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The Effect of Storage Temperature and Time on The Quality of Spray Dried Egg Powder

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THE EFFECT OF STORAGE TEMPERATURE AND TIME ON THE QUALITY OF SPRAY DRIED EGG POWDER

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ABSTRACT

Dehydration is a good approach for egg preservation. However, dried egg products may still suffer from deterioration if stored in an abused temperature environment or prolonged times. Storage conditions can induce undesirable reactions and loss of functionality and quality. The objectives of this study were to evaluate the physicochemical characteristics and functionality of spray dried egg yolk powders, which included plain whole egg (PLWE), free flow yolk (FFY), enzyme modified yolk (EMY), and free flow whole egg (FFWE) stored at 4 °C, 25 °C, 43 °C or 54 °C, over a 2-month period; and provide useful suggestions to maximize quality when used in the food industry. Functionality and physicochemical tests included solubility index, emulsion stability, viscosity and heat stability of emulsions, free fatty acid levels, lipid hydro peroxides, color change, and morphological appearance. Solubility Index results indicated that FFWE was the most soluble sample followed by PLWE, EMY, and FFY. Emulsion stability results revealed that EMY formed the most stable emulsion followed by FFWE, FFY, and PLWE. Free fatty acid levels indicated that EMY deteriorated faster, followed by FFWE, FFY, and PLWE. Lipid hydro-peroxides were most abundant in EMY followed by FFY, PLWE, and FFWE. Total color change was highest for FFY, followed by PLWE, EMY, and FFWE. All samples passed the heat test after 1 month of storage but failed after 2 months of storage at all temperatures. The morphology of the egg powder particles revealed that high processing temperatures increased porosity which resulted in greater oxidation and storage time induce agglomeration of the egg powders. Overall, physicochemical changes found during the storage of dried egg powders were more evident at 43 °C or 54 °C. Lipid oxidation was responsible for the decrease in quality of dried egg powders. Therefore, most effective approaches are required to prevent oxidation reactions. Adjusting the

storage temperature and humidity, and using an effective packaging method with an oxygen barrier is strongly suggested.

CHAPTER 1: INTRODUCTION

Food deterioration is a worldwide problem for food products during storage. Deterioration reduces the nutritional quality over time and food products become inedible. Low moisture in food products helps to inhibit the growth of pathogenic microorganisms and extends the product's shelf life. Dehydration is a good process approach for food preservation. However, dried food products suffer from significant deterioration when it is stored in abusive environments such as high temperature (>40 °C) and storage time (time >2 months). During shipping overseas, shipping temperatures typically exceed room temperature. These shipping temperatures and storage conditions can induce undesirable chemical reactions such as Maillard reaction and lipid oxidation, resulting in a significant decrease in food quality.

Dried egg yolk powder is a product that has broad applications in the food industry. It can be used as an emulsifying agent to make salad dressings, mayonnaise; infant formula, bakery products, or it can be mixed with other protein powders. Dried egg yolk powder contains about 35% protein, 50% fat and a small amount of glucose. Up to 1.8% of glucose (dry basis) may be present in egg yolk powder(USDA, 2017). Chemical reactions are highly likely to occur during storage resulting in loss of quality.

In this study, the storage stability of three commercial spray-dried egg yolk powders and one whole egg powder was evaluated. The dried egg powders were stored at four temperatures (4, 25, 43 or 54 °C) for two months. The selected temperatures were chosen to simulate refrigeration, ambient, shipping and extreme temperatures, respectively. Physicochemical changes and functionality were studied with the objective of providing useful suggestions to maximize quality when used by the food industry.

CHAPTER 2: LITERATURE REVIEW

2.1 Nutritional importance of chicken eggs

Eggs are a rich source of proteins, lipids, and other nutrients of high biological value. Egg proteins have high digestibility that makes the eggs a valuable food for people recovering from illness, most bland diets include eggs (Stadelman, 1994). Egg proteins contain all ten essential amino acids including arginine, phenylalanine, leucine, lysine, valine, threonine, methionine, isoleucine, histidine, and tryptophan as well as three non-essential amino acids, which are alanine, aspartic, and glutamic acid. Semi-essential amino acids arginine, cysteine, glycine, proline, serine and tyrosine are also present in eggs. The yolk contains unsaturated fatty acids including oleic, linoleic, palmitoleic and linolenic acid, saturated fatty acids including palmitic, stearic and myrisic acid. Docosahexanoic acid (DHA, C22:6n-3) is found in high levels and used in infants formula because it is essential for retinal function (Carlson et al., 1991). DHA has been found to be important in the prevention and treatment of chronic degenerative diseases including atherosclerosis, cancer, rheumatoid arthritis, psoriasis, age-related macular degeneration, and Alzheimer's disease (Smit, Oelen, Seerat, Boersma, & Muskiet, 2000). Similarly, egg yolk is a good source of arachidonic acid (AA, C20:4n-6) needed for infant nutrition.

Phospholipids make approximately 9% of egg yolk. Phospholipids are of amphiphilic character and have excellent emulsifying properties. Important phospholipids including phosphatidylcholine and phosphatidylethanolamine which are important for brain function (Zeusel, 2013). Egg yolk is also rich in iron, phosphorus, trace minerals, fat-soluble vitamins like A, D, E, and K and many of the water-soluble B vitamins. Antibodies found in egg yolk have potential antibody application as a natural microbial agent for preservation and bacteria inhibition (Selvan, Sentila, & Michael, 2012). Overall eggs provide a balanced source of nutrients for persons

of all ages as they significantly contribute to the body's nutrient needs during rapid growth. They are also suitable for nutritional improvement of several kinds of foods since they have four major nutritional components: proteins, lipids, all necessary vitamins (except Vitamin C), and minerals (Mikova, 2006). On average a large egg contains around 213 mg of cholesterol. Studies have demonstrated that eating two eggs per day had almost no effect and that four eggs per day had only a slight effect on human serum cholesterol (Ginsberg et al., 1994). The most recent American Heart Association guidelines no longer include a recommendation to limit egg consumption (Kritchevsky, 2004). Profile and composition of egg yolk also makes it a high value raw material for the development of value-added ingredients for use in food and pharmaceutical industries.

2.2 Stability of fresh egg products

The term stability refers to the ability of a substance to resist change over a specific period of time (Barbosa et al.,2008). The length of time a food product remains acceptable is known as storage stability or shelf life. Eggs have a shelf life of 4 to 5 weeks when stored at refrigeration temperatures < 4 °C. Raw whole eggs beaten and egg yolks can last up to 2 days and raw egg whites up to 4 days at refrigeration temperatures of 4 °C. Eggs stored at room temperature have a shelf life of approximately 2 weeks (Bhale et al., 2003). Problems encountered during storage of eggs include weight loss, interior quality deterioration and microbial contamination. Certain microorganisms can penetrate into the interior of eggs and contaminate the internal content (Bhale et al., 2003).

2.3 Stability of dried egg products

To enhance shelf life, water content of eggs is reduced by dehydration methods. Methods that influence the quality and storage stability of dried eggs have been studied extensively. The principal behind dehydration methods is the control of the water content by removal or binding to protect the food against microbial and chemical deterioration (Labuza, 1980). Water content is the total water present and water activity indicates the water that is bounded structurally and chemically. Water activity indicates the vapor pressure of water in a food product divided by the standard state partial vapor pressure of pure water at the same temperature. Water content alone is not a reliable predictor of stability; therefore, water activity and water content concepts are used together to specify the status of a food sample. As the portion of the water that is chemically bound, a_w is a thermodynamic term related to the chemical potential of water. Analyzing it thermodynamically, kinetic reactions are studied to predict the shelf life of food products. The study of the kinetic reactions of deteriorating food products is shown in Figure 2.1.

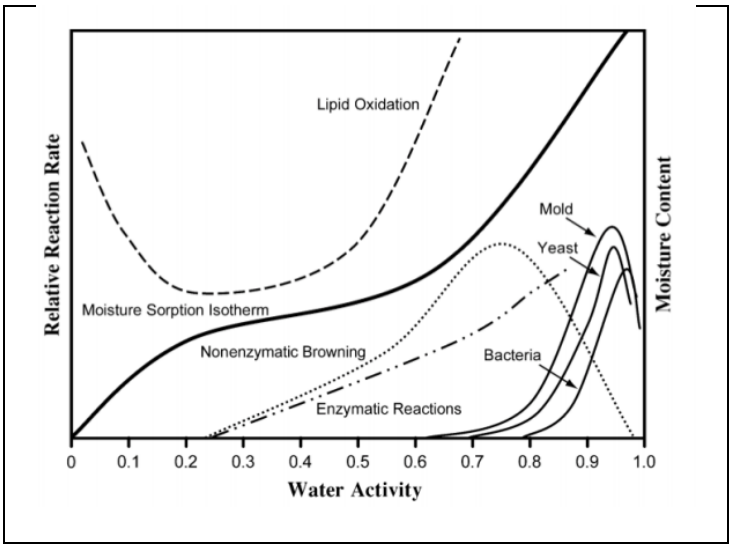

Figure 2.1 Reactions occurring at given water activity and moisture content (Labuza, 1984)

Food deterioration is a combination of enzymatic and chemical deterioration, non-enzymatic browning, lipid oxidation, vitamin loss, enzymatic activity , sensory changes, and physical deterioration (Labuza, 1984). Egg powder products of water content of 3-6% and a_w of 0.22 to 0.27 are susceptible to deterioration reactions (Koç et al. 2012). Kog reported that powdered egg stored in environments with a_w up to 0.577 showed structural changes such as sticking, collapse, caking, and agglomeration (Koç et al. 2012).

Another important concept in food deterioration is the glass transition temperature T_g , which is defined as the temperature at which an amorphous system changes from a glassy to rubbery state (Koç et al., 2012) . Water is responsible for depressing T_g in a food system. It has been reported that moisture reduction had positive effect on T_g (Koç et al., 2011). The T_g of egg powder was reported to be 114 °C, physical properties of powdered egg worsened if processed or stored above this temperature (Koç et al., 2012). Figure 2.2 displays the change in equilibrium moisture content of spray dried powdered eggs to a_w at 25 °C.

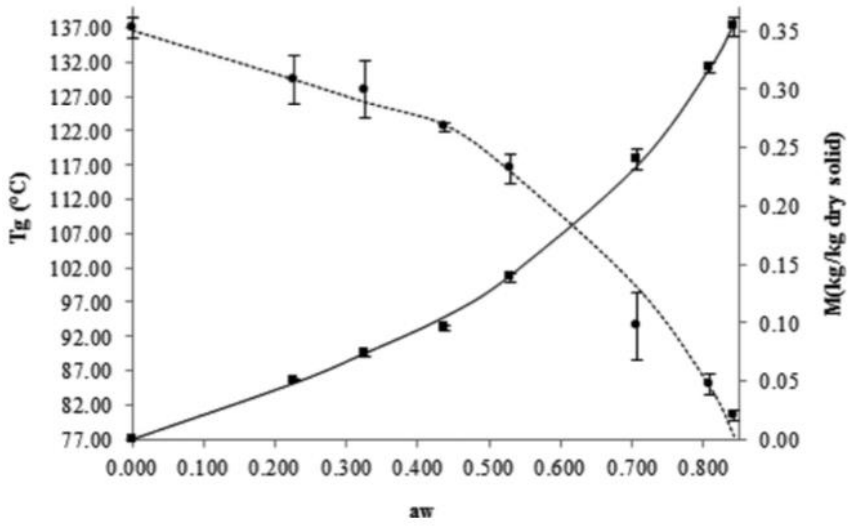

Figure 2.2 Relationship between a_w water content % and T_g of egg powders (Koç, et al., 2012)

2.3.1 Egg yolk powder

Egg yolk powder is a product with many advantages including shelf stability and low transportation costs. The low moisture content prevents the growth of microorganisms and reduces chemical reactions. Dried egg yolk powder has many applications in the food, medical, pharmaceutical, cosmetic and biotechnological industries due to its functionality and bioactivity. Egg yolks are best known for their emulsification properties in mayonnaise and other dressings. In the bakery industry, yolks impart a rich color that comes from carotenoids present in the lipid portion of the yolk. One hundred grams of dried egg yolk contains 33.7g of proteins, 58.1 g of lipids, 2.7 g of water, and 2.1g of carbohydrates (American Egg Board, 2017). It contains a large number of nutrients including lipids, amino acids, minerals and vitamins. Moisture level, storage temperature, particle size, acidity, carbonate additives and gas packaging have been considered individually and in combination as to their influence in the stability of dried egg yolk products (Stadelman, 1994). Karel and Yong (1981) suggested that the water content is the major factor controlling lipid oxidation in dehydrated foods, but effects are complex. While each of these factors can be utilized in some beneficial manner, none has proven as successful in producing a stable egg product as the removal of glucose from liquid egg prior to drying (Stadelman, 1994).

