RHEOLOGY AND MICROSTRUCTURE OF READY-TO-EAT RETORTED EGG PRODUCT

by

JEGAN S DAMODARASAMY

(Under the Direction of Romeo T. Toledo)

ABSTRACT

Breakfast eggs are an important item in the military ration program. An improved formula consisting of pasteurized liquid egg, liquid margarine, xanthan gum, pre-gelatinized starch, calcium caseinate, and water was developed which did not exhibit syneresis and a rubbery texture when the ingredients were adequately blended before thermal processing. To test the effectiveness of different blending procedures, the microstructure of the liquid before retorting and the retorted product was evaluated using a Confocal Laser Scanning Microscope (CLSM) and a cryo-stage scanning electron microscope (C-SEM). Preliminary test showed the dramatic change of texture as result of re-heating the eggs. This phenomenon was studied using a controlled stress Rheometer. The conditions of the Rheometer testing were also simulated on a Sterilmatic retort simulator and the texture of the resulting product was examined using the TAX-T2 texture analyzer. The microstructure evaluation showed that Megatron, a high speed homogenizer yielded the best homogeneity for both fat distribution and hydrocolloids. The textural and rheological studied showed that the hardness and visco-elastic properties of the product increased as result of re-heating

INDEX WORDS: Rheology, Microstructure, SEM, CLSM, Texture, MRE,

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JEGAN S DAMODARASAMY

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JEGAN S DAMODARASAMY

Major Professor:	Romeo Toledo
Committee:	Manjeet Chinnan William Kerr

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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DEDICATION

I dedicate this work to two people who have made me what I am today. My Parents

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Chapter 1

Introduction

"Commercially sterile, plain scrambled eggs in half-steam table trays and quad laminate pouches are the poorest quality in the military ration program" Kandala and others (2005). One of the primary complaints about these products is their poor texture. A formulation was developed by and Kandala and others (2005) which was shown to have acceptable flavor, color, texture. In the sensory tests conducted by Kandala and others (2005), more than 80% of the consumers thought that the product was acceptable as a part of their daily meal. The formulation consisted of pasteurized liquid whole eggs, water, margarine, salt, citric acid and egg flavor. In addition to the above ingredients a mixture of Xanthan gum, pre-gelatinized starch and calcium caseinate were added as an effective means of controlling syneresis and providing a better texture to the final product. Initial studies conducted exhibited a drastic change in the quality (texture) of the egg as result of re-heating. The effect of re-heating was further examined in detail using a dynamic stress controlled Rheometer. The liquid egg formulation was heated, cooled and re-heated in the Rheometer and the corresponding G' and G" values were recorded. A similar heating profile was also conducted on the eggs packaged in quad laminate pouches in a Sterilmatic retort simulator. This allowed us to see if the results of the Rheometer could be related to the textural hardness of a retorted product.

One of the primary hurdles faced during the preparation of the formulated liquid egg mix was homogeneity. When the mixture was non-homogeneous the functionality of the ingredients was lost. When the hydrocolloids and starch were not distributed uniformly throughout the liquid egg mix, they did not effectively control syneresis, which led to an unpleasant appearance (visible lumps) and texture. So various mixers and homogenizers were tested including an industrial scale kettle mixer. The specific objectives of this study can be summed up as follows:

1. To study the viscoelastic properties of the egg mix during a heat-cool-reheat cycle in a dynamic stress controlled Rheometer.
2. To determine if the changes in viscoelastic properties observed in the three stage heat-cool-reheat process in the Rheometer can be observed in the texture of the gelled egg mix subjected to a similar temperature cycling process in a retort simulator.
3. To examine the three-dimensional microstructure of the retorted egg product under a scanning electron microscope so as to be able to observe the component distribution in the matrix as a result of various homogenizing techniques.
4. To examine the liquid egg mix under a Confocal Scanning Laser microscope to determine the appearance of the fat globules when the liquid egg mix is subjected to different homogenizing procedures.

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CHAPTER 2

THEORY AND REVIEW OF LITERATURE

Egg Gelation:

"The coagulation or gelation of proteins-in particular, the irreversible, heat-induced coagulation or gelation of egg proteins-often controls the success of certain cooked food products" Gossett and others (1984). Gelation is an orderly aggregation of proteins, which may or may not be denatured, forming a three-dimensional network (Hermansson 1979). Polymer-polymer and polymer-solvent interactions, as well as attractive and repulsive forces are balanced such that well-ordered matrix can be formed (Schmidt 1981). The gel may be turbid or translucent; in the latter case, the gel may be thermoreversible (Shimada and Matsushita, 1981). The coagulation, i.e., denaturation of proteins can be brought about by heat, high pressure, salts, acids, alkalies, alcohol, or denaturing agents such as urea (Mirsky and Pauling 1936). The physical and chemical properties of egg albumen proteins are listed in table 1.1

Table 1.1 Properties of Egg Albumen Proteins[a]

Protein	% in albumen	Molecular weight	Characteristics
Ovalbumin	54		Phosphoglycoprotein, denatured easily, has four sulfhydryls
Ovotransferin	12-13	6.1-6.6	Glycoprotein, complexes iron and other materials
Ovomucoid	11	3.9-4.3	Glycoprotein, Trypsin inhibitor
Ovomucin	3.5	4.5-5.0	Glycoprotein, fibrous, viscous

Lysozyme	3.4-3.5	10.7	Spherical protein, four disulfide bonds, lytic action
Ovoinhibitor	1.5	5.1-5.2	Inhibits trypsin and chymotrypsin
Ovoglycoprotein	1.0	3.9	Glycoprotein
Ovoflavoprotein	0.8	4.0-4.1	Binds riboflavin
Ovomacroglobulin	0.5	4.5-4.7	Glycoprotein
Avidin	0.5	9.5-10	Binds biotin

[a] Adapted from Gossett and others (1984)

Methods to assess denaturation are based on different principles which can be classified into five groups (Kanzmann 1959); a) thermodynamic, b) optical or chemical, c) NMR and electron magnetic resonance (ESR) d) surface phenomena and e) Rheology.

Rheological Analysis:

Rheology can be defined as an attempt to establish/define a relationship between the stress acting on a given material and the resulting deformation and/ or flow that takes place. It is a study of the flow and deformation of substances and in particular, to their behavior in the transient state between solids and fluids (Munizaga and Barbosa-Canovas 2004). Rheological properties are measured by quantifying the deformation under an applied force over a specified period of time. The rheological properties and changes in these properties under various conditions of a test are relevant in many fields of study.

Food rheology can be considered as the material science of food. As Steffe (1996) has shown there are numerous areas in the food industry where rheological data is needed. For example,

a) Process engineering calculation involving a wide range of equipment such as pipelines, pumps, extruders, mixers, coaters, heat exchangers, homogenizers and on-line viscometers
b) Determine ingredient functionality in product development
c) Intermediate or final product quality
d) Shelf-life testing
e) Evaluation of food texture by correlation of rheological properties with sensory data
f) Analysis of rheological equations of state or constitutive equations.

Several distinctive rheological properties of various foods have been discussed in books by Rao and Steffe (1992) and Weiper and others (1993)

Rheology deals with how all materials react to applied forces and deformations. Basic concepts of stress (force per unit area) and strain (deformation per length) are relevant in all rheological evaluations (Munizaga and Barbosa-Canovas 2004). As stress is a function of the force applied, the direction of the force determines the type of stress induced on a test material. When the direction of the force is perpendicular to the area of application then a normal stress is realized whereas when the applied force is parallel to the area of application a shear stress is realized. Strain is the dimensional change in the test material in the direction of the applied force expressed as dimensionless quantity relative to the original dimension.

The sol-gel transition is an important physical phenomenon with many applications in foods, cosmetics and pharmaceutical products. The formation of a gel from a liquid (sol) has been extensively studied through rheological techniques. Most of these rheological studies have

been carried out on food hydrocolloids and proteins due to the importance of the gelling ability of these food ingredients and how they confer appropriate textural and mechanical properties to the final product (Gallegos and Franco 1999). Nishinari (1997) reviewed literature on the use of dynamic viscoelasticity measurements for the study of the gelation phenomenon of different gelling polymer solutions.

Egg contains high biological value proteins compared to other dietary proteins. Egg proteins are characterized by their desirable functional and nutritional properties (Ahmed and others 2003). Textural properties of food products like cakes, creams, omelet etc., are primarily due to the egg constituents. The unique textural characteristics are a result of coagulation/gelation effects, initiated by the action of heat, although in products of mixed composition other ingredients may also contribute to the development of texture and structure (Kiosseoglou 2003). The addition of hydrocolloids like xanthan and starch may greatly influence the texture and rheological properties of any product and the effects can be studied by conducting rheological measurements on the raw, intermediate and final processed product or continuously and non-destructively on the same sample in a rheometer that can be programmed to mimic anticipated processing conditions. Rheological studies on hydrocolloids have been conducted by Gallegos and Franco (1999) but these studies were on model systems not in the context of their performance in an egg product.

One of the major groups of additives used in food industry today is hydrocolloids (Rosell and others 2000). Dziezak (1991) reported that these compounds commonly called gums are capable of controlling both rheology and texture of aqueous systems through the stabilization of

emulsions, suspensions and foams. Xanthan gum is a non-linear anionic microbial hetero-polysaccharide produced by aerobic fermentation of *Xanthomonas campestris*. It consists of repeated pentasacchride units formed by two glucose units, two mannose units, one glucoranic acid units, in the molar ratio of 2.8:2.0:2.0 (Garcia-Ochoa and others 2000). The gum exhibits pseudoplaticity with high viscosity at low concentrations (Fox 1997; Urlacher and Nobel 1997). Ramaswamy and Ahmed (2001) have shown that concentration has considerable effect on rheological characteristics of xanthan gum while temperature and pH did not.

Rheological Instruments for Fluids:

Common instruments, capable of measuring fundamental rheological properties of fluid and semi-solid foods may be placed into two broad categories: rotational and tube type. Most are commercially available; others (mixer and pipe viscometers) are easily fabricated. Costs vary tremendously from the inexpensive glass capillary viscometer to a very expensive rotational instrument capable of measuring properties under dynamic or steady shear conditions. Solid foods are often tested in compression (between parallel plates), tension, or torsion. Instruments which measure rheological properties are called rheometers. Viscometer is a more limiting term referring to devices that only measure viscosity of fluids.

Rotational instruments may be operated in steady shear (constant angular velocity) or oscillatory (dynamic) modes. Some rotational instruments function in the controlled stress mode facilitating the collection of creep data, the analysis of materials at very low shear rates and the investigation of yield stress. This information would be of value in obtaining the internal structure of materials. Rotational systems are generally used to investigate time-dependent behavior while tube systems only allow one pass of material through the apparatus. There are

advantages and disadvantages associated with each instrument. Typical rotational viscometer test fixtures include cone and plate, parallel plate and concentric cylinder operated under steady shear conditions. Some instruments are capable of operating in oscillatory mode.

A rheological test can be conducted in several ways: shear, extension and bulk compression. It is possible to conduct tests in all three methods of deformation, under steady state or dynamic conditions, and compare the resulting moduli and compliances (Ferry 1980). All foods have unique flow curves and as discussed above have numerous industrial applications. Steady shear viscosity is a property of all fluids regardless of whether or not they exhibit elastic behavior; however, many phenomenon cannot be described by the viscosity function alone and elastic behavior must be taken into consideration (Steffe 1996)

Textural analysis:

Steffe (1996) describes texture as a human sensation of food derived from its rheological behavior during mastication and swallowing. Since texture is a highly human dependent function it is very difficult for any one machine to exactly mimic the actions of the human mouth. Even if a machine could mimic the movements of the human mouth, designing a sensor like a human tongue to get all the feed backs would be almost impossible. Overall there are two methods used to evaluate food texture: sensory and instrumental. The texture profile development by sensory analysis (Munoz and others 1992) uses a human taste panel and provides the most reliable tests, which as discussed above cannot be completely duplicated by any instrumental procedure. Instrumental texture analysis is much less costly and time consuming than sensory tests.

