0.5% alginate. Emulsions were reacted in petri dishes in the manner described for cross-linker studies applying emulsion to pH adjusted solution.

Combination effects of GRAS (Generally Regarded as Safe, F.D.A.) crosslinkers and egg white reactive salts were also tested by mixing 15 ml of each of two solutions in 50 ml beakers. Eight ml of emulsion (without added salt) was then injected with a syringe into the mixtures which include:

1) Tannin (2%) and sodium polypectate (1.8%).
2) Tannin and sodium alginate (1.5%).
3) Alginate and polypectate.
4) Potassium aluminum sulfate (2%) and tannin.
5) Ammonium aluminum sulfate (2%) and tannin.
6) Ammonium aluminum sulfate and potassium aluminum sulfate.

Polysaccharide and Salt Solutions

Algin and pectin solutions ranging from 0.5 to 5.0% were formulated either by dry addition or after mixing with glycerine - as dispersing agent and plasticizer, to 1.5

times the polysaccharide weight, to water during stirring with a magnetic stirrer. Occasionally, mild heat was applied while mixing to aid solubilization. Samples were then held at $4^{o}C$ until uniformly dispersed.

Pectins used include LM AB pectin and DD slow-set pectin (Hercules, Inc.), and 1.8% sodium polypectate solution in 0.02 M citrate buffer, pH 5.0 and 0.005% sorbate (courtesy Dr. R.F. McFeeters). Algins used were sodium alginate, Type "CA" (Meer Corporation), and Kelgin XL sodium alginate and Kelmar KR potassium alginate (Kelco Co.).

U.S.P, N.F. or reagent grade salts used in emulsions or as solutions to determine effectiveness in film formation and taste include: aluminum sulfate - $Al_2 (SO_4) \cdot 18H_2O$, aluminum potassium sulfate - $ALK (SO_4)_2 \cdot 12H_2O$, aluminum ammonium sulfate $ALNH_4 (SO_4)_2$, calcium acetate - $Ca (C_2 H_3 O_2)_2 \cdot H_2O$, calcium chloride dihydrate - $Ca Cl_2 \cdot 2H_2O$, calcium gluconate - $Ca (C_6H_{11}O_7)_2$, calcium lactate - $Ca C_6H_{10}O_6$, calcium phosphate dibasic - $CaH PO_4 \cdot 2H_2O$, and calcium phosphate monobasic - $Ca (H_2PO_4)_2$. Calcium chloride dihydrate was the most frequently used salt and the one used as a control in comparing other salts.

Artificial Yolk Emulsion Manufacture

Artificial yolk emulsions for algin encapsulated yolks were manufactured with various salt concentrations and pH adjustments. The range of ingredients and method of

formulation were: salts as listed above up to 2.0%, lecithin 1.0 - 1.3%, whey solids 8.0 - 8.5%, oil (peanut) 32 - 33%, liquid egg white up to 58.5%, 30% β carotene in vegetable oil (Hoffman LaRoche, Inc.) sufficient to give the desired color up to 0.05%, artificial egg flavor (Stepan Chemical Co.) 0.2%. pH adjustments were accomplished with either acetic or citric acid. Small amounts of CMC were incorporated into selected emulsions to aid spherical formation in algin solutions.

Sample mixing was accomplished with either - or a combination of the Tekmar Super Dispax, a model M62 electro mortar and pestle (Torsion Balance Co.) until completely mixed or by means of a homogenizer operated at up to 1400 psi. Most homogenizations were at 500 - 1000 psi.

Polysaccharide Film Formation

Emulsions were injected into algin or pectin solution in 50 ml beakers by means of plastic syringes (discardit, Arthur H. Thomas Co.), with the tips removed, leaving a ¼" diameter hole on the end. Injections of emulsion into solutions were made with the tip of the syringe at or just below the liquid surface, Figure 2. Steady pressure was applied to the plunger until all emulsion was injected. Eight to fifteen ml samples were used - 12 ml is about the volume of a small yolk. Reaction time was also varied, ranging from one to thirty minutes. Upon removal from film-forming solutions, "yolks" were allowed to drip to

remove excess solution before further evaluation.

Encapsulated emulsions were analyzed by measuring the yolk index (height of the yolk divided by the average width) and compared with actual yolk values (Funk, 1948; Sharp and Powell, 1930), Figure 3. Real yolks used for comparison were separated from the white before testing. Yolk and emulsion membrane rupture strengths were compared by a method similar to that suggested by Haugh (1937). A Chatillon puncture tester, Model DPD-500, (John Chatillion and Sons, New York) was mounted to a ring stand. A 3.2 cm diameter acrylic disc was mounted on the bottom of the pressure rod. Yolks in petri dishes were slowly raised against the disc by means of a "little jack" (Precision Scientific Co.). The pressure gauge was read in 0.1 units at the point of membrane rupture.

Whey Dialysis to Increase Protein Concentration

ENRPRO - 50 whey protein isolate was dialyzed in regenerated cellulosic tubing exclusion limit 10,000 to 12,000 M.W. (American Cyanamide Corp.) in distilled water. Two hundred grams of whey solids were mixed with either 600 or 1200 ml of water. The dialysis reaction was allowed to run for about five days with at least two water changes per day.

Emulsion Stability after Salt Incorporation

In order to evaluate the effect of salts and homogenization treatments on emulsion stability, "yolks" were manufactured according to the scheme listed in Table VI.

Figure 2. Method of inserting emulsion into an algin solution to form "yolk".

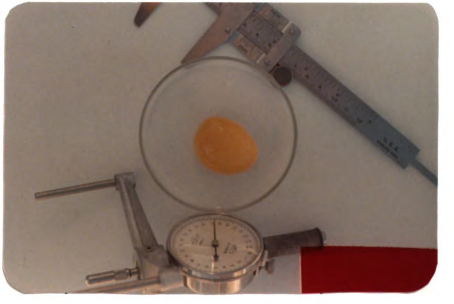


Figure 3. A formed "yolk" with calipers and height gauge used to measure yolk index.

Table VI. Emulsion Ingredients and Special Conditions Used to Establish the Effect of Salts and Homogenization Treatments on Stability. All emulsions listed contained 1% lecithin and 0.2% egg flavoring.

Treatment		Homogenization	Ingredients (%)
A.	pH adjusted to 6.1 with 1N acids 1) control, 3.0 ml H_2O added 2) 3.6 ml lactic acid 3) 3.0 ml lactic acid 4) 3.1 ml citric acid 5) 3.0 ml phosphoric acid	2 x 500 psi	calcium acetate - 1.2 whey solids - 8.3 liquid egg white - 57.9 oil (peanut) - 31.4
B.	1) unadjusted pH 2) pH adjusted to 6.85 with 10% citric acid	2 x 800 psi	calcium acetate - 1.2 whey solids - 8.5 liquid egg white - 57.1 oil (peanut) - 32.0
C.	Homogenization variations	1) 2 x 500 psi 2) 1 x 500 psi and 1000 psi	calcium acetate - 1.0 whey solids - 8.5 liquid egg white - 59.3 oil (peanut) - 30.0
D.	All of oils used in original oil tests 1) After 4 days cold storage 2) After stirring and salting 3) pH adjusted to 6.0 4) pasteurization at 60°C in water bath (took up to 2 hrs. in covered containers)	2 x 700 psi	calcium acetate - 1.0 whey solids - 8.5 liquid egg white - 57.3 oil (peanut) - 32.0
E.	Whey isolate and safflower oil as ingredient variations	500 psi and 1250 psi	calcium acetate - 1.0 whey isolate - 8.5 liquid egg white - 57.3 oil (safflower) - 32.0

Egg White and "Yolk" Binding

To aid in adherence of the formed yolk to egg white the following methods were used:

1) 2% calcium carrageenan (Marine Colloids, Inc.) as a crosslinker or as a dip after reaction in alginate.

2) 2% sodium carrageenan in the same manner.

3) Incorporation of 1% calcium or sodium carrageenan with 2.25% alginate.

4) Algin-glycerin mixed into liquid egg white as the film forming solution.

5) 1% and 2% dried egg white mixed with 2.25% alginate.

6) AL $(SO_4)_3 \cdot 18H_2O$ at 0.11% mixed with the egg white outside the formed yolk.

7) Calcium acetate at 1% level in egg white.

The formed yolks were placed in egg white for several hours or overnight and then poached over steam to observe for desired adherence between egg white and "yolks".

Taste Panel

The simulated yolk emulsion was manufactured using whey isolate and safflower oil (Condition D., Table VI). β-carotene coloring yielding 8 on the Roche yolk color fan was added by incorporating 1 g of 30% β-carotene to 9 g of safflower oil, mixing and adding this to the oil fraction of the emulsion. The emulsion was homogenized at 500 then 1000 psi. Yolks were formed using approximately 15 ml of emulsion in 2.25% Meer algin with 2% added powdered egg

white, for 4 min. Yolks were removed from algin, allowed to drip for a short time, then placed in beakers containing the separated white from an egg. After overnight storage in the cold room eggs were poached for 3½ to 4 min.

Yolks were served with a slice of buttered toast in the Food Science taste panel room to 33 randomly selected students, professors, and staff of MSU. Michigan State University Committee on Research Involving Human Subjects approved consent forms specifying ingredients used, were presented to panel members along with evaluation score sheets. The score sheets were used to establish degree of like and dislike for overall yolk appearance, yolk flavor, and general yolk acceptability. Yolk texture and color were also evaluated. All scores were based on ranking from 1 to 7. Copies of these forms are in the Appendix.

RESULTS

Evaluation of Exploratory Observation

Emulsions

Fresh liquid egg white emulsified with 32% corn oil demonstrated instability even after being homogenized three times at 1000 psi, and began to separate after one day of cold storage. Mildly stirring or shaking the emulsion yielded quick resuspension. When heat coagulated in test tubes, some oiling off was observed as solidification approached completion. However, most of the oil is firmly held within the framework of coagulated egg white, without significantly weakening the coagulum. Emulsion samples prepared with lecithin as part of the lipid fraction also separated under storage conditions, regardless of the amount of lipid in the system. Reducing the pH to 6.0 did not improve stability.

In the model system much less separation occurred during cold storage, perhaps partly due to increased viscosity from added solids. Separation which did occur consisted primarily of egg white at the bottom of the containers. Coagulation in test tubes revealed continuous firming throughout the emulsion without oil loss. Varying emulsion ingredients, followed by coagulation in the metal form

resulted in the following observations:

1) The greater the percentage of egg white in the emulsion, the firmer the coagulum.

2) Gelatin, even at very low concentrations produced samples too viscous to work with.

3) Lecithin and milk proteose-peptone fractions resulted in soft coagulums.

4) Addition of whey solids enhanced emulsion stability, oil retention and uniform gel structure.

Protein Crosslinkers

Tannin solutions reacted readily with egg white or the formulated emulsions, forming a fragile film at the protein-tannin interface. As tannin concentration (above about 0.5%) and reaction time (longer than 10 min.) were increased, the film thickened and became brittle. Glutaraldehyde concentrations above 0.5%, likewise showed reactivity with the emulsion forming a film strong enough to encapsulate the emulsion, but had a leathery look and texture. The extent of interaction and film thickness were time and glutaraldehyde concentration dependent. Of other albumin-reactive compounds, only aluminum sulfate, aluminum potassium sulfate, succinic acid, and 95% ethanol, exhibited film-forming potential. All of these films, however, were quite fragile and/or brittle. Combinations of reactants did not improve the quality of the films with the possible exception of glutaraldehyde and tannin mixtures. In this instance a more elastic membrane

was formed. It was also observed that higher concentrations of crosslinkers, or the addition of thickeners such as carboxymethylcellulose or glycerin aided in the formation of spherical glubules when the emulsion was extruded into crosslinker solutions.

Selection of the Oil Phase

Oil-Drop Spread

With the oil drop spread test, it is believed that the less the drop spreads the better the solution emulsifying properties. Based on this concept peanut oil was selected as the oil of choice for further emulsion preparations. Of the oils investigated peanut oil yielded the most consistant results, ranking as one of the best three in most of the trials. It responded favorably with formulations containing 1% lecithin and whey solids; both of which were selected for continued emulsion studies. Also it was noted that peanut oil had less tendency to continue spreading on the surface of solutions after measurements were made, than the other oils.