Cholesterol oxidation as well as Maillard reaction are more advanced when egg powders are stored at room temperature than storage at 4°C (Bhandari, 2013). Storage at room temperature also generates loss of retinol and tocopherol content, whereas storage at 4°C does not further damage powder properties (Bhandari, 2013). An increase in viscosity of whole egg or egg yolk products can occur during storage, especially at temperatures greater than 38 °C (Bhandari, 2013). Whole egg or egg yolk powders stored for 3 months at 35 °C lose part of their emulsion stabilizing

properties, whereas storage for 6 months at 24 °C or less did not have any adverse effect (Bhandari, 2013)

2.3.2 Maillard browning

The interaction of reducing sugars with amino acids was first studied by Maillard, thus the reaction bears his name (Stadelman, 1994). The Maillard reaction is a non-enzymatic browning reaction that also occurs during drying and storage conditions. Color loss due to oxidation of carotenoids and Maillard browning are responsible for the main sensory defect in powdered egg (Guardiola, Codony, Miskin, Rafecas, & Boatella, 1995). In the production of dried egg powder, this reaction causes undesirable browning and formation of unwanted flavor (Wong, Wong, & Chen, 2008). The initial bonding between the glucosidic hydroxyl groups of sugars and the amine groups of peptides on proteins is followed by other changes which result in the formation of brownish-colored product (Stadelman, 1994). Amino acid degradation products result in aldehydes, also known as Strecker degradation products. Flavor is the major factor determining the acceptance of products by consumers, therefore, reaction temperatures must be controlled to obtain the least amount of oxidation in egg powders. Undesirable formation and increase of 1-octen-3-ol (green earthly scent) was observed during the storage of egg products due to oxidation (Cécile Rannou et al., 2013). This compound is a result of enzymatic and non-enzymatic degradation of ω_6-fatty acids (Cécile Rannou et al., 2013). Maillard compounds such as pyrazines, furans and pyrroles were identified in egg powders, these compounds are greater in whole egg powder than egg yolk powder, in both cases they increase with storage time and higher storage temperature. To limit color changes from Maillard reaction desugarisation is sometimes performed prior to spray drying (Stadelman, 1994). It has been suggested that the discoloration of the dried whole egg was due, at least in part, to a reaction between cephalin amino group and aldehydes

(Sproston & Akoh, 2016). To monitor for color changes due to Malliard reaction, egg samples can be monitored using LAB color space system that measures the lightness of a sample and two color channels, a and b, and plots color-metric distances in a XYZ coordinate system.

2.3.3 Formation of lipid oxidation products

Egg products have a high lipid content that can range from 45 to 64 % in spray dried powders (Obara et al., 2006) . All fat containing foods are susceptible to spoilage through auto-oxidation. During the initiation phase, external energy, such as light, acts on the unsaturated fat in the presence of catalysts such as heavy metal ions, to produce free radicals. After, lipids go to peroxidation, the process during which they are deteriorated in a free radical autocatalytic oxidation reaction with atmospheric oxygen (Blaszczyk et al., 2013) . Lipid oxidation is a cascade phenomenon ensuring continuous delivery of free radicals, which initiate continuous oxidation (Blaszczyk et al., 2013). Peroxides can then catalyze the formation of more free radicals or decompose into aldehydes, ketones and alcohols (Depree & Savage, 2001). This process results in food rancidity which manifests itself as the change of taste, scent and color as well as decrease in shelf life of the product (Blaszczyk et al., 2013).

In many foods, water acts as medium of many reactions and it significantly influences lipid oxidation. Moisture content in food products can either inhibit lipid oxidation reactions or promote them (Obara et al., 2006). The inhibition of lipid oxidation can be achieved by lowering the oxygen diffusion to the sites of oxidation, lowering catalytic properties of metals that cause the metals to chelate and have a binding effect on hydroperoxides. When the water content corresponds to the monolayer of the food product minimum lipid oxidation had been observed (Obara et al., 2006). In foods with very low a_w, the increase in a_w may slow down the rate of lipid oxidation. It can be assumed that water acts as a barrier to oxidation sites. Cholesterol oxidation may undergo the same

free radical mechanism as lipid oxidation. During storage, cholesterol content is reduced, consequently there is an increase in cholesterol oxidation products, hence there is significant reduction in the quality of the egg product (Morgan & Armstrong, 1992). Previous studies have found that during the drying process the increase in oxysterol levels is greater in whole egg product than egg yolk powders (Obara et al., 2006). Oxidation products are associated with human diseases; research evidence links some cholesterol oxides to coronary artery disease and certain types of cancers (Obara et al., 2006). Cholesterol oxidizes spontaneously when it is exposed to air and factors such as heat, light and oxidative agents act as catalyzers (Yang and Chen, 2011). All these factors need to be taken into account during the preparation of spray dried egg products and its storage conditions. Oxidation results in product loss which has a significant economic impact, in addition, oxidation also leads to the generation of toxic substances (Mehta, Darji, & Aparnathi, 2015). Encapsulation materials such as gelatin, pullan, or lactose have been used to cover the egg fat further and prevent the fat from leaching out from the powder particle to the surface (Koç et al., 2011).

2.4 Egg powder processing

The egg industry has been processing egg to remove water since 1900. The egg industry in the United States expanded rapidly during World War II due to the demand for egg products at that time. The United States Department of Agriculture (USDA) estimated egg consumption to be 250.1 eggs per person in the year 2015 and the egg industry is estimated to be worth over $96 billion in the United States. It is projected that the per capita consumption of eggs will reach 258.3 in 2016 (Statista, 2017). Egg products refer to the eggs that have been separated from their shells to produce other products. The FDA estimates that 17% of eggs are sold as such. Egg products

act as a functional ingredient used in the preparation of several food products, therefore dehydrated egg products have been made available to egg consumers to substitute for shell eggs. The benefits of dehydrated egg products go beyond extending its shelf life. Moisture removal in egg yolk products is important for egg product stability and to reduce costs. Recently, dehydrated egg yolk products have also been used by egg consumers instead of shell egg as raw material because of its longer shelf life, lower transport and storage cost, and specific functional properties (Koç et al. ,2011). Dehydrated egg yolk powders serve as raw materials for other products. Recently, there has been an increase in the use of dried egg powders that represents a growing importance of the ready-for-use packages industry (Franke & Kießling, 2002). By reducing the moisture content of egg products, microbial stability is achieved. Moisture is removed by a combination of heat and mass transfer; the system used to obtain dried egg products determines its quality.

There are a variety of mass transfer systems, among the most common are pan drying, freeze-drying and spray drying. For industrial purposes pan drying is not easily scalable; freeze-drying is not very popular due to its high initial cost although it provides the best quality. Although freeze drying is considered one of the best practices for reducing thermal damage, spray drying is more advantageous in terms of cost, energy and throughput (Behboudi-Jobbehdar et al., 2013). Spray drying is commonly used in the egg industry to remove water and prepare powdered egg products; it is also used for dairy products, blood plasma, organic and inorganic chemicals, rubber latex, ceramic powders and detergents(Mujumdar, 2015). According to the USA Emergency Supply, powdered eggs are non-perishable when stored in an airtight container, absence of oxygen and placed in a cool storage environment, under these conditions, powdered eggs have a storage life of 5 to 10 years. Other advantages besides extended shelf life include not worrying of breaking eggshells; representing lower handing costs. USA Emergency supply states that a dozen fresh eggs

take up about 122 cubic inches in their carton, but when powdered they are reduced to 22 cubic inches per dozen powdered eggs so transportation costs are drastically reduced.

For the egg industry, the major microorganisms of concerns are *salmonella, campylobacter* and *staphylococcus aureus*. The Egg Product Inspection Act requires that all egg products distributed into commerce be pasteurized. High temperature-short time (HTST) is commonly used in industry before the liquid egg products go through the drying process. Spray drying alone does not kill microorganisms, however it extends the product shelf life. The spray drying process leads to changes in sensitive egg components, egg proteins and affects the functional properties of whole egg after reconstitution (Franke & Kießling, 2002). The formation of oxysterols has been widely confirmed, and their formation depends on two main factors: first, the air heating system used, direct or indirect and second, the inlet and outlet temperatures (Guardiola et al., 1995).

2.4.1 Spray drying technique for stabilization of egg products

Figure 2.3 depicts a standard typical spray drier chamber. A pumpable slurry is prepared with egg yolks that will pass by the feed pump (a). The spray drying process begins by feeding the liquid slurry and hot gas, processed by a heating unit into the inlet of the spray drier (b). Inside the spray drier chamber with a conical bottom, the egg slurry is dispersed by atomization into a stream of hot gas in the form of fine droplets (c). Droplets are atomized by various spray nozzles including two-fluid, ultrasonic, rotary (high speed spray disk) or pressure (hydraulic) nozzles at 5,000 to 10,000 revolutions per minute. Pressure nozzles are often used for their simplicity and ease of tuning (Dobry et al., 2009). A hot gas flow (up to 100 °C) comes in contact with the fine droplets of slurry and mass transfer occurs at their interphase (d). The flow of slurry and hot gas may be co-current, countercurrent or a combination of both. Residence times vary from 3 to 6 seconds in concurrent dryers and 25 to 30 seconds in countercurrent dryers. Cooled gas is drawn out by an

exhaust fan through a discharge line close to the bottom of the cylindrical section (e). Moisture is rapidly vaporized from the droplets leaving residual particles of dried solids. These particles are cooled by evaporation. Most of the dry solids settles out in the gas and are separated from the gas stream (f) and collected usually by a cyclone separator (g) ideally into a conveyor belt to allow product to finish the cooling process. Tiny dust particles are collected in the baghouse or dust bag (h). In spray drying the evaporation from the surface drops leads to initial deposition of solute on the surface before the interior of the drop reaches saturation. The rate of diffusion of solute back into the drop is slower than the flow of water from the interior to the surface and the entire solute content accumulates at the surface. The final dry particles are hollow and porous.