Moreover, they can be co-related to critical sensory attributes which allow some measure of consumer acceptability (Steffe 1996).

Texture profile analysis was first proposed in 1963, and was studied using an instrument known as the General Foods Texturometer (Friedman and others 1963; Szczesnaik and others 1963). Later the technique was adopted and improvised by Bourne (1968 and 1974) to be extended to the Instron Testing Machine, where a food sample is compressed two times. Two parallel plates, one moving and the other stationary are used to achieve the compression of the bite size samples (about 1 cm cube). As the test was intended to mimic the human perception of texture, the first and the second compression cycles were referred to as the first and second bites. Instrumental textural studies have been conducted on various products like tofu, chips, peanut butter, egg yolks, soya gels, egg white gels, meats and meat based products. Many characteristics like fracturability, hardness, adhesiveness, springiness, cohesiveness etc., could be obtained from a TPA graph.

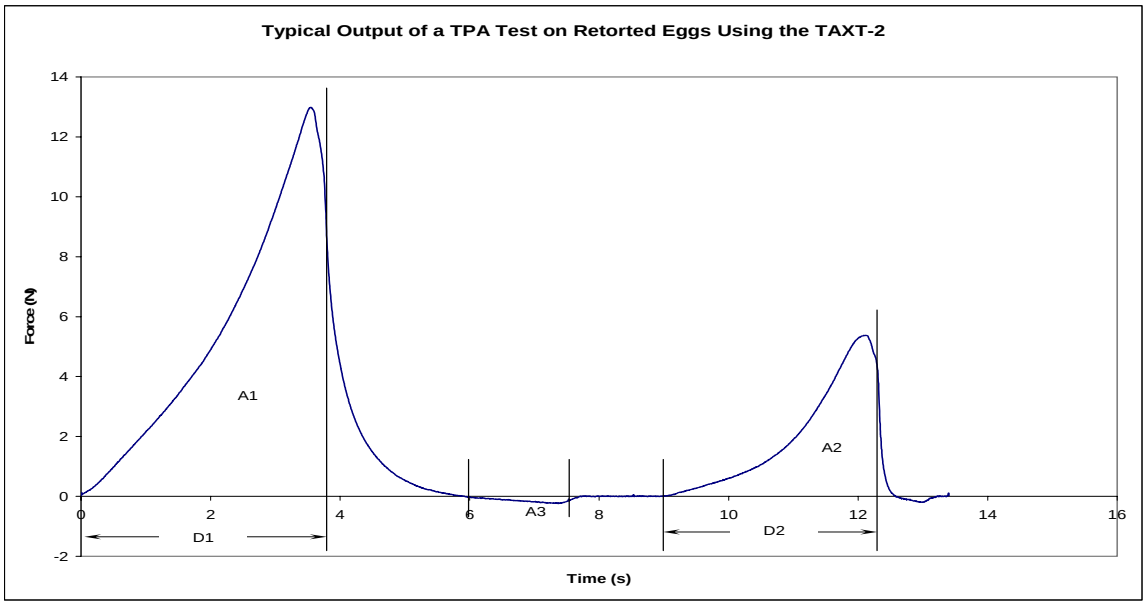

Figure 2.1 Typical output of a TPA test on Retorted eggs using the TAXT-2 apparatus (From: Kandala and others 2005)

Sampling the gels is primarily by cutting them into standard sized cubes or cylinders or by allowing the sol to set into gel in molds of desired shape and size. Pons and Fiszman (1996) have shown that most researchers use samples of 1-3 cm side for cubes or the same as diameters for cylinders with a sample height of 1.5-3 cm in both cases. Pons and Fiszman have also shown that recent studies on TPA have predominantly engaged the use of compression devices larger than the cross-section of the sample in order to measure only the uniaxial compression force. They also found that the TPA on gel foods systems have been conducted at cross head speeds in the range of 10-250 mm/min.

Microstructure Analysis:

Aguilera and Stanley (1999) defined food technology "as a controlled attempt to preserve, transform, create or disrupt structures imparted by nature or processing". A primary 20^{th} century development in the field has been in the design of process and equipment, and in adapting unit operations to transform and preserve foods (Aguilera 2004). Over the last decade, advances in various fields especially in the field of microscopy and image analysis have led us to further understand food structure on a much smaller or basic level. The more we learn about the microstructure of food the more it becomes evident that "functional properties" are primarily dependent on the make-up of their fine structure (deMan 1982). The chemical composition of a food gives rise to its structure which in turn defines its physical properties. No food is an exception to this rule.

Hydrocolloids are water soluble polysaccharides with a wide range of functional properties that make them very useful in food technology (Barcenas and Rosell 2005). However, hydrocolloids have a tendency to agglomerate affecting consumer acceptability of a retorted egg product. The presence of distinct agglomerates of gums and/or free oil creates a product with undesirable mouthfeel (Kandala, et al 2004). . Improper mixing/homogenization of the ingredients could lead to syneresis and most importantly an undesirable texture. Leviton and Pallansch (1959) have shown that in ice cream, repeated homogenization under the same conditions (multipass homogenization) decreases the fat globule size with each pass.

Scanning Electron Microscopy:

Scanning electron microscopy has been long recognized by food scientist as a tool to relate the detailed structure of food products to parameters such as shelf life and texture (Sargent 1988). At ambient/room temperature most fats would melt under the electron beam (Kalab 1983, 1984) and the vacuum inside the microscope would rapidly evaporate water creating artifacts in the image and contaminating the viewing chamber. Traditionally, before examination, hydrated samples would have to undergo either freeze drying or critical-point drying which is not only time consuming but also tend to produce artifacts in the sample (Sargent 1988). These problems can be avoided by a cold stage electron microscopy (Pawley and Norton 1978). Commercial equipment available today provides means for rapid specimen cooling and transfer to SEM in a way which avoids frost contamination of the viewing area (Beckett and Read 1986).

Robards and Sleytr (1985) have thoroughly reviewed the practice of rapid freezing. In addition, specimens can be freeze-fractured or cryo-honed with a cooled steel or tungsten carbide

blade which passes through the frozen specimen at a height set by a micrometer screw. A suitable metallic or carbon film is applied to the specimen by diode sputtering or evaporation at low temperature. Controlled heating of the cooled stage to a pre-determined temperature (usually -80°C) permits the sublimation of naturally occurring surface film of water or etching of a fractured face by careful removal of water. Since soluble components are immobilized at low temperature, greater confidence can be placed in elemental distribution data gained this way (Echlin 1984, Marshall 1988).

Of the many advantages realized by the cryo-fixing of SEM samples, an important one for most researchers is the speed of the procedure (Sargent 1988). Sargent (1988) also adds that specimens are commonly frozen, freeze-fractured, coated and prepared for examination within 8 minutes. If they are to be examined uncoated at low accelerating voltages which do not cause charging problems, preparation times are shorter.

Confocal Laser Scanning Microscopy:

Confocal Fluorescence microscopy provides a means of obtaining three-dimensional images of the microscopic network structure of gel-like food products (Kempen and others 1999). Generally, droplet size is not easy to measure in emulsions, even though a large number of methods exist (Dalgleish 1996). In late 60's Walstra and others (1969) summarized the difficulties of measuring the size of emulsion droplets on Laser Microscopy images. Blonk and others (1995) have used CLSM based methods to study the phase separation of the Na-alginate/Na-caseinate system. Heertje and others (1987) have used CLSM to measure the size of droplets in a commercial mayonnaise product.

Components of most food products do not fluoresce therefore they have to be stained with some fluorochrome that can be excited by a laser. Kempen and others (1999) have showed that nile red staining could make the lipid network visible under fluorescence microscopy. Blijdenstein (2004) used fluorescein isothiocyanate (FITC) to stain proteins and Nile Red to stain lipids in his research on "Microstructure, rheology and demixing in emulsions flocculated by polysaccharides". In addition to offering a good control in the z-direction, CLSM can also perform optical cutting in both xy-direction and xz-direction due to its very thin and adjustable focal plane (Wilson 1990). Three-dimensional images of the sample can be constructed with the help of a computer by combining a set of images taken within a specified location or at the same location but in the xy-direction at different depths of the sample (Loren and others 1999). CLSM connected to both a digital camera and a computer allows easy acquisition of images for analysis.

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Chapter 3

Rheological Changes in Eggs Subjected to Heat –Cool and Re-heat Cycles [1]

[1] Damodarasamy, J.S and Toledo, R.T. To be submitted to the Journal of Food Science

Abstract:

The texture of thermally stabilized eggs is one of the most important factors affecting its acceptability. Dynamic rheological measurements conducted on two liquid egg blends subjected to heating, cooling and re-heating cycles using a Rheometric Scientific SR5000 stress controlled rheometer showed differences in responses of the blends to heating, cooling, and reheating. Blends consisting of pasteurized raw egg, margarine, salt, citric acid and egg flavor with or without xanthan and pre-gelatinized starch were subjected to a three- step dynamic temperature ramp test consisting of heating to the gelation temperature, cooling to ambient, followed by reheating. The storage (elastic) modulus, G' and the loss (viscous) modulus G" increased with gelation and leveled-off followed by a rise to a peak and drop-off on cooling. Re-heating of the mix without the hydrocolloids resulted in a drop in both G' and G" to about the same level as in the first heating step. In the presence of hydrocolloids in the mix, G' increased sharply to the gelation temperature and continued to increase during the temperature hold at the gelation temperature while the G" values leveled off during the hold at the gelation temperature. After cooling and re-heating the G' and G" values increased to about double the values at the end of the hold at the gelation temperature. When packaged in laminate pouches, thermally processed, cooled and reheated, instrumental texture profile analysis on the final product showed no difference between the heated/cooled blend without the hydrocolloids compared to those heated/cooled/ re-heated/ cooled. In contrast, values of the texture profile parameter of hardness of the blend with hydrocolloids heated/ re-heated/ cooled, was almost double that of the heated/cooled sample.

Introduction:

Rheological properties of foods convey information on physical structure, texture and responses of the food to processing conditions. Rheology is the study of the relationship between stress and the deformation (strain) and/or flow that results. Elastic and viscous properties are exhibited by foods which are considered as viscoelastic materials (Munizaga and Barbosa-Canovas 2004). During dynamic rheological measurements the sample is subjected to small oscillatory stresses and is non-destructive therefore, changes on the same sample can be measured as the sample goes through heating and cooling process.

"The United States military is the largest consumer of Meals –Ready to Eat (MRE) products today, but the convenience of these products is also attractive to the civilian market" Kandala and others (2005). These shelf-stable products are usually heated in a heating pouch before consumption. In the case of eggs, changes in viscoelastic properties occur during the initial heat-induced gelation, sterilization and cooling and re-heating of gelled eggs.

Egg proteins have one of the highest biological value compared to other dietary proteins. The proteins in egg collectively have desirable functional and nutritional properties (Ahmed and others 2003). Textural properties of food products such as cakes, creams, scrambled eggs, omelets are set by the egg component in their formulation. The unique textural characteristics are a result of heat induced coagulation/gelation although in products of mixed composition other ingredients also contribute to the texture and structure (Kiosseoglou 2003). Kandala and others have shown that adding the hydrocolloids xanthan gum and pre-gelatinized starch to a liquid egg

mix consisting of liquid whole eggs, liquid margarine, salt, citric acid, egg flavor, effectively eliminated syneresis and prevented the development of a rubbery texture in the sterilized product. A number of rheological studies have be done on the effect of hydrocolloids on the gelling ability of polysaccharides and proteins used as texture modifying ingredients on food products (Gallegos and Franco 1999). Nishinari (1997) has reviewed the use of dynamic viscoelasticity measurements to study the gelation phenomenon of various gelling polymer solutions.