Each oil demonstrated some advantages under specific circumstances except olive oil, which consistantly displayed the least potential for emulsification.

Emulsification Potential of Lecithin and Protein Solids

The best overall emulsification properties appeared to be in solutions containing 3% lecithin, where very little difference was noted in drop-spread between oils. At 0 and

0.5% lecithin the best emulsification properties were displayed by solutions comprised entirely of egg white as the protein reactant. Solutions containing proteose-peptone as an emulsifier in conjunction with lecithin exhibited optimum results with solutions containing non-fat dry milk. At 1% lecithin both milk and whey solid solutions show more promise for emulsification than those with only egg white. Oil-drop spread measurements are listed in the Appendix.

Effect of pH on Oil-Drop Spread

Peanut oil spreading on pH-adjusted egg white was minimum at pH values of 5 and 6 with a 1.9 cm diameter spread. Below pH 5 and above pH 7 the drop size increased, indicating that pH adjustments could be significant for emulsion stability.

Centrifugation Verification of Oil-Drop Spread Test

When placed on liquid egg white soy and olive oil had exhibited the greatest oil drop spread; soy blend and peanut oil least. To confirm oil-drop spread results these four oils were emulsified at 17% with egg white. Exact separation volumes were difficult to measure due to phase color similarity, but greater separation was evident for emulsions containing olive and soy oils than those containing peanut oil and soy blend. This correlated with the findings of the oil-drop spread test.

Emulsion Stability

For emulsions listed in Table V, (emulsion conditions) centrifugation was the primary method of analysis. Observations of emulsions stored at 4 C, and microscopic evaluations were used to verify these results. In most emulsions, phase separation occurred between liquid egg white and the rest of the system, not between oil and emulsion. Stability of emulsion conditions listed in Table V are presented in Figure 4.

Emulsions with dried egg white as a solid additive (Figure 4A) are less stable than those with milk or whey solids. The emulsions formulated with whey solids showed the best physical stability. Differences based on emulsifier levels were not as clearly differentiated by phase separation as by the oil spread test.

The effect of pH over a wide range is demonstrated by data in Figure 4B. Characteristics of the emulsion containing only oil, lecithin, and pH-adjusted egg white demonstrate the instability of emulsions formulated without stabilizers. When whey solids were added at a 10.75% level, the stability improved. Emulsions with incorporated whey solids show enhanced stability at lower values of pH, those without added solids show instability.

Figure 4C demonstrates the stability enhancing effects of whey solids. The same is true of increases in oil concentration (Figure 4D). However above 50% incorporation, oil began separating from the emulsion. The effect of adjusting

Figure 4. Centrifugally induced phase separation in formulated emulsion as influenced by ingredients and pH. (See Table V for formulations).

A - lecithin percentage as emulsifier in emulsions containing liquid egg white with -- added dried egg white (O), NFDM (□), whey solids (△), and an emulsion with only reconstituted egg white in addition to oil and emulsifier (●);

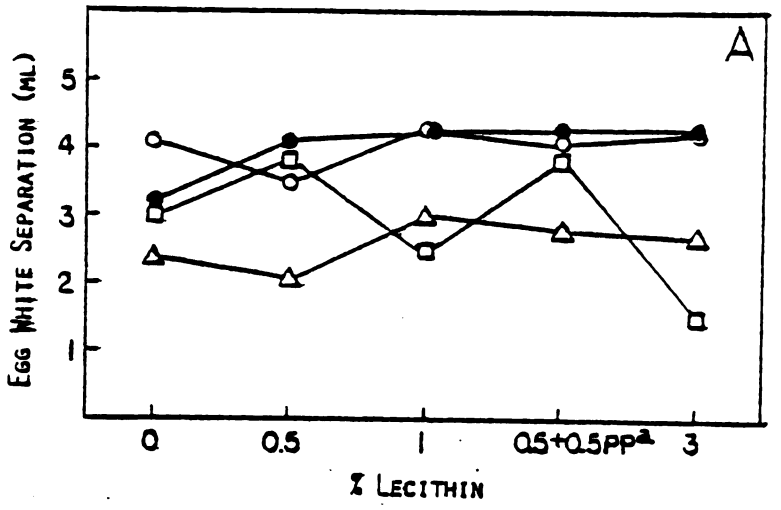
B - pH adjustment over a wide pH range with and without whey solids;

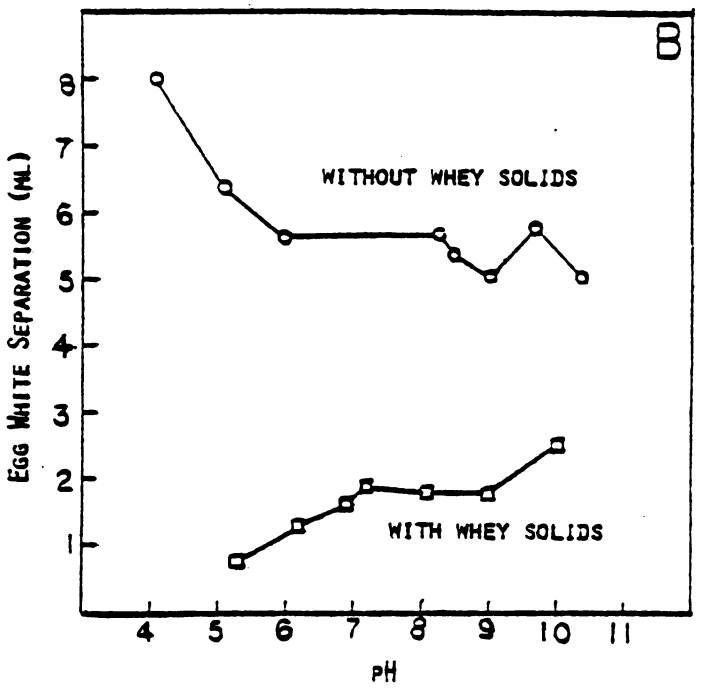
C - varying concentrations of whey solids;

D - varying concentrations of peanut oil;

E - pH adjustments over narrow pH ranges, and

F - calcium chloride incorporation along with 0.5% added stabilizers, control - 1% lecithin (a) additional lecithin (b) sodium hexametaphosphate (c) sodium trimetaphosphate (d) sodium tripolyphosphate (e) carboxymethylcellulose (CMC) 7LF (f) CMC-7MF (g) and slow-set pectin (h).


A
EGG WHITE SEPARATION (ML)
1
2
3
4
5
0
0.5
1
0.5+0.5PP[a]
3
% LECITHIN


B
EGG WHITE SEPARATION (ML)
1
2
3
4
5
6
7
8
WITHOUT WHEY SOLIDS
WITH WHEY SOLIDS
4
5
6
7
8
9
10
11
pH

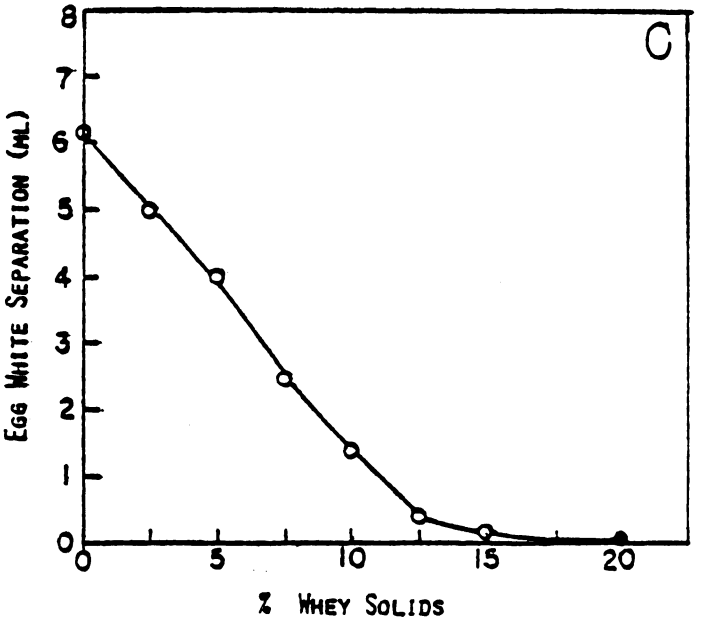
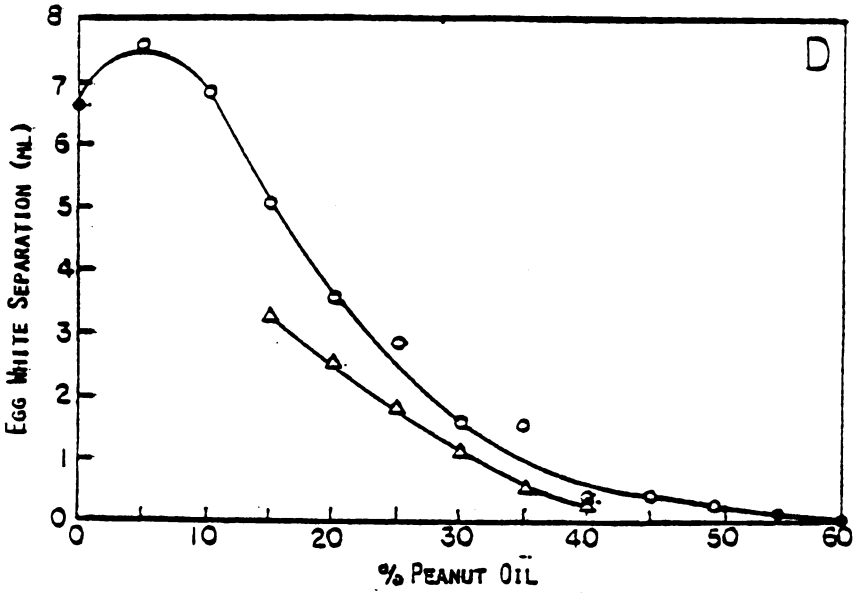
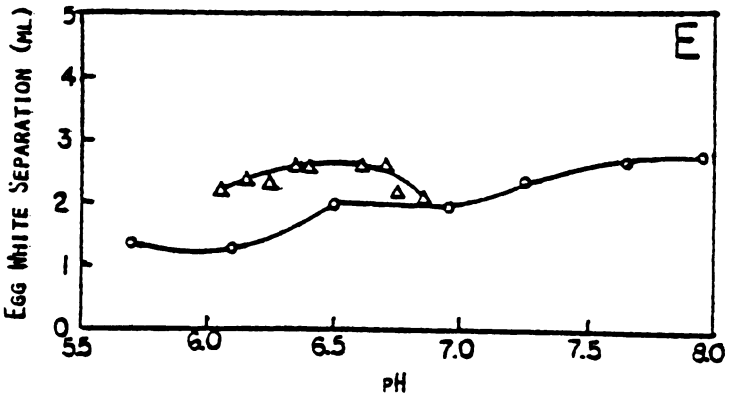
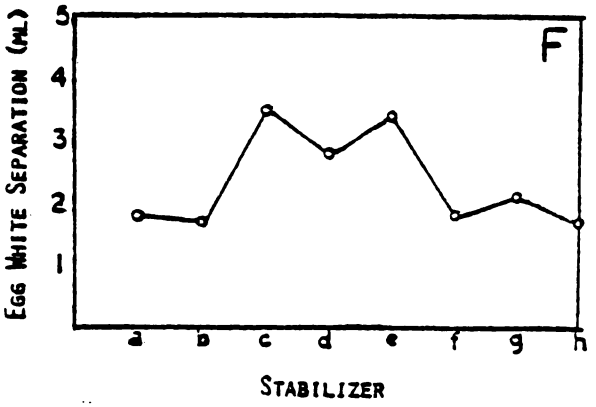

C
EGG WHITE SEPARATION (ML)
0
1
2
3
4
5
6
7
8
0
5
10
15
20
% WHEY SOLIDS

Fig. 4 Cont.


D
EGG WHITE SEPARATION (ML)
% PEANUT OIL
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60


E
EGG WHITE SEPARATION (ML)
pH
0
1
2
3
4
5
5.5
6.0
6.5
7.0
7.5
8.0


F
EGG WHITE SEPARATION (ML)
STABILIZER
1
2
3
4
5
a
b
c
d
e
f
g
h

the pH within narrower ranges in emulsions containing 8.5% whey solids is shown in Figure 4E. A pH below 7.0 was preferred for acceptable emulsion stability, with greatest stabilities obtained between pH 6.0 - 7.0. Apparently slight changes in emulsification or pH play an important role in the stability of emulsions.