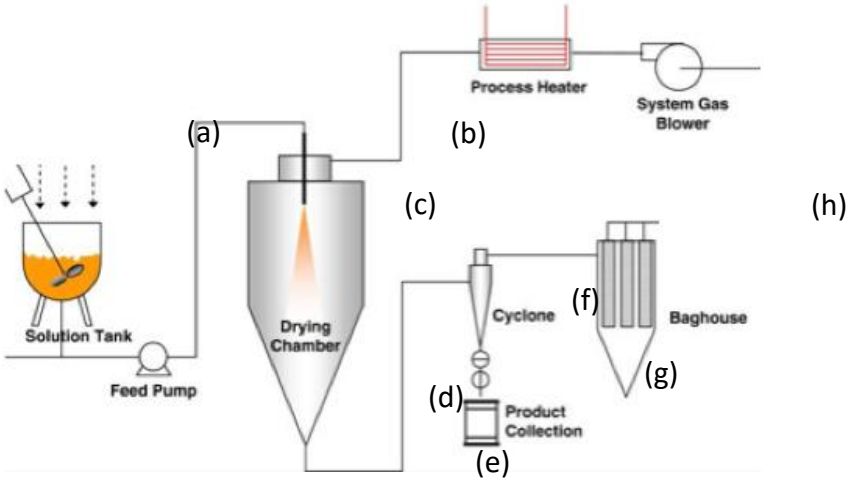

Figure 2.3 Spray drier schematic (Gea, 2015)

Key advantages of spray drying are the very short drying times which permit drying of heat sensible materials and the production of solid or hollow spherical particles. Heat transfer coefficients for individual drops may be estimated, then parameters like average drop diameter, atomizer speed, flow rates, and operating temperatures may be manipulated to obtain desired

consistency, bulk density, appearance and stability. The final product has smaller dimensions that represent economic profit (Mc Cabe, 2005).

Quality changes in spray dried egg products include chemical modifications such as the Maillard reaction, carotenoid oxidation, oxysterol formation, lipid oxidation and loss of essential polyunsaturated fatty acids and fat-soluble vitamins, and formation of thiobarbituric acid-substances occur or are initiated during spray drying (Guardiola, Codony, Miskin, et al., 1995). The flavor characteristics of food products containing unsaturated fatty acids can be drastically affected when these lipids are in direct contact with oxygen (Nijhuis et al., 1998). Lipid oxidation in spray-dried eggs is currently monitored by determination of the peroxide value and thiobarbituric acid-reactive substances (Caboni et al., 2005). Oxidative rancidity measured by peroxide value develops an off flavor usually described as "fishy". Depending on storage conditions of temperature and time, these reactions can accelerate or continue at slow rate (Guardiola, Codony, Manich, Rafecas, & Boatella, 1995).

2.4.2 Egg yolk processing

Egg yolk is the main ingredient of mayonnaise and sauce emulsions, ice cream, and bakery items. This is due to the formation of stable emulsions by decreasing the interfacial tension between the water and oil surrounding the oil droplets. Egg yolk represents one third of the egg's total weight, it contains 34% lipids and 16% protein. The functional properties of egg yolk come from the phospholipid and protein components. To enhance the functional properties in egg powder, eggs are processed to separate the yolk from the whites and produce egg yolk powder. The process for producing dried egg yolk products is to separate the yolk from the albumen, de-glucose by fermentation or enzymatic means with glucose oxidase, spray dry and application of an additional heat treatment. Spray dried egg products are stored at 54.4 °C for 7 to 10 days to

pasteurize the product and improve its functionality meeting Title 9 Code of Federal Regulations (CFR) 590.575. Then the product is tested for *Salmonella* and if it tests negative it can be packaged and sent to the market.

2.4.3 Whole egg processing

Whole egg is used in bakery applications for its foaming, gelling, binding and coloring properties. When whole egg is combined with sugar it is capable of producing stable foams. Whole egg is used for its binding properties to create firm pastries and crusts. Whole egg is used in pasta to add color and texture, the egg whites increase firmness and the egg yolk aids pasta formation during cooking (Bhandari, 2013). In 2012, 70.4 million cases of eggs were processed in the egg industry (American Egg Board, 2017) . Eggs can be processed to a dry or liquid form. To extend the shelf life of eggs they must be processed to remove or minimize deterioration factors. One of the first factors that play a role in deterioration is high level of water activity and microbial activity. For dry whole egg products, moisture is removed in a sanitized matter that avoids cross-contamination. Dried egg products are widely used in food preparations because of their microbiological safety and their reduced volume with respect to unshelled or liquid eggs (Caboni et al., 2005) The process of egg products includes receiving shell eggs, and washing/sanitizing the eggs. Eggs are mechanically rotated several times over a bright light to examine the internal quality of the egg. The function of the candling process is to remove dirty, cracked and ineligible eggs before the breaking step. Ineligible eggs are defined for having traces of blood. Then the eggs are broken and liquid egg products are separated, filtered, blended, and mixed. Then products are cooled and pasteurized. The appeal of dried eggs is their convenient and long shelf life; in fact, this product is stored without particular care (Caboni et al., 2005). Drying dehydrates eggs and later, the eggs are packaged, stored and shipped. The safety and quality of powdered whole egg

depend on two critical steps: the drying process and storage (Caboni et al., 2005). Unfortunately, high drying temperatures might initiate unwanted oxidation reactions that can continue during storage. Chemical modifications, such as oxidation and non enzymatic browning with formation of undesirable compounds can occur during these processing steps (Caboni et al., 2005).

2.4.4 Enzymatic hydrolysis

Removal of glucose from the egg slurry before dehydration either enzymatically using glucose oxidase or by fermentation with yeast can reduce browning during dehydration and subsequent storage (Sankaran, Godbole, & D'Souza, 1989). Glucose removal also has the added benefit of longer shelf-life and enhanced microbial tolerance (Wong et al., 2008). One of the ways to remove glucose from egg is adding glucose oxidase before spray drying takes place, as allowed under FDA regulations (Wong et al., 2008). An enzyme system composed of glucose oxidase and catalase, an enzyme which catalyzes the decomposition of hydrogen peroxide to water and oxygen, was developed for use in the deoxygenation of foods and beverages (Figure 2.4).

$$\beta - \text{D glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconolactone} + H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{spontaneous/catalase}} 2H_2O + O_2$$

$$\text{D} - \text{gluconolactone} + H_2O \xrightarrow{\text{spontaneous/lactonase}} \text{gluconic acid}$$

Figure 2.4. Enzymatic hydrolysis mechanism (Wong et at., 2008)

From the net reaction, it is apparent that the enzyme system can be used to stabilize foods by the removal of oxygen in the presence of excess glucose and the removal of glucose in the presence of readily available oxygen (Stadelman, 1994).

2.4.5 Phospholipase A2

Egg yolk is rich in lecithin, which is a polar phospholipid. Emulsifying properties of phospholipids found in eggs can be improved by enhancing the amphiphilic character through the specific cleavage of a fatty acid from the diglyceride moiety. Phospholipase enzyme modifiers, such as phopholipase A2 are typically added to egg products available in the market which aim is to convert the phospholipid into stable lyso-phopholipid by hydrolysis; causing the specific release of fatty acid linked to C-2 glyerol. A study in transesterification of soy lecithin by lipase and phospholipase revealed the replacement of fatty acids such as palmitic, linoleic and linolenic improved oxidative stability of the resulting product. According to DSM Bright Science Better Living, commercial enzymes like MAXAPAL® A_2 convert about 85% of the egg yolk's phospholipids which results in improvement in emulsifying properties (Sanovo, 2017). The use of egg processing enzymes help consumers to produce dressings and mayonnaise with better emulsification, greater viscosity and improved thermo-tolerance and stability.

2.4.6 Free flowing agents

Egg powders tend to stick or cake due to environmental factors such as humidity. Flow ability of egg powders can be improved by adding a free-flowing agent. Either sodium silico-aluminate or silicon dioxide is permitted to be added at levels of less than 2% and 1%, respectively (Stadelman, 1994). The addition of a free-flowing agent resolves the caking problem resulting in higher quality egg powder products.

2.5 Effects of spray drying on whole egg and egg yolk products

The entire process of spray drying exposes egg yolk to heat during pumping, concentration, filtering and drying. Spray drying affects the egg yolk structure. Proteins denature at least partially

while they are subjected to heating, shearing and air-liquid interphase (Lechevalier, Jeantet, Arhaliass, Legrand, & Nau, 2007). Egg yolk and whole egg powders are highly oxidizable products and their storage under vacuum conditions and in darkness significantly prevents the loss of polyunsaturated fatty acids. The cholesterol oxidation of whole egg is higher than in egg yolk powder when stored for 3 months in darkness at room temperature (Bhandari, 2013). Processing steps such as pumping, filtering, adjusting pH, concentration and enzymatic hydrolysis have a significant effect on egg white foaming properties (Lechevalier et al., 2007). Van der Plancken has shown that the high water content increases protein denaturation during heating, therefore the powder must stay lower than 6.8% to preserve the foaming properties of whole egg products.

2.6 Functional properties of dried whole egg powders

Eggs are a functional ingredient that can be used in the preparation of several food products including bakery foods, bakery mixes, mayonnaise, salad dressings, confections, food coloring, ice cream, pasta and much more. The three most well-known uses of eggs are based on the fact liquid eggs coagulate or solidify when heated (cakes, breads, crackers). Whipping of egg white produces lighter airier products (meringues, angel cake) and emulsifying egg yolk phospholipids and lipoproteins produce mayonnaise, salad dressing and sauces (Lomanika and Mikova, 2006) . One of the main uses of spray dried egg powder is as an emulsifier. The amphipathic nature of proteins, because of the mixture of polar and non-polar amino acid residues, induces their adsorption at the surface of fat globules to reduce interfacial tension (Hung & Zayas, 1991). Reduced interfacial tension between oil and water droplets lead to stronger emulsions. It has been studied that emulsion, foaming stability, water holding capacity of gels and color change are significantly affected by storage time (Koç, Koç, Susyal, et al., 2011). Proteins contain many

amino acids with polar and non-polar heads that aid the formation of bonds that hold the water and oil together at the interphase. Lipoproteins and phosvitin are key to the formation of a good emulsion due to their amphiphilic state that allows them to absorb at the W/O interphase. (Guo, 2016). Lecithin, a phospholipid is important to form an emulsion because its hydrophobic tail attaches to the fat droplets and the hydrophilic head sticks out of the droplet surface into the surrounding water. Other emulsifiers in egg powder such as livetins aid in the reduction of interfacial tension between the two phases. Livetins are divided into 3 sub groups, α- and β- and γ, which are serum albumin, α-glycoprotein and IgY. Serum albumen is very sensitive to high ionic strength so it aids in emulsion formation (Guo, 2016). Livetins are heat sensitive due to their globular structure, therefore heat treatment disturbs emulsions (Guo, 2016). When the strength of the bonds is strong a very viscous desirable emulsion is obtained.

2.7 Functional properties of dried egg yolk powders

The phospholipids, lipoproteins and proteins found mainly in the egg yolk are surface active ingredients that enable the formation of emulsions from immiscible liquids such as oil and water. Proteins have hydrophilic and hydrophobic regions that enable emulsification. Egg yolk fortifies egg blends to increase emulsifying action. Egg yolk is an efficient emulsifying agent, for this reason it is an essential ingredient in mayonnaise and salad dressings, and plays an important role in foods such as cake batter containing shortening, cream puffs and hollandaise sauce (Stadelman, 1994). Factors such as viscosity over time and amount of emulsifier or additives affect the stability of emulsions. The determination of the emulsifying characteristics of proteins has evolved via two main approaches: emulsion capacity and emulsion stability measurements (Tornberg & Hermansson, 1977). Emulsion capacity is defined as the amount of oil emulsified by

a certain unit of sample at the point of emulsion collapse (Hung & Zayas, 1991). Emulsion capacity measures the maximum amount of fat emulsified by a protein dispersion. Emulsion stability is the ability of the emulsifier to stabilize an emulsion following its formation and sometimes following certain stress conditions, i.e., incubation, blending, centrifugation, or high temperature (Hung & Zayas, 1991). Emulsion stability refers to oil separation, it measures the ability of a product to remain durable and unchanged. The stability of emulsions is determined by measuring changes in characteristics of the emulsion with time. These changes include resistance to coalescence of oil drops and the appearance of a water layer, referred to as creaming (Stadelman, 1994).