One of the major groups of additives used in food industry today is hydrocolloids (Rosell and others 2000). Dziezak (1991) has shown that these compounds commonly called gums are capable of controlling both rheological and textural properties of aqueous systems through the stabilization of emulsions, suspensions and foams. Xanthan gum is a non-linear anionic microbial hetero-polysaccharide produced by aerobic fermentation of *Xanthomonas campestris*. It consists of repeated pentasacchride units formed by two glucose units, two mannose units, one glucoranic acid units, in the molar ratio of 2.8:2.0:2.0 (Garcia-Ochoa and others 2000). The gum exhibits pseudoplaticity with high viscosity at low concentrations (Fox 1997; Urlacher and Nobel 1997). Ramaswamy and Ahmed (2001) have shown that concentration has considerable effect on rheological characteristics of xanthan gum while temperature and pH have little effect.

Steffe (1996) describes texture as a human sensation derived from the rheological behavior of food during mastication and swallowing. Since texture is a human sensation it is not possible for any mechanical device to exactly mimic what occurs in the human mouth. Even if a machine could mimic the movements of the human mouth the feedback generated by a sensor

like a human tongue is not possible by mechanical or electronic means. The two methods used to evaluate food texture is by sensory and instrumental. The sensory texture profile (Munoz and others 1992) uses a human panel and if conducted by trained panelists will provides the most reliable results. Instrumental texture analysis is much less costly and time consuming than sensory tests and some parameters can be correlated to critical sensory attributes which then allow some measure of consumer acceptability (Steffe 1996).

Texture profile analysis (TPA) was first proposed in 1963, and was studied using an instrument known as the General Foods Texturometer (Friedman and others 1963; Szczesnaik and others 1963). Later the technique was adopted and improvised by Bourne (1968 and 1974) to be carried out with the Instron Testing Machine. The test consists of compressing a bite sized sample (1 cm cube) two times between two parallel plates, one moving and the other stationary. As the test was intended to mimic the human perception of texture, the first and the second compression cycles were referred to as the first and second bites. Instrumental textural studies have been conducted on various products like tofu, chips, peanut butter, egg yolks, soya gels, egg white gels, meats and meat based products. Many characteristics like fracturability, hardness, adhesiveness, springiness, cohesiveness etc., could be obtained from a TPA graph.

Samples of gels for instrumental TPA are prepared by cutting them into standard sized cubes or cylinders or by allowing the sol to set into gel in molds of desired shape and size. Pons and Fiszman (1996) have shown that most researchers used samples with 1 to 3 cm sides for cubes or 1 to 3 cm diameters by 1.5 to 3 cm height for cylinders. Pons and Fiszman have also shown that recent studies on TPA used compression devices larger than the cross-section of the

sample in order to measure only the uniaxial compression force. They also found that the TPA on food gels typically used cross head speeds in the range of 10-250 mm/min

The objective of this study is to determine the effects on rheological properties when eggs are heated to heat-set the gel and re-heating the gelled egg after cooling. The effect of hydrocolloids in the liquid egg formulation on the Rheological changes and instrumental TPA parameters during the heating/cooling and reheating processes was also determined.

Methodology

Materials:

Liquid egg was pasteurized whole egg with no additives purchased from Sonnstegard Foods in Gainesville GA. Product was obtained form the plant on the same day it was processed, transported to Athens, GA, transferred to 4-liter polyethylene closable bags and frozen in a horizontal position in a blast freezer (-20 C) to give a thickness of about 5 cm after freezing. This facilitated thawing out when the eggs were used later.

Xanthan gum was obtained from DeGussa Texturant Systems in Atlanta Ga. Pre-gelatinized starch was Ultra-sperse M (National Starch, Bridgewater,NJ). Liquid margarine was manufactured by Glenview Farms, Columbia MD and obtained from the University of Georgia Food Service commissary. Food grade anhydrous citric acid was from Tate and Lyle, Decatur IL. Egg flavor was hydrolyzed whole egg obtained from Summit Hill Flavors, Middlessex NJ. Salt was food grade Culinox 999 from Morton Salt Co, Chicago IL. All formulations were prepared with deionized water

Egg mix composition:

Work by Kandala, et al 2004 showed that liquid whole eggs by itself when thermally processed resulted in a gel with hard rubbery texture and green color. Additional water and margarine was added to modulate the texture and citric acid was added to prevent green color formation. The addition of more water and oil resulted in syneresis therefore a modification was made on the formulation by adding hydrocolloid binders. Salt and liquid egg flavor was added to improve flavor acceptability of the retorted product (Kandala and others 2005). We conducted the rheological measurements on Kandala and others (2005) formula to better explain the textural changes observed by these authors. Two formulations were tested, a liquid egg mix with (HCE) and without hydrocolloid binders (E).

Table 3.1: Formulas for retorted egg product for rheological and textural analysis

Ingredients	**Formula E**	**Formula HCE**
	Percent by weight	Percent by weight
Liquid egg	74.35	73.45
Water	21.50	21.50
Liquid Margarine	3.00	3.00
Salt	0.50	0.50
Citric acid	0.15	0.15
Natural Egg Flavor	0.5	0.50
Pre-gelatinized Starch	-	0.50
Xanthan Gum	-	0.40

Note: Xanthan and Pre-gelatinized starch numbers reflect the percentage of the rest of the ingredients

Mixing:

Formula E (E for basic egg formulation) was made by mixing all the ingredients (liquid margarine which was stored in a refrigerator was pre-heated before mixing with the rest of the ingredients) together and then homogenizing in a high speed gear homogenizer called the Megatron (Kinematica Inc., OH, USA). The Megatron is fitted with a high speed rotating element inside a slotted stator. The variable speed drive was operated at 9000-11000rpm. For the formula HCE (HCE for hydrocolloid egg) the xanthan gum and pre-gelatinized starch were first hydrolyzed in the specified amount of water. Salt and citric acid were added to the water prior to mixing of the gums. Liquid margarine which was preheated was added to the liquid eggs and all the ingredients were homogenized in the Megatron. Small amounts of both the mixes were taken immediately to the Rheometer. The rest of the mix was packaged in quad-laminate pouches and were hand-sealed using a pneumatic sealer (Toss Machine Components, Inc., PA).

Rheological Analysis:

Preliminary test done during the initial stages of the project showed a dramatic change in texture when the product was re-heated. One set of experiments was done to test the effect of heating the eggs in the pouches under atmospheric steam to permit its expansion, followed by transferring to another retort for thermal processing. The delay in the transfer resulted in cooling of the gelled product prior to thermal processing. The texture of the retorted product was rubbery as opposed to the soft/moist texture of the product gelled in atmospheric steam. This observation led to further investigation of the product rheology and texture as affected by the ingredients and temperature. A Rheometric Scientific (SR 5000, Rheometric scientific Inc., Piscataway, NJ) controlled stress rheometer was used to study the rheology of the samples. A

cone and plate geometry with 0.6mm gap was used for all the tests. The diameter of the top plate was 40 mm and the cone angle was 0.04 radians. The bottom/static plate was equipped with a Peltier heating/cooling system which permitted precise control of sample temperature. The temperature variation in the peltier plate was ±0.2°C from the set point. Since the peltier plate can heat or cool depending upon the deviation from set-point, rapid increase and decrease in temperature could be achieved without overshoot. Temperature of the Peltier system was controlled by a computer using the software provided by the rheometer manufacturer. A frequency sweep was conducted to establish the region of linear viscoelasticity (where the complex viscosity id independent of the imposed strain) where ratio of G' to G" was constant over a range of frequencies and strain (Lisa and others 1992). All the tests were carried out at a frequency of 1Hz and a strain value of 1%. A 3 step ramp process was used to study the changes in the egg mix with increase and decrease in temperature. The first step was to heat the sample from 25°C to 90°C at a rate of 2°C/min. Once the sample equilibrates at 90°C a hold time of 30 min was given before the temperature was brought back to 25°C. The first ramp set the gel and the second ramp cools it down to room temperature to mimic sterilization, cooling and storage of thermostabilized eggs. When sample reached 25°C it was held for 30 min before being re-heated to 70°C. This re-heating step mimics the re-heating of the product before consumption or serving. The measurement chamber was kept at saturated moisture conditions to help prevent moisture loss during the test. Also the edges of the plates were lubricated with silicone oil as suggested by Szczesnaiak and others (1983).

Thermal Processing:

Formulations were packaged in quad laminate pouches with 8 oz (227 g) precisely. The pouches were then placed in a custom made perforated steel rack to be processed in the retort. The thermal process was carried out in a Sterilmatic retort simulator (Steritort, FMC Food Tech, Madera, CA, USA). A centrifugal pump was plumbed into an intake port located at the lowermost point in the retort and the discharge from the pump was directed to a manifold at the uppermost point in the retort where multiple openings directed a steady stream of water over the laminate pouch rack. Thus the retort operation simulated a cascading water retort. A thermal process schedule was set to simulate the temperature profile as conducted in the Rheometer. The pouches were first heated to 90°C and held at the same for about 30 min. After 30 min hold the pouches were cooled down to room temperature. Once the pouches were cool enough they were taken out of the retort and stored overnight at room temperature. The following day re-heating was done by loading the pouches in the retort and heating to 90°C. The re-heating step also had a hold time of about 30 min. Even though the set point on the retort was 90°C the temperature was between 90-94°C. After the re-heating step the pouches were cooled down before pulling out of the retort and being analyzed in the TAXT-2. A similar process was also carried out with an over pressure of 2.8 bars from the beginning till the end of the thermal process to see the effect of pressure on the development of the texture.

Textural Analysis:

TPA analysis on the retorted product was done at room temperature on the TAXT-2 texture analyzer. The cross head speed was set at 2mm/s after running some preliminary tests. After the test the cross-head was set to retract at a sped of 10 mm/s. A time interval of 1 second

was set between the bites (cross-head plunges). A No.12 cork-borer (17 mm diameter) was used to produce uniform cylindrical sections. The height of the cylindrical section was 10mm. The plunger used to compress the samples had a diameter of 25.4mm. The samples were subject to 60% deformation of the original height. The above compression was chosen because most of the samples stayed intact with a 50 % deformation even after the second bite. A macro was used to compute the textural parameters from the TPA graphs.

Results and Discussion:

Rheological analysis:

Rheological data are reported as G' (elastic modulus) and G" (viscous modulus) at various times and temperatures. Both G' and G" are expected to increase with gelation in the first heating cycle due to the liquid to solid gel transformation and immobilization of the liquid component within the gel matrix. In Figure 3.1 a and b. for Formula E values of both G' and G" exhibited up and down scatter in the first one minute of the process indicating gelation of a film on the surface of the heated plate before the bulk of the liquid had fully gelled. As the gel firmed up, there was a sharp increase in both G' and G" and both parameters leveled off when the temperature reached 90 C. Figure 3.1a and 3.1b show the curves of G' and G" vs. time and temperature vs. time for formula E. The liquid formula E had a viscosity of 0.025 Pa.s measured on the rheometer at 25 C. 1 Hz, and 1% strain. .