In emulsions with calcium chloride the addition of stabilizers and thickness in addition to lecithin showed no improvement on stability; and phosphate compounds decreased stability (Figure 4F).

Increases in oil or whey solids decreased the foaming tendencies of egg white. It was observed that the final volumes in the centrifuge tubes more closely approximate the initial 15 ml when whey solids or oil concentration are increased. Adjusting the pH did not influence final volume. The phosphates and pectin caused a measurable solid precipitant in addition to egg white separation, due to reaction with calcium ions.

Additional Stability Studies

Reduction in the particle size of the emulsion as an indicator of increased stability demonstrated fair agreement with centrifugation but lacked consistancy in overall results. A possible exception was with emulsions varying in concentration of whey solids, where observed oil-droplet sizes decreased in the same manner, as phase separation decreased with increased whey. Results of microscopic analysis

are listed in the Appendix (Figure A1).

When samples were stored for several days at 4 C to observe phase separation, emulsions usually demonstrated tendencies toward instability in agreement with the centrifugation studies.

Coagulated Emulsion Texture

Compressibility

Kramer texture analysis was used to study coagulated emulsion characteristics and to identify modifications in formulations which most closely correlated with the texture of hard-boiled egg yolks. The results from each test may be compared by converting peak heights to compressibility. The equation for this parameter in total force applied in lbs. is as follows:

$$\text{Compressibility} = \frac{\text{Ring value x range x peak height}}{100 \times 100}$$

For the egg and emulsion samples compressibility is total force applied/yolk.

Compressibilities of coagulated emulsions are compared to hard-boiled yolks and illustrated in Figure 5. Figure 5A demonstrates the variety of textures obtained under various emulsifier levels and solids content. In general, added egg white solids cause a much higher compressibility than do NFDM or whey solids. Also increases in lecithin concentration yield significantly softer textures.

Figure 5. Compressibility of coagulated emulsions as influenced by ingredients and pH. (See Table V for formulations).

A - lecithin percentage as emulsifier in emulsions containing liquid egg white with - added dried egg white (O), NFDM (□), whey solids (△), and an emulsion with only reconstituted egg white in addition to oil and emulsifier (●);

B - pH adjustment over a wide pH range with and without whey solids;

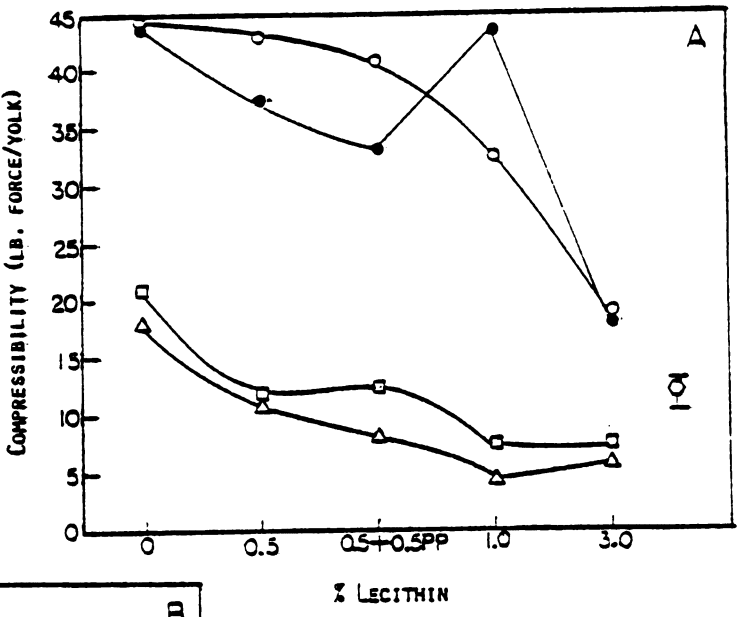
C - varying concentrations of whey solids;

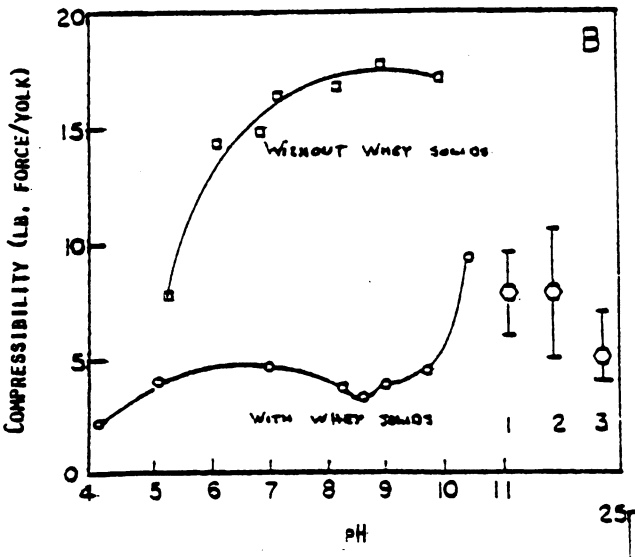
D - varying concentrations of peanut oil;

E - pH adjustments over narrow pH ranges; and

F - calcium chloride incorporation along with 0.5% added stabilizers, control - 1% lecithin (a), additional lecithin (b), sodium hexametaphosphate (c), sodium trimetaphosphate (d), sodium tripolyphosphate (e), carboxymethylcellulose (CMC)7LF (f), CMC-7MF (g), and slow set pectin (h).

Compressibility ranges are averaged for authentic yolks are indicated by ⊖. For Figure 5A this is for an average of 6 large yolks; and for B-F, one dozen sets of - fresh yolks (1 and 2) and 10 week old eggs (3).


A
COMPRESSIBILITY (LB. FORCE/YOLK)
45
40
35
30
25
20
15
10
5
0
0
0.5
0.5+0.5PP
1.0
3.0
% LECITHIN


B
COMPRESSIBILITY (LB. FORCE/YOLK)
20
15
10
5
0
WITHOUT WHEY SOLIDS
WITH WHEY SOLIDS
1
2
3
4
5
6
7
8
9
10
11
pH

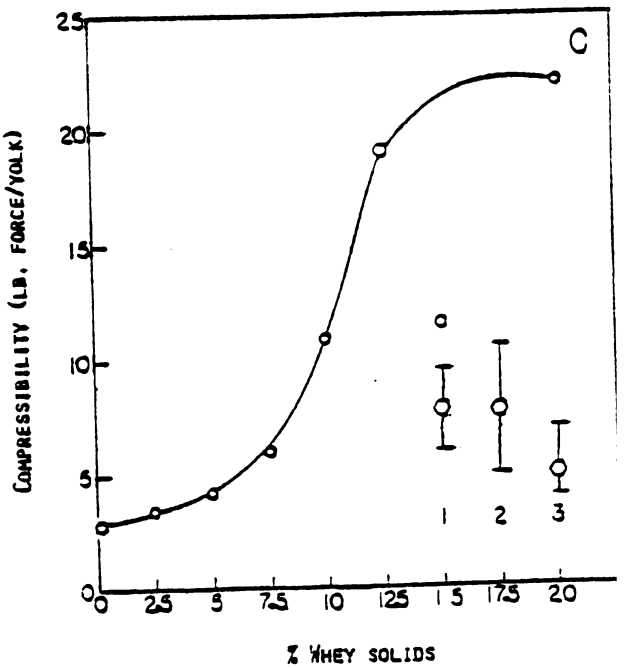
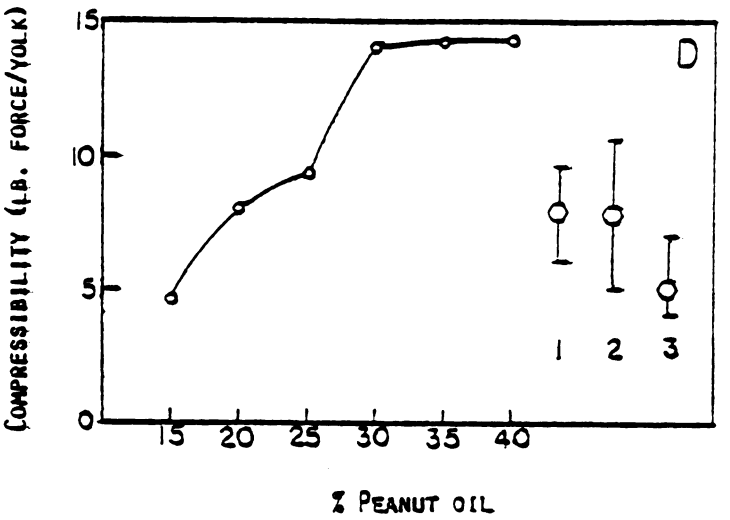
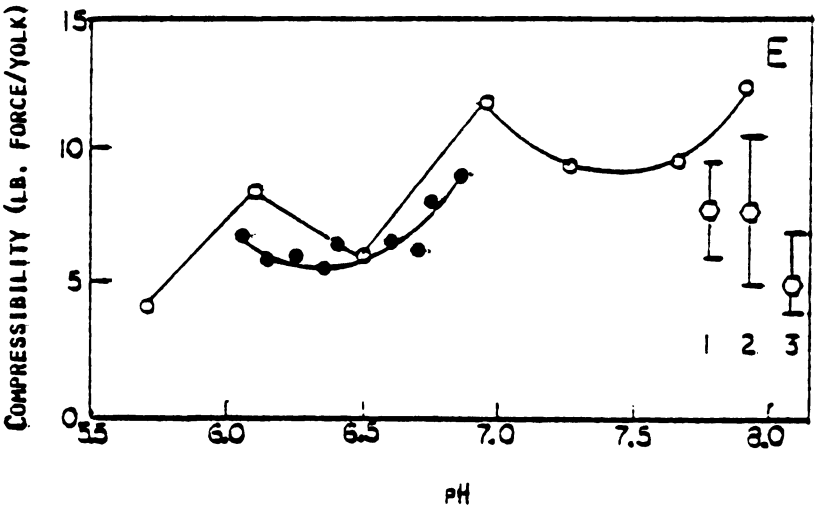
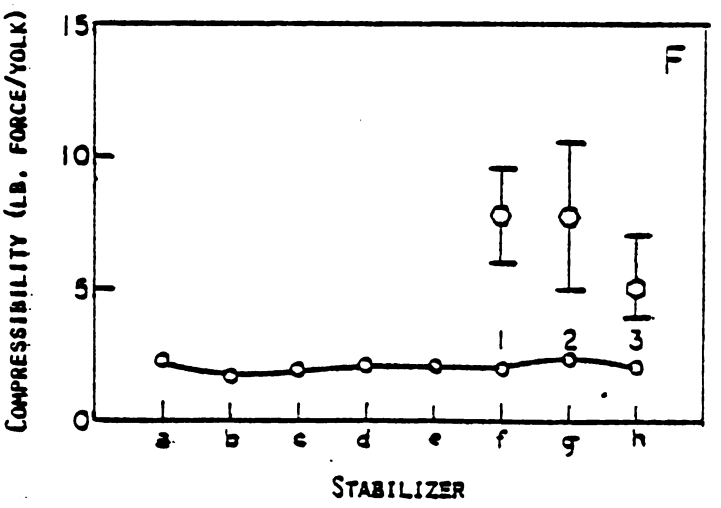

C
COMPRESSIBILITY (LB. FORCE/YOLK)
25
20
15
10
5
0
1
2
3
0
2.5
5
7.5
10
12.5
15
17.5
20
% WHEY SOLIDS

Fig. 5 Cont.


D
COMPRESSIBILITY (LB. FORCE/YOLK)
15
10
5
0
15 20 25 30 35 40
1 2 3
% PEANUT OIL


E
COMPRESSIBILITY (LB. FORCE/YOLK)
15
10
5
0
5.5 6.0 6.5 7.0 7.5 8.0
1 2 3
pH


F
COMPRESSIBILITY (LB. FORCE/YOLK)
15
10
5
0
a b c d e f g h
1 2 3
STABILIZER

The effects of pH and added whey solids are evidenced by Figure 5B. Without added solids, an egg white, oil and lecithin emulsion was quite soft. A 10.75% whey addition significantly increased the compressibility of the emulsions, and raising the pH also increases the compressibility.

