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Sensory Evaluation and Quality Indicators of Nutritionally Enhanced Egg Product with Omega-3 Rich Oils

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**Sensory Evaluation and Quality Indicators of Nutritionally Enhanced
Egg Product with Omega-3 Rich Oils.**

Helenia Dawn Sedoski

**Thesis submitted to the
Davis College of Agriculture, Natural Resources and Design
At West Virginia University
In partial fulfillment of the requirements
for the degree of**

**Master of Science
in
Animal and Nutritional Sciences**

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Abstract

Sensory Evaluation and Quality Indicators of Nutritionally Enhanced Egg Product with Omega-3 Rich Oils.

Helenia Dawn Sedoski

Heart disease is a leading cause of death in the United States. Omega-3 (n-3) fatty acids (FA) positively affect heart health while high levels of cholesterol are an indication of poor heart health. Nutritionally-enhanced low-cholesterol egg sticks fortified with n-3 FA were developed; quality indicators were measured and sensory evaluations were conducted. Store-bought eggs were separated and egg whites were mixed with oil, spray dried egg whites, annatto (color) and salt. The oil sources included: algae, menhaden, flaxseed, and canola oils. Experimental egg batters and mixed whole egg (control) were cooked in the shape of a cheese stick, vacuum packed, and stored at 4C. Quality indicators (color (L^* , a^* , b^*), texture, pH and lipid oxidation) of egg sticks were measured over a storage period of 14-d. There were no changes in color over time ($P>0.05$); however, whole egg was significantly less red and yellow compared to experimental eggs ($P<0.05$). Significant differences in texture properties were observed ($P<0.05$) but these were independent of time ($P>0.05$). A difference in pH between egg types was seen ($P<0.05$); however, the pH for each egg type did not change over time ($P>0.05$). No trend in lipid oxidation was seen over storage time ($P>0.05$). Egg sticks were evaluated by a consumer panel of 56 participants for color, odor, flavor, texture between fingers, mouth feel and overall liking with a 9-point scale (1=dislike extremely; 9=like extremely). Panelists also ranked samples from most to least liked. The texture between fingers of the whole egg was significantly more liked than the experimental eggs containing DHAS and canola oils ($P<0.05$). Odor scores were significantly greater ($P<0.05$) in whole egg compared to the experimental egg containing flaxseed oil. No

significant preference was observed in mouthfeel or flavor. All samples were well-liked, with a combined average score of 5.9. Ranking results support this conclusion with no significant differences in preference ($P>0.05$). The results indicate that the experimental eggs are accepted by consumers on the same level as mixed whole eggs and may have potential as a marketable product.

Key words: Omega-3, Egg, TBARS, texture profile analysis, functional food, egg composition, sensory evaluation

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CHAPTER I

INTRODUCTION

Most fatty acids (FA) can be synthesized by processes called desaturation and elongation (Hanebutt, et al., 2008). Humans lack a desaturase enzyme that can create a double bond on the third carbon from the omega terminal meaning that omega-3 (n-3) FAs must be consumed in the diet in order to be present in the body. Alpha-linolenic acid (ALA, 18:3 n-3) is an essential FA found in plants. ALA may be converted to docosahexaenoic acid (DHA, 22:6 n-3) and eicosapentaenoic acid (EPA, 20:5 n-3); however, this conversion is relatively low in humans (Hanebutt, et al., 2008). Therefore, it is best to consume these metabolites in the diet.

There are many health benefits with n-3 FA consumption, particularly EPA and DHA. The most extensively researched benefit is the reduced risk of cardiovascular disease (CVD). This may occur secondary to the reduction in blood pressure (BP), arrhythmias and the rate of atherosclerotic plaque buildup and platelet aggregation which decreases the number of thrombi. Blood lipid profile can also be improved. EPA and DHA have been shown to reduce low density lipoproteins (LDL: known as bad cholesterol). Omega-3 FAs produce n-3 series eicosanoids. These eicosanoids include prostaglandins, leukotrienes and thromboxins. N-3 series eicosanoids are weaker than n-2 (pro-inflammatory) series eicosanoids produced by arachidonic acid (AA), an essential n-6 FA. By the process of competitive inhibition the n-3 series reduces the production of the n-2 series. This leads to reduced inflammation of the blood vessels, making it easier for blood to flow.

The recommended intake for n-3 FAs is not clearly established. For those with a healthy heart the American Heart Association (AHA) recommends fatty fish consumption twice a week. For individuals at risk for heart disease, when consuming EPA and DHA, research suggests a range of 0.5 – 1.8 grams each day. If consuming the precursor, ALA, the recommended range is 1.5 – 3 grams. Those with coronary heart disease (CHD) should consume 1 gram of EPA/DHA. To reduce elevated triglycerides (TAG) 2 – 4 grams a day should be consumed only under a doctor's supervision to monitor signs of toxicity (Kris-Etherton, et al., 2003). Some recommendations for intake are based on a specific ration of n-6:n-3 FAs.

Currently the U.S. population consumes a diet higher in n-6 FAs and lower in n-3 FAs than in the Paleolithic period (Simopoulos, 2006). The evolutionary human diet was about 1:1 (Simopoulos, 2006) and the current ratio is about 10 - 16.75:1 (Kris-Etherton, Harris and Appel, 2002 and Simopoulos, 2006). Consumption is expressed in grams per day is estimated to be 0.05 grams of DHA, 0.08 grams of EPA and 1.2 grams of ALA (Simopoulos, 2001). The high consumption of n-6 FAs may contribute to the increase in health problems because of the n-2 series eicosanoids that promote platelet aggregation and inflammation. This imbalanced intake reflects availability in the food supply and dietary intake.

It is estimated that the beneficial n-6:n-3 ratio is a 1:1 consumption ratio and that the current consumption is higher due to the increased use of n-6 rich oils (Kris-Etherton, Harris and Appel, 2002). To correct the imbalance in the American diet, n-3 supplements can bring the ratio closer to the recommended 1:1.

Supplements containing EPA and DHA, such as fish oil supplements, are known to cause some gastrointestinal discomfort for some. A supplement containing ALA may be a better option for those experiencing the discomfort (Escott-Stump, 2008). It is estimated that a higher amount of ALA, 1.5 – 3 g/d, would need to be consumed to achieve the same benefits as the EPA/DHA supplement (Kris-Etherton, Harris and Appel, 2002).

Omega-3 FAs are also essential for development of the eyes and brain of a fetus. A deficiency of n-3 FAs it may lead to cognitive dysfunction and poor mental health. Adequate consumption of n-3 FAs during pregnancy will aid in the development of the fetus. To continue the proper development, infants should consume n-3 FAs. If an infant is breast fed it is important that the mother continues to consume n-3 FAs. If the infant is formula fed, a formula with n-3 FAs should be selected.

EPA and DHA are naturally found in fatty fish such as mackerel, albacore tuna and salmon. ALA is a plant-based source of n-3 fatty acids. Tofu, walnuts, flaxseed and canola oil all contain ALA which can be converted to EPA and DHA in the body. Due to the limited sources of naturally occurring EPA and DHA, food products with added n-3 FAs are being developed and are part of a market section called functional foods.

Functional foods are defined as “foods with a physiological function or activity” that are “used for products containing biological active components at levels that may confer specific health benefits” (Knovel, 2005). Although production of functional foods is increasing there is no universal definition. Many organizations have provided definitions of functional foods but all agree that functional foods provide a health benefit

(Hasler, 2002). EPA and DHA derived from fatty fish are the n-3 fatty acids most researched to be used as a functional food because of the benefits for heart, brain and eye health they appear to promote. One animal derived functional food containing n-3 FAs is the egg.

An average large egg contains provides around 70 calories and 13 essential vitamins and minerals (Incredible!, 2010). Eggs are a good source of protein that is inexpensive. One large egg has 7 g of protein, 4 of which are within the egg white. However, a whole egg also contains 5 g of fat and 213 mg of cholesterol in the egg yolk (Brown, 2008). The Therapeutic Lifestyle Change (TLC) Diet recommends no more than 200 mg of cholesterol a day to maintain a healthy heart (American Heart Association, 2006). One large egg a day would exceed the cholesterol limits of the TLC diet.

Feeding chickens a diet rich in n-3 FAs, such as menhaden oil, fish meal, flaxseed or canola oil, will allow for incorporation of the n-3 FAs into the egg itself and the cholesterol concentration may be reduced; however, cholesterol is not eliminated from the egg. The purpose of this study was to evaluate the sensory and quality parameters of a nutritionally-enhanced, low-cholesterol, post-laying, cooked egg product fortified with n-3 FAs over storage time. It is important to examine the proximate composition, pH changes, lipid oxidation, color changes and consumer likeability.

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CHAPTER II

REVIEW OF LITERATURE

HEALTH BENEFITS OF OMEGA-3 FATTY ACIDS

Heart disease is the number one cause of death for men and women in the United States. One possible indication of inflammation that can increase the risk of myocardial infarction is high levels of C – reactive protein (C-RP) (Kelley, et al., 2008). Moderate risk levels are 1.0 – 3.0 mg/L and high risk levels are greater than 3.0 mg/L omega-3 (n-3) fatty acids (FA). It is possible to observe a significant reduction in C-RP levels in 6 months when consuming 6 grams of n-3 fatty acids daily (Bowden, et al., 2009).