Figure 3.1a: G' vs Time for Formula E Egg Sample

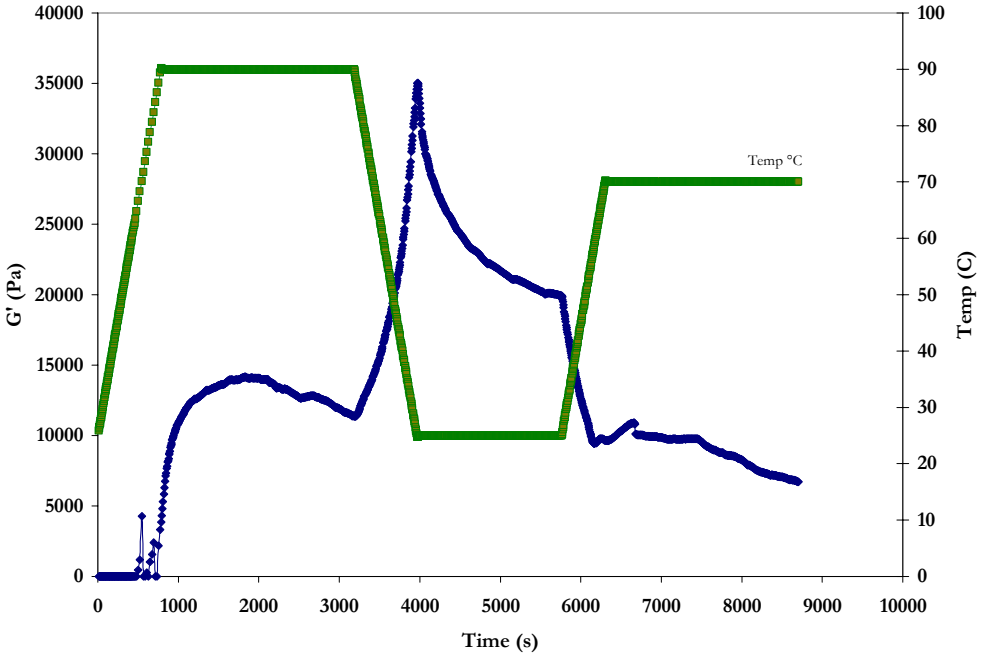
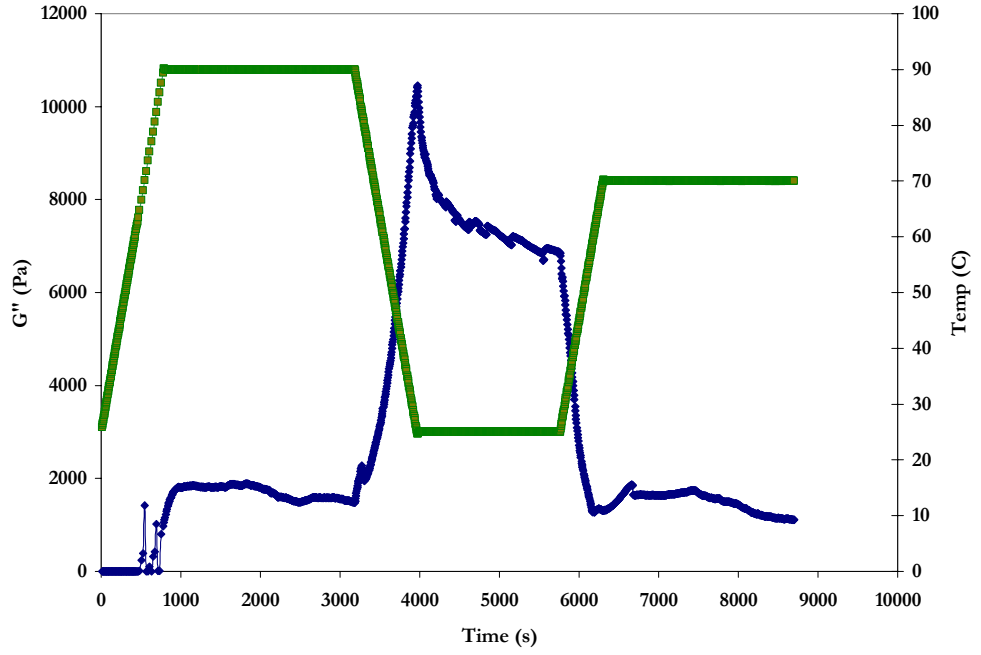


Figure 3.1b: G" vs Time for Formula E Egg Sample



G' and G" increased during the cooling step however during the hold at 25 C both parameters decreased. In preliminary studies on the rheometer, when the sample was held at each

of the steps longer than 30 min. G' and G" always leveled off with prolonged hold at the constant temperature. The pattern of plateauing of the viscoelasticity has also been reported by Cordobes and others (2004) in their study on Rheology and microstructure of heat induced gelled egg-yolks. The increase in G" with gelation and cooling and the parallel change with G' indicate that as the gel was firming up, the gel matrix also bound the liquid stronger resulting in an increase in G" along with G'. G' values were one order of magnitude larger than the G" values indicating that the gel was more solid-like than fluid-like.

. An interesting feature of the data is the behavior of G' and G" during the re-heating step. The values of the both G' and G" dropped rapidly after an initial rise at the start of cooling and continued to decrease until the end of the 30 min. hold at 25 C. However, on re-heating to 70 C values of G' and G" continued to decrease until they leveled off during the 70 C hold to coincide with the values of G' and G" during the first hold at 90°C. Thus for the egg blend without the hydrocolloids (formula E) values of G' and G" did not change very much when the cooled, and the solid gel was re-heated. A downward trend of G' and G" with prolonged hold at 70°C indicated breakdown of the gel matrix with prolonged re-heating. This breakdown of the gel matrix results in syneresis and the free water decreased the resistance of the matrix to deformation.

Instrumental TPA data of retorted samples of formula E are shown in Figure 3.2. . The hardness values of these samples were not significantly different between a single stage (heat/cool) and a double stage process (heat/cool/re-heat/cool) and whether the first heating process was done at atmospheric pressure or with a 2.8 bar air overpressure. The single stage

process with no pressure consisted of processing in the retort at 90°C for 30-35 min and then cooling to room temperature. The internal temperature of the product reached about 90°C. This is because the retort temperature stayed between 90-94°C even with the set point at 90°C. In the double stage process the product which had undergone the single stage process was stored overnight at room temperature and then re-heated back to 90°C for 30 min. The single stage process with overpressure consisted of processing eggs at 135 C with an over- pressure of 2.7 bars and cooling. The double stage process with overpressure consisted of re-heating the product after the single stage process at 90 C and 1 bar for 30 min. and cooling. The hardness values of formula E eggs as measured by the TAXT-2 did not have any significant differences ($p<0.05$) between the double stage and single stage with or without overpressure. A TPA test was conducted to examine the differences in texture. Of all the properties measures like fracturability, Springiness, chewability, adhesiveness, cohesiveness only hardness values showed differences before and after heating. For this reason only hardness values have been discussed below even though a TPA was conducted. These results parallel the changes in G' with heating/cooling/re-heat in the dynamic rheological tests for formula E eggs.

Figure 3.3 a and b. show the changes in G' and G" of the HCE blend with heating cooling and re-heating. The rheometer recorded the viscosity of the liquid formula HCE to be 0.273 Pa.s at 25 C, 1 Hz and 1% strain. The presence of the hydrocolloids increased the viscosity of the liquid about ten times that of formula E. It can be seen that the Initial part of the curve looks very similar to that of formula E.

Figure 3.3a: G’ vs Time for formula HCE egg sample

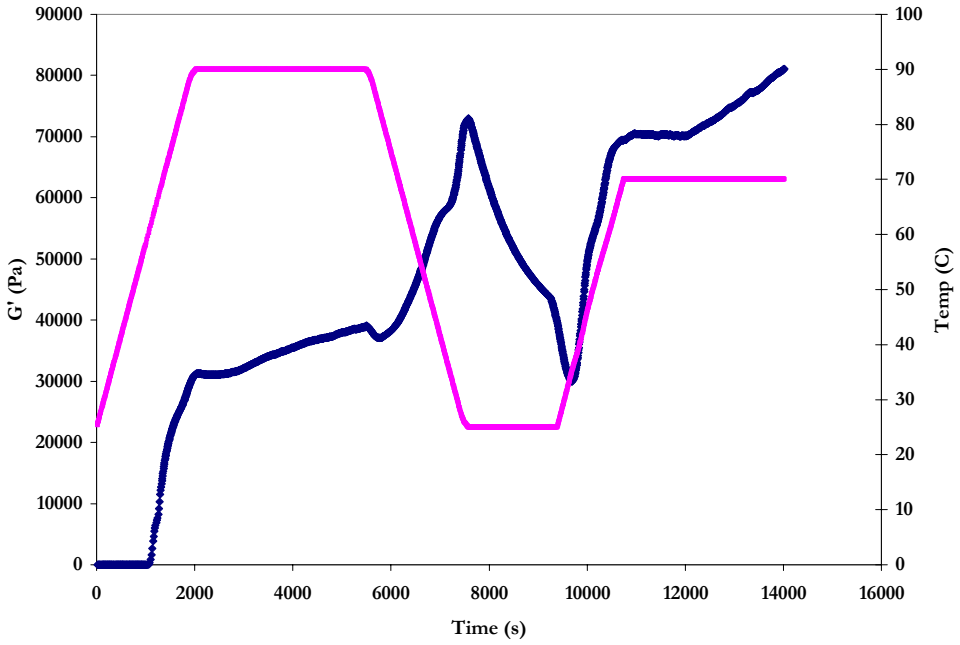
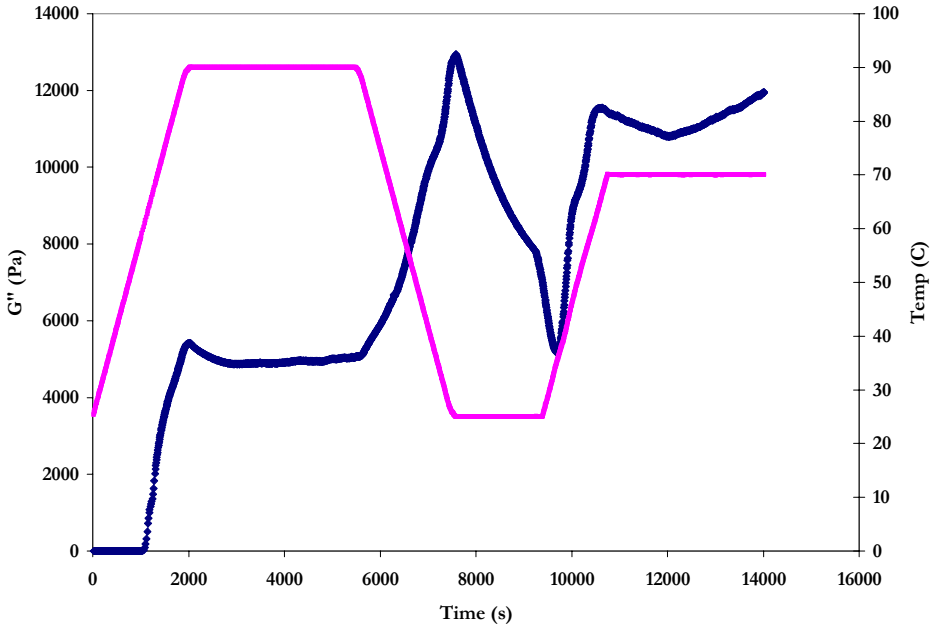


