FORMULATION AND ACCEPTABLITIY OF CHICKEN BREAST MEAT ENRICHED BY

AN OIL AND WATER EMULSION MARINADE CONTAINING OMEGA-3 FATTY ACIDS.

by

BENJAMIN C. WILLIAMS

(Under the Direction of Dr. Romeo T. Toledo)

ABSTRACT

Long chain Omega 3 fatty acids have been linked to a multitude of health benefits if incorporated into the human diet. Finding new avenues of introducing these healthy fatty acids is a pressing goal for the food industry. A deodorized menhaden oil(Omega Pure) containing 20-26% omega 3 fatty acids were combined with whey protein concentrate, salt, and phosphate to formulate a stable emulsion marinade for chicken breasts. Creaming, viscosity, and oil droplet particle size indicated that marinade was stabile and therefore suitable for infusion. Yield and pH values revealed effective incorporation of the marinade in the muscle system. Rancidity measurements (TBA and headspace sampling) of the product containing the menhaden oil marinade , revealed a gradual, not steep increase in lipid oxidation products with storage time. Sensory testing was done to assure commercial acceptability of the product. The trained panel found ($P < 0.05$) no significant development of off flavors. A triangle test indicated a difference in the enriched and conventionally marinated chicken meat at $P < 0.001$. Demographic data showed that no significant gender, consumption frequency, or health education impacts the willingness to purchase the ω-3 fatty acid enriched chicken meat. In a blind acceptance test, the willingness to purchase the control chicken was greater than the enriched product.

INDEX WORDS: Omega-3 Fatty Acids, Chicken, Marination, TBA, Hexanal, Oxidation

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DEDICATION

I dedicate this thesis to my parents Warren and Sandy Williams for raising me to be the person I

am today.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

According to the United States Department of Agriculture's most recent statistical report, Georgia produced 6.3 billion pounds annually (2003) of chicken with a market value of over $2.1 billion dollars(USDA-NASS 2005). Chicken growth, production, and marketability have and will continue to be one of Georgia's largest economic bases. For food companies, the search for new and improved ways of adding value to chicken is a consistent goal. A trend which started twenty years ago and is still continuing is marination. Marination was originally invented by chefs as a way to improve the flavor, juiciness, texture, and overall enjoyment of a product. The USDA's definition of marination is a mixture of ingredients in which a food is soaked, massaged, tumbled or injected to improve taste, tenderness or other sensory attributes.

Food companies have taken full advantage of marination. By integrating staged ingredient addition into the process, marination can improve product flavor and juiciness but more importantly overall yield. The most prevalent marinade functional ingredients are phosphates, salts, proteins, and starches. All of these ingredients may be combined to form a functional marinade system. When the Food and Drug Administration approved a health claim for Omega-3 fatty acids in food products (FDA 2004), addition of oils containing high levels of Omega-3 fatty acids presented a means for adding a health benefit to the consumption of red meat and poultry products. Thus, work was initiated to test the feasibility of incorporating omega-3 fatty acids into a meat marinade.

The scope of this research project covers all aspects of commercial development of a marinade to include stability and sensory of the marinated product. The defined objectives of this research project are as follows:

i)   Define marinade constituents and optimize process and formula to maximize sensory acceptability and storage stability of marinated cooked product.

ii)   Determine physico-chemical properties such as pH, viscosity and particle size of the optimized marinade.

iii)   Test and modify chemical test methods available in the literature for measuring rancidity and use an appropriate test method to measure stability of the ingredients, prior to, as well as the finished product after marination ..

iv)   Determine the sensory attributes of the finished product using consumer and trained panels.

To achieve these objectives, an optimized marinade was developed and injected into chicken breast. Since the component of interest in this work is an oil, viscosity, creaming index, particle size, and zeta potential measurements were performed to characterize emulsion stability. It is important that the oil does not separate form the bulk of the marinade otherwise the level that is incorporated into the meat will not be uniform in all pieces. Infusion parameters such as pickup, pH, and cook yield were used to determine effectiveness of the marination process. Oxidative changes were monitored using gas chromatography and 2-thiobarbituric acid assay. Product sensory properties were examined using a triangle difference test between the test product and a conventionally marinated product, a test of acceptability measured by a willingness to purchase, and a trained panel to characterize magnitude of individual defined attributes.

The hypotheses of this research are:

i)   That a marinade containing an Omega-3 fatty acid enhanced oil can be made stable to avoid oil separation in the marinade reservoir during the inject marination process.

ii)   That a marinade high in Omega-3 fatty acid content will not develop rancid off flavors in the frozen marinated meat faster than product marinated with a conventional marinade. .

iii)   That although chicken meat with high Omega-3 fatty acid oil incorporated in the marinade may show detectable sensory differences from a control; consumers will still be willing to purchase the product because of the perceived health benefit.

Results from this research will provide the industry with scientific data on proof of concept and feasibility of commercial adoption of this type of product. Additionally, data and observations can be applied in the development of marinades for and other muscle products.

1.2 References

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CHAPTER 2

LITERATURE REVIEW

2.1 Chicken Marination History

According to the United States Department of Agriculture's most recent statistical report, Georgia produced 6.3 billion pounds annually (2003) of chicken with a market value of over $2.1 billion dollars(USDA-NASS 2005). For food companies, the search for new and improved ways of adding value to chicken is a consistent goal. A trend which has been continuing over the last twenty years has been the production of marinated products. Marination was originally invented by chefs as a way to improve the flavor, juiciness, texture, and overall enjoyment of a product. The USDA defined a marinades as a mixture of ingredients in which a food is soaked, massaged, tumbled or injected to improve taste, tenderness or other sensory attributes.

Food companies have taken full advantage of marination. By integrating staged ingredient addition into the process, marination can improve product flavor and juiciness but more importantly overall yield. The most prevalent functional ingredients are phosphates, salts, non-muscle or non-meat proteins, and starches. All of these ingredients may be combined to form a functional marinade system. However, although functional from the standpoint of marinade retention, these ingredients do not address a vital concern, that is, the total benefit to the consumer of the food. Arguably, a most pressing goal is to improve the health promoting benefit of the food.