Increasing the level of whey solids incorporated into emulsions elevated compressibilities (Figure 5C). When fresh yolk averages were considered as optimum for compressibility; whey solid incorporation at about 8.5% was considered ideal. Compressibility also increased with an increase in oil percentage. Figure 5D demonstrates the effects of increasing the oil concentration, with whey solids held constant at 10%. Increases in compressibility appear to level off at about 30% oil.

Compressibility values for pH adjusted emulsions with 8.5% whey and 32% oil are shown in Figure 5E. This graph demonstrates that pH even in narrow ranges plays a significant role in textural properties. And also indicates that the best ranges for comparison with yolks are between pH 6 and 7; probably optimum at pH 6.1 and pH 6.8 or 6.9.

The addition of calcium chloride appears to both lower the compressibility below a desirable level, and to negate any effects which the phosphate or polysaccharide compounds might impart (Figure 5F).

Taste

Tasting samples that were used in the Kramer analysis helped establish guidelines for further adjustments of formulation. Emulsions comprised primarily of egg white did not display the mealy texture of yolks; and emulsions incorporating milk were mushy. Whey solid addition most closely approximated yolk texture. Lecithin at a 3% level resulted in a sticky, mushy mouth feel. Below pH 6 emulsions were slightly sour and above pH 9 somewhat rubbery. The most desirable flavor considering crumbliness and a less distinct whey influence in taste were between pH 6.5 and 7.5.

The mouth-feel and flavor of whey modified emulsions was optimum in the 7.5 - 10% range. However, the higher the concentration of whey the more sweet and whey flavored the yolks became. When percent oil was lowered below 20%, the formed yolks were mushy and bland. Above 35% oil concentration, the texture and flavor became oily and somewhat harsh. The addition of calcium chloride caused the emulsions to become mushy and slightly astringent.

Relative Viscosities

Viscosity comparisons were primarily between real yolks and pH adjusted emulsion or emulsions with added stabilizers. Real yolks when containing a small amount of egg white, had an average pipette emptying time of 1.9 min. Fresh yolks with all white removed were timed between

6.4 and 9.1 min. Yolks from eggs stored eight weeks had an average time of 4.9 min.

Emulsion viscosity, in general, increased at lower pH values. At pH 7.9 the dropping time was 0.60 min., but at pH 5.7 was 10.3 min. The addition of carboxymethylcellulose and pectin increased the dropping time to over 30 min. Additional lecithin also increases viscosity, but the rate of increase depends on other emulsion conditions. The emulsion with apparent viscosity nearest that of fresh yolk contains 1% $CaCl_2$ and 1.3% lecithin. Compiled results are listed in the Appendix.

Yolk Weights

The weights of coagulated emulsion samples with pH adjustment between 5.7 and 7.9 ranged from 11.20 to 12.36 g with an average of 11.73 g. For the pH range 6.05 to 6.85, weights were 11.54 g to 12.10 g with an average of 11.86 g. Those with calcium chloride and stabilizers incorporated into emulsion weighed 12.23 to 12.65 g (ave. 12.47 g). For actual yolks, one dozen yolks from fresh eggs ranged from 7.88 g to 16.75 g and averaged 12.55 g. Weights for 10 week old eggs were 9.78 g to 14.31 g (ave. 11.64 g). Based on both weight and size there is a good correlation between coagulated emulsion and yolks, thus the two may be compared for compressibility.

Crosslinkers

The crosslinkers responding with the most potential were tannic acid, glutaraldehyde, aluminum potassium sulfate and aluminum ammonium sulfate. Of all the compounds used only glutaraldehyde supported the emulsion without rupture while remaining at an acceptable thickness. Glutaraldehyde is not, however, a GRAS food constituent, precluding it from direct food use. A summary of potential crosslinker results is listed in the Appendix.

Polysaccharides

Algin Selection

In view of the lack of adequate or desirable reactivity with crosslinkers, another method of encapsulation was investigated, utilizing polysaccharides (algins and pectins) that develop solid gels or films in the presence of appropriate cations. Since the results of algin and pectin response are based primarily on observations, a summary of these observations is included.

When pectin or algin were incorporated into emulsion, the emulsion became quite viscous. And when the emulsion is placed in contact with calcium ions the resulting film became quite thick as the calcium migrated inward, even after emulsion was removed from the calcium solution. When calcium chloride or other suitable salt was incorporated into the emulsion, then placed in algin or pectin solution, a desirable encapsulating film was formed.

Without calcium or other suitable cations, algins and emulsions do not react as a result of the pH adjustment, unless the pH is lowered below 4. At this pH the response appeared to be more of a coagulation than an emulsion - algin interaction. At low calcium ion levels (i.e. 0.15%) a lowered pH of either algin or emulsion aids in film formation. But. unless allowed to react until quite thick the films were fragile. At higher calcium ion concentrations (i.e. p.3%), pH effects became less significant.

Slow-set pectin doesn't yield desirable film formation, irregardless of calcium content. The low-methoxy pectin and sodium polypectates produced films with excellent yolk membrane-like sheen, but readily ruptured, except when the encapsulating film was judged to be too thick.

Kelco algins responded much like the pectins. Low viscosity alginate would not support the emulsion and the potassium alginate formed nice appearing films, which split under very mild pressure. Therefore, most encapsulation was done with the Meer Co. type "CA" sodium alginate.

Mixtures of pectins and algins only weaken the film potential of the algin. Also, mixtures using tannins, potassium, or ammonium aluminum sulfate in conjunction with pectin or algin do not improve the film response of any of the solutions, or show any advantage over alginate alone.

Glycerin aids somewhat in the dispersal of algin upon mixing, but has little effect on film formation.

Salt Selection

Both reactivity and taste had to be considered when selecting polyvalent cations to incorporate into emulsions. Cations that are both algin reactive and GRAS food additive are limited to the calcium and aluminum salts. All of the aluminum salts employed yield adequate films, but at salt levels sufficient for reactivity an objectionable astringency developed. Calcium phosphates impart the least objectionable taste, but do not readily ionize at the pH conditions compatible with emulsion or algin utilization.

Calcium gluconate ionized too slowly to produce a thin, strong film and imparts an undesirable flavor. Calcium lactate yielded a gritty textured emulsion and produced a thick film before attaining strucutral integrity. Initially, calcium chloride dihydrate was found to be the salt demonstrating superior results, when incorporated into emulsions. Finally, however, calcium acetate was observed to display equal or superior results both in cation availability and taste.

When placed in contact with boiling water, the film surrounding an encapsulated emulsion becomes thinner and turns a milky white, but does not soften. At least 1% salt (by weight in an emulsion), in the form of calcium chloride dihydrate or calcium acetate was necessary to provide available calcium adequate for a supportive encapsulation, without the film becoming too thick.

Alginate Concentration

For adequate emulsion globule and film formation, alginate concentration should be greater than 2.0%. A 2.25% solution demonstrated the most desirable encapsulations. Algin concentrations below 2.0% produced elongated structures when the emulsion was injected into algin - containing solution. The best "yolks" were formed when emulsion was injected just at or below the algin surface followed immediately by an addition of algin to layer over the top of the emulsion. Emulsion inserted much below the algin surface (i.e. 1 cm) left a protrusion that readily ruptured; and if the algin was greater than 2.5% in concentration a large flat spot developed on top upon injection, or an irregularly formed "yolk" resulted.

Emulsion Stability and Function After Calcium Salt Addition

Mechanical emulsification with the Dispax, incorporated air in amounts sufficient to cause the emulsions to float in the algin solution before a uniform film could be obtained. Homogenized samples performed better.

Acid and pH Effects on Emulsions Containing Calcium Acetate

When calcium acetate was selected as the preferred salt, emulsions formulated according to conditions and from ingredients listed in Table VI, were centrifuged to ascertain the effects of calcium salt, pH, and homogenization

interactions on stability. Different acids didn't vary significantly in their effects on emulsion stability; and in the presence of calcium acetate, actually lowered the stability.

Centrifugation of emulsions adjusted to pH 6.0 resulted in greater egg white separation (3.5 to 3.7 ml separation) than that of unadjusted emulsion at pH 7.3 (3.3 ml separation). This was a reversal of the pH effects observed on emulsions, before calcium salt was incorporated. Figure 6 demonstrates that increased homogenization pressures and the use of dialyzed whey and safflower oil aided stability.

Stability changes based on various oils in emulsions with calcium acetate, are demonstrated in Figure 7; the influence of pH adjustment and pasteurization are also shown.

Yolk Rupture and Yolk Index Comparisons

Emulsions can be encapsulated with alginate at a level that yields comparable membrane strengths to that of authentic yolks. Both egg yolks and artificial yolks demonstrate a wide range of rupture strengths. The Chattilon values from real yolks ranged from 6.0 to 18.3 for a dozen large eggs; three of which were one week old, the rest fresh. The artificial yolks ranged from 0 to 88.7 depending on time of reaction, method and shape of yolks formed, and pH and concentration of alginate solution. It can be seen

Figure 6. Stability increasing effects of some emulsion ingredients and emulsification variables. (Table VI):

A - 1.2% calcium acetate, pH 6.85 homogenized twice at 800 psi.

B - 1.2% calcium acetate, no pH adjustment, homogenized twice at 800 psi.

C - 1% calcium acetate, homogenized at 500 and 1000 psi.

D - 1% calcium acetate, incorporation of safflower oil and dialyzed whey, homogenized at 500 psi and 1250 psi.

Figure 7. Stability comparison of emulsions using all of the oils originally tried in the drop-spread tests; 1% calcium acetate is incorporated and the emulsions are homogenized twice at 700 psi. Oils are: a) peanut oil, b) safflower oil, c) soy oil, d) olive oil, e) Hollywood blend, f) corn oil and g) soy blend. Conditions or treatments include: after 3 days storage (O); after remixing with a magnetic stirrer (●); pH adjustment to 6.0 (△); followed by pasteurization (□).

Fig. 6

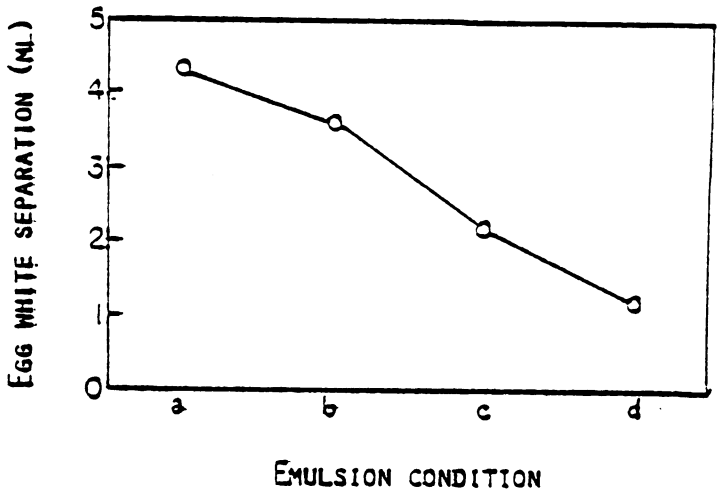


Fig. 7

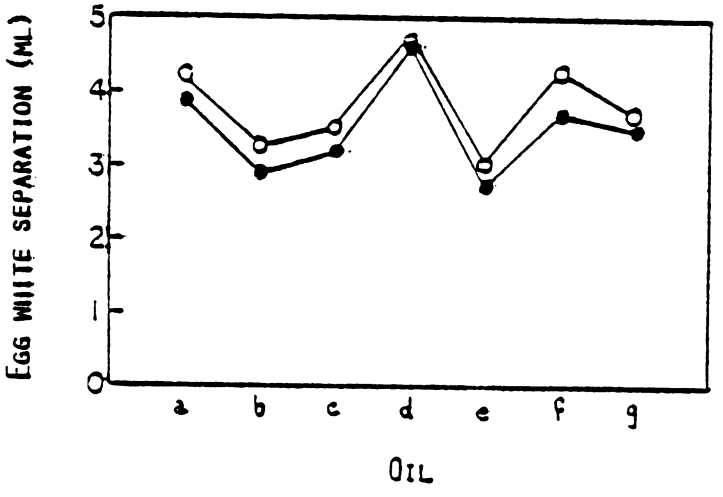
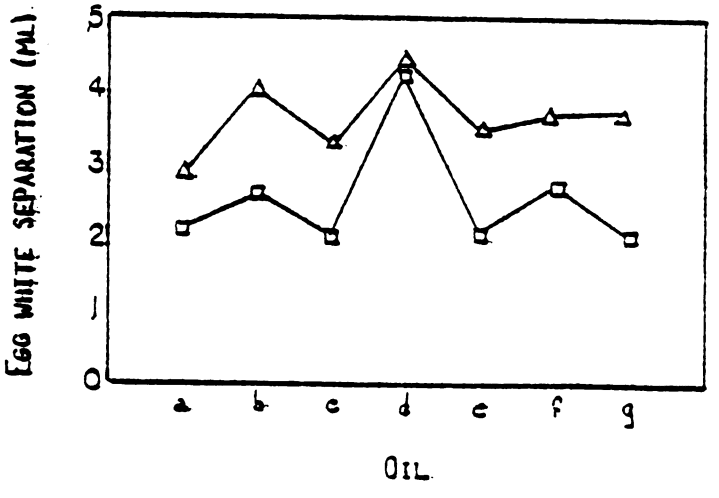


Fig. 7



from Table VII that films with acceptable resistance to rupture were formed in very short periods of time. However, film formation is usually not adequate unless reaction is allowed to continue for at least three minutes. Most of the emulsions listed contain 1% calcium chloride. Emulsions with calcium acetate at a 1% level respond similarly. The pressures required to break similarly formed "yolks" often were widely varied. Measured yolk index values for artificial yolks are also listed in Table VII. A more complete set of pressure and yolk index data are listed in the Appendix.