Figure 3.3b: G” vs time for Formula HCE egg sample

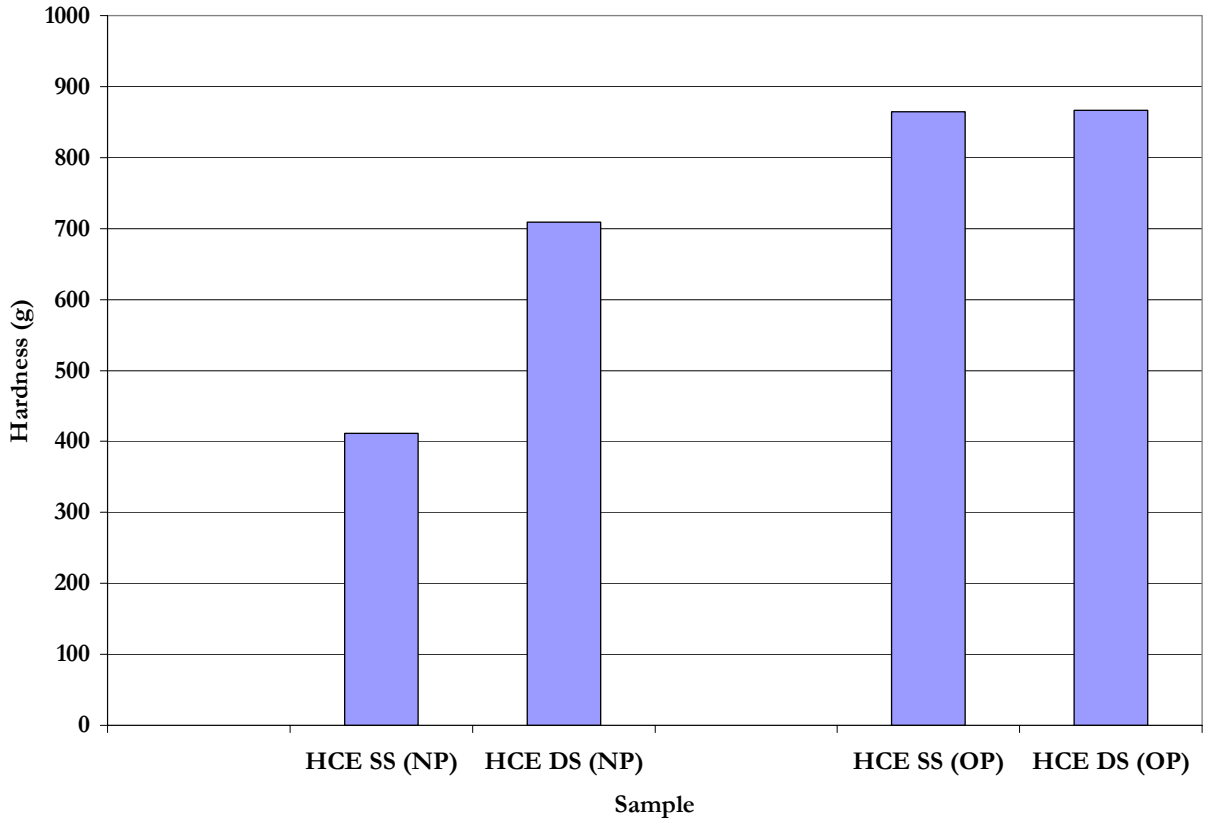


The large difference in the viscosity of the liquid formula E and formula HCE is manifested in the values of the G" during the first heating phase. G" values reached about 5000 Pa after gelatinization for formula HCE compared to 2000 Pa for formula E. In the case of formula HCE the scatter observed in the first min. of heating of formula E was not exhibited. The G' curve of formula HCE did not exhibit the leveling off exhibited by Formula E when the constant temperature of 90°C was reached. G' of formula HCE continued to increase during the whole 30 min. period of the first heating stage as opposed to G" which leveled off once the temperature was constant at 90°C. Eggs with hydrocolloids have much higher G' values compared to G' of the Formula E, this may be caused by syneresis of heated Formula E. Due to syneresis the top plate my "slip" instead of actually deforming the sample. In addition, these values indicate that the hydrocolloids may have maintained the liquid in the matrix.

The change in loss and storage moduli with cooling was also different in formula E compared to formula HCE. Both G' and G" continued to drop at the end of the cooling phase and in the second heating phase for formula E, while for formula HCE the drop in the moduli stopped at the end of the hold time at 25 C and increased with heating in the second heating phase. G' increased to values greater than the peak value during the cooling phase while G" also continued to increase although values were less than the peak at the end of the cooling phase. In preliminary experiments (data not shown) we observed G' to increase with a constant slope of about 5.6-5.7 Pa/s with prolonged hold at 70 C in the re-heating phase. These observations indicate that reheating and prolonged hold of cooked eggs at 70 C can be detrimental to texture.

The rheological data is supported by data on the TPA parameter of hardness obtained with the TAXT-2.. Figure 3.4 below shows the hardness values of processed formula HCE.

Figure 3.4: Hardness of HCE egg samples as measured at 60% deformation



Note: SS- Single Stage process; **DS**- Double stage process, **NP** – Process with no over pressure; **OP** – process with 2.8 bars over pressure

Formula HCE processed with no over pressure had the most remarkable change in hardness when re-heated. The hardness of the heated/cooled/re-heated sample was almost double that of the heated/cooled sample and the difference was significant statistically ($p<0.05$). On the other hand, processing with over-pressure also resulted in higher hardness values which are significantly different from those processed without overpressure. Re-heating pressure processed/cooled sample did not result in a significant ($P<0.05$) increase in hardness. By

comparing results for formula HCE to formula E, the primary reason for the increase in hardness with re-heating is due to the interaction of the egg proteins with the hydrocolloids xanthan and pregelatinized starch. This might be due to the fact that the 2.8 bar overpressure had compacted the product so much that re-heating did not induce any more protein re-arrangement or re-orientation.

Conclusions:

The presence of hydrocolloids in an egg blend not only influences the firmness and liquid-like properties of the gelled egg but also influences the rheological properties when subjected to heating, cooling and re-heating. Eggs with hydrocolloids have much higher G' values compared to G' of the Formula E, this may be caused by syneresis. Due to syneresis the top plate my "slip" instead of actually deforming the sample. In addition, these values indicate that the hydrocolloids may have maintained the liquid in the matrix. The G" values for samples with hydrocolloids are marginally higher indicating higher loss component (Viscosity component), this may be due to the interactions of hydrocolloids in the mix. However, hydrocolloids also affected the textural stability making the product firmer on re-heating after the first gelatinization process. Thermal processing in a flexible pouch with 2.6 bars overpressure also compressed the gel resulting in a higher hardness value for the product compared to processing without overpressure.

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Chapter 4

Micro-Structure of Ready-to-Eat Retorted Egg Product:

Role of Ingredients and Mixing Techniques[2]

[2] Damodarasamy, J.S, Kandala, R.N and Toledo, R.T. To be submitted to Journal of Food Science

Abstract

Breakfast eggs are an important item in the military ration program. An improved formula consisting of pasteurized liquid egg, liquid margarine, xanthan gum, pre-gelatinized starch, calcium caseinate, and water was developed which did not exhibit syneresis and a rubbery texture when the ingredients were adequately blended before thermal processing. To test the effectiveness of different blending procedures, the microstructure of the raw product was evaluated using a Confocal Laser Scanning Microscope (CLSM) and that of the retorted product was examined using a cryo-stage scanning electron microscope (C-SEM). Nile red and flourescein were used to stain fat red and protein green, respectively under the CLSM. CLSM images of a hand-mixer mixed liquid showed a homogeneous liquid matrix with a uniformly green background. Green protein globules predominated the field of view with only a few distinct fat globules visible. CLSM images of liquid mixed using an in-line high speed rotary homogenizing mixer showed an orange background and very few green protein globules indicating that the fat and protein molecules were intimately mixed. Under C-SEM, images of the cooked product showed small cell-like structures of hydrocolloid dispersed within the protein matrix. The size of the hydrocolloid structures was highest in the hand-mixer mixed samples and smallest in the samples mixed through an on-line high speed rotary homogenizing mixer. Samples mixed in a kettle with a turbine mixer showed large areas in the of field of view occupied by starch structures. In-line mixing with the high speed rotary homogenizer 10,000-11,000 revolutions per minute, yielded the most homogenous dispersion of hydrocolloids and lipids within the water and protein matrix.

Introduction

Aguilera and Stanley (1999) defined food technology "as a controlled attempt to preserve, transform, create or disrupt structures imparted by nature or processing". A primary 20th century development in the field has been in the design of process and equipment, and in adapting unit operations to transform and preserve foods (Aguilera 2004). Over the last decade, advances in various fields especially in the field of microscopy and image analysis have led us to further understand food structure on a much smaller or basic level. The more we learn about the microstructure of food the more it becomes evident that "functional properties" are primarily dependent on the make-up of their fine structure (deMan 1982). The chemical composition of a food gives rise to its structure which in turn defines its physical properties. No food is an exception to this rule. This work was conducted to elucidate the microstructure of retorted Ready-to-Eat egg as affected by ingredients and homogenizing techniques. The 3-dimensional microstructure of a retorted egg mix was analyzed using a cryo scanning electron microscope (C-SEM) and the size and distribution of fat and protein globules in the liquid mix before processing was evaluated using a confocal laser scanning microscope (CLSM).

Many operations in food processing are aimed at creating a microstructure that provides the desirable traits and functional properties of a product (Aguilera 2004). A desirable shelf-stable egg product should have the odor and flavor characteristics of fresh-scrambled eggs. Color should be light yellow. Product should be moist and slightly spongy, but not rubbery, contain no agglomerates and no free liquid because of syneresis/weeping. (Kandala and others 2005). Kandala and others (2005) developed the formula for an acceptable, quality, ready-to-eat egg product using liquid eggs, water, margarine, salt, citric acid and natural egg flavor. They found

that this base formula would lose around 5% of liquid by weight by syneresis. Variations in the base formulation by addition of pre-gelatinized starch and hydrocolloids prevented syneresis and improved product texture. Of the starches and hydrocolloids tested, a mixture of Xanthan gum and pre-gelatinized starch yielded the best sensory characteristics. Xanthan gum was shown to be very effective in controlling syneresis but did not provide adequate firmness. The firming effect was obtained by incorporating pre-gelatinized starch or pre-gelatinized starch and calcium caseinate.

Hydrocolloids are water soluble polysaccharides with a wide range of functional properties that make them very useful in food technology (Barcenas and Rosell 2005). However, hydrocolloids have a tendency to agglomerate affecting consumer acceptability of a retorted egg product. The presence of distinct agglomerates of gums and/or free oil creates a product with undesirable mouthfeel. Improper mixing/homogenization of the ingredients could lead to syneresis and most importantly an undesirable texture. Leviton and Pallansch (1959) have shown that in ice cream, repeated homogenization under the same conditions (multipass homogenization) decreases the fat globule size with each pass.

Scanning electron microscopy has been long recognized by food scientist as a tool to relate the detailed structure of food products to parameters such as shelf life and texture (Sargent 1988). At ambient/room temperature most fats would melt under the electron beam (Kalab 1983, 1984) and the vacuum inside the microscope would rapidly evaporate water creating artifacts in the image and contaminating the viewing chamber. Traditionally, before examination, hydrated samples would have to undergo either freeze drying or critical-point drying which is not only

time consuming but also tend to produce artifacts in the sample (Sargent 1988). These problems can be avoided by a cold stage electron microscopy (Pawley and Norton 1978). Commercial equipment available today provides means for rapid specimen cooling and transfer to SEM in a way which avoids frost contamination of the viewing area (Beckett and Read 1986).