Nutraceuticals are a way to do this. Nutraceuticals are food products or ingredients which in addition to having nutritional and caloric value also provide a medical benefit.

Nutraceutical products, in 2002 have an estimated world wide value of $65 billion dollars(Lachance 2002). Currently in the United States, the functional foods market for 2004 is $10.4 billion dollars and is estimated to increase to $12.8 billion by 2009(Mintel 2004). A marination process can be used to easily incorporate a nutraceutical into a meat product, as the product is already being infused with a marinade solution. Probably the best choice of a health functional ingredient for meats would be lipid based since the meat already contains lipids but not the high Omega-3 fatty acids.

Walter Willett MD, PhD, Chair, Department of Nutrition at Harvard University, stated that it has been long known that certain types of fat are essential to health and can reduce the risk of cardiovascular disease(Willett and Stampfer 2003). The highlighted triglycerides with nutraceutical value are those that contain the essential fatty acids linoleic acid(18: 2n-6) and linolenic acid(18:3n-3), Conjugated linoleic acid(CLA), Alpha-linolenic acid(ALA), and Phophatidylcholine(PC). Additionally, the longer chain icosapentanenoic(EPA) (C20:5 n-3), Docosahexaenonic (DHA) (C22:6 n-3), and Docosapentaenoic (DPA) (C22:5 n-3) have been shown to posses these cardiovascular disease lowering characteristics. A further examination of each of these will be discussed later after a discussion of the marinade system from ingredient to ingredient.

2.2 Marinade Constituents

Briefly, phosphates perform the most critical task of breaking the actomyosin linkage in the myofibrillar proteins(April and others 1972). Salts work to improve water holding capacity, yield, and ionic strength (Hamm 1960; Kristinsson and Hultin 2003). Proteins form protein

matrices, which add to product firmness. And last, starches during hydration form a gel, which reinforces the protein matrix(Kokini and others 1992).

2.2.1 Phosphates & Salts

The current five major phosphates allowed in meat products are disodium pyrophosphate (DSPP), tetrasodium pyrophosphate (TSPP), tetrapotassium pyrophate(TKPP), sodium tripolyphosphate (STPP) and glassy phosphate. The optimum meat pH for marination is about 6.0 and may increase to 6.5 as a result of marination(Lewis and others 1986). Each phosphate, depending on the degree of alkalinity can positively affect water holding capacity, which in-turn can influence cooking yields and textural properties(Schmidt 1986). The phosphates by virtue of their modification of product pH affect the charge of the proteins which leads to increased water binding sites both within the muscle filament and between muscle filaments(April and others 1972). Phosphates additionally act as ion chelators and have been documented to retard lipid oxidation(Ang and Hamm 1986).

Phosphates supplied commercially as ingredients consist of othrophosphate (monophosphate) as the primary form of phosphate. All phosphates eventually degrade to the orthophosphate.   Salts with sodium or potassium are available The orthophosphate is tribasic and is commercially available with all acid groups substituted with sodium or potassium (TSP or TKP) or as the mono basic or dibasic forms where one or two acid hydrogen remain. Pyrophosphates contain two phosphate groups and are tetrabasic. They are available in the form of TSPP, TKPP, DSPP, or DKPP.   They have an extremely weak solubility constant. Metaphosphates(Glassy) have a non-crystalline structure which gives them an extremely high solubility.   Another form of phosphate that is commonly used in marination is the tripolyphosphate (STPP or KTPP). The tripolyphosphates contain 3 phosphate groups but the

middle phosphate does not contain the acid group therefore the tripolyhosphate is tetrabasic. Tripolyphosphates are available only with all the acid hydrogens fully substituted with sodium or potassium. Rates of hydrolysis are critical to the functionality of phosphates since it is the diphosphate which is most functional and the monophosphate (orthophosphate) is non-functional. TSPP, TKPP are completely converted to the monophosphate within 1.5 hr, STPP in 3 hrs, DSPP in 5.75 hrs. and Glassy over 6 hrs(Li 1998). Researchers in the UGA food process development laboratory found that TSPP injected breast had the highest yields. STPP and TSPP led to no measurable differences in moisture content, expressible moisture content, net weight increase or shear. Falling last, glassy phosphate injected breasts had the highest purge, lowest pick-up and highest expressible moisture(Zheng 2000).

Another vital part of a brine, salt provides a vital compliment to phosphates(Froning and Sackett 1985). Salt is used as a primary ingredient to extract muscle proteins(actin and myosin) (Barbut and others 1996). Additionally, the Cl^- ions can improve the disruption of myofibril cross linkages, which otherwise would remain linked as in the untreated muscle (Li 1998). The basic theory is that the filament lattice spacing is the principle determinant in water retention and water uptake, caused by (a)electrostatic repulsive forces, (b)restraining forces by structural proteins, and (c)chemical potential of the fibrils/fibers(Kristinsson 2003). On evidence to support this theory is the observation that salt is not needed in significant amounts to achieve the desired water binding by marinated muscle. Chang and others (2001) found that strong gels with good water holding capacity could be formed at low salt concentration. This points to the Donnan potential between proteins in the gel as the basis of strong gels (Feng and Hultin 2001). This type of gel structure allows more water to be held via capillary forces.

2.2.2 Lipids

Lipids are biological molecules that are soluble in organic non-polar solvents, such as petroleum distillates (hexanes), chloroform, ethers and alcohols(Duncan 2000). From the public's view, lipids are a primary reason for 65% of American adults being overweight, up from 47% 25 years ago(CR 2004). The American Heart Association recommends that no more that 30% of total caloric intake be from fat, with saturated fat not more than 10%(AHA 2000). However currently, saturated fat represents 40% of all fat in the diet consumed in the US(Willett 2003). All fats are not equal, some present health benefits, and the nutritionists' goal is to change this ratio(Hunter 1999). Marinades present a unique opportunity to improve this ratio, but choice of the right kind of fat and the benefits to be delivered are two hurdles to overcome.