2.8 Effect of storage temperature and time on egg powder quality

The purpose of this study is to study the effects of storage temperature and time on the quality of for commercial spray dried egg powders. It has been documented that adverse storage temperature such as > 40 °C has adverse effects on the quality and functional properties of spray dried egg products. An important characteristic of egg yolk products is the ability to dissolve into the solute. Protein solubility is required to fulfill functional properties and it is used as an indicator of egg product quality. Protein solubility also influences other properties such as gelatinization. (Assis, Garcia Rojas, Souza, Giraldo-Zuniga, & Melo, 2010). Solubility parameters can be measured using refractive index. Values of 23 to 25 correspond to 91.67% and 95.29% which are common practices in egg industry. By monitoring color change, lipid oxidation, free fatty acid percentages, emulsifying and solubility properties for two months, the quality of spray dried egg products can be analyzed.

Objectives:

- To evaluate the functionality and physicochemical characteristics of spray dried egg powders over a 2 month period.
- Provide suggestions to Michael Foods to improve the quality of their dried egg products

CHAPTER 3: MATERIALS AND METHODS

3.1 Spray dried egg powder proximate analysis

To carry out this research, 4 different types of commercial spray dried egg products were provided by Michael Foods (Gaylord, MN). The types of egg yolk powder included: Enzyme modified Yolk (EMY) , Free Flow Yolk (FFY) , Plain Whole Egg (PLWE), and Free Flow Whole egg (FFWE). Moisture content was measured according to the AOAC official method 930.15 (AOAC,1999) in triplicate. To extract the fat from the spray dried egg powder the solvent 3:2 Hexane:Isopropyl (HIP) alcohol was prepared (Michael Foods, 2016) . First, 40ml of HIP were placed in a 150ml beaker with 1g of egg powder; it was covered and incubated at room temperature for 30 minutes. A small amount of magnesium sulfate was added to the sample to bind the water. The sample was then vacuumed filtered into a 125mL Erlenmeyer flask using a Butcher Funnel and Whatman #2 filter paper. Then the solution was transferred to a pre-weighed 100mL beaker and the HIP was evaporated in a fume hood on a hot plate at low setting. When the fat turned golden yellow it was placed in a vacuum oven at 105ºC for 30 min. Then the sample was transferred to cool off and the weight of the flask was subtracted from the weight of the flask plus fat to calculate the fat extracted from the sample in triplicate. Crude protein content was determined according to AOAC official method 992.15 (AOAC, 2006) using a Perkin Elmer Nitrogen Analyzer (Model 2410, Perkin Elmer Instruments, Norwalk, CT). The crude protein (%) was reported as 6.25 times the nitrogen content (%) in triplicate. Ash content was determined according to official methods 942.05 (AOAC 1999) in tripicate . Approximately 5 g of dried egg powder was placed in a Thermolyne Type 600 muffle furnace (Thermo Scientific, Lawrence, KS) at 550 °C for 5 h and the ash content was weight.

3.2 Storage assessment

The spray dried egg powders were stored at 4°C, 25 °C, 43 °C or 54 °C in incubators at the LSU Food Incubator and Food and Animal Science Laboratory Building. The sample bags were stored under no light and packaged in transparent polyethylene plastic bags. The bags were closed with bag wrist ties.

3.3 Solubility

The solubility index was determined by refractometry using Michael's Food protocol for determining the Haenni Value (C. Rannou et al., 2015). A 5% NaCl solution was prepared and 5ml were added to 1g of egg powder sample into a 15mL centrifuge tube. Samples were centrifuged at 3,000 rpm for 15 minutes and then inverted 10 times twice. Samples were centrifuged for 5 additional minutes and a pipet was inserted through the upper layer and a small volume of the aqueous phase beneath was collected. One or 2 drops of the extract was transferred to a refractometer and the refractive index value was read. The refractive index of the 5% NaCl solutions was also measured. To calculate the solubility percentage, the Haenni Value was calculated using Eq.3.2. and the solubility percentage was calculated using equation 3.3.

$$Haenni\ Value = (sample\ refractive\ index - 5\%\ NaCl\ refractive\ index)x\ 1000 \qquad (3.2)$$

Where "y" is the Haenni value and "x" is the percentage solubility in sodium chloride. For convenience, the following table may be referred in order to convert Haenni Value to the solubility percentage.

$$log_{10}y = 0.445 + 0.01x \qquad (3.3)$$

Table 2.1 Solubility percentage and Haenni Value

Haenni Value	Solubility Percentage	Haenni Value	Solubility Percentage
17	78.55	23	91.67
18	81.03	24	93.29
19	83.36	25	95.29
20	85.60	26	97.00
21	87.72	27	98.64
22	90.00	28	100.00

3.4 Emulsion preparation

Emulsions were prepared following Michael Foods Protocol. The ingredients: 1.8 % egg powder, 10.1% water, 0.5% sugar, 1.3 % salt, 7.3% white vinegar (5% acidity), and 79.0% oil were processed. The dried egg powder was combined with water, sugar, and salt in a 250mL beaker. The solution was stirred and left standing for 10 min with occasional stirring to promote hydration of the egg powder. The egg mixture was then transferred to a food processor with a blade (Hamilton Beach Prep Star 350W max, used at setting #2). The egg mixture was blended for 1 min, and then 60% of the oil was added through a plastic funnel. The remaining oil (19%) was mixed with the 7.3% vinegar to acidify the emulsion and incorporated into the blend. Once the oil/vinegar mixture was incorporated it was blended for an additional one minute.

3.4.1 Viscosity test

The viscosity test was performed using a protocol provided by Michael Foods. In brief, the emulsion was incubated at room temperature for 17 h to allow the emulsion to stabilize. A DV-E Brookfield viscometer Spindle s64 was immersed 30mm into the sample. It was tested at 5 rpm for 1 min and recoded every 15 s. Results were reported in centipoise.

3.4.2 Emulsion stability

Emulsion stability is the ability of the emulsion to remain stable and unchanged against coalescence (Hung & Zayas, 1991). The stability of the emulsion was determined by calculating the stability according to Yang and Cotterill (1989) and Hung and Zayas (1991) with some modifications. After preparing the emulsion, it was left standing at room temperature for 17 h to allow the emulsions to reach equilibrium. One gram of emulsion was weighted into a 15mL centrifuge tube and heated at 95 ºC for 30 min in water bath. The method of heat transfer for this stability test was conductive heat. The heated emulsion was centrifuged at 1500 x g at 4 ºC for 30 min (Eppendorf Centrifuge 5810, Westbury, NY, USA). Centrifugation was at low speed so that proteinaceous material was not removed from the fat globule interphase (Yang & Cotterill, 1989). The oil layer on top was removed carefully using a pasteur pipet. The emulsion stability (%) was calculated as follows:

$$ES\% = \frac{Weight\ of\ emulsion\ after\ oil\ separation\ (g)}{Initial\ weight\ of\ emulsion\ (g)}\ x\ 100 \qquad (3.1)$$

3.4.3 Heat stability

A Protocol referenced by Michael Foods was used to test for heat stability. Fifty grams of emulsion were placed in a 250mL beaker. The sample was microwaved on high for 15 s, the method of heat transfer for this stability test was microwave. The sample was observed for signs of oil separation. The case where there was no oil separation in the sample represents a “pass” heat stability test. A failed sample formed an oil layer on top. After the first heat treatment, the sample was heated for an additional 10 s and heat stability was again reported as “pass” or “fail”.

3.5 Free fatty acids

Free fatty acids were calculated as % Oleic Acid following Michael Food's Protocol. Briefly, the free acid determination step was accomplished by adding 30 mL of a 2:1 toluene-methanol solution + 2% distilled water to the sample. The sample was manually titrated to a pH of 8.8 endpoint using standardized 0.05 N potassium hydroxide in methanol. Free fatty acid (FFA) was expressed as the number of mL needed to neutralize the free fatty acids in one gram of crude fat using equation 3.5.

$$FFA = V/m \quad (3.5)$$

Where V is the total volume of KOH required to neutralize the sample and m is the mass of egg fat collected by extraction

The fatty acids were expressed as an equivalent percent oleic acid, calculated using equation 3.6, where Mn is the molecular weight of oleic acid, 282 g/mol. N is the normality of KOH solution standardized with KPH and 0.1 is the factor to convert mg/g to %.

$$\% \text{ Oleic acid} = FFA \times Mn \times N \times 0.1 \quad (3.6)$$

3.6 Lipid hydroperoxides

The ferric-xylenol orange (FOX) method for measurement of hydroperoxides was used with some modifications (Fukuzawa et al., 2009). FOX is a technique that employs reduction of peroxides in acidic condition by Fe^{2+} and formation of the colored ferric-orange product with a peak at 560 nm. Egg powder (0.5 g) was weighed into a 50 mL centrifuge tube and homogenized with 15 mL of cold (−20 ºC) HPLC grade methanol using an homogenizer. Homogenates were centrifuged at 1,400 × *g* for 3 min and the supernatants were removed for assay. Five hundred

microliters of 0.25 mM $FeSO_4$, 200 µL of 25 mM H_2SO_4, 200 µL of 0.1 mM xylenol orange, 1,025 µL of water (volume of water needed to make up the final volume to 2 mL), and 75 µL of the methanolic extract were added sequentially to 10-mL a screw-capped tube. After incubation at room temperature under light for 5 h, absorbance was read at 560 nm with a UV spectrophotometer. The LHP value of the samples was expressed as mEq O_2/kg egg lipid. The cumene hydroperoxide was determined with reference to a linear regression curve performed with cumene hydroperoxide as the standard.

3.7 Color change

The color of egg powder samples (L*, a*, and b*-value) was measured with a colorimeter and results were expressed in accordance with the CIE Lab system. L* value stands for the brightness with 0 for black and 100 for white. The a* value covers from green to red with negative values for green and positive values for red. The b* value covers from blue to yellow with negative values for blue and positive values for yellow. The total color change (ΔE* value) of egg powder with respect to storage conditions was measured using the Minolta Chroma Meter BC-10 (Baking Contrast Meter, Osaka, Japan). Prior to analysis, the Chroma meter was calibrated using a white calibration plate. Δ*E**, calculated with equation 3.7 is a single dynamic parameter that considers the differences between the three-color coordinates (*L**, *a**, and *b**) of the sample as compared to the same sample at zero time at room temperature.

$$\Delta E^* = \sqrt{\left(L^*_{sample} - L^*_{standard}\right)^2 + \left(a^*_{sample} - a^*_{standard}\right)^2 + \left(b^*_{sample} - b^*_{standard}\right)^2} \quad (3.7)$$

3.8 Morphological appearance

The microstructure of the egg powders was evaluated by scanning electron microscopy using the JSM-6610LV, SEM (JEOL Ltd. Japan) working with a voltage of 10kV. High-resolution images of the egg powder surfaces using SEM were obtained to evaluate the effect of drying and storage condition on the microstructure of egg powder. Samples of egg powders were mounted on aluminum SEM stubs, and then sputter-coated with a gold: palladium (60:40) layer in an Edwards S150 sputter coater (Edwards High Vacuum International, Wilmington, MA) to produce the conductive surface and observed at 1000x magnification.