High levels of serum triglycerides are also a risk factor for cardiovascular disease (CVD). Increased consumption of docosahexaenoic acid (DHA) and eicosanpentaenoic acid (EPA) enriched foods will lower triglyceride levels (Rose and Holub, 2006, Dawczynski, et al., 2010). The American Heart Association states that intake of n-3 FAs may also slow atherosclerotic plaque build-up, reduce abnormal heart rhythms and reduce the risk of blood clots that can lead to a stroke (Kris-Etherton, et al., 2010). High serum cholesterol has also been associated with increased risk of CVD. Though n-3 FAs only slightly decrease serum cholesterol levels (Kris-Etherton, et al., 2010) dietary cholesterol can increase serum cholesterol levels. Consuming approximately 120 g of standard whole eggs daily for less than 4 weeks can increase serum cholesterol (Roberts, McMurry and Conner, 1981).

Omega-3 FAs are beneficial when consumed by women prior to and during pregnancy. During pregnancy docosahexaenoic acid – lysophosphatidyl choline (DHA-

LPC) is released from the liver. DHA-LPC delivers DHA to the placenta to help in fetal brain, central nervous system (CNS) and eye development (Valenzuela et al., 2010, Hanebutt et al., 2008). The most rapid fetal brain growth occurs during the final trimester of pregnancy. Preterm deliveries do not have the exposure to n-3 FAs at the crucial time period which leads to inferior development (Hanebutt et al., 2008).

Breast fed infants rely on the mother's consumption of n-3 FAs (Jensen, 1999). Maternal FA stores can be diminished during late pregnancy as the fetus develops (Bergmann et al., 2008). If there is no intake of DHA, breast milk could contain very little DHA. Normal breast milk contains 0.2 – 0.4 % DHA (Jensen, 1999). With 200 mg of DHA supplementation each day beginning in mid-pregnancy and continuing through lactation, breast milk can contain an average of 0.5 % DHA (Bergmann et al., 2008). The group that did not receive the supplement in Bergmann's study had an average of only 0.25 %. This supports that theory that supplemental DHA intake can enhance the n-3 status of both the mother and the infant.

NUTRITIONAL ENHANCEMENT OF EGGS

Traditionally, changes in the nutritional composition of eggs were accomplished through diet manipulation. In an attempt to increase the n-3 content of eggs, hens were fed diets enriched with n-3 rich oils from marine, algae or oilseed sources. Up to 200 mg of n-3 FAs were deposited in the yolk of the egg when fed a diet with 15 – 30 mg of marine oil for each kilogram of feed (Van Elswyk, 1997). When the hens were taken off the high n-3 FA diets, a 20% decrease in n-3 FA content of the egg yolks 1 week after n-3 FAs were not added to the hen feed. During the second week following removal of n-3 FAs from the feed, another 20% decrease was observed (Van Elswyk, 1997). The

n-3 fatty acids are incorporated into the egg at the expense of some n-6 fatty acids (Van Elswyk, 1997) not the cholesterol.

Marshall and others reported an increase in n-3 FAs (Linolenic acid, DHA and EPA) with a decrease in n-6 FAs (arachidonic acid) when feeding hens 1.5% menhaden oil for 4 weeks (1994). The n-3 increase included a two-fold increase of alpha-linolenic acid (ALA) and a six-fold increase in DHA. EPA was not found in the control hen eggs but the treatment hen eggs contained 16 mg per yolk. A reduction in the saturated fatty acids, palmitic (16:0) and stearic (18:0) was also observed in the treatment eggs (Marshall, Sams and Van Elswyk, 1994).

When flaxseed enriched feeds are given to hens, more n-3 fatty acid are incorporated into the egg yolks (24 mg/g yolk when ground and 18 mg/g yolk when whole) than 15 mg marine oil/kg (9 mg/g yolk). The incorporation of n-3 fatty acids seems to reach its maximum point within 4 weeks when fed the higher levels of n-3 rich diets. When the diet is discontinued, n-3 fatty acid levels reduce back toward base line (Van Elswyk, 1997).

Lipids in eggs function in flavor, mouthfeel, texture and emulsification capacity. When the fat level is reduced those parameters will be altered. When the lipid content of cheese is reduced a firmer, more rubbery texture is produced (Drake, et al., 1998). Increasing the amount of unsaturated fatty acids in an egg causes the yolk to become less firm when subjected to compression tests (Iglesias, et al., 2007). Reducing fat content in chocolate causes a higher viscosity and poor melting in the mouth (Do, et al., 2007). Overall changing the lipid composition changes the function and sensory

attributes of food products. However, n-3 enriched eggs would contain the same amount of fat as a standard egg, maintaining consumer acceptability.

A nutritionally-enhanced cooked egg product was developed by Kassis and others (2010 A and 2010 B). The experimental eggs were prepared by removing the egg yolks, and then adding n-3 rich oils (flaxseed, menhaden, algae and krill oils), annatto, freeze dried eggs and salt to the egg whites to mirror the proximate composition, color and texture of whole eggs. The liquid batters were poured into polycarbonate tubes and cooked in a microwave oven.

Proximate composition (crude protein, total fat, moisture and ash) of the experimental eggs matched that of a whole egg ($P < 0.05$). Though the total fat content of the experimental eggs matched that of a whole egg, the whole egg control had a much higher cholesterol content than the experimental eggs. Fatty acid analysis showed that the experimental eggs had a much higher n-3 FA content. The texture of the experimental eggs was not different than the whole egg ($P > 0.05$) but color differences were seen between the whole egg and the experimental eggs ($P < 0.05$) (Kassis, et al., 2010 A). This method of nutritional-enhancement could yield a better result nutritionally than traditional enhancement by lowering cholesterol and adding high amounts of n-3 FAs.

LIPID OXIDATION

The change in lipid composition of an egg may affect the shelf life of the product, due to a different rate of lipid oxidation. The breakdown of lipids changes many aspects of food, from the color to pH. Rancidity and off-odors and flavors may result. Egg yolks

naturally contain vitamin E, avidin and phosphatine which make a whole egg resistant to lipid oxidation (Pike and Peng, 1985). Whole eggs that are nutritionally-enhanced via chicken feed may be more susceptible to lipid oxidation because the eggs may contain a higher amount of very long-chain polyunsaturated fatty acids (VLCPUFAs), which are more readily oxidized than saturated fats (Marshall, Sams and Van Elswyk, 1994).

The first step in lipid oxidation is the formation of a free radical. Foods with unsaturated fatty acids are more susceptible to free radical formation. The available hydrogen ions bond with oxygen and peroxides are formed. Another unsaturated fatty acid donates a hydrogen atom. This produces a hydroperoxide with the initial fatty acid and another free radical from the donation of the hydrogen atom (McWilliams, 2005). The primary products of lipid oxidation include lipid hydroperoxides (LHP), which are further broken down into secondary products. To measure lipid oxidation of n-3 enhanced egg batters the secondary oxidation products, such as malondialdehyde (MDH), should be measured (Galobart, et al., 2001).

Testing thiobarbituric acid reactive substances (TBARS) is a method commonly used to assess the amount of malondialdehyde (MDA) in a product. MDA is commonly used to measure lipid oxidation because it has a much longer half life than LHP. A higher value means more lipid oxidation has occurred. Over a 6-week storage time eggs of chickens fed varying levels of flaxseed and fish oil diets had increasing TBARS values (Scheideler, Froning and Cuppett, 1997). However, past week 6 of storage, TBARS levels decreased perhaps due to reactions with other chemical components within the egg (Scheideler, Froning and Cuppett, 1997).

SENSORY EVALUATION

Consumers are displaying more demand for foods with health benefits (Childs and Poryzees, 1997). As consumers age they tend to become more concerned with the health benefits of foods than taste, opposite of young consumers (Roininen, Lähteenmäki and Tuorila, 1999). Sensory evaluations are a method to evaluate the acceptability of a food product. Just as consuming different types of feeds changes the lipid content of a hen's egg, taste, smell and appearance may also be altered.

When hens were fed 30 mg of marine oil/kg of feed the hen eggs were perceived as fishy when eaten scrambled but not when eaten hard boiled at room temperature. Hens fed a 15 mg marine oil/kg feed diet did not produce eggs that were perceived as fishy and were stable during storage time (Van Elswyk, 1997). A diet consisting of 1.5 % menhaden fish oil or 5 % flaxseed to enrich egg yolks produce an egg that is still acceptable by consumers (Scheideler, Froning and Cuppett, 1997 and Marshall, Sams and Van Elswyk, 1994). However, when flaxseed is increased to 10 % and 15 % of feed, a negative response to appearance and flavor was seen (Scheideler, Froning and Cuppett, 1997).