Currently, 98% of all fatty acids in meats, fish and vegetable oils are in the form of glyceryl esters. Glycerol forms a 3-carbon alcohol backbone which is esterified at the sn1, sn2 and sn3 position with fatty acids. The larger molecule, triacylglycerols constitute 90% of the lipids in the body(Duncan 2000). Other lipids include phospholipids(amphipathic glycerol molecules with a phosphate group in the polar head), glycolipids(have sugar residue and a sphingosine moiety), and sterols(four fused rings with the alcohol group esterified to fatty acids)(O'Brien 2004).

Whether the fatty acid is attached to a ring in a sterol, held in a phospholipid, or part of a triglyceride, it is the fatty acid that contributes either a nutritional benefit or nutritional concern. The structure of the hydrocarbon chain(bond type & location) can determine the benefit. One of the newer fatty acids linked to health benefits is conjugated linoleic acid(CLA). CLA which is primarily a by product of biohydrogenation in ruminate animals, was first reported by Michael Pariza to have anticarcenogic properties(Ha and others 1987). Overall conjugated linoleic acid

has been reported to inhibit tumors, reduce atherosclerotic risk, and reduce body fat(Ha and others 1989). Researchers in Germany, found that women given 2.1g of racemeric(1/1) CLA had a positive increase of CLA in phospholipids and total lipids. Triglyceride levels stayed the same and no significant change in body fat was noticed(Petridou and others 2003). The *trans*10-cis*12* isomer has been claimed to help impact weight in humans; however recently, Swedish researchers found that *trans*10-cis*12* caused significant impairment of the peripheral insulin sensitivity as well as blood glucose serum lipid levels(Riserus and others 2003). The article concluded that more research was needed but clearly stated the benefits of the isomer.

The current most publicized group of fatty acids is the ω-3 fatty acids. Omega or n-3 fatty acids have repeatedly been cited for their positive health benefits. The currently known ω-3's are alpha-Linolenic acid(C18:3 n-3), Eicosapentanenoic(EPA) (C20:5 n-3), Docosahexaenonic (DHA) (C22:6 n-3), and Docosapentaenoic (DPA) (C22:5 n-3). Alpha-linolenic is the principle n-3 fatty acid which is found in oil seeds and is the major polyunsaturated fatty acid (PUFA) in chloroplast lipids(Hwang 2000). EPA, DHA, and DPA are commonly found in marine animals' fat tissues and in marine microalgae. Health benefits of ω-3's include preventing coronary heart disease, hypertension, type 2 diabetes, renal disease, rheumatoid arthritis, colitis, chronic obstructive pulmonary disease, and aiding brain development and growth(Simopoulos 1999). Most of these health benefits can be traced to the effect of n-3 fatty acids to suppress eicosanoid synthesis.

Eicosanoids are the oxygenated derivatives of C_{20} polyunsaturated fatty acids, which with prostaglandins(eicosaniods with cyclopentane ring) act as mediators for various physiological processes(Horton and others 2002). There are many forms of an eicosanoid, of which prostaglands only constitute a moderate fraction. Other forms include thromboxane, leukotrine,

hydroxy- and hydroperoxy-eicosatetraenoic acid, and lipoxin. Table 2-1 provides a functional

description for most of the eicosanoid classifications. [As stated earlier, as moderators, the

successful regulation and prevention has been shown to have direct health benefits.] Some of the

benefits that the n-3's have against eicosanoids are as follows. First discovered in the 1960's, n-

3 PUFAs can competitively inhibit conversion of 18:2n-6 to arachidionic acid, which was first

thought to be the origin of prostaglandins(Machlin 1962). Later the primary and more direct

pathway from 20:3n-6 and 20:4n-6 was discovered; however the suppression and competitive

substitution ability in the synthesis chain was still thought to apply. Following countless studies

have linked n-3 fatty acids with correct regulation of this group of biologically active

compounds(Lands and others 1973; Gryglewski and others 1979; Needleman and others 1979;

Ochi and others 1983; Lee and others 1984). Every year new studies link this characteristic with

a new health benefit, which explains why n-3 fatty acids can be of much value in the human diet.

With all the nutritional aspects considered, a selection of the best oils to use in a

marinade can be made. Among the commercially available oils, linseed/flax oil has naturally

high levels of ω3 fatty acid(52.7% α-linolenic, 15.9% linoleic, 19.9% oleic) but equally

accompanied with a high level of oxidation in processing. Possible alternatives are the

Australian Linola™ which is specially modified flax with (2% 18:3, 72% 18:2)(Haumann

1990). Additionally, Canada is pioneering the development of *solin*(flax oil with less than 5%

linolenic acid). Researches stay that they plan to have *solin*(2% 18:3, 70% 18:2) available in

two years. A petition for GRAS(generally recognized as safe) status has been submitted to the

Food & Drug Administration(Malcolmson and others 1998).

Safflower oil is another oil that has an incredible lipid profile(77.7% linoleic, 13.1%

oleic). Safflower oil is derived from the oil seed of *Carthamus tinctorius*(Fedeli 1983). Of all

the oils, safflower oil has the largest amount of 18:2 of any of the non-GMO oil seeds, and consumers are willing to purchase the oil at a premium for this fatty acid (FA) profile(Smith 1985). Oxidative stability is comparable to other oils(cottonseed, corn, soybean, and sunflower), because the high tocopherols levels (266.9mg/kg)(Muller-Mulot 1976) in Saffower oil compensates for the high 18:2 FA content.

The following oils are closely related and offer good health-benefit: Corn oil(0.9% linolenic, 57% linoleic, 27.5% oleic), Sunflower(0.5% linolenic, 68.2% linoleic, 18.6% oleic), Soybean(7.8% linolenic, 53.2% linoleic, 23.4% oleic). Corn, although slightly less in linoleic acid than Sunflower and Soybean, offers the advantage of its ubiquinone content. Ubiquinone is a potent antioxidant that helps prevent oxidative rancidity in the oil, and corn oil is the only oil which has a significant amount of it (200 mg/kg oil)(Folkers 1974). It should also be noted, that there is another tier of healthy oils(Canola, Olive, and Peanut) which offer high monounsaturated fatty acid profiles; however, although the oils have more oxidative stability, the preceding high linoleic oils would serve better for a nutraceutical product.