3.9 Statistical analysis

All data were analyzed using SAS software version 9.2. Means and standard deviations of the data are presented. Q-test was performed to remove outliers. A 1x4x3 factorial experiment was designed to perform an ANOVA analysis of variance. The factors in this experiment were the types of sample, the 4 storage temperatures and 3 time period of storage. A Tukey test was carried out to determine differences among treatments at the significant level of $p < 0.05$.

4. RESULTS AND DISCUSSIONS

4.1 Composition of dried egg products

Egg yolk powders, including Enzyme Modified (EMY), Free Flow Yolk (FFY), Free Flow Whole Egg (FFWE), and Plain Whole Egg (PLWE) were analyzed for protein, lipids, and ash content. Carbohydrates were calculated by subtraction using the definition of proximate analysis, equation 4.1. Moisture contents values were obtained from Michael Foods (Gaylord, MI, USA). The proximate composition of egg powders is listed in Table 4.1

$$100\% = Carbohydrates + Lipids + Protein + Mosisture + Ash \quad (4.1)$$

Table 4.1 Proximate analysis of egg yolk and whole egg powders

% in dry basis	Enzyme Modified Yolk	Free flow yolk	Free flow whole egg	Plain whole egg
Moisture	2.7 ± 0.05	2.9 ± 0.05	2.8 ± 0.09	3.3 ± 0.06
Total lipids	46 .0 ± 0.05	50.0 ± 0.00	40.0 ± 0.10	41.0 ± 0.10
Crude Protein	34.2 ± 0.08	33.9 ± 0.07	45.6 ± 0.07	46.3 ± 0.08
Ash	2.4 ± 0.6	2.4 ± 0.74	2.5 ± 0.34	2. 4 ± 0. 65
Carbohydrates (g/100)	14.2 ± 0.1	10.8 ± 0.2	9.1 ± 0.09	7.0 ± 0.09

Results for proximate analysis of egg powders were similar to other commercial egg powders produced in the United States. Their proximate analysis for dried egg powder was 36% protein, 60% fat and 2.10% moisture (Guo, 2016)

4.2 Solubility of egg powders in water

The Haenni value is commonly used in the egg industry as an indicator of egg quality. In this study the Haenni Value was used to calculate egg powder solubility. Table 4.2 indicates the solubility of each sample stored at different temperatures. The Haenni value is a good indicator of egg yolk solubility. Hanni values of 23 to 25 which correspond to 91.67% to 95.29% solubility,

are in an acceptable range that compares egg powder with the original properties of fresh egg. Anova and Tukey indicate a significant interactive effect of time and temperature ($p<0.05$) for each individual sample, presented in the superscripts in Table 4.2.

Table 4.2 Solubility % of egg powder samples with temperature x time significant effect

Sample	Month	Solubility %			
		4 C°	25 C°	43 C°	54 C°
Enzyme modified yolk	0	100 ± 0.0^{A}	100 ± 0.0^{A}	85 ± 4.0^{B}	85 ± 4.0^{B}
	1	95 ± 2.9^{A}	97 ± 2.4^{A}	59 ± 2.3^{C}	36 ± 0.6^{D}
	2	85 ± 1.8^{B}	85 ± 3.7^{B}	40 ± 0.8^{D}	23 ± 0.2^{E}
Free flow yolk	0	98 ± 4.2^{A}	98 ± 1.3^{A}	86 ± 2.5^{AB}	86 ± 4.4^{AB}
	1	96 ± 2.6^{A}	91 ± 1.9^{A}	77 ± 4.4^{ABC}	72 ± 2.4^{ABC}
	2	$89\pm.42^{A}$	86 ± 4.4^{AB}	70 ± 7.3^{BC}	51 ± 1.4^{C}
Free flow whole egg	0	92 ± 2.8^{A}	98 ± 3.8^{A}	91 ± 2.8^{A}	91 ± 2.8^{A}
	1	$91\pm.86^{A}$	91 ± 1.6^{A}	77 ± 2.6^{BC}	$57\pm.94^{CD}$
	2	$91\pm.67^{A}$	$90\pm.89$	58 ± 1.5^{CD}	$40\pm.45^{D}$
Plain whole egg	0	100 ± 0.0^{A}	100 ± 0.0^{A}	96 ± 2.6^{A}	96 ± 2.2^{A}
	1	98 ± 1.5^{A}	97 ± 2.3^{A}	73 ± 2.3^{B}	$53\pm.77^{BC}$
	2	$96\pm.84^{A}$	$96\pm.45^{A}$	56 ± 2.1^{BC}	$40\pm.67^{C}$

*Superscripts indicate the hierarchy of the best performing samples when the effects of time and month are studied. Different subscripts indicate the sample is significantly ($p>0.05$) different from the others, when subscripts match the samples are not significantly different.

Synergistic effects of month and temperature indicate month 1 had the best results, followed by month 0 and 1. Egg powders stored at 4 C° during month 0 are significantly($p<0.05$) different than month 1 but not from month 2. At storage temperature of 43 C°, all months are significantly ($p<0.05$) different.

The trend line suggests that the solubility of samples stored at 4°C, or 25°C for 2 months decreased slowly but the solubility of samples stored at 43°C and 54°C had a drastic drop. For example EMY dropped from 85% to 23% when the egg powder was stored at 54° (Table 4.2).

Samples stored at 43°C or 54°C were more clumpy and harder to solubilize. Studies on spray dried egg powder showed that higher air temperatures during drying decreased protein solubility and foaming power (Koç et al., 2011). High air temperatures combined with storage time can cause a decrease in solubility of dried egg powder.

In Table 4.2 it can observe that the solubility was higher for samples stored at 4 or 25°C and significantly lower for samples stored at 43°C or 54°C. EMY was the best performing sample in other experiments which include viscosity and stability, but in solubility FFWE and PLWE retained higher solubility.

Temperature had a big impact on solubility because high temperatures denatured the egg proteins and oxidized the lipids in the egg powders making them harder to solubilize. Egg proteins denature at 65 C° to 84 °C (Donovan, Mapes, Davis, & Garibaldi, 1975).

4.3 Emulsification

Emulsions were successfully prepared with Enzyme Modified Yolk powder stored at 4, 25, 43 or 54 °C during month 0 and 1 only. During month 2 emulsion was unable to form using EMY powder stored at 54°C as seen in Figure Table 4.2.

Emulsions prepared with Free Flow Yolk powder were successfully prepared with powder stored at 4, 25, 43 or 54 ° during month 0 and month 1. In month 2 a stable emulsion was not achieved with FFY powder stored at 43 °C and at 54 °C which failed to form an emulsion.

When using Plain Whole Egg powder stored at 4, 25, 43 or 54 °C, emulsion were successfully produced during month 0 and 1. Emulsion formed with powder stored at 54 °C produced a soft or creamy emulsion in month 1. The emulsions tend to cream, owing to the density difference between the aqueous and oil phases and the presence of flocculated droplets (Opawale

& Burgess, 1998) .When using powder stored at 43°C or 54°C the sample failed to emulsify during month 2.

Emulsions prepared using Free Flow Whole Egg powder were less firm than the rest of the powders. For month 1 and 2 emulsions were successfully prepared with powders stored at 4, 5 or 43°C. During month 2, emulsions prepared using powder stored at 54°C failed to form a stable emulsion.

			
Failed emulsion prepared with Enzyme Modified Egg Yolk stored at 54 °C	Failed emulsion prepared with Free Flow Yolk powder stored at 54 °C	Creamy emulsion prepared using Plain Whole Egg powder stored at 43 °C	Failed emulsion prepared using Free Flow Whole Egg powder stored at 54 °C

Figure 4.2 Emulsions prepared with samples after 2 months of storage at various temperatures

Emulsions are thermodynamically unstable systems because of the positive free energy needed to increase the surface area between the oil and water phases and because oil and water have different densities (McClements & Decker, 2000). Factors affecting the stability of food emulsions include the emulsifying agent, droplet size and net charge on the discontinuous phase, interfacial tension, ionic strength, viscosity of the droplets, gravitational fields, temperature, weight ratio of the two phases, structure of the emulsifying agent, and pH (Hung & Zayas, 1991). It has been shown that the type of emulsifying apparatus being used strongly influences the

creaming stability of protein emulsions (Tornberg & Hermansson, 1977). For the entire duration of the experiment the Food Processor Hamilton Beach Prep Star 350W was utilized. Studies have shown that the stability of emulstion made with dried-whole egg and dried egg yolk was decreased when these egg products were stored at 35 °C for 3 months (Lieu, Froning, & Dam, 1978). Data collected in this study follow the same deteriorating trend.

4.4 Viscosity of emulsions

Viscosity was measured in centipoise (cP) to observe the bond strength between the proteins and emulsifiers in the egg powders binding the oil and water molecules. The emulsion prepared was a water in oil (W/O) emulsion, meaning the water droplets are trapped inside the oil droplets in an emulsified state, as seen in Figure 4.3.

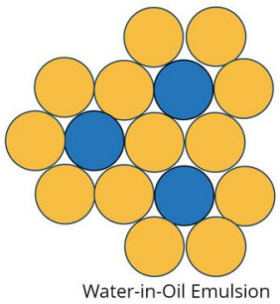

Figure 4.3 Water droplets trapped inside oil droplets forming a W/O emulsion.

ANOVA tests indicate the effect of time and temperature was significant. ($p<0.05$). There are 2 way interactions between month and temperature ($p<0.05$).

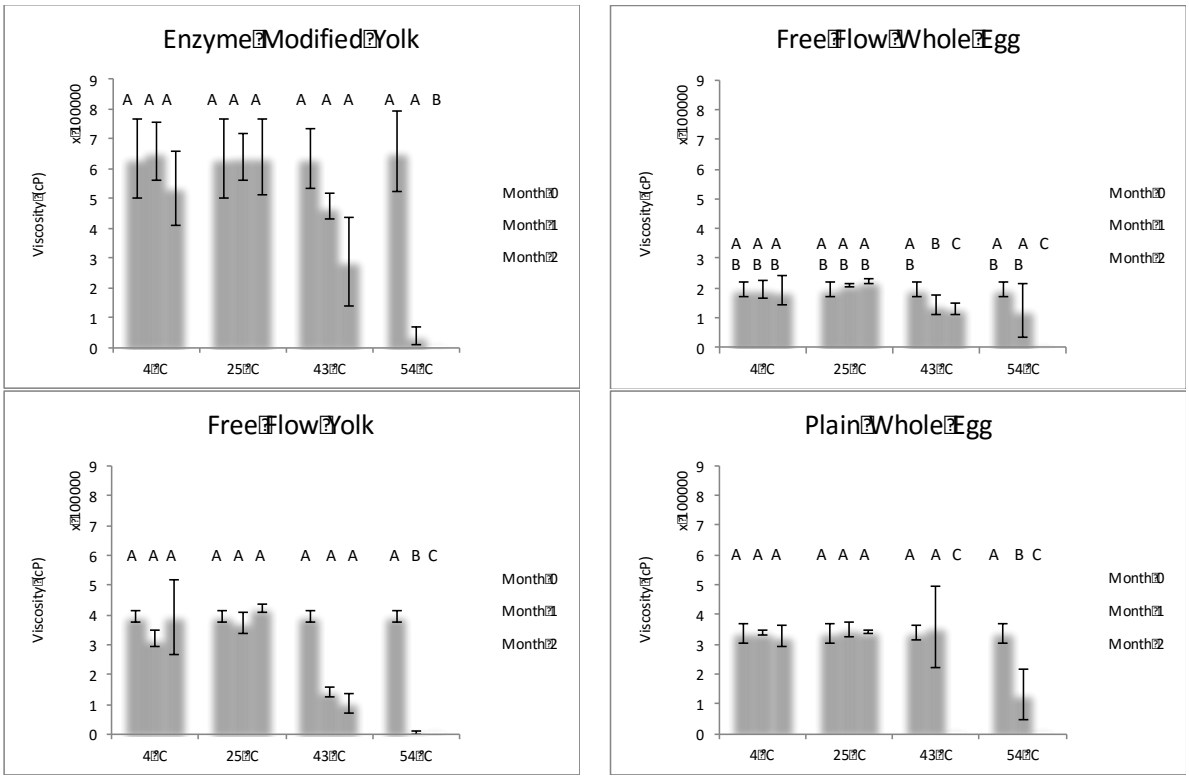

Figure 4.4 Viscosity of egg powder samples stored at 4, 25, 43 or 54 °C for 0,1 or 2 months

*Letters indicate the hierarchy of the best performing samples when the effects of time and month are studied. Different subscripts indicate the sample is significantly ($p>0.05$) different from the others, when subscripts match the samples are not significantly different.