Some theorize that adding an antioxidant will reduce the off flavors that are associated with n-3 fatty acid enhancement. Eggs from hens fed 10% of their diet from flax seed and 10 mg vitamin E/kg of feed were perceived as fishy when compared to a control egg. However, when eggs from hens fed 20% flax and 10 mg vitamin E/kg of feed were compared to eggs of hens fed 20% flax and 100 mg vitamin E/kg of feed the off flavor was not noted (Leeson, Caston and MacLaurin, 1998).

Tocopherol was used by Cherian and other (1996) to examine the extent of reducing lipid oxidation in eggs from hens fed diets containing different lipids. There was no difference in lipid oxidation over time between eggs from hens fed tocopherols and those that were not. However, hens that were fed tocopherols in addition to a diet rich in n-3 FAs had a higher amount of n-3 FAs than hens not fed tocopherols with the n-3 FAs. A possible explanation is that tocopherols protect the n-3 FAs from deterioration or tocopherols enhance the desaturase pathway and increases the synthesis of n-3 FAs (Cherian, Wolfe and Sim, 1996).

Hayat and others (2010) used 100 mg/kg feed butylated hydroxytoluene (BHT) or 100 IU/kg feed vitamin E (α -tocopherol) as antioxidants in feeds that contained 10% flax seed to maintain sensory qualities. Untrained sensory evaluators were unable to distinguish a difference among any of the samples taste or aroma. Trained sensory evaluators did find the enhanced eggs to have an off-flavor and a different aroma than the control eggs. The addition of antioxidants did not prevent off-flavors and aroma associated with lipid oxidation (Hayat, et al., 2010).

MAINTAINING QUALITY PARAMETERS

Raw eggs sold in the market are typically thermally pasteurized to prevent *Salmonella* enteritidis. However, about 19000 cases of food-borne illness still occur annually due contamination (Kamotani, et al., 2010). The majority of eggs sold as an already prepared item are packaged under modified atmospheres (MAP). These egg items have a pH around 7.7, a level suitable for bacterial growth (Clair, et al., 2004). The quality of this ready to eat, MAP eggs decreases over storage time and have a chance of post production contamination from handling during packaging and

preparation (Clair, et al., 2004). Weight loss and changes in color and pH are important indicators quality deterioration (No, Prinyawiwatkul and Meyers, 2005), all which can decrease more rapidly if conditions allow bacterial growth.

A variety of packaging methods are used to prevent spoilage of already prepared hard boiled eggs. One method used is dipping prepared eggs, in shell or peeled, into edible chitosan-lysozyme composite coating (Kim, Daeschel and Zhao, 2008). Coating the eggs, with or without the shell, may maintain the color and slow loss of moisture and changes in pH (Kim, Daeschel and Zhao, 2008). Proper refrigeration of prepared egg products may be more important in maintaining quality than the packaging process (Clair, et al., 2004).

TEXTURE PROFILE ANALYSIS (TPA)

The application of heat or cooking an egg denatures the proteins and causes a gel to form. The temperature of the egg will increase very rapidly until the gel begins to form at 60-65°C. At this point, approximately 4 minutes into cooking, the protein ovotransferrin will denature. The temperature of the egg will increase very slowly until it reaches 80-85°C. Ovalbumin will denature and strengthen the gel causing the entire egg to firm and hold a shape (Woodward and Cotterill, 1987 and Croguennec, Nau and Brulé, 2002).

Many factors contribute to the texture profile of a cooked egg. When an egg is hard-boiled in shell without disrupting the yolk, the yolk texture will be dry and crumbly. If an egg yolk is disrupted and mixed with the egg while the texture will be springy and hard when the egg is cooked (Woodward and Cottrill, 1987). Woodward and Cottrill

(1987) found that the hardness of an egg will increase if pH, temperature, time exposed to heat, concentration of protein or concentration of salt is increased (Woodward and Cotterill, 1987). As an egg ages, it becomes more basic, meaning older eggs will naturally form a harder gel (Croguennec, Nau and Brulé, 2002). A pH ranging from 7 to 9 will form a more uniform gel due to the smaller spaces between the aggregates, negative net charge and slower thermocoagulation leading to higher water-holding capacity and more viscoelasticity (Croguennec, Nau and Brulé, 2002). Many have stated that as salt concentration increases the gel will become harder up to 0.05M NaCl (Woodward and Cotterill, 1987 and Croguennec, Nau and Brulé, 2002). Concentration levels above this have very little additional effect.

SUMMARY

Nutritional enhancement of eggs via alterations in chicken feed is likely to increase n-3 FA content as long as feeding is maintained. Sensory changes from nutritional enhancement eggs using flax is possibly the least detected source of n-3 fatty acids. It is unclear if the use of antioxidants will stave off lipid oxidation and preserve the sensory qualities of the eggs. The amount of fat is not altered just the type of fats allowing them to function the same as a standard egg (Pankey, 1969). It appears that the incorporation of the n-3 FA is in place of n-6 FA and not of saturated fats or cholesterol (Marshall, Sams and Van Elswyk, 1994). For that reason, altering feed to enhance chicken eggs may not provide maximal health benefits.

Enhancement of the eggs after laying by removal of egg yolks may be a better method. This method would eliminate the saturated fat and cholesterol from the egg and add n-3 FAs. Eggs are a staple item; being one of the top 25 foods to contribute to daily

calorie consumption, and in the top 15 of foods that contribute to saturated fat and cholesterol consumption in the average U.S. American diet (National Cancer Institute, 2010), a lipid altered egg product could lead to a decrease in saturated fat and an increase in n-3 FA consumption on a population level. Therefore, the purpose of this study was to evaluate the sensory and quality parameters of a nutritionally-enhanced, low-cholesterol, post-laying, cooked egg product fortified with n-3 FAs over storage time. It is important to examine the proximate composition, pH changes, lipid oxidation, color changes and consumer likeability.

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CHAPTER III

Sensory Evaluation and Quality Indicators of Nutritionally Enhanced Egg Product with Omega-3 Rich Oils.

Abstract

Heart disease is a leading cause of death in the United States. Omega-3 (n-3) fatty acids (FA) positively affect heart health while high levels of cholesterol are an indication of poor heart health. Nutritionally-enhanced, low-cholesterol egg sticks fortified with n-3 FA were developed; quality indicators measured and sensory evaluations conducted. Store-bought eggs were separated and egg whites mixed with oil, spray dried egg whites, annatto (color) and salt. The oil sources included: algae, menhaden, flaxseed, and canola oils. Experimental egg batters and mixed whole egg (control) were cooked in the shape of a cheese stick, vacuum packed, and stored at 4°C. Quality indicators (color (L^* , a^* , b^*), texture, pH and lipid oxidation) of egg sticks were measured over a storage period of 14-d. There were no changes in color over time ($P>0.05$); however, whole egg was significantly less red and yellow compared to experimental eggs ($P<0.05$). Significant differences in texture properties were observed ($P<0.05$) but these were independent of time ($P>0.05$). A difference in pH between egg types was seen ($P<0.05$); however, the pH for each egg type did not change over time ($P>0.05$). No trend in lipid oxidation was seen over storage time ($P>0.05$). Egg sticks were evaluated by a consumer panel of 56 participants for color, odor, flavor, texture between fingers, mouth feel and overall liking with a 9-point scale (1=dislike extremely; 9=like extremely). Panelists also ranked samples from most to least liked. The texture between fingers of the whole egg was significantly more liked than the experimental eggs containing DHAS and canola oils ($P<0.05$). Odor scores were significantly greater ($P<0.05$) in whole egg compared to the experimental egg containing flaxseed oil. No significant preference was observed in mouthfeel or flavor. All samples were well-liked,

with a combined average score of 5.9. Ranking results support this conclusion with no significant differences in preference ($P>0.05$). The results indicate that the experimental eggs are accepted by consumers on the same level as mixed whole eggs and may have potential as a marketable product.

Key words: Omega-3, Egg, TBARS, texture profile analysis, functional food, egg composition, sensory evaluation

Introduction

Animal protein is an excellent source of amino acids essential to building body proteins due to their high bioavailability. However, sources of animal protein tend to be high in saturated fat which is associated with higher risks of cardiovascular disease. The trend of consumer demands is heading toward foods with more health benefits, such as those foods containing omega-3 (n-3) fatty acids (FA) because of the association with lower risks of several health complications such as inflammatory diseases (Bou, et al., 2009).

Though a current recommendation does not exist for the amount of n-3 FAs that should be consumed, a suggested ratio of omega-6 (n-6):n-3 FAs does. A 1:1 ratio is thought to be the most beneficial to human health; however, the current n-6:n-3 FA ratio consumed by U.S. American's is much higher, about 10 - 16.75:1 (Kris-Etherton, Harris and Appel, 2002 and Simopoulos, 2006). This is likely due to the availability of more n-6 FA containing foods for consumption.

Eggs are a staple protein source in the American diet; they are one of the top 25 foods eaten and contribute 11% to daily calorie consumption (National Cancer Institute, 2010 and Drewnowski, 1995). Though they are a good source of protein, eggs are also in the top 15 of foods that contribute to saturated fat and cholesterol intake (National Cancer Institute, 2010). Given the high contribution to a typical diet, formulating an egg product that is low in saturated fat, cholesterol-free and nutritionally enhanced with more n-3 FAs could be beneficial.