Marine oils also may prove to be useful as an ingredient. Consumption of fish oils began before the 1940's with the usage of cod liver oil as a vitamin A and D supplement. The cod oil had distinct off flavors and after the vitamins could be synthesized, consumption decreased. Later fish oils gained great attention with the Greenland Eskimo study done in 1970's by Danish researchers(Dyerberg and others 1978). The Eskimos had remarkable health despite a high fat but heavy fish based diet, which prompted discovery of the benefits of ω-3 intake. Now some fish varieties are specifically harvested for reduction to fish meal and oil, due to a high percentage of oil and or bones. The largest fish commercially landed for these purposes is menhaden at 1,497 million pounds yearly(NMFS 2004). Menhaden *Brevoortia* sp is categorized

as a pelagic fish which is small, oily herringlike similar in appearance to the alewife and shad(Bimbo 1990). The refined deodorized oil has an excellent lipid profile consisting of 10.21% EPA, .10% HPA, 2.3% DPA, and 12.99% DHA, amounting to roughly 25% long chain omega-3 fatty acids, which are naturally present(OPC 2003). On June 5, 1997, the Food and Drug Administration issued a final rule affirming GRAS status for menhaden oil as a direct human food ingredient(62 FR 30751), with the stipulation of intake not exceeding 3.0 grams/per person/per day. With this FDA approval, menhaden oil provides a better, more functional oil than plant oils for introduction of ω-3 FA into food systems. The question is at what dietary levels should the oil be used.

Effective usage levels have been reported in a various literature sources. Currently the FDA recommends that consumers not exceed more than a total of 3 grams per day of EPA and DHA omega-3 fatty acids, with no more than 2 grams per day from a supplement(FDA 2004). A structured method of outlining usage and benefits was proposed by the International Association of Fish Meal Manufacturers(Barlow and others 1990): Levels of intake are recommended to (1) sustain normal healthy life and (2)correct certain diseases. Subdivisions of the levels for sustaining health (i) for the development of a fetus and a young child and (ii) for normal adult. In developing animals, studies have linked DHA levels with proper brain and nervous tissue development (Crawford 1987; Neuringer and others 1986). Moreover, DHA requirements in the neural tissue have been shown to double for a human fetus in the third trimester(Martinez and others 1974). Estimates of consumption of DHA for pregnant and lactating women are 80 mg/day on average, but intakes of 200-300 mg/day are necessary to achieve a minimal level of 0.4% in breast milk(Holub 2001). Normal adult intake levels suggested are 0.4% (Bjerve and others 1989), 0.54% (Holman and others 1982), and 0.2 to 0.3% of calories(Bjerve and others

1987a; 1987b). Studies done in both Norway(Bjerve 1989) and Germany(Kromhout 1989) point to an intake level of 1 g/d necessary for proper lipid balance in adults(Barlow and others 1990). Mention in multiple sources point to an effective ratio of the short chain and the long chain omega-6 fatty acids. Neuringer and others (1994) suggest an n-6/n-3 ratio of 4:1- 10:1. The Western diet is estimated at 10:1-11:1(Simopoulos 1996); where as, the Japanese diet is estimated to contain a ratio of 4:1-5:1(Okuyama and others 1997). The Greenland Eskimos diet is reported to have a ratio of 1(Weber 1989). A diet rich in omega-6 fatty acids has been cited to shift the physiology of the human body to a prothrombotic and proaggregatory state, which increases blood viscosity, vasospasm, and vasocontrition(Simopoulos 2001).

Intake levels have also been associated with the correction and prevention of certain diseases categorically grouped as either (i) blood vessel related or (ii) inflammatory related, reflective of the regulator function of lipids. Simopoulos(1989b) reported that 800-1100 mg/day of 18:3n-3 and 300-400 mg/day of 20:5n-3 and 22:6n-3 were required to prevent depletion of liver and brain phospholipids. Supplementation of 750-1500 mg/day DHA has been found to exhibit anti-thrombotic and anti-arrhythimic potential *in vitro* (Conquer and Holub 1998). Cross validated research points indicate that 18:3n-3, EPA, and DHA have individual specialized functions in the retina and central nervous system(Wheeler and others 1975; Bivins and others 1983; Hoffman and others 1993; Neuringer and others 1994). The American Heart Association has a recommended standard target of 1 g/p/d for combined EPA and DHA levels for persons with documented coronary heart disease(AHA 2006). Persons without documented coronary conditions are recommended to consume a variety of fish and nuts, with fish consumption at least twice a week. With the literature values used as a basis the target levels for incorporation of long chain PUFA to be used in this research will be targeted at 1/g/d total for a normal adult.

2.2.3 Proteins

Lipids(TG) are primarily non-polar due to their hydrocarbon tail. On the other hand, water is polar. Thus, lipids in water will have a tendency to separate and form lipid pools on the water surface. A hydrocolloid stabilizer(gum/starch) would help in preventing lipid segregation, but a protein, which could act as an emulsifier, would be more beneficial. Proteins contain hydrophobic and hydrophilic amino acid residues, which in solution, reduce the interfacial tension. Laplace pressure(despersability of droplets) is accordingly decreased. Additionally, recoalescence is thwarted by adsorption at the lipid-water interface. Some interfacial tension gradients are created that diminish recoalescence(Maragoni Effect)(Tornberg and others 1997).

The steps in emulsion formation are as follows: (1) The protein is adsorbed at the oil-water interface and (2) the polypeptide chain may unfold to a certain degree, which could also be called surface denaturation. (3) Polypeptide residues interact with neighboring residues possibly forming a continuous network (two-dimensional gel) or a precipitate(coagulate). Surface denaturation is theorized to be confined to only the quaternary and tertiary structure(Cumper and Alexander 1950).