Enzyme Modified Egg Yolk (EMY) samples stored at temperatures 4°C or 25°C, had significantly greater viscosity ranging from 5 to 6 cP ($p<0.05$) in comparison to the other three types of egg powders PLWE, FFWE, FFY that range from 1.96 to 4.8 cP (Figure 4.4). This indicates EMY was the best performing sample. During month 0 and 1 all the samples were able to emulsify and produce stable emulsions During enzymatic modification, phosphatidyl choline, the most prevalent phospholipid (80%), is turned into lysophosphatidic acid which is negatively charged and imparts negative charges to emulsion droplets and keeps the droplets apart. The enzyme modification can enhance emulsion's thermal stability (Guo, 2017). It can be observed

that emulsion prepared with egg powder stored at 43 °C had a drastic reduction in viscosity. In month 2 samples stored at 54 °C began to fail to form emulsion.

Some of the failures experienced with the emulsions prepared with powders stored at 43 °C in month 1 and 54 °C in month 2 were coalescence, creaming, flocculation and breaking as diagramed in Figure 4.5.

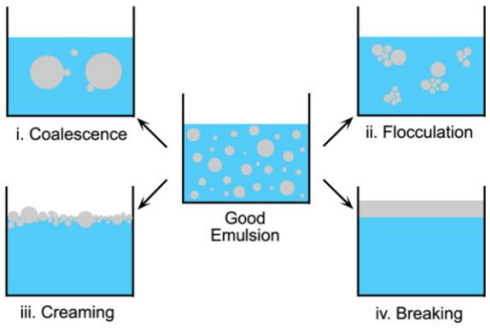

Figure 4.5 Good emulsion and emulsion failures.

Egg powders stored at 43 °C or 54 °C would not be able to emulsify properly because they would break out of emulsion after 17 hours of elaboration, meaning they were not stable therefore, viscosity readings were not recorded. The failure to create an emulsion indicates some protein had been partially denatured by heat and can't emulsify properly, hence lower viscosity readings indicate unsatisfactory emulsion quality.

4.5 Emulsion stability

During the drying process, egg proteins denature and lipids oxidize thereby affecting the egg's functional properties (Koç, et al., 2011). ANOVA tests indicate the significant effect of time and temperature ($p<0.05$). There are 2 way interactions between month and temperature ($p<0.05$).

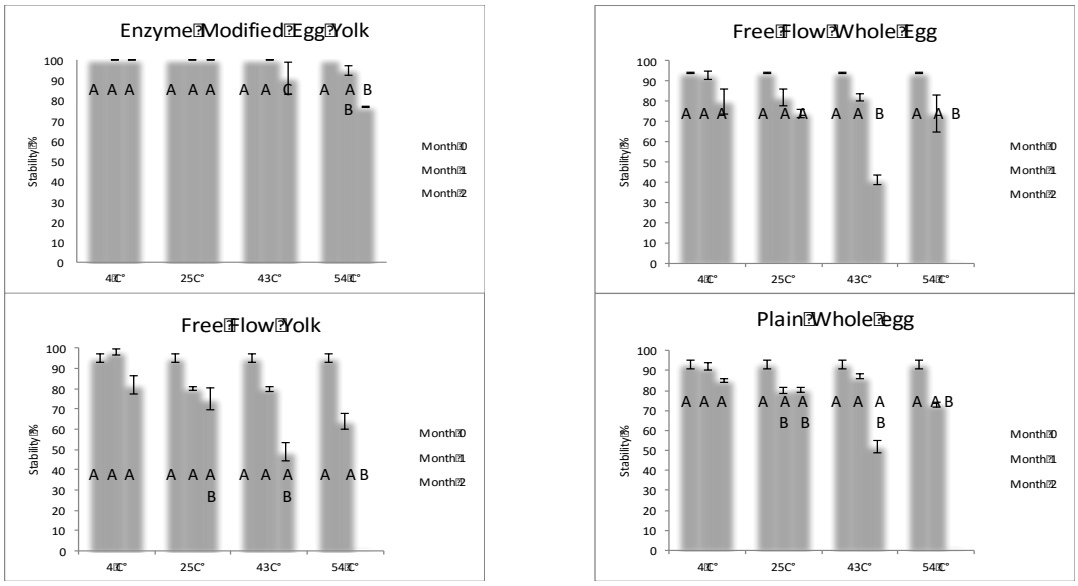

Figure 4.6 Emulsion Stability % of spray dried egg powders stored at 4, 25, 43 or 54 °C for 0,1 or 2 months

* Letters indicate the hierarchy of the best performing samples when the effects of time and month are studied. Different subscripts indicate the sample is significantly ($p>0.05$) different from the others, when subscripts match the samples are not significantly different.

The results of emulsion stability remained above 80% for all dried egg samples stored at 4°C, or 25°C for the entire duration of the study (Figure 4.6). Emulsion stability did not significantly decrease for emulsions prepared with EMY powder ($p<0.05$). Emulsion stability significantly decreased for FFY, PLWE, and FFWE samples stored at 43 °C after 2 months of storage and at 54 °C after 1 months of storage. Emulsion stability is negatively affected by the storage temperature of the egg powders, temperatures over 43 °C should be considered as temperature of abuse. Stadelman and Cotterill reported that emulsion stability can be affected negatively by storage conditions (Stadelman,1994 and Cotterill, 1979). Emulsion stability for samples prepared with EMY powder had 91% ($p<0.05$) stability at 54 °C after 2 months of storage suggesting the emulsifying properties of this sample had slightly declined after being exposed to heat, this is due to the enzymatic modification that makes this sample different from the other 3 egg powder samples.

To improve emulsion stability some studies suggest that gel firmness and emulsion stability of whole egg powder were improved at high inlet air temperature, 165 °C, and low outlet temperature, 70 °C, and low liquid flow rate during the spray drying process (Zimmerman, Hayes, & Skerlos, 2004).

4.6 Heat stability

Statistics on heat stability results reveal the type, time and temperature effects did not yield a statistically different significant effect between treatments. EMY sample was the best performing sample, being able to resist microwave heating after being stored at 43 °C for up to 2 months and possibly longer. In Table 4.4 it can be observed that only EMY sample was still heat stable at month 2 at storage temperature 4°C or 25 °C. Emulsions prepared with EMY were not heat stable when stored at 54 °C for 1 month or stored at 43 °C or 54 °C for 2 months. Emulsions prepared with FFY, FFWE and PLWE powder lost heat stability when subjected to more than 10 seconds of microwave heat during month 1. FFY, FFWE and PLWE emulsions were not heat stable after 2 months of storage at any temperature. Figure 4.7 shows an emulsion that failed the heat stability test.

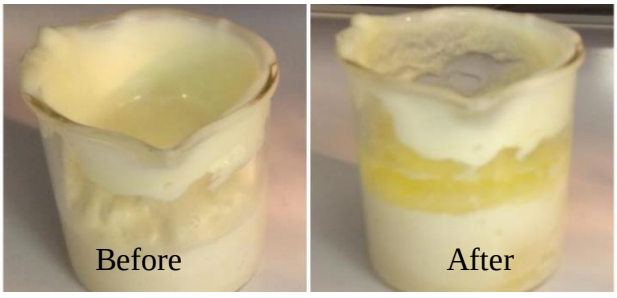

Figure 4.7 Emulsion stability before and after a failed heat stability test

Table 4.4 Heat stability results of dried egg powders stored at 4°C, 25 °C, 43 °C or 54°C.

Month	0		1								2							
Temperature (°C)	25 °C		4 °C		25 °C		43 °C		54 °C		4 °C		25 °C		43 °C		54 °C	
Time (seconds)	15	25	15	25	15	25	15	25	15	25	15	25	15	25	15	25	15	25
Enzyme	✓	✓	✓	✓	✓	✓	✓	✓	✖	✖	✓	✓	✓	✓	✖	✖	✖	✖
modified	✓	✓	✓	✓	✓	✓	✓	✓	✖	✖	✓	✓	✓	✓	✖	✖	✖	✖
egg yolk	✓	✓	✓	✓	✓	✓	✓	✓	✖	✖	✓	✓	✓	✓	✖	✖	✖	✖
Free flow	✓	✖	✓	✖	✓	✖	✓	✖	✖	✖	✖	✖	✖	✖	✖	✖	✖	✖
yolk	✓	✓	✓	✖	✓	✖	✓	✖	✖	✖	✖	✖	✖	✖	✖	✖	✖	✖
	✓	✖	✓	✖	✓	✖	✓	✖	✖	✖	✖	✖	✖	✖	✖	✖	✖	✖
Plain	✓	✖	✓	✖	✓	✖	✓	✓	✖	✖	✓	✖	✖	✖	✖	✖	✖	✖
whole	✓	✖	✓	✖	✓	✖	✓	✖	✖	✖	✖	✖	✖	✖	✖	✖	✖	✖
egg	✓	✖	✓	✖	✓	✖	✓	✖	✖	✖	✖	✖	✖	✖	✖	✖	✖	✖
Free flow	✓	✖	✓	✖	✓	✖	✓	✖	✓	✖	✖	✖	✖	✖	✖	✖	✖	✖
whole	✓	✖	✓	✖	✓	✖	✓	✖	✖	✖	✖	✖	✖	✖	✖	✖	✖	✖
egg	✓	✖	✓	✖	✓	✖	✓	✖	✖	✖	✖	✖	✖	✖	✓	✖	✖	✖

FFY, PLWE, and FFWE were not heat stable after 1 month of storage at 43 °C or 54°C. This indicates that the most suitable temperature to store FFY, PLWE, and FFWE egg powder is 4 °C or 25 °C to retain the emulsion's thermal stability. EMY can be stored at 43 °C for 1 month without losing thermal stability. Emulsions prepared with egg powders stored at 54 °C were not heat stable for the duration of this study.

4.7 Lipid Hydroperoxides

The peroxide number is a measurement of the concentration of peroxide groups in edible oils, it is used in the oil industry during the production and storage to check for preservation. Its value in egg powder indicates the degree of decomposition in an egg powder. The results for lipid hydroperoxides were calculated in mEq O_2/kg, which is the amount of active oxygen or peroxide

per kilogram of oil. Peroxide values of fresh oils are less than 10 mEq O_2/kg. When values range from 30 to 40 mEq O_2/kg, a rancid odor is noticeable, and the product is unfit for human consumption (Chakrabarty, 2003). ANOVA tests indicate the significant effect of time and temperature ($p<0.05$). There are 2 way interactions between month and temperature ($p<0.05$).