Enriching eggs with n-3 FAs is typically accomplished by increasing the n-3 FA content in the feed given to hens. Hens are able to incorporate the dietary n-3 FAs into the fat composition of the egg yolks (Bout, et al., 2009). Typical oils added to an egg-laying hen's diet include: marine algae (Van Elswyk, 1997), menhaden oil (Marshall, Sams and Van Elswyk, 1994, Scheideler, Froning and Cuppett, 1997 and Van Elswyk, 1997) and flaxseed oil (Van Elswyk, 1997 and Scheideler, Froning and Cuppett, 1997), with each differing in the amount of n-3 FA incorporation into the egg yolk.

When feed supplemented with 24 g marine algae/kg feed was fed to hens for four weeks, 9.5 mg n-3 FA/g yolk was incorporated into the egg (Van Elswyk, 1997). The maximum beneficial amount of marine algae is suggested to be between 24 and 48 g/kg feed because when hens were fed 48 g/kg feed the total n-3 FA incorporation did not double but increased to 12.2 mg n-3 FA/g yolk (Van Elswyk, 1997). Eggs of hens fed 25 - 30 g menhaden oil/kg feed reached a plateau of n-3 FA incorporation, 200 mg/yolk, after 3 weeks of feeding (Van Elswyk, 1997). The menhaden oil contains more EPA than DHA; however DHA was the primary n-3 FA deposited in the egg yolk. About 89% of the consumed DHA was incorporated into the egg yolk while less than 5% of the EPA consumed was in the egg yolk. N-3 FAs were incorporated; however, significant reductions of n-3 FAs in the egg yolks were reported 4 weeks after the diet ceased. The increase in n-3 FAs was at the expense of n-6 FAs, not cholesterol (Van Elswyk, 1997). Consumer's found eggs from hens receiving 30 g menhaden oil/kg feed acceptable when hard boiled, but reported fishy flavors when eggs were scrambled (Van Elswyk, 1997). When 150 g of ground flaxseed/kg feed was given to hens, each gram of yolk contained 24 mg n-3 FAs (Van Elswyk, 1997). The primary n-3 FA incorporated into the

yolk was linolenic acid, which is considered less bioactive (Van Elswyk, 1997). Flaxseed oil incorporated into a hen's diet at a level of 5% of the feed were acceptable to consumers, but when the feed was 10 – 15% flaxseed oil, negative sensory attributes were seen in appearance and flavor (Scheideler, Froning and Cuppett, 1997).

Adding n-3 FAs to eggs after production may yield more consistent results. Kassis and others developed an n-3 enriched egg product (2010b) by egg yolk removal and incorporation of n-3 FA rich oils into the egg whites. Freeze-dried egg whites were included to replace the protein lost with the yolk. The proximate composition (crude protein, total fat, moisture and ash) were similar to that of a whole egg control (Kassis, et al. 2010b). The total fat of the experimental eggs contained a greater amount of n-3 FAs and significantly less cholesterol and saturated fat than the whole egg ($P < 0.05$) (Kassis, et al., 2010b). Therefore, the purpose of this study was to evaluate the sensory and quality parameters of a nutritionally-enhanced, low cholesterol, ready-to-eat (RTE) egg product similar those developed by Kassis et al. (2010 a and b) over storage time. Proximate composition, pH changes, lipid oxidation, color changes and consumer likeability will be examined.

MATERIALS AND METHODS

Preparation of egg products

Nutritionally-enhanced egg sticks were formulated based on the findings of Kassis and others (2010a, 2010b). The yolks of fresh eggs purchased at a local grocery store in the refrigerated section were hand-separated to yield 430 ml of egg whites. The target macronutrient composition of the batters was to be similar to a whole egg; therefore, to replace the protein removed with the egg yolk, the egg whites were hand-mixed for 10 min with 15 g of powdered egg whites (Honeyville Farms Powdered egg whites, Sherrco Products Honeyville, Utah). The mixture was stored in a foil covered beaker overnight at 4 °C to allow for complete hydration of the powdered egg whites. Once the dried egg whites were rehydrated and dispersed in the egg white mixture, 50 ml of the following oils were used in the formulation of the experimental egg batters:

1. Canola oil from Kroger (Morgantown, WV).
2. Flaxseed oil from Jedwards International, Inc. (Quincy, MA).
3. Menhaden oil (Omega Pure 8042TE) from Omega Pure (Reedsville, VA).
4. Algae oil (DHAS) from Martek Biosciences (Columbia, MD).
5. Blend of 30 ml flaxseed, 10 ml DHAS and 10 ml krill oil (4225F) from Enzymotec USA, Inc. (Springfield, NJ).

In addition to the oils, 750 µl Annatto (for color) and 5 g of NaCl (table salt) were added to the egg whites. The volume of the batters was approximately 500 ml. The batters were stirred on a stirring plates (Fisher Scientific Thermix Stirrer model 120S)

set at medium speed with a magnetic stir bar for 2 h in a standard 1000 ml glass beaker. Temperature was maintained at 4°C by wrapping the beaker with bags of ice.

Cooking of omega-3 enhanced egg batters

Immediately after stirring, approximately 50 ml of the batter were poured into polycarbonate tubes (length = 18 cm, internal diameter = 1.90 cm, wall thickness = 0.635 cm) lightly sprayed with cooking spray (Kroger brand Vegetable oil cooking spray) to prevent sticking, and sealed with screw caps. Tubes were submerged in a standard stainless steel stockpot of boiling water for 10 min. After cooking, tubes were cooled in ice water, the cooked egg sticks were removed from the tubes, patted dry with disposable towels, vacuum-packed and stored at 4 °C until analyses were conducted.

Analysis on Cooked Egg Sticks

Proximate Composition: Proximate composition (moisture, fat, protein and ash) of cooked experimental egg stick containing the oil blend was determined according to the Association of Official Analytical Chemists methods (1995) and is be expressed as g/100g (wet basis). Moisture was determined by spreading 2 g of sample in aluminum dishes (Fisher Scientific Co., Fairlawn, NJ) and drying them at 105°C for 24 h. The Soxhlet extraction method and Kjeldahl assay were used to determine fat and crude protein content, respectively. Ash was determined by ground sample being placed in porcelain crucibles and incinerated at 550°C for 24 hours.

pH: Changes in pH were measured over a two-week storage period, based on a targeted retail shelf-life. The pH of each prepared egg stick was tested using the pH/ion

analyzer (Model 350, Corning Inc.; Corning NY, USA) by placing the probe against the surface of the egg stick. The samples were tested on days 0, 2, 4, 6, 8 and 14.

Color: Color differences and changes of each egg stick were measured over time using a chromameter (Model CR-300; Minolta Camera Co. Ltd., Osaka Japan) calibrated with a standard white plate No.21333180 (CIE L 93.1; a* 0.3135; b* 0.3198). Color was measured based on lightness (L), amount of red (a*) and amount of yellow (b*). The samples were tested on days 0, 2, 4, 6, 8 and 14.

Syneresis: The amount of water loss from the cooked egg products, or syneresis, was calculated using the following equation by Juliano and others (2006a):

$$\% \text{ weight loss} = [(\text{initial weight} - \text{final weight}) / \text{initial weight}] \times 100\%$$

The % weight loss was calculated for each sample on each day of the storage period (days 0, 2, 4, 6, 8 and 14).

Testing thiobarbituric acid reactive substances (TBARS): TBARS were completed in order to assess the lipid oxidation of the unsaturated fatty acids within the egg sticks. The oxidation of unsaturated fatty acids creates carbonyl compounds giving an unpleasant or rancid taste. Lipid oxidation was assessed by measuring the color absorbance at 535 nm. The specific compound created by oxidation that will be tested using TBARS is malondialdehyde, a dicarbonyl compound as explained by Yu and Sinnhuber (1957).

Texture Profile Analysis (TPA): Texture profile analysis (TPA) was performed on the egg sticks in triplicate at room temperature. Samples (length = 2.54 cm, diameter

= 1.90 cm) were brought to room temperature and subjected to a two-cycle 50% compressions using a texture analyzer (Model TA-HDi, Texture Technologies Corp., Scarsdale, NY) in likeness of Cheret and others (2005). A 70-mm TPA compression plate was attached and was set to move at 120 mm/min. Hardness, springiness, cohesiveness, gumminess, chewiness and resilience were determined from the recorded force-time curves (Chen and Jaczynski, 2007a; 2007b; Taskaya et al., 2009a; 2009b; 2009c). Hardness is the peak force needed to compress the sample. Springiness is the ability to recover the original form after force ceases. Cohesiveness is the degree the sample can be deformed before it breaks. Gumminess is hardness x cohesiveness. Chewiness is springiness x gumminess. Resilience is how much the sample opposes the force to return to its original shape (Kassis, et al., 2010a).