Artificial Yolk-Egg White Adherence

When egg white was incorporated into the alginate solution used to encapsulate emulsion it was found that the resulting film adhered to egg white much better than for other methods tried. Calcium or aluminum ions incorporated into egg white, migrated into the algin increasing the algin firmness. Carrageenan solution, either as a dip after film formation or incorporated with algin as a film forming ingradient, did not aid in algin-egg white interaction. These observations were made when yolks were placed in egg white and poached or fried.

Taste Panel

Taste panel results as an indication of acceptance of an artificial egg yolk product are listed in Table VIII.

Table VII. Artificial Yolk Pressure and Yolk Index Evaluations[a]

Method of Emulsification	Time of Algin Film Formation (min.)	Yolk Index	Chattilon Reading (total Pressure = Reading x 10^1 g)
Homogenized	15	.491	42.3
2 x 500 psi	10	.477	24.0
pH 7.45	8	.463	11.9
	8	.422	16.3
	7	.414	20.0
	6	.430	16.7
	6	.457	7.0
Homogenized	6	.556	0[b]
2 x 500 psi	6	.498	13.6
pH 6.85	4	.496	6.6
CMC added	4	.488	19.2
Homogenized	4	.527	10.9
2 x 700 psi	4	.471	4.8
pH 6.9	3.5	.488	24.1
CMC added	3.5	.492	17.1
	3	.468	23.1
	2.5	.418	15.4
	2.5	---	0
	2	.461	21.9
	1.5	---	0
	1.5	.456	

a) Emulsions contain 1% $CaCl_2 \cdot 2H_2O$, injection samples are from 12-15 ml and formed in 2.5% alginate.
b) 0 is assigned to emulsions which were already leaking before pressure was applied.

Table VIII. Summary of Taste Panel Evaluation Scores for Soft Poached Eggs with Emulsion "Yolks"

Evaluation Categories and Score Distribution									
	Overall yolk appearance	Yolk flavor	General Yolk Acceptability		Yolk texture			Yolk color	
1. Like very much	4	1	0	1.	Very thick	1	1.	Very dark	2
2. Like moderately	5	4	4	2.	Moderately thick	11	2.	Moderately dark	6
3. Like slightly	5	8	8	3.	Slightly thick	8	3.	Slightly dark	9
4. Neither like nor dislike	6	1	7	4.	Typical	3	4.	Typical	9
5. Dislike slightly	7	10	7	5.	Slightly thin	7	5.	Slightly light	6
6. Dislike moderately	4	8	5	6.	Moderately thin	0	6.	Moderately light	1
7. Dislike very much	0	1	1	7.	Very thin	2	7.	Very thin	0
Total Responses	31	33	32			32			33
Average	3.6	4.3	4.1			3.4			3.4

Since no comparison was made with real yolks, a statistical interpretation was not applied. Responses were widely varied, and although most panelists did not indicate a neither like not dislike score, it was the overall average.

DISCUSSION

The development of an artificial egg yolk was undertaken to provide an alternative to low cholesterol scrambled-style egg replacements. To develop a substitute with the appearance of an intact egg yolk required both the development of a stable yolk-like emulsion and surrounding yolk-size portions of emulsion with an edible membrane or film.

Such a product might be fabricated either by inserting emulsion into an edible sack or by evolving a film around the emulsion. It was decided that forming the film around the emulsion would perhaps be more feasible.

Exploratory experiments indicated that a reasonably stable, liquid yolk-like emulsion could be formulated. Other observations indicated that, if an appropriate cross-linking or film forming agent could be found, emulsion globules could be macro-encapsulated, resulting in a non rigid spherical product.

Selection of Emulsion Ingredients

Oil

Peanut oil, containing 29.3% polyunsaturated glycerides, was selected as the most desirable oil for incorporation into artificial yolk emulsions. But because a major

purpose for developing an artificial egg yolk was to appeal to individuals wishing to decrease cholesterol and saturated fatty acid consumption, other oils were studied. Safflower, corn, soy, and cottonseed are more desirable, since they contain 72.1, 52.9, 52.1 and 50.0 percent polyunsaturates, respectively (Church and Church, 1975). In comparison the lipid fraction of natural yolks contain only 9.3% polyunsaturated glycerides (Cotterill, 1973). In general, other oils including safflower, resulted in equal or greater emulsion stability than observed with peanut oil.

Other Ingredients

Whey solids and lecithin were selected as emulsion ingredients for the following reasons: Both have been utilized extensively in egg emulsion systems, are readily available and imparted appropriate functionality to emulsions.

Major emphasis was directed to the encapsulation technology, utilizing only ingredients which were readily accessible and acceptable.

Lecithin

Lecithin is native to egg yolk and has been promoted because of its potential to bind dietary cholesterol. As a commercial compound, lecithin is a mixture of phospholipids including phosphotidyl choline, phosphatidyl ethanolamine, and phosphatidyl inositol (Central Soya, Chemurgy Div.).

This mixture is actually more comparable to egg yolk phospholipids than is phosphatidyl choline alone. A one percent (w/w) concentration of lecithin in the emulsion was most satisfactory based upon the following observations:

a) Centrifugation results indicated that higher concentrations of lecithin did not significantly increase emulsion stability;

b) Textural properties of the emulsion were optimal at this level; and

c) Slight variations above and below the one percent level greatly increased or decreased emulsion viscosity.

Whey Solids

It was apparent early in the project that a thickener was needed to achieve emulsion stability. Whey solids, particularly ENRPRO-50 (Stauffer) protein concentrate was selected as the most satisfactory product for the following reasons:

a) Viscosity was enhanced

b) Desirable modifications in the texture of coagulated emulsions were produced; and

c) Emulsion stability was enhanced.

Citric Acid

Citric acid is frequently employed to make pH adjustments in food systems and was used for this purpose in this study. It is highly soluble, imparts less harsh flavor than most acids, loosely sequesters and lessens the

astringency of salts, and may aid in emulsification (Gardner, 1975).

Liquid Egg White

Liquid egg white was utilized throughout the project to provide the aqueous phase in the formulated emulsions. It is employed in nearly all simulated egg products, contributing the essential characteristics of heat coagulation to the ingredient system.

Emulsion Stability

Centrifugation

Centrifugation appears to be an adequate method of studying emulsion stability to determine the effect of both ingredients and method of homogenization on final stability. It is quick, simple, and quantitative, and correlates with observations made on emulsion stability as a result of short term storage at 4 C.

There are some latent disadvantages to this method for determining stability. Phase separation is often difficult to observe where color differences between the separated egg white and emulsion are not distinct. An oil rich layer at the top, and an oil poor layer at the bottom may develop without being visible (Puski, 1976). In addition destabilization mechanisms may be altered during the centrifugation process and produce atypical results (Petrowski, 1976). However, these problems did not appear to limit the method

for detecting the characteristics of stability.

Centrifugation also indicates the amount of air incorporated into the emulsion by comparing initial and final volumes. And ingredients which denature proteins may be identified if a precipitant is present.

Microscopic Analysis

Groves and Freshwater (1968) state that emulsion stability and viscosity are dependent upon particle size or particle size distribution of the dispersed phase. Measuring changes in particle distribution reveal slight changes in emulsion stability, even before phase separation is visualized. Therefore, comparing droplet distribution and their change with time should give the most accurate indication of emulsion stability.

However, in this study microscopic analysis was not found to be an adequate indicator of emulsion stability. This may be attributed to:

a) Different ingredients produced equal stabilities with different droplet sizes due to altered viscosity and emulsification effects;

b) Occasionally, the small size of emulsion samples may not have been representative;

c) Results were reported in terms of relative observations rather than as a direct measurement; and

d) Specific numbers of particles or a specific emulsion area was not considered.

Microscopic analysis did make it possible to visualize the destabilizing processes. Particle floculation was frequently observed, particularly in less stable emulsions. Also visual comparisons of emulsions, differing only in the method of mixing, are valid indicators of emulsion stability. An example of this is the effect of homogenization pressures on emulsion stability (Fig. 8). The observed decrease in droplet size with homogenization at 1000 psi resulted in increased stability; an observation substantiated by centrifugation. The stability of native yolk is also illustrated. Under centrifugation conditions identical to those employed on the formulated emulsions no phase separation was apparent; only a slight amount of precipitant was detected.

Correlation Between Stability and Textural Studies

Centrifugation and Kramer texture analysis were the primary evaluations in selecting the proper levels of various ingredients. Emulsion viscosity, and the organoleptic characteristics of coagulated samples were also considered.

After the selection of whey and lecithin as previously described, the effect of pH adjustments was considered. Initially the pH range was limited to between 6.0 and 9.0. Above and below this range, viscosity, texture, and taste were not acceptable.

At this point 8.5% (w/w) whey solids was selected as most ideal from the standpoint of taste and texture.

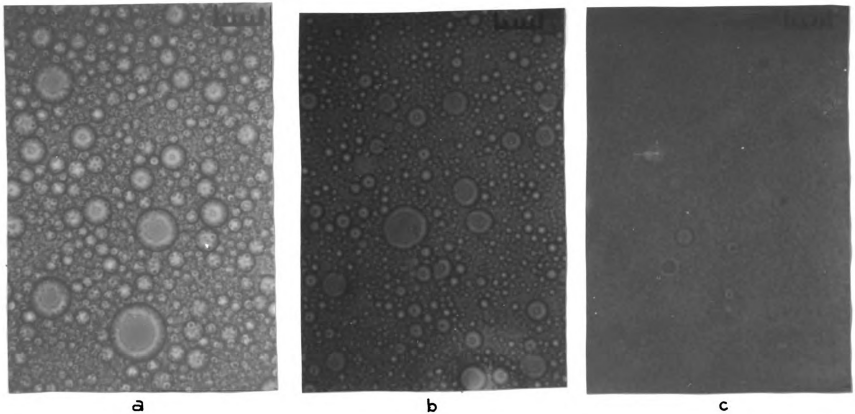


Figure 8. Homogenization influence on particle size and emulsion stability in comparison with authentic yolk. a) homogenized twice at 500 psi; b) homogenized once at 500 psi and once at 1000 psi; c) yolk. The legend in the upper right hand corner is 25 μ in units of 5.

Stability was not optimum, yet was acceptable as a compromise between stability, flavor and texture. It most closely approximates the characteristic crumbliness of yolks.

Increases in oil concentration also increased stability, but at cencentrations of over 40%, with constant whey solids content, emulsions became overly viscous. The texture of coagulated emulsion samples, containing 30-40% oil, were similar indicating that above 30% oil, whey solids became the major factor contributing to compressibility. In terms of flavor, less than 25% oil in emulsion produced a soft or mushy mouth feel; over 35%, an oily taste was apparent. A final oil content of 32% was selected in combination with 1% lecithin to simulate the lipid content of natural yolks.