Currently the two predominant proteins used in marinades are whey and soy. Whey proteins are generally recognized as the soluble protein fraction of milk at a pH of 4.6 at 20°C. Among the proteins found are β-lactoglobulin(mw 18,600), α-lactalbumin(mw 14,400), and immunoglobulins(mw 160,000). The approximate composition is β-lactoglobulin(51%), proteose-peptones(20%), biologically active proteins(13%), α-lactalbumin(11%), and Serum albumin(5%)(Modler 1985). Some noteworthy minor constituents include lactoferrin, lactoperoxidase, lysozyme, lipoprotein lipase, plasmin, and phosphatase(Walsh and others 2000). The exact composition will fluctuate depending on the source of the coagulation(enzyme or acid

based). Rennet(chymosin), an enzyme first isolated from suckling mammals, disrupts the casein micelles and causes phase separation in the milk. Benefits of using rennet include higher pH and better flavor development due to the amount of protein fragments generated. Acidic based coagulation shifts the caseins to the isoselectric point at which coagulation forms. The major drawback is that little to no proteose-peptones are recovered in the whey. Hence, this process is used for cottage cheese.

Whey protein is available in either concentrate(<80% protein) or isolate(92-97%) powders. Isolates are cost prohibitive for most products, while concentrates are more widely used as the only difference is slightly less protein coupled with increased lactose, fat, and ash. The amino acid profile of whey concentrate contains not only essential amino acids but also a wide variety of branched chained amino acids.   The most predominant acids are glutamic acid(16.9%), aspartic acid(10.6%), lysine(10.4%) and leucine(10.2%) (HI 2005).   Lysine and leucine both are essential, and leucine is branched.   The other essential acids are threonine(6.2%), isoleucine(6.1%), valine(5.6%), phenylalanine(3.0%) and methionine(2.0%).

Whey protein has been well documented as having superior emulsification and stabilization properties(Djordjevic and others 2004, McClements and Decker 2000, Singh and Dalgleish 1998, and Dickinson 1997). Solubility is not critically dependant upon pH or ionic strength, except at the isoselectric region near 4.5(Hermansson 1973). Gelation properties are very useful for a variety of applications. Lipids and carbohydrates maybe introduced into the protein gel with little loss of structure(Bottomely and others 1990). Additionally whey protein gels have remarkable rigidity at temperatures greater than 100°C or frozen temperatures around -18°C. Whey protein is therefore an ideal choice as a marinade ingredient for most applications.

Soy proteins are a major component of the oil rich soy bean. The proteins are separated from carbohydrates and fiber present in the soybean meal, the residue after removal of the oil. Ultracentrifugal analysis of the extracted protein originally identified the 2S, 7S, 11S, and 15S protein fractions that generate beneficial interactions with meat proteins. Various fractions have properties beneficial for specific applications and soy protein preparations are recommended for specific uses. Soy protein isolates are used in the meat industry to this day(Wolf and Briggs 1956). The majority (37% of total protein) of the protein is found in the 7S fraction, a molecule 180,000 to 210,000 Daltons. (Wolf 1972). The 7S is primarily composed of β-Conglycinin, a large glycoprotein with a carbohydrate moiety(Koshiyama 1969). Multiple subunits (α, ά, β) of 7S can interact to form six isomeric forms of the fraction (B1-B6)(Kilara and Harwalkar 1996). Each isomer has been report to have differing properties. The larger molecular weight fractions are the 11S (350,000 Daltons) and 15S (600,000 Daltons separations. The 11S fraction, Glycinin, is the other majority fraction constituting 31% of the total soybean protein. The 11S protein, due to a complex quaternary structure, is highly influenced by salt, pH, or temperature, which may cause difficulties depending upon the product used (Wolf 1972). The remaining 2S and 15S fractions make up respectively 22 and 11% of the remaining protein of the bean. In gelation properties, each fraction subunit gels at different temperatures and denature from 80°C to even over 100°C (Kilara and Harwalkar 1996).

With the above stated high denaturation tendencies and ionic sensitivity, soy will be tested in this research but probably not used for an emulsion application.

2.2.4 Starches

Plant starches, native or modified, are one of a few ingredients commonly used as marinade enhancers. Commercial starches come from corn, waxy corn, high-amylose corn,

wheat, rice, and tubers and roots(potato, sweet potato, and tapioca). Starches have been referenced for a wide variety of food uses including adhesion, binding, clouding, dusting, film forming, foam strengthening, antistaling, gelling, glazing, moisture retaining, stabilizing, texturizing, and thickening applications(Bemiller and Whistler 1996). In meat applications, starches are commonly used for their ability to gelatinize during cooking and capture free water expressed from the protein lattice in the various myofibrillar layers. For example, Zhang and Barbut(2005) found that adding modified tapioca and potato starches to PSE chicken improved G´ and yield as a partial remedy to protein exudation. In terms of specifications, careful attention is paid to the ratio of amylose to amylopectin chain as it does affect the thermodynamics of the gelatinization process(German and others 1992). Additionally, the size of the various starch granules have been observed to change the peaks in the rheological profile such as the storage G' and loss G'' moduli(Singh and Kaur 2004). Amylopectin chain length is also attributed to be a contributor to preventing starch retrogradation(Kohyama and others 2004). The individual starch characteristics are well documented in the literature; however these previously mentioned characteristics are worthy of consideration when evaluating a starch for marinades. The emulsion type marinade hypothesized in these experiment could easily have a starch in the recipe; however the starch was not used as yield was not the primary focus of the project.

2.3 Characterization of the Marination Process

A marination system has three major components: the meat substrate, the marinade, and the unit operation(s) done to incorporate the marinade into the meat. The type of meat, state of rigor, handling conditions, and resultant pH before and after slaughter, can affect meat quality. The marinade must be formulated to make the conditions in the raw meat optimum for marinade

retention. More specifically, the ionic strength of the marinade, the salt level, the phosphate level, and the percent binders must be individually considered. Last, the type of operations(i.e. injection, tumbling, or massaging) used must achieve the result desired.