Microbial Analysis: The microbial load of the egg sticks were assessed over the storage period. Ten g of prepared egg sample were placed in a filtered stomacher bag with 90 mL of deionized, distilled water. Samples were pulverized using a stomacher until evenly distributed in the water. Using a micropipette 0.1 mL was spread-plated onto Xylose Lysine Deoxycholate (XLD; Remel, Lenexa, KS, USA) agar plate and 0.1 mL was spread-plated to a Trypticase Soy Agar (TSA; Difco, Becton Dickinson, Sparks, MD) plate. XLD was used specifically to monitor for *Salmonella* spp. contamination and the TSA was used to determine if any other bacterial contamination has occurred. Microbial analysis was conducted on days 0, 2, 4, 6, 8 and 14. The plates were incubated (Fisher Scientific low temperature incubator) at 35° C for 24 – 48 hours and the number of colonies were counted.

Sensory evaluation

Sample preparation: Egg sticks formulated with menhaden oil had a noticeable fish odor and egg sticks formulated with the oil mixture (1% DHAS, 1% krill oil and 8% flaxseed oil) presented with an unnaturally red color due to astaxanthin in the krill oil. Therefore, only egg sticks formulated with DHAS oil, flaxseed oil, canola oil and whole egg were tested during the sensory evaluation. Samples were prepared as described above, cut into 1-cm “coins” and placed into soufflé cups with lids coded with a random 3-digit code. The concept of the egg product was to mimic a hard-boiled eggs, which are often eaten cold; therefore samples were held at 4 °C until sensory evaluation was conducted.

Consumer panel participant selection: Fifty six adults over the age of 18 were recruited from the West Virginia University Davis College of Agriculture, Natural Resources and Design and were screened for food allergies.

Evaluations: Sensory evaluations were held in a well-lit, white colored room in the Test Kitchen and Foods Lab at West Virginia University. Prior to testing, participants were given a brief orientation on the testing methods and were required to read an informational hand-out (Appendix A) approved by the Institutional Review Board at West Virginia University. Participants were then asked to complete a pre-testing questionnaire (Appendix B) designed to assess the panelist demographics and to evaluate egg consumption.

Attribute testing: After completing the pre-testing questionnaire, participants were presented with one set of 4 samples in a balanced randomized order and asked to rate each sample on a 9-point hedonic scale where 1 = dislike extremely and 9 = like

extremely (Appendix C). Panelists were asked to evaluate the samples in the order they were laid out for them from left to right. They were asked to evaluate the sample odor when the lid was first removed, followed by visual color, texture between fingers, then flavor, mouth feel and overall liking. Space was provided on the scorecards for comments on each attribute. Panelists were instructed to cleanse their palates between samples by tasting unsalted crackers and rinsing their mouth with room temperature water.

Ranking test: A second set of the same 4 samples coded with different 3-digit codes, was presented to the panelists in a balanced, randomized order. They were instructed to taste all sample in the order provided and to rank them from most liked to least liked (Appendix D). Panelists were offered the option of going back and repeating the evaluation of each sample in the order of presentation, while the test was in progress.

Statistical analysis: The storage study was replicated three times. All storage evaluations (color, pH, texture profile; TPA) were performed in triplicate. Two measurements were completed for proximate analysis (protein, total fat, ash and moisture) and fatty acids. Thiobarbituric acid reactive substances (TBARS) analysis was completed by blending triplicates and three measurements were taken of each sample. A 6x6 factorial experiment in a completely randomized design was used and the data were analyzed using ANOVA where the two factors were Time with levels 1, 2, 4, 6, 8 and 14 days and Egg Type with 6 levels (whole egg, canola, flax, menhaden, DHAS algae and blend). The relationship between color, surface pH, texture and TBARS and time was examined via simple linear regression. Sensory evaluation

responses from 56 participants were also analyzed. Tukey's Honestly Significant Differences Test ($P < 0.05$) was completed to analyze mean separation. All data were analyzed using JMP 8 software (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Proximate analysis

The moisture content (77.7 ± 0.0), crude protein (12.8 ± 0.5), ash (0.7 ± 0.0) and fat content (10.3 ± 0.5) of the experimental egg containing the oil blend (Flaxseed, DHAS and Krill oils in an 8:1:1 ratio) was consistent with the findings of Kassis and others (2010a).

Surface pH

The pH of each sample did not significantly change over the storage time ($P > 0.05$) (**Table 1**). Fresh eggs have a pH ranging from 7.6 – 8.5 with saturated levels of CO_2 , mainly in the form of bicarbonate (Banerjee, Keener and Lukito, 2011 and Kim, Daeschel and Zhao, 2008). Bicarbonate can act as an acid or a base and contributes to pH levels. Over time, CO_2 escapes from the shell resulting in less bicarbonate in the egg and pH increases. The release of CO_2 seems to effect the pH of hardboiled eggs as well. When a peeled hardboiled egg was coated with a chitosan-lysozyme composite a slower change in pH than an uncoated hardboiled egg was observed over a 10 week storage period (Kim, Daeschel and Zhao, 2008). Peeled and vacuum packed hardboiled eggs also had less change in pH over time (Kim, Daeschel and Zhao, 2008). Both methods reduced the amount of CO_2 lost (Kim, Daeschel and Zhao, 2008). The experimental eggs were vacuum packed in a non-gas permeable package that likely contributed to the pH stability over the two week storage time by controlling the loss of CO_2 .

Color analysis

Color values (L^* , a^* , b^*) on prepared egg samples over storage time are reported in **Tables 2 – 4**. No significant changes were seen over the two week storage time ($P>0.05$); however, significant differences in color were seen between the samples ($P<0.05$). A grey scale value, L^* , represents the measurement of light reflected and light absorbed. L^* values range from 0 to 100; where 0 = black and 100 = white. The experimental egg containing a blend of oils had a significantly lower L^* value than all other eggs throughout the storage time ($P < 0.05$), meaning they were darker in color than all other eggs. This was consistent with the findings of Kassis, et al. that the experimental egg containing krill oil was the significantly darkest sample (2010a).

A positive a^* value represents reddish hues and a negative a^* value, greenish hues. Kassis and other showed a very high a^* value when using krill oil (2010a). The amount of krill oil was reduced in attempt to eliminate the unnatural appearing red color. However, the experimental egg containing the blend of oils (krill, DHAS and flax) had a significantly higher a^* value ($P<0.05$) than all other samples throughout the storage time. By reducing the amount of krill in the experimental egg the redness of the sample was reduced to 23 or less from that of Kassis, et al. at 37.5 (2010a). The whole egg was significantly ($P<0.05$) different than all experimental eggs with a negative a^* value, numerically showing the greenish tint to the whole egg sample. This is a common color produced in hard-boiled, in shell eggs that are cooled slowly around the surface of the egg yolk due to ferrous sulfide production.

A positive b^* value represents yellowish hues and a negative b^* value, bluish hues. On days 0, 2, 4 and 8 all experimental eggs had a significantly higher b^* score than the whole egg ($P < 0.05$). This gives a numeric value to the visibly more yellow

color in experimental eggs than the whole egg. On days 6 and 14 DHAS experimental eggs were not significantly different from other experimental eggs or the whole eggs ($P > 0.05$). This could be explained by the random sampling and inconsistent distribution of oils and annatto throughout the samples.

Syneresis

Syneresis is the loss of water from a gel. The experimental eggs had no significant changes in water loss over time ($P > 0.05$) but there were changes with the whole egg over time ($P < 0.05$) (**Table 5**). Linear regression showed no interaction between time and percent of weight loss ($P > 0.05$); however, significant differences were seen in the percent weight loss between samples ($P < 0.05$). Given that the whole egg generally had the least amount of weight loss; it is likely that a natural emulsifier, lecithin, which is present in the yolk, had an effect on the water holding capabilities. Addition of an emulsifier to the experimental egg batters may have allowed for better distribution of water, protein and fat leading to less water loss over time. Juliano and others (2006b) reported that xanthan gums reduced the amount of water loss in cheese; the water holding capabilities of the experimental eggs may also be improved with the addition of xanthan gum.

Testing thiobarbituric acid reactive substances (TBARS)

Lipid oxidation typically occurs at a much faster rate in n-3 FA containing foods resulting in a higher concentration of MDA. Although there were significant changes in MDA concentration for each experimental egg product ($P < 0.05$) over the course of the 14-day period, linear regression showed no correlation of time and lipid oxidation (**Table**

6). Reactions with other chemical components within the egg may have caused the inactivation of oxidation products resulting in the observed decreases of MDA on days after day 0 (Scheideler, Froning and Cuppett, 1997).

Texture Analysis (TPA)

Texture profile analysis data are presented in **Tables 7 – 12**. Data show that textural integrity (hardness, springiness, cohesiveness, gumminess, chewiness and resilience) at day 0 was not significantly different at the end of the storage period experimental egg products ($P > 0.05$). The texture was likely maintained because the factors that influence texture changes (pH and water loss) (Croguennec, Nau and Brulé, 2002, Banerjee, Keener and Lukito, 2011, Kim, Daeschel and Zho, 2008) remained the same over the 2 week storage time.

There were differences with the “blend” experimental egg ($P < 0.05$) that yielded a poor texture. These samples contained krill oil which is high in phospholipids (Gigliotti, et al., 2011 and Schneider, 2001). High levels of phospholipids can create textural and viscosity changes in food products (Schneider, 2001) and could explain the differences seen in this sample.