Confocal fluorescence microscopy provides a means of obtaining three-dimensional images of the microscopic network structure of gel-like food products (Kempen and others 1999). Generally, droplet size is not easy to measure in emulsions, even though a large number of methods exist (Dalgleish 1996). In late 60's Walstra and others (1969) summarized the difficulties of measuring the size of emulsion droplets on Laser Microscopy images. Blonk and others (1995) have used CLSM based methods to study the phase separation of the Na-alginate/Na-caseinate system. Heertje and others (1987) have used CLSM to measure the size of droplets in a commercial mayonnaise product.

In this study we examined the size and distribution of fat and protein droplets in liquid egg using CLSM. Components of most food products do not fluoresce therefore they have to be stained with some fluorochrome that can be excited by a laser. Kempen and others (1999) have showed that nile red staining could make the lipid network visible under fluorescence microscopy. Blijdenstein (2004) used fluorescein isothiocyanate (FITC) to stain proteins and Nile Red to stain lipids in his research on "Microstructure, rheology and demixing in emulsions flocculated by polysaccharides". In addition to offering a good control in the z-direction, CLSM can also perform optical cutting in both xy-direction and xz-direction due to its very thin and adjustable focal plane (Wilson 1990). Three-dimensional images of the sample can be

constructed with the help of a computer by combining a set of images taken within a specified location or at the same location but in the xy-direction at different depths of the sample (Loren and others 1999). CLSM connected to both a digital camera and a computer allows easy acquisition of images for analysis.

Materials and Methods

Materials:

Liquid egg used in the scaled-up process was pasteurized whole egg with no additives purchased from Michael Foods (Gaylor, MN) Product was shipped frozen to the Department of Defense Demonstration Facility in Piscataway NJ. The frozen eggs were thawed at 2 C for one week prior to use. Liquid pasteurized eggs used in laboratory prepared samples were obtained from Sonnstegard Foods (Gainesville, Ga)

Xanthan gum was obtained from DeGussa Texturant Systems in Atlanta Ga. Pre-gelatinized starch was Ultra-sperse M (National Starch, Bridgewater,NJ). Liquid margarine was manufactured by Glenview Farms, Columbia MD and obtained from the University of Georgia Food Service commissary. Food grade anhydrous citric acid was from Tate and Lyle, Decatur IL. Egg flavor was hydrolyzed whole egg obtained from Summit Hill Flavors, Middlessex NJ. Salt was food grade Culinox 999 from Morton Salt Co, Chicago IL. Deionized water was used in all formulations.

Preparation of the mix

The basic formulation consisted of liquid eggs, water, liquid margarine, salt, citric acid and natural egg flavor. Xanthan proved very effective against syneresis even though it made the texture softer. In addition to the above ingredients pregelatinzed starch and a mixture of pregelatinized starch and calcium caesinate were also tried to firm product texture. The egg formulations used in the study of ultrastructure are shown in Table 1.

Table 4.1: Formula of egg product for analysis of microstructure

Ingredients	**Formula E**	**Formula HCE**	**Formula HCE-CS**
	Percent by weight	Percent by weight	Percent by weight
Liquid Egg	74.35	73.45	73.45
Water	21.50	21.50	21.50
Liquid Margarine	3.00	3.0	3.0
Salt	0.50	0.50	0.50
Citric Acid	0.15	0.15	0.15
Natural Egg Flavor	0.50	0.50	0.50
Starch	-	0.50	0.25
Calcium Caesinate	-	-	0.25
Xanthan Gum	-	0.40	0.40

Laboratory batches were prepared by hydrating the xanthan gum and pregelatinized starch in water which contained the salt and citric acid. The powdered ingredients were added to the aqueous phase slowly while mixing with a hand mixer. Liquid margarine which was stored in

a refrigerator at a temperature of 4-5° C was pre-heated in a microwave to 30 C and added to the liquid pasteurized egg fraction and stirred slowly by hand using a ladle. The egg-margarine fraction and the aqueous fractions were then combined and mixed using either a hand mixer or a high-speed in-line rotary mixer.

A scale-up of the process was conducted at the Department of Defense Demonstration Manufacturing Facility at the Center for Advanced Food Technology (CAFT) Rutgers University, Piscataway, NJ. The scaled-up process was designed to simulate procedures used by US defense contractors, therefore the ingredients were added in a slightly different manner. First the required amount of water was added to the kettle followed by the slow addition of xanthan gum and pregelatinized starch. Once all the starch and gum were added and hydrated, pasteurized liquid eggs followed by rest of the ingredients were added to the kettle while mixing continuously with the kettle mixer.

Mixing

Obtaining a homogenous mix was one of the most challenging aspects of pre-processing the liquid egg formula prior to filling and thermal processing. Mixing equipment used included a hand held kitchen mixer (Braun Mutiquick MR 400), a high speed kettle mixer (CAFT) and Megatron (Kinematica, Inc., OH, USA). The megatron is an in-line, high speed rotary gear homogenizer consisting of a high speed rotating element and stator with side slits. The megatron was operated between 10,000-11,000 RPM. The CAFT kettle mixer consisted of a high speed turbine mixer at the center of the kettle and a slower speed surface scraper at the wall of the kettle. While the center mixer was instrumental in breaking down the bigger particles, the surface

scraper helped move the entire mix through the high speed mixer. Both the surface scraper and the high speed mixer had individual speed controls. After all the ingredients were added they were mixed for about 15 minutes in all three types of mixers. A non- homogeneous mix leads to separation of fat (oil) in the retorted product. Moreover since the hydrocolloids and starch are pre-hydrated, inadequate mixing would give rise to big particles of starches and gums unevenly distributed in the final product. This not only leads to an undesirable texture but renders the hydrocolloids ineffective in preventing syneresis.

Packaging and Thermal Processing:

The mixture was filled into laminate pouches and hand sealed using a pneumatic sealer (Toss Machine Components, Inc., PA). Each pouch was carefully filled to contain 8 oz (227 g) of the mix. Pilot plant thermal processing was carried out in a Sterilmatic retort simulator (Steritort, FMC Food Tech, Madera, CA, USA) at the University of Georgia. A custom built perforated steel rack was used to hold the pouches inside the retort. A five stage thermal process as described by Kandala and others (2005) was employed for the processing of the pouches. The five stages included an initial come-up to 100° C, a hold at 100° C until the product temperature reached close to 70° C, increase of retort temperature form 100° C to a processing temperature of 130° C, followed by a hold at processing temperature until the desired lethality was reached and finally the cooling step. The Sterilmatic retort used for processing was equipped with a centrifugal pump which was plumbed in such way as to enable pumping the water from the lower most point in the retort to a manifold at the uppermost point in the retort where multiple openings directed a steady stream of water over the laminate pouch rack. This helped us simulate a cascading retort and ensured heat transfer rates. Packaging at CAFT was done in a horizontal

form and fill packaging machine followed by vacuum sealing. A similar five stage retort process as described above was employed in a semi-industrial scale cascading retort (one cage Stork 1100 retort).

Scanning Electron Microscopy (SEM)

Processed samples were examined using a LEO® 982 Field Emission Scanning electron microscope (SEM) (Leo Electron Microscopy Ltd, Cambridge, England). The SEM at the Center for Ultrastructural Research, University of Georgia was equipped with an Oxford® 6901 detector (Oxford Microanalysis Group, England) and Gatan Alto 2500 Cryostage and cryoprep chamber (Gatan UK, Ferrymills 3, Osney Mead, Oxford, OX2 0ES, UK) was used to view the samples. The sample to be observed was glued onto a holder using a mixture of Tissue Tek® (Sakura Finetek U.S.A., Inc.) and carbon. It was then instantaneously frozen by plunging into a "nitrogen slurry". The frozen sample was first sublimated to remove any ice or nitrogen on the surface and then sputter-coated with gold for 120 seconds before being placed inside the viewing chamber of the microscope.

Confocal Laser Scanning Microscope (CLSM)

A Leica® TCS SP2 Spectral Confocal Microscope (Leica Microsystems Inc., Suite 107, 410 Eagleview Blvd., Exton PA 19341) with Coherent Ti:sapphire multiphoton laser (Mira Optima 900-F) was used to study the distribution of the lipid and protein droplets inside the unprocessed liquid samples. Nile Red was used to stain lipids and fluorescein isothiocyanate (FITC) was used to stain the proteins. A stock solution of Nile red was made with 0.5mg/ml in acetone. A working solution of Nile red was made by mixing 0.10 ml of stock solution to 100 ml

of a 75:25 glycerol water mixture. A stock solution of FITC was made with 0.25% FITC in water. The working solution was obtained by further diluting the stock solution to 1% in water. Samples analyzed with the confocal laser scanning microscope were commercially sterilized eggs. A thin section of the sample of about 1mm thickness and 10mm X 10mm was cut using a surgical scalpel. The sample was placed on a pre-cleaned glass micro slide and stained with FITC first for 15 minutes followed by Nile red for another 15 minutes. The sample was washed twice with deionized water in between the two stains and also after the last stain.

Results and Discussions:

Scanning Electron Microscopy

Different structural networks were present in the retorted egg product as result of the various ingredients and different mixing techniques. Distinct differences in 3-dimesional structures (networks) were exhibited by the Formula E (basic egg formulation), formula HCE (hydrocolloid egg), and formula HCE-CS (hydrocolloid with Caseinate egg) egg samples. A dense network of proteins with fat globules distributed uniformly is one of the many possible microstructures expected from the Formula E sample. The C-SEM image of the Formula E sample shown in Fig 4.1 showed the protein matrix as a uniform cell-like structure. The dense protein network was predominant with small fat globules spread throughout the entire protein matrix. Different views showed the same kind of network because no other components exist. Figure 4.1a shows the dense protein network of the Formula E sample. Figure 4.1b shows the same sample at a higher magnification where the small fat globules can be observed. The PE sample was mixed by Megatron therefore the fat was uniformly dispersed.

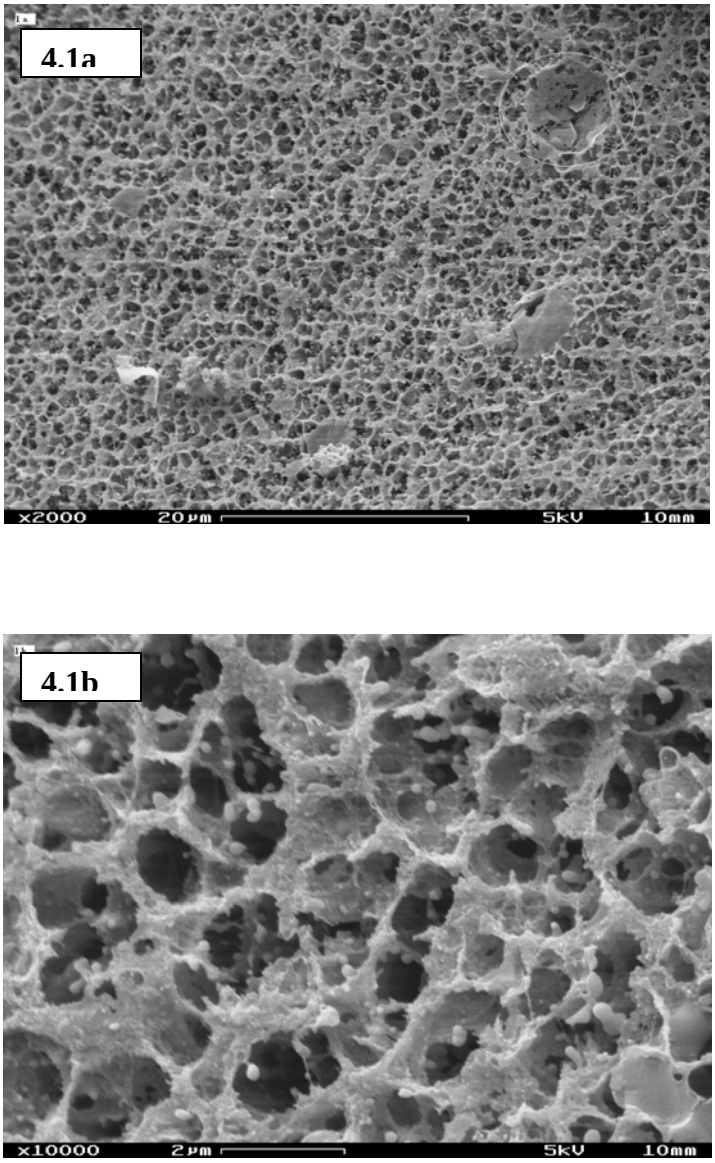


Figure 4.1(a), (b) - Scanning electron micrographs of retorted Formula E sample mixed by Megatron.