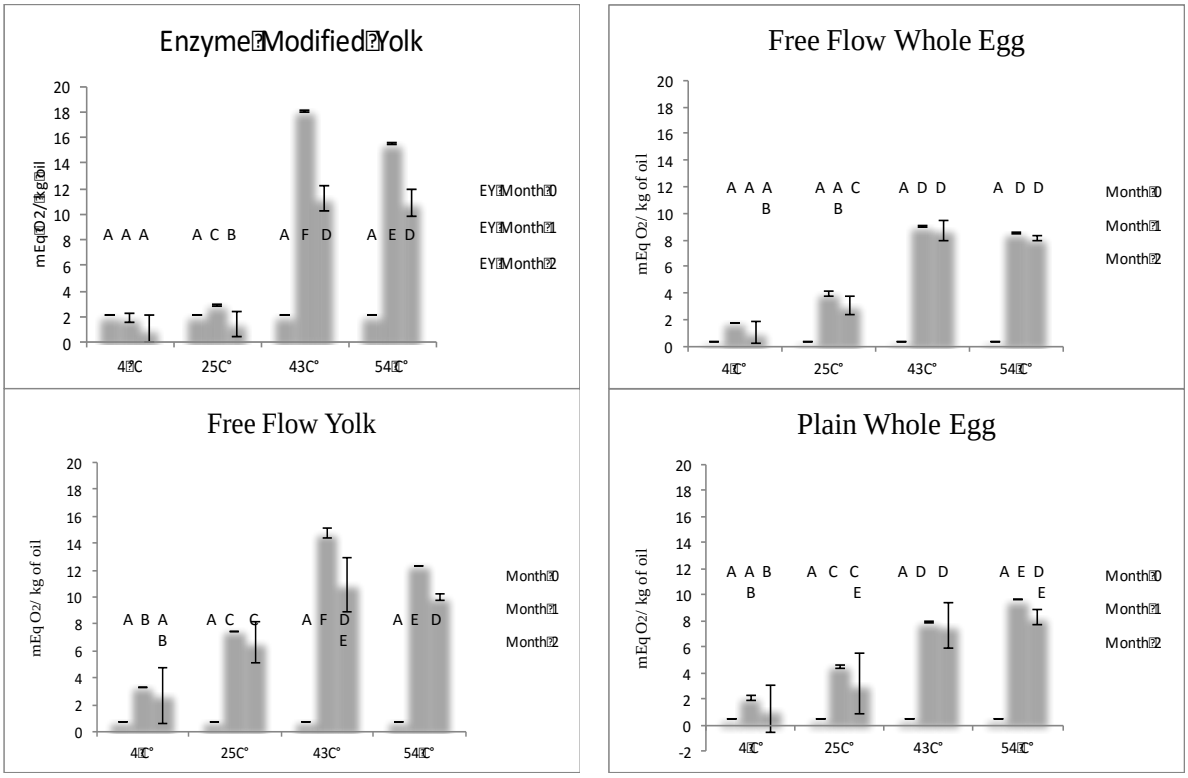

Figure 4.8 Peroxide values of egg powder stored at 4, 25, 43 or 54 °C for 0, 1 or 2 months

*Letters indicate the hierarchy of the best performing samples when the effects of time and month are studied. Different subscripts indicate the sample is significantly ($p>0.05$) different from the others, when subscripts match the samples are not significantly different.

During month 0 all the lipid hydro peroxide values for all samples were below 2 mEq O_2/kg. At month 1 changes were observed, higher values indicate that the free radicals were being formed. For samples stored at 4°C or 25°C peroxide value ranged from 1 to 3 mEq O_2/kg, indicating the samples were going through oxidation but were still fresh. For temperatures 43 °C or 54 °C, peroxide value of 7 to 18 mEq O_2/kg was measured during month 1 and peroxide value

of 7 to 15 mEq O_2/kg was measured during month 2. This decrease in peroxide value for samples stored at 43 °C or 54 °C indicates oxidation was advancing and the peroxide compounds had reacted to form aldehydes and other compounds that could be measured by other methods such as the TBA test. Samples stored at 43°C had slightly higher peroxide values than samples stored at 54°C meaning that the accelerated aging occurring at samples stored at 54°C was moving towards the termination step. This can lead to an underestimation of the real oxidative status of the egg product. The increase in peroxide value from month 0 to month 1 confirmed the occurrence of lipid oxidation. Then the peroxide values decreased, which is explained by the increased rate of peroxide interaction. The decrease in peroxide value does not mean the decrease of lipid oxidation, but indicates the progress of lipid oxidation has moved to the secondary oxidation period. Other studies on the quality of egg yolk products at different storage temperatures also reported the rise and drop of peroxide during storage time (Guo, 2016).

Protection against lipid oxidation is a critical factor for food quality and the shelf life of egg powder. Egg contains over 11% lipids, mainly concentrated in the yolk (33% to 35%) (Koç, Koç, Susyal, et al., 2011). Guardiola and others reported that high temperature and large surface area due to atomization and forced oxygen flow during spray drying are the factors promoting the double bonds of unsaturated fatty acids to oxidative deterioration (Guardiola, Codony, Miskin, et al., 1995). Other studies reveal micro encapsulation of egg fat can be adopted as an approach to prevent oxidation of the egg (Koç, 2011). Using spray drying as an encapsulation technique, the use of gelatin had shown favorable results. Gelatin serves as a wall material due to its properties of emulsification, film formation and water solubility. The addition of a small amount of gelatin can coat the egg particles and help prevent the migration of oily components to the surface. Other chemicals that serve this purpose are hydrocarbon compounds. The idea is to enhance the

formation of spherical and smooth-surfaced microcapsules. In other studies they applied microencapsulation process to improve the oxidative stability of egg powder and found that the peroxide value of the encapsulated egg powder was lower than that of egg powder without the wall material (Koç, Koç, Susyal, et al., 2011) The food industry applies microencapsulation for a number of reasons such as stabilization of active substances, controlled release of active substances, masking unpleasant tastes and smells, and protecting ingredients from oxidation (Koç, Koç, Yilmazer, et al., 2011). Another option to reduce lipid oxidation is to bring the water content of the egg powder to the corresponding water content of the monolayer of egg powder. In this way no diffusion of water can occur; minimum lipid oxidation had been observed at this conditions (Koç, Koç et al. 2011). Research in storage conditions of spray dried egg products had shown that vacuum packaging and absence of light were highly effective in preventing oxidation during storage (Guardiola et al., 1997).

4.8 Free fatty acids

In spray dried egg products free fatty acids form and react with other compounds such as proteins. Egg is also high in cholesterols, and they oxidize faster when they are in the presence of other lipids. This makes quantitative analysis of these compounds very difficult. ANOVA tests indicate the significant effect of time and temperature ($p<0.05$). There are 2 way interaction between month and temperature ($p<0.05$).

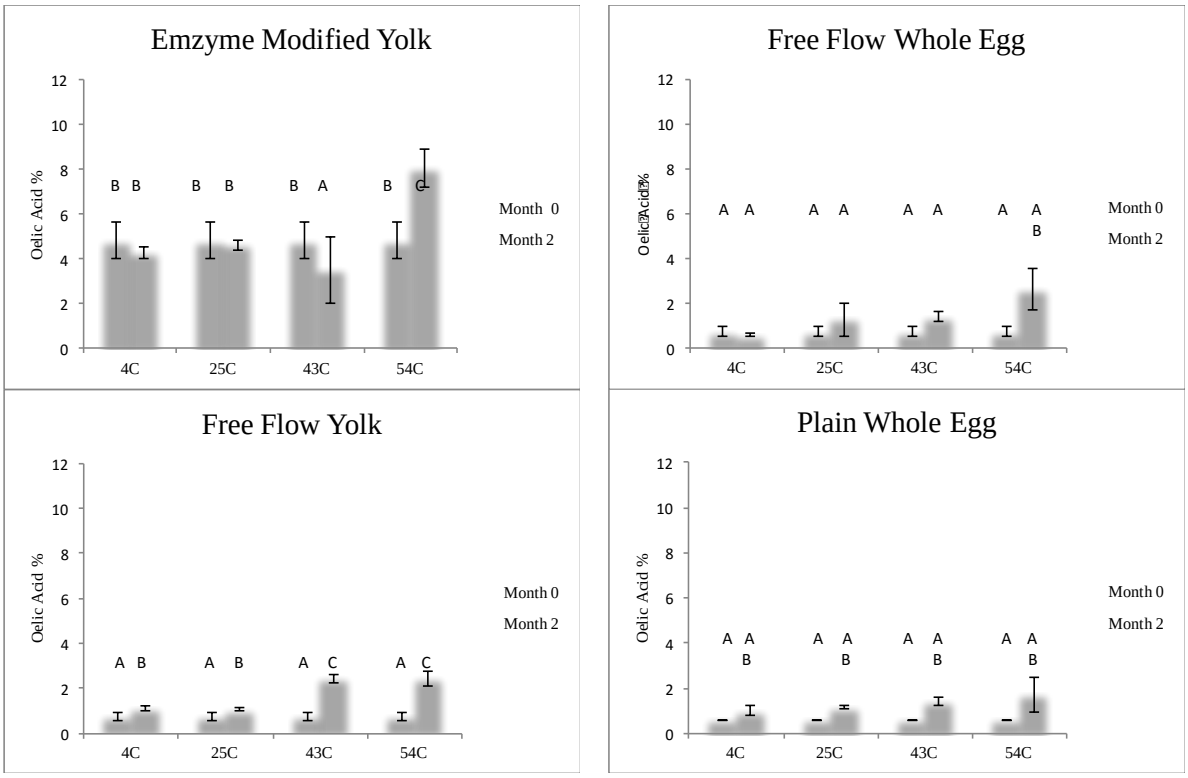

Figure 4.9 Free fatty acids as Oleic Acid % of spray dried egg samples stored at 4, 25, 43 or 54 °C for 0,1 or 2 months

*Letters indicate the hierarchy of the best performing samples when the effects of time and month are studied. Different subscripts indicate the sample is significantly ($p>0.05$) different from the others, when subscripts match the samples are not significantly different.

When spray drying temperature is high, vacuum packaging and no light exposure is effective in avoiding the loss of fatty acids during storage. (Guardiola, Codony, Manich, et al., 1995). As shown in Figure 4.9 samples were still under the acceptable range for the entire study. Free fatty acids are released during enzyme modification therefore EMY has higher values for oleic acid %.

4.9 Color change

The net color change ΔE during storage was calculated as change in Hunter L, a and b values during the storage period of each egg powder. Color changes of samples with storage time are

shown in Figure 4.10. ANOVA tests indicate the effect of time and temperature ($p<0.05$). There are 2 way interactions month and temperature ($p<0.05$) for each sample.