A final set of pH adjustments indicated that the most desirable stability and compressibility traits were obtained in the range of 6.05 and 6.85. As the texture of coagulated emulsions approached yolk-like texture, the distinctive flavors imparted by whey and/or egg white were minimized. When one percent $CaCl_2 \cdot H_2O$ was incorporated into emulsions, compressibility was substantially reduced. The addition of phosphates or polysaccharides as potential stabilizers did not improve either texture or stability.

Relative Viscosities

Emulsion viscosities were affected by minor changes in composition, pH, and method of emulsification. Yolks were

affected by age and the amount of adhering white. Viscosity measurements were not a major consideration in selecting or modifying emulsion ingredients. They did, however, demonstrate that slight adjustments particularly in the level of lecithin added, would alter the fluidity of emulsions. Desired viscosities (i.e. near that of fresh egg yolk) could be obtained.

Texture Comparisons Between Hard-Boiled Yolks and Heat-Coagulated Emulsions

Although the texture of egg yolk depends largely on the method of cooking, hard-boiled yolks were compared with heat-coagulated emulsions. The rationale for this comparison was as follows:

1) This represents the most reproducible method of comparing yolks and emulsions on a size and shape basis.

2) The texture of hard-boiled yolks was more consistant from egg to egg than was observed for other cooking methods.

3) The possibility of developing a yolk substitute for tube-style hard-boiled eggs became apparent.

4) Adjustments in the composition of the emulsion formulation to approximate the texture of yolks could be predicted.

Shear press data indicated the major comparison between yolks and coagulated emulsion was the compressibility parameter. The shapes of graph peaks, however, indicated

differences between textures of samples possessing identical compressibilities.

Coagulated emulsions with a high egg white content had a tendency to yield split peaks; a characteristic encountered only occassionally in yolks or other emulsion formulations. For both formed emulsions and real yolks, relatively narrow peaks were obtained. Ten-week old yolks, and emulsions with calcium chloride and polysaccharides or polyphosphates added demonstrated a flatter peak profile. The shoulder at the trailing side of the peak resulted from shear blade drag against the shear cell (Szcazesniak, Humbaugh and Block, 1972). This was an indication of sample adherence or stickiness. That authentic egg yolk and coagulated emulsions may be adjusted to very similar peak profiles and consequent texture may be seen in Fig. 9.

Size Comparison of Yolk and Formed Emulsion

Comparison between yolks and formed emulsions was based on texture relative to shape and surface area. The inside surface area of the sphere used for most coagulations was approximately 26.0 cm^2. Assuming that authentic yolks are spherical and the fresh yolk density is 1.035 (Berquist, 1973), the surface area for a fresh hard boiled yolk of 12.55 g - average yolk weight for fresh small eggs - would be 25.5 cm^2. This value is within 2% of the area for the formed emulsions. Therefore differences in compressibility between yolks and formed emulsion primarily indicate

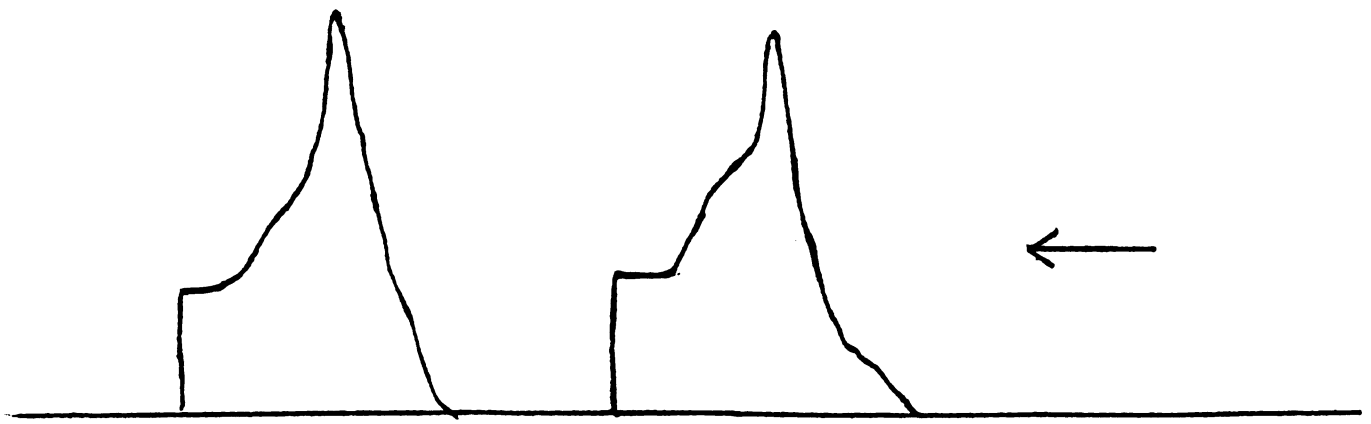


Figure 9. Allo-Kramer Shear Press peak profile comparing a hard boiled yolk and coagulated emulsion. On the left is the peak profile of coagulated emulsion with 1% lecithin, 8.5% whey solids, 32% oil, 58.5% liquid egg white, and pH adjusted to 6.1; Compressibility is 8.3. The profile at the right is from an egg yolk with a compressibility (7.9) about average for fresh yolks.

textural differences. The emulsion yolks contain some incorporated air which seems to affect texture readings. It is of interest to note that real yolks once broken and coagulated in the spherical form yielded a much firmer, rubbery coagulation than those excised from hard-boiled eggs.

Emulsion Encapsulation with Alginate and Comparison with Authentic Yolks

Alginate Films

After it became apparent that none of the protein crosslinkers were satisfactory for film or membrane formation, polysaccharides were investigated for encapsulation of emulsions. Due to their unpredicatable composition, pectins demonstrated limited potential for desirable film formation. Of the algins used for this study, the Meer type "CA" sodium alginate displayed the most satisfactory results.

Calcium-Alginate Reaction

At a given algin concentration, film thickness and strength are based primarily on time and the calcium ions availability in the emulsion. Calcium ions migrate from the emulsion into the algin as a function of sample composition, temperature, and time (Luh et al., 1976). The film thickens as ions continue to migrate. Therefore, sufficient calcium ions must be present at the emulsion-algin

interface to establish a thin, strong film. Calcium acetate at 1% (w/w) incorporation in emulsion was selected as the salt most capable of providing an adequate film formation, yet minimizing an objectionable taste.

Encapsulated Emulsion

Films

The film necessary to encapsulate emulsions was much thicker than the yolk vitelline membrane. It is, however, transparent enough to allow for a good yolk-like color and sheen with properly colored emulsions. It became translucent and blended well with egg white upon heating (Fig. 10).

Yolk Membrane Strength

The Chattilon pressure tests on yolks and encapsulated emulsions demonstrated that algin films can be formed with rupture strengths comparable to those of fresh yolks. Under proper conditions films can be formed quickly which are sufficiently thin and of adequate strength.

Yolk Index

The yolk index, i.e. yolk height divided by average width, was employed as an indicator of freshness in eggs; the higher the value the fresher and more desirable the yolk. A summary of yolk index values was prepared by Funk (1948). Fresh yolks were found to have average index values of 0.48 when measured in their natural position in the egg, and 0.43 when separated from the albumen. When

Figure 10. Comparison of raw yolk vitelline membrane and algin films. Both yolk and encapsulated emulsion were broken and the membranes rinsed with water. At the top is the vitelline membrane. The white algin film has been heated in boiling water. To compare these to appearance with yolk inside see Fig. 3.

properly formed, simulated yolks compared with these values, particularly with values of yolks remaining in their natural environment. This demonstrates that in addition to good color and membrane strength, adequate physical shape of emulsion yolks is obtainable.

Cooked Simulated Yolks

Once yolks with adequate algin films were obtained, their response as simulated eggs was examined. Simulated yolks were added to egg whites previously separated from fresh eggs and poached or soft fried and compared to natural eggs. Examples of these processed eggs are shown in Figures 11 and 12.

Two major problems in physical appearance were evident:

1) the simulated yolks did not adhere well to the egg white, and

2) the liquid centers had a slightly curdled appearance.

At this point whey solids were substituted with whey isolate resulting in yolks possessing a smoother appearing liquid center when soft-cooked. Dried-egg white incorporated into the alginate was found to effectively increase the attachment between egg white and algin. Finally, peanut oil was replaced with safflower oil to increase the polyunsaturated lipid fraction.

Figure 11. Poached eggs. The artificial egg is in the center of both pictures. The real egg at the left was separated and then returned before poaching. It shows comparable lack of adhesion between white and yolk to that of the artificial yolk. The bottom picture with yolks displays the difference in color hue and the curdled appearance of the liquid center. These yolks were manufactured before textural improvements were made.

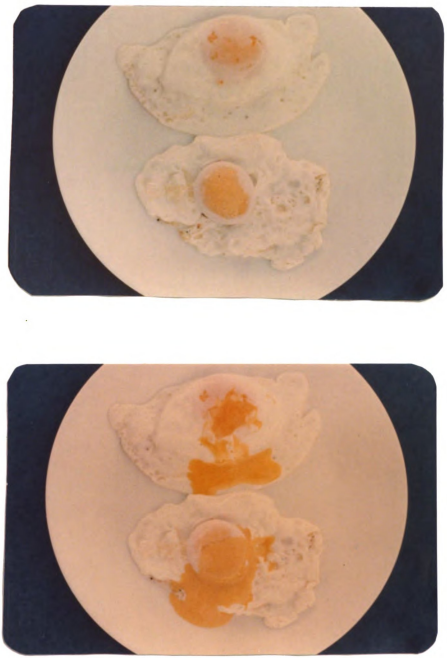


Figure 12. Fried eggs. These pictures better demonstrate the tendency for the artificial yolk (at the bottom of each picture) to separate from the egg white. The inconsistency of emulsion yolk is also apparent for the artificial egg yolk.

Stability of Emulsions with Safflower Oil and Whey Isolate

Yolks manufactured with calcium acetate, whey protein isolate and safflower oil showed stability equal or superior to that of yolks manufactured with undialyzed ENRPRO-50 and peanut oil. A major difference in stability was encountered; decreasing the pH of the new emulsion decreased stability rather than the previously observed increase.

Kramer texture evaluations were not performed on emulsions with the new ingredient formulation. It had previously been observed that calcium chloride addition eliminated otherwide similar textures between yolks and coagulated emulsion. The new emulsion partially achieved the requirements for a better appearing liquid emulsion when manufactured as liquid-centered, cooked eggs.

Taste Panel Responses

Perhaps the best test of success or failure for the algin encapsulated yolks was the response of the taste panel. Because panel members were asked to compare an artificial yolk product with an ideal for authentic poached eggs, high scores were not anticipated. The scores were widely distributed with essentially as many panelists expressing varying degrees of "like" as those expressing degrees of "dislike". In most cases a neither like or dislike answer was avoided by panelists. Overall scores were good enough to indicate that with minor improvements

in yolk flavor and consistancy as well as algin film texture and thickness, yolks could be much more acceptable. This would be true particularly for individuals seeking to restrict consumption of natural eggs, yet desiring eggs as part of their diet.

The comments on the evaluation forms were as useful as the scores. Initial appearance before breaking the yolk was quite well received. However, after breaking the membrane several persons indicated that a better color and sheen was needed. The liquid yolk looked "mustardy". The color imparted by β-carotene can easily be adjusted or modified with slight changes in concentration, and/or the addition of xanthophylls or other colorings. The glossy yolk appearance perhaps could be improved by better homogenization or the utilization of a protein source, i.e. such as vegetable proteins, with coagulation characteristics similar to egg yolk.

Some individuals noticed the calcium acetate as a slightly "bitter" or "chemical" flavor. Otherwise "blandness" of flavor was the primary expression. Calcium acetate might be masked and the blandness overcome by addition of an appropriate egg flavoring other than the one utilized.

With the realization that it is impractical and difficult to reproduce the flavor and texture of a natural yolk, the responses of individuals disliking the product were balanced by those expressing good acceptability as a substitute product.

Texture responses were also widely varied. The presence of the algin film imparted a "chewiness" or slight "toughness" unnatural to vitelline membranes. Simulated yolks were also slightly difficult to puncture and did not always display a yolk-like fluidity from the liquid center.