2.3.1 Physiological Consideration of the raw meat

Raw poultry breast muscle typically contains roughly 74.76% water, 23.09% protein, 1.24% fat, and 1.02% ash(USDA Nutrient Database 2005). Therefore in 100 grams, the chicken lipid content is 1.24 g   and consists of   330 mg saturated, 300 monounsaturated, 280 mg polyunsaturated, 25 mg trans, and 58 mg cholesterol. The remaining lipids can be attributed to glycerol, phosphate, sugar or sterols which are chemically bonded to the fatty acids but are not fatty acids themselves. The starting pH of a post-mortem chicken muscle is roughly around 7 with shifts to 5.5 and then ends between 5.4 to 6.8, post-rigor . The pH depends upon the post-mortem glycogen conversion in the muscle(Honikel and Hamm 1994). Muscle physiological conditions such as pale, soft, and exudative(Alvarado and Sams 2003) or dark firm and dry(Barbut and others 2005) have presented difficulties in effective marination and are conditions not easily remedied. PSE has been characterized to be protein denaturation linked to a rapid pH decline at elevated temperatures with stress at slaughter a contributing factor(Ludvigsen 1954). The resultant muscle exudes protein and has limited water holding capacity.

Dark, firm, and dry is characterized by high pH, sticky texture, and high water holding capacity(Cornforth 1994). DFD meat is undesirable to most processors because at the high pH shelf life is dramatically diminished. Optimal final muscle tissue should be in the pH range 6.0-6.4 to optimize protein gelling characteristics (Foegeding and others 1996). At the higher mid range pH values the protein gel matrix becomes swollen because of the Donnan potential

generated by the fixed charge. Increasing the pH above the pI of the muscle proteins leads to increased charged repulsion between the proteins in the gel network(Kristinsson and Hultin 2003). Therefore, getting a muscle to the optimal pH range is a noteworthy attribute to achieve, and can be mediated by the the added marinade.

2.3.2 Considerations for the Marinade

A typical white marinade contains both salt and phosphate as a foundation for the ingredient system which may contain other water binding agents and flavoring agents. Salt levels are set at levels to meet consumers' desired levels for taste and also to build the ionic strength. Ionic strength defined as $i = \frac{1}{2} \sum z_i^2 \; m_i$ for each ion in the solution, where z is the valence factor and m the molar concentration. The total of all the ions in the solution result in the total ionic strength. The mitigator for water uptake and water retention in muscle systems is filament lattice spacing(Offer and Knight 1988).   Therefore, phosphates, salts, and muscle minerals are all interact since each affects the lattice. Ionic strength is an osmotic driver generated by the dissolved solutes of a marinade to efflux into the fiber bundles to alleviate the pressure (Hedrick and others 1989). The muscle develops some ionic strength naturally through *rigor mortis*, whereby natural minerals and ions are released(Feidt and Brun-Bellut 1999). The marinade must provide a strength greater than this to allow for the marinade to be absorbed. Also, as mentioned earlier the pH of the marinade should be on the alkaline side as to increase the pH of the meat. The last consideration for the marinade would be the most desirable operation for infusion.

2.3.3 Unit operations necessary for processing.

A vital component of successful infusing involves either injection(McGee and others 2003), tumbling(Deumier and others 2003), or massaging(Lachowicz and others 2003).

Injection involves using stainless steel needles in varying diameters to pressure inject a mass fraction of marinade into a meat. Injection can be done at up to the legal limits for percent added water(varies with meat), phosphate(0.5%), salt(self limiting), or binders(2-3.5% depending on type). Done at the correct levels, the injection process can yield an excellent quality finished product as the infusion is penetrated deep into the interior of the meat. Tumbling and massaging both serve the same purpose to mechanically provide work to extract myofibrillar proteins to the surface of the meat, while giving the marinade an opportunity to incorporate into the meat. Massaging and tumbling has been used for decades in the red meat industry with only recent addition into the white meat plants. Most effective of all the methods is a combination of injecting and tumbling/massaging, which can offer superior yield and marinade diffusion.

Eicosanoid	Response
Prostaglandin E_1	Inhibits platelet aggregation
Prostaglandin E_2	Vasodilation; increases cAMP levels; decreases gastric acid secretion; suppresses immune response; lutotropic action
Prostaglandin I_2	Relaxes smooth muscle; vasodilation; inhibits platelet aggregation; raises cAMP levels
Thromboxane A_2	Contracts smooth muscle; causes platelet aggregation; bronchoconstriction
Prostaglandin D_2	Inhibits platelet aggregation; raises cAMP levels; causes peripheral vasodilation
Leukotrine B_4	Neutrophil and eosionphil chemotaxis; leakage in micro circulation; raises cAMP levels; causes neutrophil aggregation
Leukotrine C_4-D_4	Contracts smooth muscle; constricts peripheral airways; leakage in microcirculation; decreases cAMP levels
12-HETE[*] 12-HPETE[**]	Neutrophil chemotaxis; stimulates glucose-induced insulin secretion
15-HETE	Inhibits 5- and 12-lipoxygenases
Lipoxin A	Superoxide anion generation; chemotaxis; activates protein cell activity
Lipoxin B	Inhibits natural killer cell activity

[*]Hydroxyeicosatetraenoic acid
[**]Hydroperoxyeicosatetraenoic acid

Table 2.1: Eicosaniod biophyisical responses adapted from (Hwang 2000).

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CHAPTER 3

FUNCTIONAL PROPERTIES AND STABILITY OF AN OIL AND WATER EMULSION

CONTAINING ω-3 FATTY ACIDS USED FOR MARINATION IN CHICKEN BREAST

MEAT[1]

Abstract

Long chain Omega 3 fatty acids have been linked to a multitude of health benefits when incorporated into the human diet. Finding new avenues of introducing these fatty acids with known health benefits is a pressing goal for the food industry. A deodorized menhaden oil(Omega Pure) containing 20-26% omega 3 fatty acids was combined with whey protein concentrate, salt, and phosphate to formulate a stable emulsion marinade for chicken breast meat. Creaming, viscosity, and oil droplet particle size indicated that marinade was stabile and therefore suitable for infusion. Yield and pH values revealed effective incorporation of the marinade in the muscle system. Rancidity measurements (TBA and headspace sampling) of the product containing the menhaden oil marinade, revealed a gradual, not steep increase in lipid oxidation products with storage time.