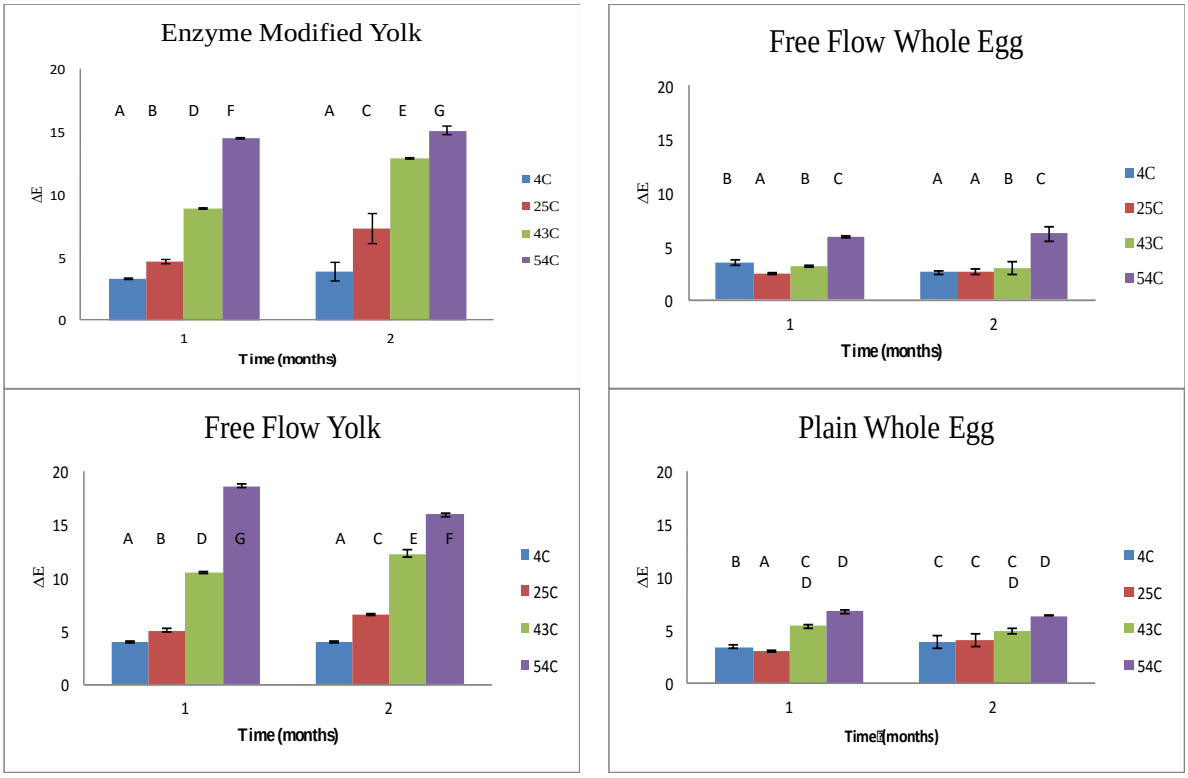

Figure 4.10 ΔE of egg powder samples stored at 4, 25, 43 or 54 °C for 0, 1 or 2 months

*Letters indicate the hierarchy of the best performing samples when the effects of time and month are studied. Different subscripts indicate the sample is significantly ($p>0.05$) different from the others, when subscripts match the samples are not significantly different.

As storage temperature increases the ΔE become more evident. It is well known that long time drying at high temperatures can significantly decrease the quality of dried materials, for example, change in color (Koç, Koç, Susyal, et al., 2011). During storage conditions these reactions continue to deteriorate the product at a slower rate. Other newly formed compounds can arise during the processing and storage of egg products due to Maillard reactions, which lead to the browning of the egg-containing product (Caboni et al., 2005).

At refrigeration and ambient storage temperatures, 4 °C or 25 °C respectively, the color change of the egg products was less obvious than 43 °C or 54°C. At higher storage temperature, 43 °C or 54°C, the color change significantly increased ($p<0.05$). As the color change became more evident, off flavors developed by oxidation were also perceived. (Bornstein & Bartov, 1966) The pigments in eggs are very sensitive to oxidation, and excessive exposure to heat in a dryer the pigments will tend to change (Stadelman, 1994). Values above ΔE 3.5 are noticeable by the human eye. Studies showed that the addition of carbohydrates and protein can prevent the change in egg powder (Koç et al., 2011). Consumer acceptability depends on the visual impression, which is not always well correlated with chemical determinations because the human eye is not very sensitive to shades of yellow (Bornstein & Bartov, 1966).

4.10 Morphological appearance

Egg powders were examined by Scanning Electron Microscopy (SEM). Studies demonstrate that egg powder with $a_w<.59$ shows structural changes such as sticking, collapsing, caking and agglomeration (Koç et al., 2012).

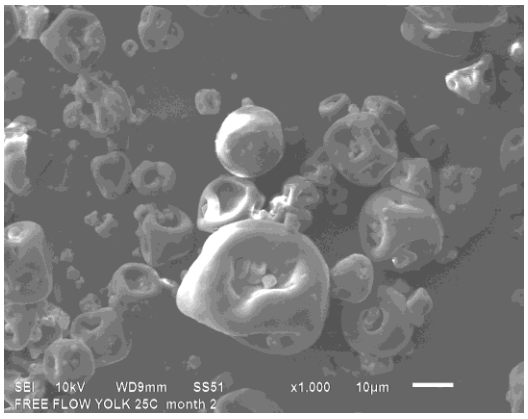

Figure 4.11 Shriveled appearance of spray dried Free Flow Egg powder stored at 25 °C after 2 time periods of storage

The egg powder particles in Figure 4.11 were observed as amorphous and shriveled. This is due to the spray drying; when part of the surface remains moist, the particles deflate and shrivel as they cool (Koca, Erbay, & Kaymak-Ertekin, 2015). Fast formation of particles cause damage in the surface of the particles. By SEM inspection, all the powders seem to have had moisture on their surface present after spray drying due to their deflated and porous appearance as seen in Figure 4.11. The porous surface aids solubility and at the same time it exposes the product to air and it's a great possibility that this causes product oxidation resulting in reduced storage stability. The bulk properties of the egg powder such as particle size and distribution are dependent on the spray drying conditions (Koç et al., 2012).

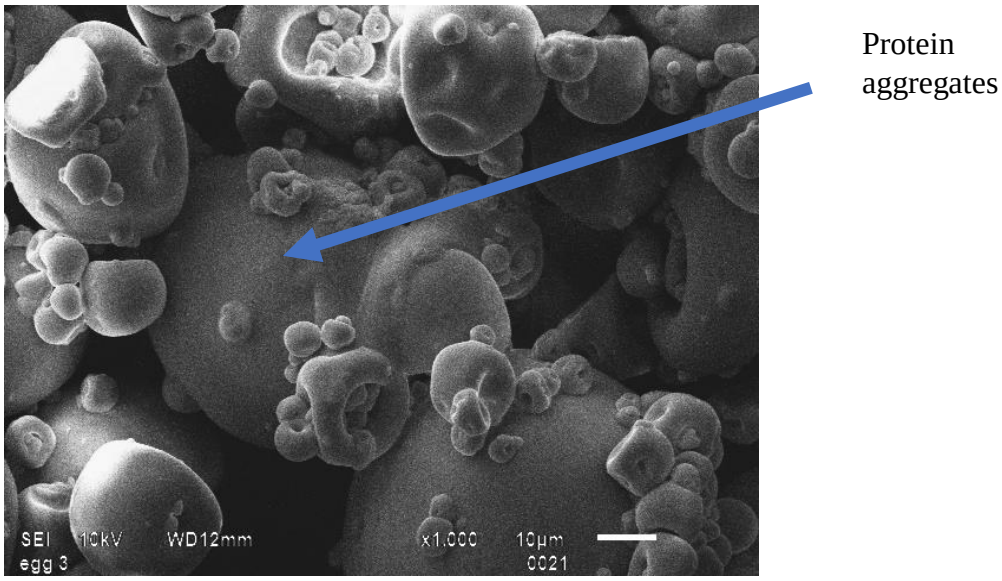

Figure 4.12 Enzyme modified egg powder protein aggregates sticking when stored at 43 °C for 2 months

Figure 4.12 shows that at storage temperature above 43 °C we can begin to observe this changes such as sticking and collapsing. In Figure 4.12 protein aggregates are observed in egg powder stored at 43 °C after 2 months. High storage temperatures over 43 °C leads to the occurrence of several chemical reactions that results in denatured proteins that expose its potential

binding sites. When these sites such as thiol group, ligand and fatty acid binding sites are exposed, other proteins interact with each other and form protein aggregates (Guo, 2016). Partial denaturation of proteins also promotes lipid oxidation and Maillard reactions (Guo, 2016). Changes in protein structure resulting from the formation of protein aggregates alter the solubility and emulsion ability as measured in this study.

4.11 Recommendations

-Oxidation reactions must be minimized

Sealing the bag and completely or partially removing oxygen will extend the shelf life of dried egg products. Methods to control oxygen content such as modified atmosphere packaging (MAP) optimizes the nitrogen-to-oxygen ratio to produce shelf stable egg powders. Another method is vacuum packaging which removes the oxygen completely. The removal of glucose had also shown to reduce oxidation reactions such as Maillard that requires a reducing sugar to form oxidation compounds.

-Light also plays a role in oxidation reactions

A dark or solid color should be selected for packaging. When transporting the egg overseas avoid shipping in plastic bags and replace with white 6 gallon buckets. Plastic bags allow oxygen and other components such as ink to permeate and contaminate the product.

-Control the storage temperature

Studies have shown temperatures above 38 ° C speed up the rate of reactions in egg powder, in this 2-month study, storage at 43 ° C was enough to oxidize and lose thermal stability in egg powder.

-Packaging

Film packaging such as NYLEX-PAS6AS from ANTISTAT should be considered. NYLEX-PAS6AS is a clear high gas barrier Nylon and EVOH coex designed for use in form-fill and pouch applications requiring anhanced sealant and anti-static properties. The anti-static property is important to reduce the combustible nature of the dry product. This type of packaging also has puncture resistance.

CHAPTER 5: CONCLUSIONS

From the storage study of commercial egg powders, we can conclude that high storage temperatures and long storage time induced changes in the physicochemical properties of dried egg products. Dried egg powder samples stored at 43°C lost emulsifying capacity at month 2. EMY powder was the best performing egg powder when studying emulsion stability, viscosity and thermal stability. Emulsion stability for FFWE, FFY and PLWE was reduced to 40-60% with powders stored at 43 °C and 0% at 54 °C during month 2. EMY was heat stable at 25 °C but not 43 °C or 54°C during month 2. FFWE, FFY and PLWE lost heat stability at storage temperature 54 °C in month 1. In month 2 all samples lost heat stability at storage temperature 43 °C.

Lipid oxidation was greater for EMY > FFY > FFWE > PLWE. Peroxide values for powders stored at 43 °C were higher than peroxides for powders stored at 54°C indicating that oxidation compounds are being converted into other oxidation compounds at a faster rate. Free fatty acid loss is another indicator of lipid oxidation and it was observed that EMY had higher values but still in an acceptable range for Michael Foods. Color increased for EMY>FFY>PLWE>FFWE, as storage temperature increased the change was more evident. Protein agglomeration was observed for samples stored at 43 °C. Lipid oxidation and Maillard reactions are the two main reasons for the deterioration of dried egg yolk.

Possible approaches to increase the shelf life of dried egg yolk include the removal of reducing sugars. With low reducing sugar levels, such as low glucose, the extent of Maillard reactions would be reduced. Adding antioxidants such as ascorbyl palmitate, dl-α-Tocopherol and propyl gallate would reduce oxidation in egg powders. Vacuum packaging is strongly recommended to reduce the amount of oxygen inside the packing that is available that will promote oxidation. Other preservation methods such as MAP show promising results in extending the shelf

life of perishable food items. In conclusion temperatures > 43 °C are not recommended for the storage of egg powder.

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