Thinner films might be achieved by using one or a combination of the following methods:

1) selection of another alginate more specific to the desired response,

2) slight acidification of the alginate to enhance calcium reactivity,

3) rinsing the yolk in water upon removal from the algin bath to reduce adhering and unreacted alginate. Allowing the yolk to drip momentarily and then placing it directly into egg white was not a completely satisfactory procedure.

Some incorporation of air was noted producing a sponginess in the coagulated portion of the emulsion. Most of the defects might be alleviated by careful mixing of ingredients and homogenization. Perhaps the use of a commercial defoaming agent would be helpful.

Although the overall consensus was that liquid centers are too thick or pudding-like, some panelists felt they were not viscous enough. No doubt this disparity was based on differences in individual perferences for poached eggs. Very minor adjustments in egg white or lecithin content can be employed to adjust viscosity.

Stability of Stored Samples

Emulsion stability did not appear to be a major problem for the artificial yolk systems. Yolks identical to those used in the organoleptic evaluations were examined after three weeks of storage at 4 C. All had maintained good structural integrity and showed little if any emulsion separation. They did, however, tend to float in the egg white.

More conclusive evidence of stability is revealed by the emulsion containing whey protein isolate, calcium acetate and Wesson oil (i.e. a soy blend). This preparation was stored seven months at 4 C following pH adjustment to 6.0 and pasteurization. No visual signs of separation occurred during this time and a yolk possessing a satisfactory appearance was formed. Instability problems which develop with emulsions might be eliminated by use of additional emulsifiers such as mono-diglycerides.

CONCLUSION

The manufacture of an artificial egg yolk for application in "poached" or "fried egg" usage appears to have commercial potential. As a feasibility study in macro-encapsulation of a lipid-water emulsion, the research objectives were met.

Minor manipulations in ingredient formulation, homogenization or pH adjustment could be utilized to optimize emulsion characteristics.

In addition to the goal of a non-rigid, self-contained liquid yolk, it was found that "hard-boiled" yolk texture could be duplicated.

APPENDIX A

Table A1. Results of oil spread studies on solutions with potential use in artificial yolk emulsions

Solutions	Oils						
	Corn	Olive	Soy Blend	Soy	Peanut	Hollywood Blend	Safflower
	Diameter (cm) of oil drop spread						
Distilled water	7.0	7.5	6.5	3.1	8.0	9.0	3.5
1% lecithin solution	1.4	1.6	1.5	1.5	1.4	1.4	1.6
Liquid egg white	2.8	5.0	2.2	4.7	2.5	2.8	3.1
NFDM (17% solids)	3.9	7.0	4.5	5.0	5.0	6.1	7.2
Whey solutions 1) 10.7%	3.6	8.0	2.8	2.7	2.4	2.6	3.9
2) 12.0% with 2% lecithin	1.8	1.8	1.6	1.9	1.8	1.7	1.6

Table A2. Results of oil drop spread studies on solutions, containing egg white with additional solids and varying lecithin percentages

Emulsifier	Solutions	Oils						
		Corn	Olive	Soy Blend	Soy	Peanut	Holly-wood Blend	Saf-flower
		Diameter (cm) of oil drop spread						
No Emulsifier	Liquid and dry egg white	2.7	4.5	2.5	2.6	2.6	2.5	2.5
	Liquid egg white and NFDM	3.5	6.5	3.3	3.9	3.8	4.0	6.5
	Liquid egg white and whey solids	4.6	9.0	2.9	2.8	2.6	3.1	3.6
	Dried egg white in water	2.1	2.0	2.2	2.2	2.2	2.0	2.8
0.5% Lecithin	Liquid and dry egg white	2.0	1.8	2.2	1.9	1.8	2.0	2.4
	Liquid egg white and NFDM	4.7	5.5	4.3	3.0	3.2	3.8	2.9
	Liquid egg white and whey solids	5.1	9.0	2.9	2.8	2.7	1.9	3.4
	Dried egg white in water	2.4	3.5	2.5	2.0	2.3	2.3	2.2
1% Lecithin	Liquid and dry egg white	2.5	5.0	2.4	3.6	2.8	2.0	2.2
	Liquid egg white and NFDM	2.0	2.7	2.1	2.0	1.8	2.0	1.7
	Liquid egg white and whey solids	1.4	2.4	1.7	2.0	1.8	1.7	3.5
	Dried egg white in water	2.5	3.2	2.6	2.2	2.0	2.8	2.0

Table A2 (cont'd.).

Emulsifier	Solutions	Oils						
		Corn	Olive	Soy Blend	Soy	Peanut	Holly-wood Blend	Saf-flower
		Diameter (cm) of oil drop spread						
0.5% Lecithin & 0.5% Proteose peptone	Liquid and dry egg white	3.0	2.6	2.7	2.3	2.1	2.4	2.4
	Liquid egg white and NFDM	1.9	2.0	1.8	1.9	1.8	1.7	1.8
	Liquid egg white and whey solids	4.2	2.0	2.7	2.3	2.4	2.8	3.1
	Dried egg white in water	3.4	4.5	3.0	3.5	2.8	3.5	2.8
3% Lecithin	Liquid and dry egg white	1.7	1.7	1.7	1.7	1.7	1.7	1.7
	Liquid egg white and NFDM	1.8	1.8	1.9	1.8	1.8	1.8	1.8
	Liquid egg white and whey solids	1.8	2.0	1.8	1.8	1.8	1.7	1.9
	Dried egg white in water	1.9	1.9	1.8	1.7	1.9	1.8	1.7

Table A3. Potential crosslinker or film forming compounds selected for reaction with emulsion at initial and various levels of adjusted pH

Compound	Manufacturer or Supplier	Initial pH, pH range and percent level of use	References
Polymers			
Carbopol (Carboxypolymethylene polymer)	B.F. Goodrich Chemical Co.	3.3-1%, 3.8-0.1%	Sternberg (1975)
934		3 to 12, 0.1%	
940			
941			
EMA (Ethylene Maleic Anhydride)	Monsanto Co.	3.0-1%, 3.6-0.5%	Sternberg (1975)
		4.4-0.2%	
		3 to 12, 0.2%	
91		3.1-1%, 3.3-0.5%	Miller (1972a)
81		4 to 12.0-0.2%	
61		3.0 to 12.0-1%	
Gantrez AN (polymethyl vinyl ether maleic anhydride)	GAF Corp.	2.8-1%, 3.15, 4 to 12,0.5%	Sternberg (1975)
		2.7-1%,3.15, 4 to 12-0.5%	
139			
169			
Goodrite K (polyacrylic acid)	B.F. Goodrich	3.6,3 to 12-1%	Sternberg (1975)
		3.4,2 to 12-1%	
702			
732			
Starch Modifiers			
Acetic Anhydride	Fisher Scientific Co.	2.5,3 to 12-2%	Whistler (1964)
Acetic Anhydride with adipic anhydride (polymer)	Pfaltz & Bauer, Inc	2.7,3 to 12-25% Acetic anhydride, 0.12% adipic anhydride	
Acrolein	Pfaltz & Bauer, Inc.	7.4,3 to 12-2%	
Epichlorohydrin	Eastman Organic Chemicals	3.6,3 to 12,3%	Radley (1965)

Table A3 (cont'd.).

Compound	Manufacturer or Supplier	Initial pH, pH range and percent level of use	References
Propylene Oxide	Aldrich Chemical Company	7.55,3 to 12-5%	
Sodium trimetaphosphate	Stauffer Chemical Co.	6.55, 3 to 12-2%	
Sodium tripolyphosphate	FMC Corp.	9.1,3 to 12-2%	
Succinic anhydride & epichlorohydrin	Pfaltz & Bauer, Inc.	0.3% epichlorohydrin & 4.0% succinic anhydride	
Succinic anhydride	Pfaltz & Bauer	2.65,3 to 12-2%	Miller (1973b)
Vinyl acetate	Eastman Organic Chemicals	3.3,2 to 12-2%	
Other Protein Reactive Compounds			
Ammonium Persulfite and sodium sulfite	J.T. Baker Chemical Co.	3.4,3 to 12-1.84% $(NH_4)_2S_2O_8$ 0.2% Na_2SO_3	Needles & Whitfield (1969)
Aluminum ammonium sulfate	Mallinckrodt, Inc.	3 to 12-2%	
Aluminum potassium sulfate	Mallinckrodt, Inc.	3 to 12-2%	
Ethanol		3,5,10,20,40,60, 80, and 100%	
Glutaraldehyde	Pfaltz & Bauer, Inc.	7.2-1.0 to 25% 3 to 12-1%	
Maleic anhydride	Fisher Scientific Co.	1.6,3 to 12-2%	Miller (1973b)
Sodium hexametaphosphate	FMC Corp.	9.1,3 to 12-2%	
Succinic acid	Mallinckrodt, Inc.	2.7,3 to 12-2%	
Tannic acid	J.T. Baker Co.	5.7,3 to 12-1% other percentages to 10% at unadjusted pH.	

Table A4. Summary of crosslinker responses (reactions include No Response (NR), Coagulation (C), Precipitation (P), and Film Formation (FF).

Compounds	Current or proposed food application	pH or pH range of greatest response	Observation reaction or response				Comments
			NR	C	P	FF	
Polymers							
Carbopol 934	Indirect Precipitation of whey, then removed	3-4		X			Decreased response with increase in pH. Only response at pH 3.0 probably from acid
Carbopol 940		3.0	X				
Carbopol 941		3.0	X				
EMA 91	Indirect (like Carbopol)	3.5		X	X		Decreases with increase in pH
EMA 81		3.0		X			No response above 3.0
EMA 61		3.0		X			No response above 3.0
Gantrez AN 169	Indirect, (same as Carbopol)	3-4			X	X	Decreases with increase in pH, but some filmlike transparent formation
Gantrez AN 139		3-4			X	X	
Goodrite K702	Indirect	3-4		X		X	Slight filmlike response
Goodrite K732	Indirect	3-4		X		X	Decreases with pH, increase filmlike response is slight
Starch Modifiers							
Acetic Anhydride		2.5-8		X			Gellike coagulation (like adipic anhydride alone)
Acetic Anhydride and adipic anhydride	Starch Modifier	2.7-8.0		X			

Table A4 (cont'd.).

Compounds	Current or proposed food application	pH or pH range of greatest response	Observation reaction or response				Comments
			NR	C	P	FF	
Acrolein	Starch Esterifier	3-9		X			Gel like coagulation
Epichlorohydrin	Starch Modification	2.5-5.0		X			
Propylene Oxide		3-4		X			Minimal reaction
Sodium Trimetaphosphate	Starch Modifier	3-5		X			Minimal
Sodium Tripolyphosphate	GRAS	3-5		X			Minimal
Epichlorohydrin with succinic anhydride	Starch Modifier	2.5-5.0		X			
Succinic Anhydride	Starch Modifier up to 4.0%	1.6-6		X			
Vinyl Acetate	Starch Modifier		X				
Ammonium Persulfite and Sodium Sulfite		3-8		X			Minimal
Aluminum Ammonium Sulfate	GRAS	3-4		X		X	Weak membrane formation, most filmlike at pH 4
Aluminum Potassium Sulfate	GRAS	3-4		X		X	Weak membrane formation, most filmlike at pH 4
Ethanol	Flavor Extractor	40-100%		X		X	Very brittle-waxy film
Glutaraldehyde	Enzyme Immobilization	3-12		X			Leathery dark membrane. Strength of membrane decreases as pH increased.

Table A4 (cont'd.).