3.2 Introduction

According to the United States Department of Agriculture's most recent statistical report, Georgia produced 6.3 billion pounds annually (2003) of chicken with a market value of over $2.1 billion dollars(USDA-NASS 2005). Chicken growth, production, and marketability have and will continue to be one of Georgia's largest economic bases. For food companies, the search for new and improved ways of adding value to chicken is a consistent goal. The nutraceutical and functional foods market has proven to be a novel avenue for food companies to offer high value added products with a market potential of $12.835 billion US by 2009(Mint*e*l 2004). Thus, a new approach to value added chicken could involve nutraceuticals.

A common industry practice for adding value to raw chicken meat is marination. Marination was originally invented by chefs as a way to improve the flavor, juiciness, texture, and overall enjoyment of a product. Traditional marination usually took optimally 24 hours. Commercially, the marination step achieves the same goals as the traditional method but with treatment time < 1 hour. The process is accelerated by using injectors, massagers or tumblers. A nutraceutical may easily be introduced into the meat via the marinade. Omega-3 fatty acids were selected as the nutraceutical of choice to add to meats because volumes of studies touted their health benefits including prevention of coronary heart disease, hypertension, type 2 diabetes, renal disease, rheumatoid arthritis, colitis, chronic obstructive pulmonary disease, and aiding brain development(Simopoulos 1999). Previously studies on improving the fatty acid profile of the chicken lipids to be more beneficial to health have focused on altering the feed composition. (López-Ferrer and others 2001; 1998; Gonzalez-Esquerra and Leeson 2000; Marshall and others 1994). Feed alteration has also been tested in other animals such as pigs or lamb to alter the muscle lipid profile(Hoz and others 2004; Cooper and others 2004). A process applied post

mortem such as marination would be a more efficient means of ameliorating the muscle lipids since there will be no feed conversion loss. Additionally, the marinade process would achieve similar established increases in tenderness, juiciness, and flavor enhancement as marinades without the added oil(Alvarado and Sams 2004; Xiong and Kupski 1999).

Recurring issues linked to marination have been proper marinade formulation and functionality of ingredients. Typical white marinades contains a minimum of a phosphate blend and salt form (organic or inorganic) to affect the Donnan potential in the filament lattice(April and other 1972; Hamm 1986). Additionally, a plant or dairy protein is commonly added to the marinade to improve texture and yield (Zhang and Barbut 2005). These fundamental steps in marinade formulation and preparation must be done correctly for the process to become commercially viable   To fortify the marinade with ω-3 fatty acids, the marinade is emulsified to allow the refined fish oil to be held in suspension. The emulsifier could be one of the proteins due to the amphiphilic nature of the amino acid residues. Proteins have been used extensively as emulsifying agents (Fomuso, Corredig, and Akoh 2002; Djordjevic and others 2004 A;B).

The focus of our experiments was to test the application of marination as a delivery vehicle for ω-3 fatty acids, specifically long chain eicosapentaenoic (C20:5, EPA) and docosahexaenoic (C22:6, DHA) acids in the nutritional fortification of meats. Menhaden fish oil has been documented to be naturally rich in both EPA and DHA(OPC 2005). Menhaden oil has been affirmed to be GRAS (21 CFR 184) and can therefore be used as a direct food ingredient. Additionally, the United States Department of Agriculture under directive 7120.1 has approved fish oil for use in various meat products. Accordingly, a deodorized menhaden fish oil was selected for our experiments.

The main objective of our study was to modify the lipid profile of chicken breast meat to contain a significantly higher level of ω-3 fatty acids when compared to the control. The other objectives of the study are to test the stability of the emulsion, evaluate performance of the marinade emulsion during infusion, and rancidity development in the ω-3 fortified meat.

3.3 Materials and Methods

3.3.1 Materials

Chicken was procured from a local chicken supplier Wayne Farms, LLC(Oakwood, GA). Individual breast meat pieces ranged from 250-350 grams. The whey protein concentrate used was Proliant's(Ames, IA) 8600 partially hydrolyzed WPC. The menhaden oil used was obtained from Omega Protein(Reedville, Virginia). Omega Pure™ refined deodorized menhaden oil with 2000 ppm added mixed tocopherals was used in all experiments. The phosphate used was Brifisol STP New by B.K. Giulini(Simi Valley, CA).

3.3.2 Marinade Preparation

Marinades were formulated with target levels in meat of 1% Omega Pure™, 1% Salt, 0.4% Sodium Tripolyphoshpate, and 1% Whey Protein Concentrate. The control marinade had the same target levels of all ingredients except the Omega Pure™ and mass balanced with water. The order of mixing was phosphate, salt, WPC, and oil. The water used in all marinades was reverse osmosis water using a Water & Power Technologies lab unit(Columbia, SC). Ingredients were mixed using a Gifford-Wood(Hudson, NH) model 2L 82 high shear mixer with an Dart(Zionsville, IN) 250 series analog variable speed drive controller. All marinades were mixed at 15,000 RPM to effectively emulsify the oil. The marinades were then cooled to 4.4°C using a Wolf-Tec (Kingston, NY), Socacel immersion cooler.

3.3.3 Injection and Tumble Process

Injection was done using a Schröder N40 injector(Wolf-Tec). The injector setup used 3 mm stainless steel needles with 1.2 mm openings at 1.0 bar head pressure. The advance was set at 40 mm. The injector was fully flushed and sanitized prior to each injection. After injection, the marinade retained was measured by weighing the meat pieces. The injected meat pieces were then transferred into a tumbler where additional marinade was added to meet the target total marinade addition. The tumble process was done using a U-MEC(Hayward, CA) tumbler under 26 inches of Hg at 8 RPM for 25 minutes.