The Formula HCE sample mixed with a hand-held kitchen mixer was not very homogenous. At the time of filling the liquid mix into the pouches, uneven viscosity was already exhibited. The same non-homogeneity was also exhibited with the hand mixed Formula E. Fat separated out of the mix when held under refrigeration 12 to 24 hrs after mixing. None of the hand-mixed Formula E or Formula HCE samples were examined under the C-SEM. C-SEM images of the Megatron mixed formula HCE samples exhibited additional networks in addition to the protein. Both xanthan and starch appeared in the same

network dispersed within the protein. The xanthan-starch network was less dense when compared to the protein network. The size of the embedded xanthan-starch network depended on the homogeneity of the mix. Fig 4.2(a) shows a Megatron mixed sample with starch network (circled in the picture). The size of the starch network is about 50µm. Fig 4.2(b) shows a sample mixed with the CAFT kettle mixer. The average size of the starch network is about 200-300µm.

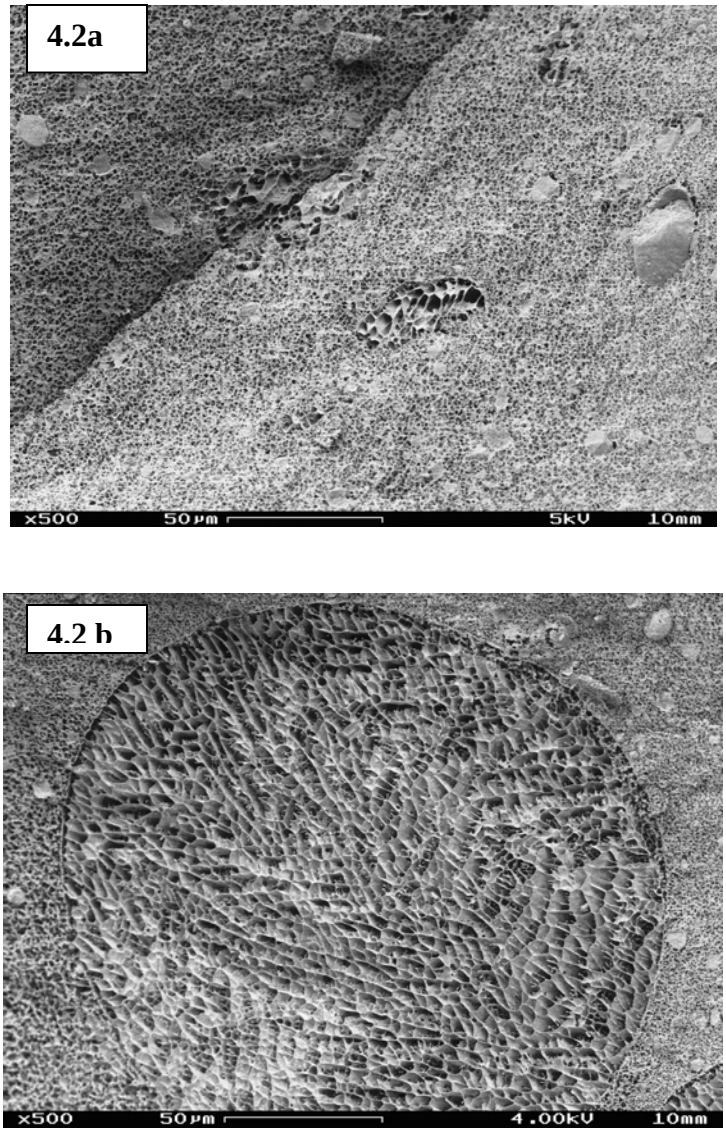
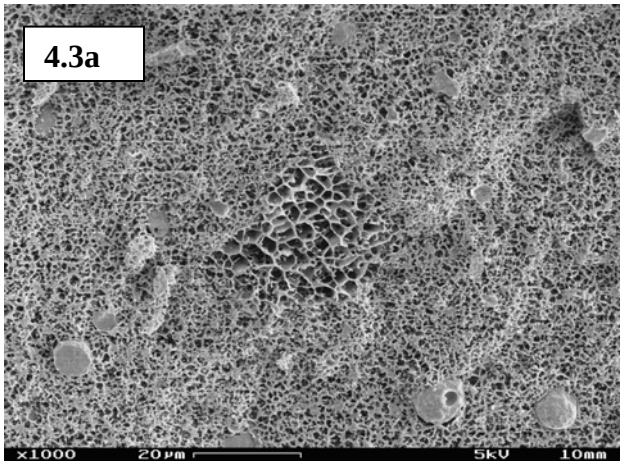
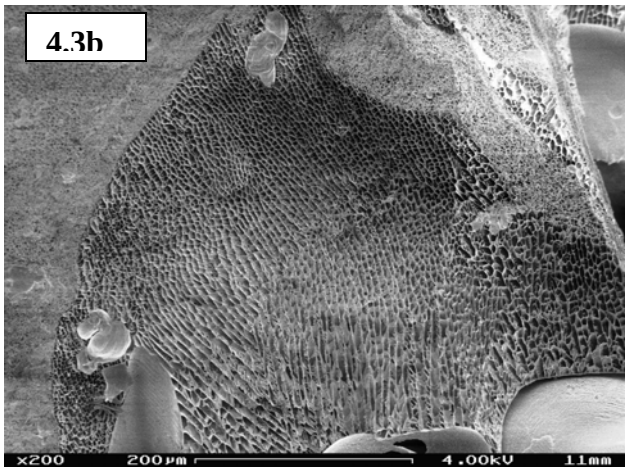


Figure 4.2 – Scanning electron micrographs of Formula HCE sample, (a)-Mixed with Megatron and (b)-mixed with Kettle mixer

C-SEM images of Formula HCE-CS samples exhibited similar hydrocolloid and protein networks exhibited by the Formula HCE sample images but a third network structure which did not resemble those of the starch or the protein was observed. This third network structure was denser than the protein and the xanthan-starch network. Fig 4.3 (a) shows the xanthan-starch and protein network in the megatron mixed sample. Fig 4.3(b) shows images of the formula HCE-CS sample mixed in the CAFT kettle mixer. Figure 4.3 (c) shows the third very dense network in the CAFT-kettle mixed formula HCE-CS sample. Figure 4.3 (d) shows the dense network of /figure 4.3c at 10,000 X magnification which shows the dense network to be a tightly packed mass of globules of the calcium caseinate.





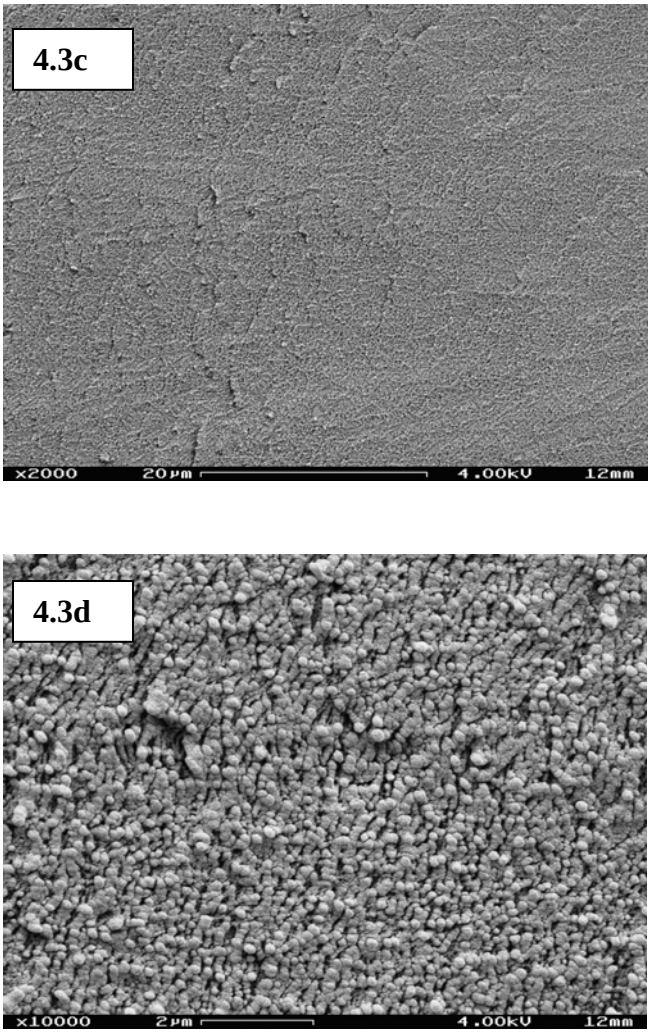


Figure 4.3 – Scanning electron micrograph of Formula HCE-CS samples
(a)-Megatron mixed sample
(b)- CAFT-kettle mixed sample
(c)- Dense network in CAFT-kettle mixed sample
(d) – Dense network at 10,000X magnification

Confocal Laser Scanning Microscopy

CLSM image of Formula E samples mixed with a Megatron is shown in Figure 4.4. The green color in the image represents the FITC stained protein. The red dots are Nile Red stained fat globules. The yellowish-orange area in the image represents a region containing both protein

and fat and the orange glow is because of the intimate mixing of green and red in a well-homogenized mixture. The primary concern during sample preparation was getting a proper dye penetration. Since the dye had to penetrate the thickness of the sample, the cross-section was cut as thin as possible. The other problem during staining is washing the sample of the extra stains. Having too much stain can cause the sample to fluoresce very brightly rendering it impossible to make any clear distinctions. Since the sample is too thin washing the sample should be done carefully so as to not rupture the sample. This leads to some "stray" stains in the image e.g.: dark red spots and green bubble in Fig 4.5 (b). In figure 4.4 individual red dots can be observed along with some orange glow. This shows that some of the fat has been mixed into the protein network but a good portion has separated from the protein matrix.