Compounds	Current or proposed food application	pH or pH range of greatest response	Observation reaction or response				Comments
			NR	C	P	FF	
Maleic Anhydride	None	2.65-6		X			
Sodium Hexametaphosphate	GRAS	3-5		X			Minimal reaction
Succinic Acid		2.7-7		X		X	Most filmlike pH 3 to 5
Tannic Acid	GRAS (precipitation)	3-6		X		X	Film formation, but fragile

Table A5. Relative viscosity comparisons of emulsions and egg yolks based on emptying times from a 20 ml pipette

Yolk or Emulsion Variable	Emptying Time (Min.)				
	Trial 1	2	3	4	Ave.
pH					
7.9	0.61	0.60			0.60
7.65	0.65	0.64			0.64
7.25	0.95	0.88	0.94		0.93
6.95	1.24	1.0	1.06		1.10
6.5	3.91	3.8			3.90
6.1	7.40	12.90			10.2
5.7	10.29				10.3
6.85	2.34	2.12	1.83		2.10
6.75	2.50	1.93	2.09		2.15
6.7	1.81	1.86			1.84
6.6	2.07	2.18			2.12
6.4	4.11	4.10			4.10
6.35	3.90	3.98			3.94
6.25	5.33	5.26			5.30
6.15	4.69	4.38			4.54
6.05	4.40	3.57	3.19		3.72
Stabilizer					
1% Lecithin	1.57	1.70			1.63
1.5% Lecithin	17.03	16.06	17.50		16.86
Sodium Hexameta-phosphate	3.25	3.13			3.19
Sodium Trimeta-phosphate	2.54	2.49			2.51
Sodium Tripoly-phosphate	6.34	7.25	4.78		6.12
CMC-7LF	19.77	19.92			19.85
CMC-7MF	-	-			30+
Slo Set Pectin	-	-			30+
$CaCl_2$ and Lecithin Variation					
0% $CaCl_2$ 1.3% Lecithin - pH 6.85	1.63	1.53	1.53		1.56
1% $CaCl_2$ 1.30 Lecithin - pH 6.85	7.32	10.22	6.95	8.94	8.36
1% $CaCl_2$ 1% Lecithin - pH 6.9	0.84	1.00			0.92

Table A5 (cont'd.).

Yolk or Emulsion Variable	Emptying Time (Min.)				
	Trial				
	1	2	3	4	Ave.
Yolks					
With some egg white	1.87	1.96	1.88		1.90
Fresh after several hours at room temp.	6.97	9.20	6.36	6.65	7.30
Fresh	8.92	8.95	8.93	9.12	8.98
4 wks. old	6.40	6.44	6.43	6.45	6.43
8 wks. old	5.16	4.96	4.54	4.98	4.91

Table A6. Chattilon pressure and yolk index values for artificial egg yolks with varied emulsification and formation times[a]

Special Algin or Emulsification Conditions	Time of Algin Film Formation (Min.)	Yolk Index	Chattilon Reading (Total Pressure = Reading x 10^1g)
Dispax	20	.335	2.6
pH 6.1	20	.339	6.0
0.5	20	.309	0
$CaCl_2$	20	.460	5.8
2.0% algin	25	.380	21.9
	25	.353	2.5
	25	-	0
Homogenized			
1400 psi	15	.398	0
	20	.474	10.9
	25	.382	36.1
	24	.507	46.0
	23	.509	22.6
Homogenized			
2 x 500 psi	15	0.491	42.3
pH 7.45	10	0.477	24.0
	8	0.463	11.9
	8	0.422	16.3
	7	0.414	20.0
	6	0.430	16.7
	6	0.457	7.0
Homogenized pH 6.85,			
CMC added	6	.556	0
	6	.498	13.6
	4	.496	6.6
	4	.488	19.2
Homogenized pH 6.9,			
CMC added	4	.527	10.9
	4	.471	4.8
	3.5	.488	24.1
	3.5	.492	17.1
	3	.467	23.1
	2.5	.418	15.4
	2.5	-	0
	2	.461	21.9
	1.5	-	0
	1.5	.456	0

Table A6 (cont'd.).

Special Algin or Emulsification Conditions	Time of Algin Film Formation (Min.)	Yolk Index	Chattilon Reading (Total Pressure = Reading x 10^{1}g)
Homogenized, pH 6.9, CMC added			
Algin at pH 4.6	3	.426	31.1
	3	.438	13.4
	3	.496	19.6
Algin at pH 4.8	3	.463	16.0
	3	.472	88.7
	5	.469	45
Algin at pH 10.4	3	.483	28.2
	3	.407	7.8
	3	.476	20.6
Homogenization pH 6.8, 2 x 500 psi 1% Lecithin	10	.425	19.4
	5	-	0
	3	.366	7.0
	3	.391	7.0
Lecithin 1.5%	5	.406	19.1
	4	-	0
	4	.423	17.0
	3	.480	4.0
	3	.462	17.8
Sodium Hexametaphosphate	15	-	0
	10	.379	2.5
	5	-	0
Sodium Trimetaphosphate	15	-	0
	15	-	0
	10	-	0
Sodium Tripolyphosphate	15	.345	3.3
	15	-	0
CMC-7LF	5	.447	4.0
	5	.481	1.4
	4	-	0
	4	.427	6.1
	6	.469	20.0

Table A6 (cont'd.).

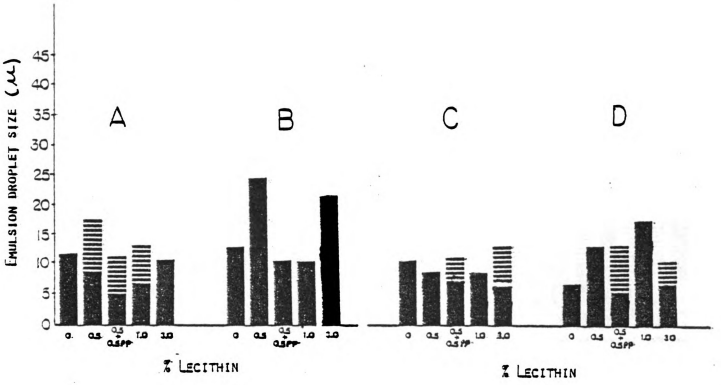
Special Algin or Emulsification Conditions	Time of Algin Film Formation (Min.)	Yolk Index	Chattilon Reading (Total Pressure = Reading x 10^1g)
CMC-7MF	6	-	0
	6	.451	23.6
	5	.500	5.0
	4	.470	5.0
	3	-	0
Pectin	8	.507	7.0
	7	-	0
	6	.472	0
	5	-	0
	5	-	0
1:2% Ca Acetate Glycerin in Alginate	20	-	15.9
	15	-	17.8
	10	-	7.5
	5	-	2.0

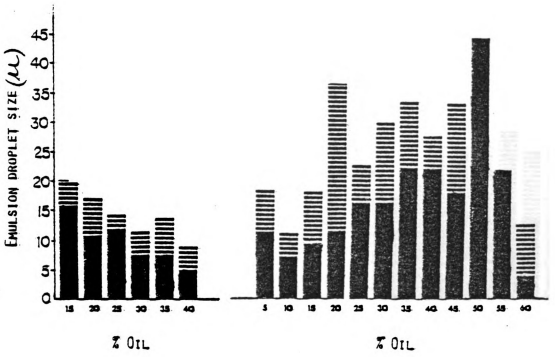
a) Unless otherwise indicated, encapsulated emulsion volumes are 10-15 ml (most 12 ml) contain 1% $CaCl_2$ and are manufactured in 2.5% algin.

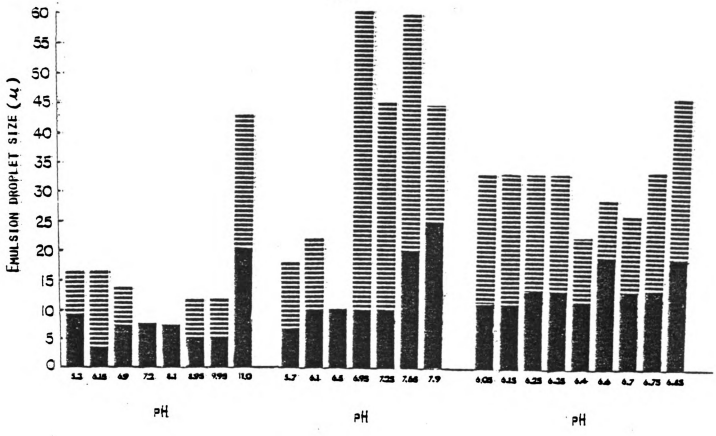
Figure A1. Microscopic Evaluation of Emulsions

The dark area represents the predominant range of droplet size. The shaded part, where added, extends to the maximum droplet size. Each graph is headed by the ingredient or condition modification by which emulsion was evaluated.
For % lecithin emulsions:
A-Liquid egg white with whey solids
B-Reconstituted dry egg white
C-Liquid egg white with added dry egg white
D-Liquid egg white with NFDM.

For stabilizers 0.5% of each compound added:
a-control (1% lecithin total)
b-additional lecithin
c-sodium trimetaphosphate
d-sodium tripolyphosphate
e-carboxymethylcellulose (CMC) 7LF
f-CMC - 7MF
g-slow set pectin


EMULSION DROPLET SIZE (μ)
A
B
C
D
% LECITHIN
% LECITHIN


EMULSION DROPLET SIZE (μ)
% OIL
% OIL


EMULSION DROPLET SIZE (μ)
60
55
50
45
40
35
30
25
20
15
10
5
0
5.3 6.18 6.9 7.2 6.1 6.95 9.98 11.0
5.7 6.1 6.6 6.95 7.25 7.66 7.9
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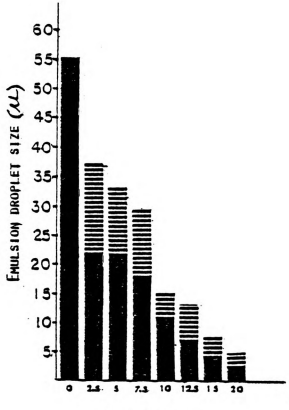
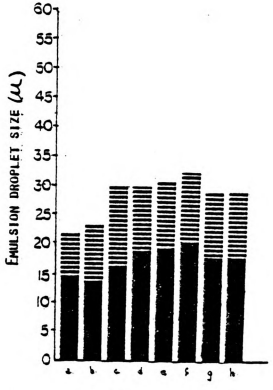
pH
pH
pH


EMULSION DROPLET SIZE (μ)
60
55
50
45
40
35
30
25
20
15
10
5
0
0 2.5 5 7.5 10 12.5 15 20
% WHEY SOLIDS


EMULSION DROPLET SIZE (μ)
60
55
50
45
40
35
30
25
20
15
10
5
0
a b c d e f g h
STABILIZER

APPENDIX B

APPENDIX B

TASTE PANEL FORM

Name_____ Date_____

Instructions:

1) Based on your concept of a soft poached egg, place the number which you feel is most appropriate from the proper Evaluation Score column in Your Results section below.

2) Limit scores to the yolk portion of the poached egg.

3) Comments are encouraged.

Evaluation Scores

Overall yolk appearance
Yolk flavor
General yolk acceptability

1. Like very much
2. Like moderately
3. Like slightly
4. Neither like nor dislike
5. Dislike slightly
6. Dislike moderately
7. Dislike very much

Yolk texture

1. Very thick
2. Moderately thick
3. Slightly thick
4. Typical
5. Slightly thin
6. Moderately thin
7. Very thin

Yolk Color

1. Very dark
2. Moderately dark
3. Slightly dark
4. Typical
5. Slightly light
6. Moderately light
7. Very light

APPENDIX B (cont'd.).

Your Results

Factors Evaluated:

_____ Overall yolk appearance

_____ Yolk flavor

_____ General yolk acceptability

_____ Yolk texture

_____ Yolk color

Comments:

Thank you.

Appendix B (cont'd.).

Information and Consent Form

This form and explanation are presented to conform to current university rules regarding use of human subjects for research purposes.

The following ingredients have been incorporated into an artificial egg yolk and cooked with fresh egg white in the form of a soft poached egg:

Fresh egg white
Whey protein Concentrate (Enpro-50)
A commercial high polyunsaturated oil
Lecithin
Calcium Acetate
Glycerin
Sodium Alginate
β-carotene coloring
Commercial egg flavoring

All ingredients are commercial or GRAS list food items. Taste panel work is necessary to determine potential acceptability of a low cholesterol natural yolk replacement.

There are no known or anticipated risks in eating this product, nor are there any specific or implied individual benefits from its consumption. Individual results will be kept in strict confidence.

Appropriate precautions have been taken to insure freshness and cleanliness in preparation of these ingredients.

I, _____, have been informed of the nature of and the ingredients used to compose the product for which I am being asked to serve as a taste panelist. Descriptions of any possible discomfort and/or risks as well as any possible benefits have been given. I agree to serve on this panel which will be conducted _____, but am free to withdraw my consent and to discontinue participation in the project at any time. I realize that although my individual results will be kept in strict confidence, compiled results may be published.

Signed_____

Date_____

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