Microbial Analysis

Salmonella spp. or other bacterial contamination was not detected on any day of the 2 week storage time after streaking agar plates with pulverized samples and incubated for 24 hours.

Sensory Evaluation

Participants. The 56 panelists were between 18 and 35 years old. Of the participants, 82% reported consuming eggs at least twice a month. “Cholesterol consumption” was a concern to 86% of the participants and all participants reported protein consumption being of importance on some level. Only 6 participants reported they would not be likely to purchase a product similar to the experimental eggs.

Attribute testing and ranking. The results of the attribute test showed some significant differences on specific attributes (**Table 13**). Numerically, the colors of the experimental eggs were more favorably rated than the whole egg; however, this was only statistically significant for the canola oil variation ($P = 0.03$). This is consistent with instrumental measurements that show color of the experimental eggs to have a more yellowish hue and the whole egg to have a greenish hue. This may have influenced panelists color ratings. The color of the whole eggs is due to the high amount of sulfuric compounds in the egg yolk. Ovalbumin is the only protein in the egg white with free sulfhydryl groups (Croguennec, Nau and Brulé, 2002) so the greenish color would not be exhibited in the experimental eggs.

Numerically, the odor, texture between the fingers, and mouthfeel of the whole egg was more favorably rated than the experimental eggs; however these differences were not statistically significant ($P > 0.05$). The differences in types of lipids could explain the differences in odor and texture ratings because fat not only aids in creating texture properties it also carries lipophilic flavors and aromatic compounds (Zuidam and Heinrich, 2010). A lipid encapsulation method could reduce unfavorable odors (Zuidam

and Heinrich, 2010). No significant difference ($P > 0.05$) was seen in the attributes of flavor, mouthfeel or overall liking. Ranking of the samples supports no significant differences in overall liking because all samples were equally preferred. The average overall liking scores show that all experimental egg variations were generally well liked and 89% percent of the participants indicated interest in purchasing a product like this.

Conclusions

The results of the sensory evaluation show that this could be a marketed product if the low scoring attributes (texture between fingers and odor) are corrected. Emulsifier and antioxidant testing to find the best solution to evenly distribute the oils throughout the sample and extend the shelf life could increase the product acceptability. The sensory evaluation was completed less than 24 hours after preparation before the samples had been vacuum packed and problems, such as the occurrence of syneresis occur. Sensory evaluations after vacuum packaging and storage time should be conducted.

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Table 1: pH of cooked whole egg and experimental eggs with addition of canola, flaxseed, menhaden, DHAS (algae) and a blend of DHAS, flaxseed and krill oils over a two-week storage period with no significant differences in each sample over storage time.

Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 14
Whole Egg	8.0±0.1 B	8.1±0.2 B	8.1±0.2 B	8.1±0.1 B	8.0±0.2 C	8.2±0.3 B
Canola	8.5±0.1 A	8.6±0.1 A	8.6±0.1 A	8.6±0.1 A	8.6±0.2 A	8.7±0.2 A
Flax	8.6±0.1 A	8.6±0.1 A	8.6±0.2 A	8.7±0.1 A	8.6±0.2 A	8.7±0.2 A
Menhaden	8.5±0.1 A	8.5±0.1 A	8.6±0.1 A	8.6±0.1 A	8.5±0.1 AB	8.5±0.2 AB
DHAS (Algae)	8.4±0.2 A	8.5±0.1 A	8.5±0.1 A	8.5±0.2 A	8.4±0.2 B	8.5±0.3 AB
Blend	8.4±0.2 A	8.5±0.1 A	8.5±0.1 A	8.5±0.1 A	8.5±0.1 AB	8.5±0.2 AB

Different letters within the same column indicate significant differences (Tukey HSD mean pair comparison, $P < 0.05$). Data expressed as mean value $\pm$ standard deviation.

Table 2: Lightness color values (L) of cooked whole egg and experimental eggs with addition of canola, flaxseed, menhaden, DHAS (algae) and a blend of DHAS, flaxseed and krill oils over a two-week storage period with no differences in each sample over time.

Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 14
Whole Egg	84.1±2.9 A	82.4±3.8 A	82.8±3.3 A	82.0±5.1 A	81.6±3.7 A	83.4±4.1 A
Canola	88.0±6.8 A	85.1±3.6 A	85.2±3.4 A	85.6±3.5 A	85.2±3.6 A	85.2±2.9 A
Flax	86.9±7.6 A	85.6±3.6 A	85.6±3.6 A	85.7±3.5 A	85.2±3.4 A	85.4±3.1 A
Menhaden	87.2±7.2 A	85.1±4.0 A	84.4±3.1 A	84.8±3.2 A	84.9±3.8 A	84.9±2.9 A
DHAS (Algae)	88.8±6.4 A	86.2±3.4 A	86.4±3.4 A	86.6±3.8 A	86.6±4.0 A	86.6±3.7 A
Blend	73.7±4.4 B	73.5±4.1 B	72.6±1.9 B	74.1±3.8 B	74.2±3.3 B	73.4±4.1 B

Different letters within the same column indicate significant differences (Tukey-Kramer mean pair comparison, $P < 0.05$). Data expressed as mean value $\pm$ standard deviation.

Table 3: a* color values (positive value = reddish hue, negative value = greenish hue) of cooked whole egg and experimental eggs with addition of canola, flaxseed, menhaden, DHAS (algae) and a blend of DHAS, flaxseed and krill oils over a two-week storage period with no differences in each sample over time.

Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 14
Whole Egg	-4.4±1.0 C	-5.0±0.6 C	-4.7±0.6 C	5.3±0.9 C	-4.8±1.2 C	-5.2±1.0 C
Canola	5.8±3.9 B	5.7±4.2 B	6.1±4.6 B	5.4±4.3 B	5.7±4.4 B	5.0±4.5 B
Flax	5.2±3.5 B	4.8±3.9 B	5.2±4.4 B	4.6±4.0 B	4.8±4.0 B	4.5±3.8 B
Menhaden	6.3±4.1 B	5.8±4.6 B	6.6±4.8 B	5.6±4.1 B	5.8±4.7 B	5.8±4.2 B
DHAS (Algae)	3.7±4.3 B	3.2±4.4 B	3.6±4.2 B	3.1±4.7 B	3.4±4.5 B	2.9±4.6 B
Blend	23.3±1.8 A	21.1±3.8 A	22.7±2.0 A	20.4±3.4 A	20.5±2.6 A	20.3±4.1 A

Different letters within the same column indicate significant differences (Tukey-Kramer mean pair comparison, $P < 0.05$). Data expressed as mean value $\pm$ standard deviation.

Table 4: b* color values (positive value = yellowish, negative value = bluish hue) of cooked whole egg and experimental eggs with addition of canola, flaxseed, menhaden, DHAS (algae) and a blend of DHAS, flaxseed and krill oils over a two-week storage period with no differences in each sample over time.

Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 14
Whole Egg	28.1±3.2 B	25.8±4.4 B	27.4±0.9 B	27.0±4.1 B	26.7±2.2 B	27.5±2.7 B
Canola	55.9±12.6 A	53.7±15.3 A	52.9±14.8 A	53.5±15.7 A	53.7±15.8 A	52.6±16.5 A
Flax	53.4±12.2 A	51.9±15.5 A	51.5±14.8 A	51.6±15.5 A	52.1±15.1 A	51.5±14.8 A
Menhaden	57.0±13.1 A	54.5±16.8 A	54.9±14.8 A	54.4±15.3 A	53.9±15.8 A	54.6±15.7 A
DHAS (Algae)	48.0±14.8 A	45.5±16.3 A	45.8±15.5 A	45.2±16.4 AB	46.3±15.8 A	45.1±16.3 AB
Blend	51.4±7.4 A	48.2±11.3 A	48.2±9.1 A	47.8±10.8 A	48.0±11.7 A	46.7±10.5 A

Different letters within the same column indicate significant differences (Tukey-Kramer mean pair comparison, $P < 0.05$). Data expressed as mean value $\pm$ standard deviation.

Table 5: Syneresis expressed as percent weight loss of cooked whole egg and experimental eggs with addition of canola, flaxseed, menhaden, DHAS (algae) and a blend of DHAS, flaxseed and krill oils over a two-week storage period.

Treatment	Day 2	Day 4	Day 6	Day 8	Day 14
Whole Egg	6.0±1.0 C, ab	5.7±1.5 B, b	6.7±0.6 A, ab	7.0±0.0 B, ab	8.0±0.0 B, a
Canola	13.0±2.0 A, a	12.3±0.6 A, a	13.0±1.0 A, a	12.3±0.6 A, a	12.7±1.5 A, a
Flax	10.3±0.6 AB, a	10.0±1.0 A, a	8.3±5.5 A, a	12.7±2.9 A, a	11.0±1.0 A, a
Menhaden	11.7±1.2 AB, a	11.3±1.5 A, a	10.3±1.5 A, a	11.3±0.6 A, a	12.0±0.0 A, a
DHAS (Algae)	10.3±0.6 AB, a	10.3±1.5 A, a	11.3±1.5 A, a	11.3±1.2 A, a	11.7±1.5 A, a
Blend	9.3±0.6 B, a	9.3±1.5 A, a	8.6±1.5 A, a	11.0±1.7 AB, a	11.3±1.2 A, a

Different capital letters within the same column indicate significant differences. Different lowercase letters within the same row indicate significant differences. (Tukey-Kramer mean pair comparison, $P < 0.05$). Data expressed as mean value $\pm$ standard deviation.