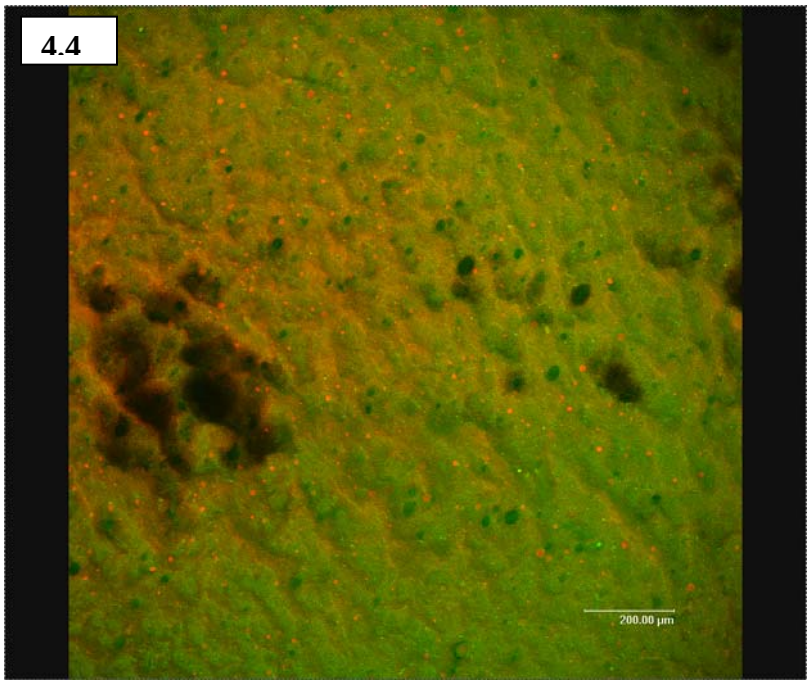
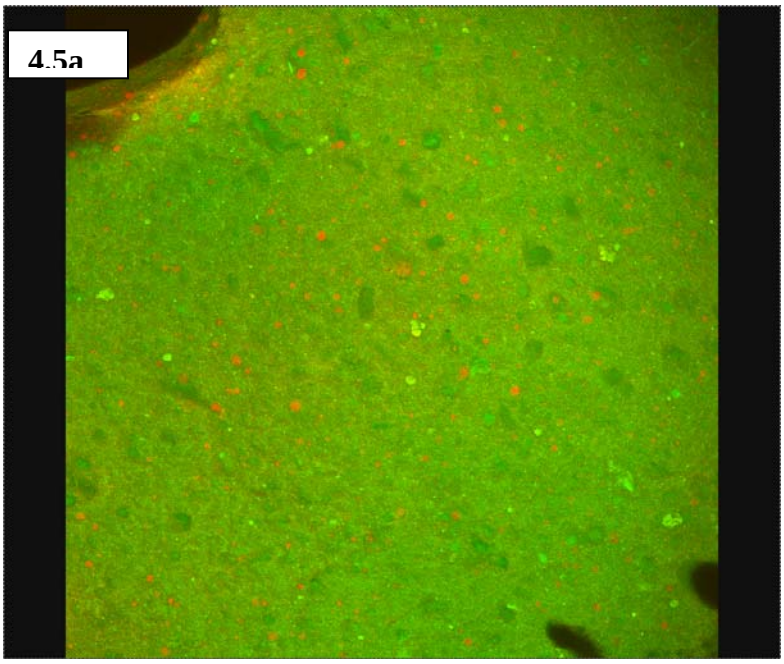


Fig 4.4 – Fluorescence image of Formula E sample stained with FITC (green) for proteins and Nile Red (red) for fats

CLSM images of unprocessed Formula HCE sample are shown in Figure 4.5(a) and (b). Figure 4.5 (a) represents the Formula HCE sample that was mixed using a hand held kitchen mixer. Figure 4.5 (b) shows the Formula HCE sample mixed in the Megatron. In figure 4.5 (a) it can be clearly seen that the fat has not mixed into the protein network at all. This could be observed even as the mix was being filled into the pouches. Since there is a delay between the time the mix is made to the time it gets packaged into quad laminate pouches, the margarine had separated to the top. The average size of the fat droplets in the kitchen mixer samples was about 5-50µm. The separate red dots in figure 4.5 (a) show the fat globules.



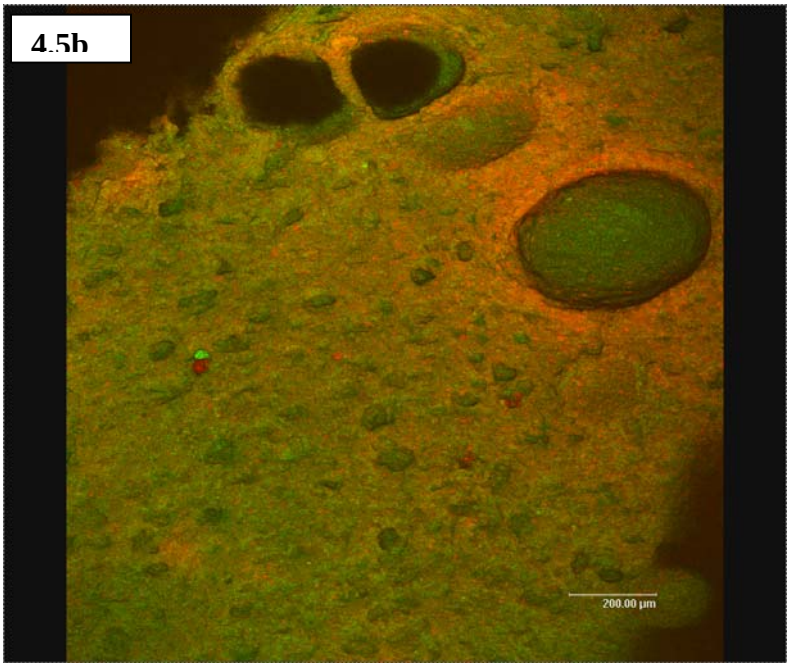


Fig 4.5 - Fluorescence image of Formula HCE sample stained with FITC (green) for proteins and Nile Red (red) for fats
(a) – Mixed with Hand held Kitchen mixer
(b)- Mixed in the megatron

Figure 4.5 (b) shows the HCE sample mixed with the Megatron. It can be seen that there are very few individual fat globules; instead we see an orange shade throughout the sample proving that most of the fat is well mixed into the protein matrix. In comparing sample HCE (fig 4.5b) and sample E (fig 4.4) it can be observed that even though both are mixed with Megatron, the one with the xanthan gum and starch had a better distribution of fat globules in the final product. The fat droplets had an average size distribution of about 0-20µm in the megatron mixed samples. Formula HCE-CS which was mixed in kettle mixer at CAFT, Rutgers can be seen in Figure 4.6. In this figure it can be seen that the fat is not well homogenized ad the Megatron HCE sample but is still better than the hand mixed sample. The size distribution of the protein globules in the CLSM image of the raw Kettle homogenized sample was between 5-25µm

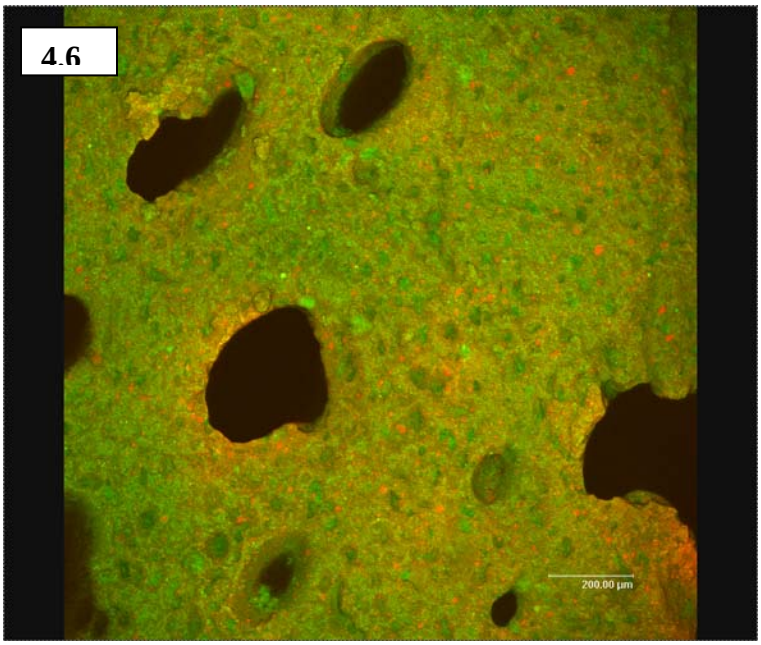


Fig 4.6 - Fluorescence image of Formula HCE-CS sample mixed in the kettle mixer and stained with FITC (green) for proteins and Nile Red (red) for fats

Conclusion

Confocal laser scanning microscopy and scanning electron microscopy were found to be useful for determining the microstructure of retorted MRE egg products. Scanning electron microscopy was instrumental in understanding the distribution of the starch and degree of homogenization. The size of the area covered by fragments of the starch network depended on the type of homogenizer used for dispersing the ingredients. Of the homogenizers used, Megatron (Kinematica, Inc., OH, USA) yielded the best homogenization. The average size distribution for a samples mixed with megatron were between 20-100µm. This can also be seen in the CLSM images. The Megatron samples had the smallest size for the fat droplets. Most of the fat was small enough not to be distinguished in the CLSM image. The fat droplets had an average size distribution of about 0-20µm in the megatron mixed samples. Samples that were mixed with the hand held mixer had the least homogeneous structure. Figure 4.5(a) shows a The

Formula HCE sample mixed with the hand held kitchen mixer showed inadequate dispersion of fat droplets did not mix within the protein matrix. The average size of the fat droplets in the kitchen mixer samples was about 5-50μm. The kettle mixer resulted in a size distribution that was between the megatron and the kitchen mixer. The average size if the starch fragments dispersed in the protein network of the retorted product that homogenized in the kettle mixer was between 50-500μm. The size distribution of the protein globules in the CLSM image of the raw Kettle homogenized sample was between 5-25μm. A unique dense network contributed by calcium caseinate was observed in the Formula HCE-CS samples. This sample did not expand as much as the Formula HCE sample and could have affected the retorted product texture.

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Chapter 5

Conclusions

The presence of hydrocolloids in an egg blend not only influences the firmness and liquid-like properties of the gelled egg but also influences the rheological properties when subjected to heating, cooing and re-heating. Eggs with hydrocolloids have much higher G' values compared to G' of the Formula E, this may be caused by syneresis. Due to syneresis the top plate my "slip" instead of actually deforming the sample. In addition, these values indicate that the hydrocolloids may have maintained the liquid in the matrix. However, hydrocolloids also affected the textural stability making the product firmer on re-heating after the first gelatinization process. Thermal processing at 90° C in a flexible pouch with 2.6 bars overpressure also compressed the gel resulting in a higher hardness value for the product compared to processing without overpressure at the same temperature.

Confocal laser scanning microscopy and scanning electron microscopy were found to be useful for determining the microstructure of retorted MRE egg products. Scanning electron microscopy was instrumental in understanding the distribution of the starch and degree of homogenization. The size of the area covered by fragments of the starch network depended on the type of homogenizer used for dispersing the ingredients. Of the homogenizers used, Megatron (Kinematica, Inc., OH, USA) yielded the best homogenization. The average size distribution for a samples mixed with megatron were between 20-100μm. This can also be seen in the CLSM images. The Megatron samples had the smallest size for the fat droplets. Most of the fat was small enough not to be distinguished in the CLSM image. The fat droplets had an

average size distribution of about 0-20µm in the megatron mixed samples. Samples that were mixed with the hand held mixer had the least homogeneous structure. Figure 4.5(a) shows a The Formula HCE sample mixed with the hand held kitchen mixer showed inadequate dispersion of fat droplets did not mix within the protein matrix. The average size of the fat droplets in the kitchen mixer samples was about 5-50µm. The kettle mixer resulted in a size distribution that was between the megatron and the kitchen mixer. The average size if the starch fragments dispersed in the protein network of the retorted product that homogenized in the kettle mixer was between 50-500µm. The size distribution of the protein globules in the CLSM image of the raw Kettle homogenized sample was between 5-25µm. A unique dense network contributed by calcium caseinate was observed in the Formula HCE-CS samples. This sample did not "Puff" up as much as the Formula HCE sample and could have affected the retorted product texture..

The results of the rheological and ultrastructural studies demonstrate that the texture of a retorted egg product will depend not only on the formulation but the manner in which the ingredients are dispersed and the thermal process employed. Re-heating of a cooled gelled egg product for prolonged periods resulted in the formation of a firmer and more elastic texture. Overpressure during thermal processing should be minimized to permit the product to expand during the process of gelling to develop the desirable fluffy texture of scrambled egg.