Table 6: mg MDA/kg sample in cooked whole egg and experimental eggs with addition of canola, flaxseed, menhaden, DHAS (algae) and a blend of DHAS, flaxseed and krill oils over a two-week storage period.

Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 14
Whole Egg	1.5±0.2 BC, b	4.6±0.2 A, a	1.6±0.3 AB, b	1.6±0.7 BC, b	1.6±0.1 B, b	2.7±1.4 A, b
Canola	2.2±0.2 AB, bc	3.0±0.6 B, ab	1.6±0.7 AB, bcd	0.7±0.3 C, d	1.5±0.7 B, cd	3.6±1.0 A, a
Flax	2.5±0.6 A, a	3.0±0.8 AB, a	2.7±0.7 A, a	2.6±0.7 AB, a	2.1±0.4 B, a	1.9±0.6 A, a
Menhaden	1.6±0.2 BC, a	2.6±0.1 B, a	1.0±0.2 B, a	2.8±0.7 A, a	3.5±2.0 AB, a	2.5±1.3 A, a
DHAS (Algae)	3.0±0.4 A, a	2.3±0.8 B, ab	2.0±0.2 AB, ab	2.7±0.7 A, a	1.8±0.7 B, ab	1.3±0.2 A, b
Blend	1.3±0.1C, c	3.5±0.0 AB, b	2.8±0.1 A, bc	3.2±1.0 A, b	6.0±0.7 A, a	1.4±0.4 A, c

Different capital letters within the same column indicate significant differences. Different lowercase letters within the same row indicate significant differences. (Tukey-Kramer mean pair comparison, $P < 0.05$). Data expressed as mean value $\pm$ standard deviation.

Table 7: Springiness of cooked whole egg and experimental eggs with addition of canola, flaxseed, menhaden, DHAS (algae) and a blend of DHAS, flaxseed and krill oils over a two-week storage period.

Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 14
Whole Egg	2.0±0.0 A, a	2.0±0.0 A, a	2.0±0.1 A, a	2.0±0.0 AB, a	2.0±0.1 AB, a	2.0±0.0 A, a
Canola	1.5±0.3 A, a	1.4±0.4 B, a	1.6±0.3 A, a	1.6±0.3 B, a	1.6±0.3 B, a	1.8±0.4 A, a
Flax	1.9±0.3 A, a	1.6±0.4 AB, a	1.7±0.4 A, a	1.7±0.6 AB, a	1.8±0.2 AB, a	1.7±0.5 A, a
Menhaden	1.4±0.3 A, a	1.6±0.5 AB, a	1.7±1.0 A, a	1.9±0.2 AB, a	1.7±0.4 AB, a	2.0±0.2 A, a
DHAS (Algae)	1.8±0.6 A, a	1.9±0.2 AB, a	2.0±0.1 A, a	1.9±0.4 AB, a	2.0±0.2 A, a	1.9±0.2 A, a
Blend	1.6±0.4 A, b	1.9±0.4 AB, ab	2.1±0.2 A, ab	2.1±0.3 A, a	1.9±0.2 AB, ab	2.0±0.4 A, ab

Different capital letters within the same column indicate significant differences. Different lowercase letters within the same row indicate significant differences. (Tukey-Kramer mean pair comparison, $P < 0.05$). Data expressed as mean value $\pm$ standard deviation.

Table 8: Gumminess of cooked whole egg and experimental eggs with addition of canola, flaxseed, menhaden, DHAS (algae) and a blend of DHAS, flaxseed and krill oils over a two-week storage period with no significant differences in each sample over storage time.

Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 14
Whole Egg	948.6±209.2 A	1041.4±210.8 A	713.3±523.9 A	1010.1±194.7 A	1010.32±173.8 A	1079.5±219.0 A
Canola	629.4±249.0 AB	769.9±147.4 AB	678.3±291.8 AB	501.8±299.7 ABC	638.2±375.4 AB	710.1±364.0 A
Flax	776.8±508.6 A	878.7±381.0 AB	718.8±415.0 A	537.8±454.9 ABC	657.0±536.4 A	701.1±439.3 A
Menhaden	900.5±157.6 A	593.3±310.1 BC	828.6±434.4 A	690.5±406.4 AB	973.0±237.1 A	644.3±475.7 A
DHAS (Algae)	501.5±415.1 AB	626.3±407.1 AB	719.1±419.6 A	480.3±307.6 BC	580.0±431.9 AB	756.4±338.2 A
Blend	195.3±67.9 B	207.1±116.1 C	107.4±48.8 B	137.0±74.4 C	161.0±76.9 B	147.4±124.8 B

Different letters within the same column indicate significant differences (Tukey-Kramer mean pair comparison, $P < 0.05$). Data expressed as mean value $\pm$ standard deviation.

Table 9: Cohesiveness of cooked whole egg and experimental eggs with addition of canola, flaxseed, menhaden, DHAS (algae) and a blend of DHAS, flaxseed and krill oils over a two-week storage period.

Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 14
Whole Egg	0.6±0.1 A, ab	0.6±0.1 A, ab	0.5±0.2 A, b	0.6±0.1 A, ab	0.6±0.0 A, ab	0.6±0.0 A, a
Canola	0.6±0.1 A, a	0.6±0.0 A, a	0.6±0.1 A, a	0.5±0.1 A, a	0.5±0.1 A, a	0.6±0.1 A, a
Flax	0.5±0.2 AB, a	0.6±0.1 A, a	0.6±0.1 A, a	0.5±0.2 AB, a	0.5±0.2 A, a	0.5±0.1 A, a
Menhaden	0.6±0.0 A, a	0.5±0.1 AB, a	0.6±0.2 A, a	0.5±0.2 A, a	0.6±0.0 A, a	0.5±0.2 A, a
DHAS (Algae)	0.5±0.2 AB, a	0.5±0.1 AB, a	0.5±0.1 A, a	0.5±0.1 AB, a	0.5±0.1 A, a	0.6±0.1 A, a
Blend	0.4±0.1 B, a	0.4±0.1 B, a	0.3±0.1 B, a	0.3±0.1 B, a	0.3±0.1 B, a	0.3±0.1 B, a

Different capital letters within the same column indicate significant differences. Different lowercase letters within the same row indicate significant differences. (Tukey-Kramer mean pair comparison, $P < 0.05$). Data expressed as mean value $\pm$ standard deviation.

Table 10: Chewiness of cooked whole egg and experimental eggs with addition of canola, flaxseed, menhaden, DHAS (algae) and a blend of DHAS, flaxseed and krill oils over a two-week storage period with no significant differences in each sample over storage time.

Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 14
Whole Egg	1931.5±429.5 A	2124.0±424.8 A	1444.5±1057.6 A	2053.7±391.1 A	1996.2±346.2 A	2175.9±439.5 A
Canola	952.8±458.6 AB	1058.4±293.5 BC	1080.0±468.3 AB	725.4±383.6 BC	990.5±579.2 AB	1211.8±575.7 BC
Flax	1482.1±1045.3 A	1498.5±942.2 AB	1262.8±895.8 AB	803.6±825.1 BC	1246.9±1097.1 AB	1229.9±965.3 AB
Menhaden	1298.1±384.9 AB	867.9±503.1 BC	1084.1±629.4 AB	1312.2±810.5 AB	1640.5±624.0 A	1215.2±890.8 BC
DHAS (Algae)	905.7±917.0 AB	1180.3±808.1 BC	1456.2±885.3 A	922.5±666.1 BC	1168.7±886.6 AB	1450.6±663.5 AB
Blend	329.4±169.0 B	369.4±205.5 C	215.8±88.3 B	289.5±154.7 C	311.0±141.8 B	271.9±235.4 C

Different letters within the same column indicate significant differences (Tukey-Kramer mean pair comparison, $P < 0.05$).
Data expressed as mean value $\pm$ standard deviation.

Table 11: Resilience of cooked whole egg and experimental eggs with addition of canola, flaxseed, menhaden, DHAS (algae) and a blend of DHAS, flaxseed and krill oils over a two-week storage period with no significant differences in each sample over storage time.

Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 14
Whole Egg	0.3±0.0 AB	0.3±0.0 B	0.3±0.0 B	0.3±0.0 B	0.3±0.0 B	0.3±0.0 B
Canola	0.3±0.0 B	0.3±0.0 B	0.3±0.0 B	0.3±0.0 B	0.3±0.0 B	0.3±0.0 B
Flax	0.4±0.1 AB	0.3±0.0 B	0.3±0.0 B	0.3±0.1 B	0.3±0.1 B	0.3±0.0 B
Menhaden	0.3±0.0 AB	0.3±0.0 B	0.3±0.0 B	0.3±0.0 B	0.3±0.0 B	0.3±0.1 B
DHAS (Algae)	0.3±0.0 AB	0.3±0.1 B	0.4±0.1 B	0.3±0.1 B	0.3±0.0 B	0.3±0.0 B
Blend	0.4±0.1 A	0.4±0.1 A	0.5±0.2 A	0.5±0.1 A	0.4±0.1 A	0.5±0.2 A

Different letters within the same column indicate significant differences (Tukey-Kramer mean pair comparison, $P < 0.05$). Data expressed as mean value $\pm$ standard deviation.

Table 12: Hardness of cooked whole egg and experimental eggs with addition of canola, flaxseed, menhaden, DHAS (algae) and a blend of DHAS, flaxseed and krill oils over a two-week storage period with no significant differences in each sample over storage time.

Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 14
Whole Egg	1521.4±257.5 A	1627.4±254.7 A	1183.4±712.4 A	1613.6±189.8 A	1589.5±213.3 A	1650.8±247.9 A
Canola	1083.5±321.2 AB	1241.2±186.7 A	1160.5±359.6 A	913.8±435.8 AB	1066.0±492.2 AB	1157.6±501.9 A
Flax	1250.5±701.0 A	1418.0±491.8 A	1160.3±571.4 A	953.7±645.7 AB	1116.8±712.8 AB	1182.5±599.7 A
Menhaden	1421.3±218.5 A	1105.4±436.9 A	1318.3±584.1 A	1155.3±530.9 A	1535.3±298.1 A	1093.8±659.5 A
DHAS (Algae)	921.3±575.0 AB	1114.0±550.8 A	1222.2±558.7 A	921.2±425.8 AB	1002.5±575.9 AB	1293.2±402.3 A
Blend	503.8±110.2 B	504.0±203.2 B	375.3±117.3 B	404.0±180.0 B	487.3±172.4 B	428.7±241.1 B

Different letters within the same column indicate significant differences (Tukey-Kramer mean pair comparison, $P < 0.05$). Data expressed as mean value $\pm$ standard deviation.

Table 13: Sensory attribute evaluation and ranking of cooked whole egg and experimental eggs with addition of canola, flaxseed, DHAS (algae).

Treatment	Color	Odor	Flavor	Texture Between Fingers	Mouthfeel	Overall Liking	Ranking
Whole Egg	5.7±2.2 B	5.6±2.0 A	6.1±2.2 A	6.3±1.7 A	6.5±1.9 A	5.9±2.0 A	2.4±1.2 A
Canola	6.7±1.6 A	5.3±2.0 AB	6.2±1.9 A	4.9±1.8 B	6.0±1.7 A	6.0±1.8 A	2.4±1.1 A
Flax	6.6±1.6 AB	4.5±2.1 B	5.3±2.0 A	5.5±1.7 AB	5.7±2.1 A	5.7±1.8 A	2.4±1.1 A
DHAS (Algae)	5.9±1.9 AB	4.9±2.2 AB	6.0±2.1 A	5.2±1.9 B	6.0±1.7 A	6.0±1.9 A	2.7±1.0 A

Different letters within the same column indicate significant differences (Tukey-Kramer mean pair comparison, $P < 0.05$). Data expressed as mean value $\pm$ standard deviation. Attributes were scored using a 9-point hedonic scale 1=Dislike Extremely, 2=Dislike Very Much, 3=Dislike Moderately, 4=Dislike Slightly, 5=Neither Like or Dislike, 6=Like Slightly, 7=Like Moderately, 8=Like Very Much, 9=Like Extremely. The samples were ranked 1 – 4, 1 being most liked 4 being least liked.

Appendices

APPENDIX A: Consent Form

West Virginia University

Davis College of Agriculture, Forestry and Consumer Sciences

Informed Consent for Participants in Research Projects Involving Human Subjects

Title of Project **Sensory Evaluation of Nutritionally Enhanced Egg Product**

Investigators Kristen Matak, Assistant Professor
Division of Animal and Nutritional Sciences

Helenia Sedoski, Graduate Student
Human Nutrition and Foods

I. Purpose of this Research/Project

The purpose of this research is to determine if there is a taste difference between nutritionally enhanced egg product versus whole egg.

II. Procedures

There will be 1 session that lasts about 30 minutes at each session. You will twice be presented with 6 samples and asked to rank certain sensory characteristics of the samples. As a panelist, it is critical for you complete your session.

The research will be conducted in the test kitchen at room 110 Agricultural Sciences Annex.

III. Benefits/Risks

Your participation in the project will provide information about sensory properties of nutritionally enhanced egg product. You will have access to the results of the panel at the completion of the study.

There are no identifiable risks associated with tasting these egg samples.

IV. Extent of Anonymity and Confidentiality

The results of your performance are strictly confidential. Individual panelists will not be referred to in any publications or reviews.

V. Compensation

There is no monetary compensation for participation in this project. However, a candy treat will be offered at the end of the session.

VI. Freedom to Withdraw

If after becoming familiar with the sensory project you chose not to participate, you may withdraw without penalty. It is essential to the success of the sensory panel that you complete each session; however, there may be circumstances under which that you may chose not to complete your session, you may also withdraw at any time without penalty.

VII. Approval of Research

This research has been approved by the Institutional Review Board for projects involving human subjects at West Virginia University. The IRB Approval is on file.

Should you have any pertinent questions about this research or its conduct, and research subjects' rights, and whom to contact in the event of a research-related injury to the subject, you may contact:

Kristen Matak (Investigator) Phone: (304) 293-2631 x 4401

Helenia Sedoski (Investigator)

Paul Lewis (Director ANS) Phone: (302) 293-2231 x 4413

Charles Craig (Director IRB) Phone: (304) 293-6094

APPENDIX B: Prescreening Questionnaire
Pre-evaluation Questionnaire

Judge _____

Instructions: For each question shade the circle beside the option that best fits your answer.

1. Age _____
2. Gender _____
3. How often do you prepare your own food?
 - ☐ Daily
 - ☐ Weekly
 - ☐ Monthly
 - ☐ Never
4. How frequently do you consume eggs?
 - ☐ Daily
 - ☐ Weekly
 - ☐ Bi-weekly
 - ☐ Monthly
 - ☐ Rarely
5. Which of the following do you prefer?
 - ☐ Whole egg
 - ☐ Egg White
 - ☐ Egg substitute
6. How important is monitoring your dietary cholesterol intake?
 - ☐ Very important
 - ☐ Slightly important
 - ☐ Not important
7. How likely are you to buy a product containing omega-3 fatty acids, known to have heart health benefits?
 - ☐ Very likely
 - ☐ Possibly
 - ☐ Not likely
8. How important is protein intake to you?
 - ☐ Very important
 - ☐ Slightly important
 - ☐ Not important
9. How likely are you to buy a ready-to-eat, cholesterol-free, omega-3 rich egg product in a stick form (YUM-EGG-A)?
 - ☐ Very likely
 - ☐ Possibly
 - ☐ Not likely

APPENDIX C: Hedonic Scale

Instructions: You will receive a total of 4 egg samples. For each sample, please indicate your opinion about the characteristics being tested (color, odor, flavor, texture, mouth feel and overall liking) by highlighting the numbered box that best describes that attribute. Feel free to include comments to describe any noticeable attributes.

1=Dislike Extremely, 2=Dislike Very Much, 3=Dislike Moderately, 4=Dislike Slightly, 5=Neither Like or Dislike, 6=Like Slightly, 7=Like Moderately, 8=Like Very Much, 9=Like Extremely

Sample # _____

Color

1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---

Odor

1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---

Flavor

1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---

Texture between fingers

1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---

Mouth feel

1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---

Overall liking

1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---

Comments: _____

Sample # _____

Color

1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---

Odor

1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---

Flavor

1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---

Texture between fingers

1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---

Mouth feel

1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---

Overall liking

1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---

Comments: - _____

Comments on Attributes

Color

Odor

Flavor

Texture

Mouth feel

Comments on Attributes

Color

Odor

Flavor

Texture

Mouth feel

APPENDIX D: Ranking Form

Ranking Form

Judge _____

Instructions: Please assign each sample a number (1 – 4). Use 1 for the sample you liked most and 4 for the sample you liked least. Use each number only once.

Sample: _____ Rank: _____

Sample: _____ Rank: _____

Sample: _____ Rank: _____

Sample: _____ Rank: _____

Judge _____

Ranking Form

Instructions: Please assign each sample a number (1 – 4). Use 1 for the sample you liked most and 4 for the sample you liked least. Use each number only once.

Sample: _____ Rank: _____

Sample: _____ Rank: _____

Sample: _____ Rank: _____

Sample: _____ Rank: _____

Judge _____

Ranking Form

Instructions: Please assign each sample a number (1 – 4). Use 1 for the sample you liked most and 4 for the sample you liked least. Use each number only once.

Sample: _____ Rank: _____

Sample: _____ Rank: _____

Sample: _____ Rank: _____

Sample: _____ Rank: _____