3.3.4 Storage and handling

After the inject and tumble process all samples were placed in Cryovac(Duncan, SC) multiple barrier cook-in bag(CN 510) and vacuum packaged. The samples were immediately frozen to -30 °C and held until used for evaluation. When ready for use the samples were cold thawed in a cold room over night and cooked to endpoint 73.8°C in a Alkar(Lodi, WI) smokehouse. The smokehouse schedule used was an electric heated, saturated steam cook at target 87.7°C dry bulb 76.6°C wet bulb setting. Immediately following cooking, the samples were cooled to 3.3°C using a liquid nitrogen freezer Martin-Baron(Irwindale, CA) . The chicken was then placed in Cryovac E2300 multilayer polyolefin oxygen permeable bags with an oxygen transmission rate 5000 (cc/m^2/24 hrs, given 1 atm) for the duration of the storage study. All samples were held at 4.4°C until sampling for the various tests.

3.3.5 Creaming Studies

Creaming or oil separation from the marinade was studied by placing samples in 10 ml graduated cylinders and storing quiescently at room temperature (22.7°C ± 0.3) for 10 days. Four replicates were done for each of the specified experimental emulsions, and the control was excluded as it only constituted a single phase. Cream separation was recorded on day

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1,2,3,4,5,7,and 10. The serum separated after creaming was recorded as a percentage of the total height of the column of liquid in the graduated cylinders.

3.3.6 Particle Size Distribution

Particle size distribution for each marinade was measured using an integrated light scattering Malvern Mastersizer S (Malvern Instruments, Southborough, MA). The refractive index for the sample and the aqueous phases were used to the select the QHD presentation code for the instrument. Measurements were done in a block of ten consecutive readings separated by purges. The average particle size diameters, $D_{3,2} = (\sum n_i d_i^3 / \sum n_i d_i^2)$, and $D_{4,3} = (\sum n_i d_i^4 / \sum n_i d_i^3)$, are both reported for comparison. D indicates diameter size and n is number of particles of diameter D.

3.3.7 Zeta Potential Measurement

Zeta potential was measured using a Brookhaven Zeta Plus(Brookhaven Instrument Corp., Holtsville, NY). The instrument utilized optical heterodyning whereby a 30 mW laser is passed through a dilute solution, scattered at 15°, and then recombined. The resultant Doppler shift caused by the sample particles is correlated with the modulated reference frequency at 250 Hz. Each sample vial was filled to the recommended 1500 μl with a target 0.05 wt% concentration. Additionally, the BI-ZR3 dark blue colloidal powder standard was obtained from Brookhaven and used to attenuate equipment variability after reconstitution in buffer as recommended by manufacturer.

3.3.8 Viscosity Measurement

Rheological measurements were performed using a controlled stress rheometer (Model SR-5000, Rheometric Scientific, Piscataway, NJ). Measurements were carried out using a Couette geometry. The cup diameter was 32.0 [mm] and the bob had a diameter of 29.5[mm]

and 44.25 [mm] length. Measurements were done at a constant temperature of 4.4°C. A steady rate sweep was performed with increasing shear 0-100 s-1 in 360 s. Determination of the flow behavior (n) and consistency index (K) were carried out by fitting the power-law model of shear stress and rate at the linear points of the curve.

3.3.9 Lipid Oxidation

Lipids oxidation was determined by measuring 2-thiobarbituric acid reactive substances(Jo and Ahn 1998) at day 0,1,3,7 and 10 days. Additionally, aldehyde formation was monitored by performing quantitative headspace volatile analysis using an headspace sampler(Aligent 7694; Aligent Technologies, Wilmington, DE). 4 grams of macerated meat was placed in 10 ml Teflon-lined headspace vials and sealed. The vials were loaded into the headspace sampler and automatically loaded into the oven(100°C) and agitated on high for 15 minutes. Upon sampling, the vial was pressurized and volatiles injected into a GC(Agilent 6890; Aligent Technologies, Wilmington, DE) During injection a cryogenic trap was used to concentrate the samples to facilitate detection(Larick and Turner 1990). The oven was held at -20°C for 1 minute, after which to return to 40°C at 40°C/min. Inlet and detector temperatures were set to 230°C and 240°C respectively. The column used was a 60-m capillary column(250 µm i.d. and 1.00 µm film thickness, SPB-5; Supelco, Bellefronte, PA). Column oven temperature was programmed from 40°C to 220°C at 6°C/min for 10 minutes and 220°C for 42.5 minutes, with a split ratio of 1:1. Hydrogen was used for the carrier gas 25.0 mL/min. Headspace sampling was done at days 0,1/2, 1, 3, 7, 10.

3.3.10 Fatty Acid Composition

Lipid composition of the control and treatment marinated chicken samples was confirmed by extracting the lipids fraction(Folch and others 1957) from the marinated sample and then methylating the free fatty acids. The fatty acid methyl esters were analyzed on a GC(Agilent 6890; Aligent Technologies, Wilmington, DE), and separated using a 100-m capillary column(0.25 –mm i.d. and 0.20 – m film thickness, SP 2560; Supelco, Bellefronte, PA). Column oven temperature was programmed at 150 to 165°C at 1°C/min, 165 to 167°C at 0.2°C/min, 167 to 225°C at 1.5°C/min, and held at 225°C for 15 min with 1:100 split. Injector and detector temperatures were maintained at 250°C. Hydrogen was used as the carried gas at a flow rate of 1 mL/min. Identification and quantification of individual fatty acids were done via comparison of retention times with known standards(Sigma Chemical, St. Louis, MO; Supelco and Matreya, Pleasant Gap, PA).

3.3.11 Statistical Analysis

Data analysis was done using SAS 9.1(SAS Institute, Cary, NC). The GLM procedure was used with the respective treatment as the experimental unit. Least square means was used to differentiate between interactions and means. Significance levels in all tests were established at $P \leq 0.05$. Correlation coefficients were obtained using the CORR function.

3.4 Results and Discussion

3.4.1 Emulsion characterization

Rheological properties of the marinades are seen in figure 3.1 plotted as viscosity at various shear rates. The viscosity of the emulsion marinade was significantly higher at 5.2 mPa s, compared to the control at 4.4 mPa s. Fluid behavior in both marinade recipes indicated classical Newtionian behavior with an average flow behavior index of [1.05,1.06] and a

consistency index of [3.7, 4.0 mPa.s], respectively control to experimental. Accordingly, using high shear in preparation of marinades does not cause thinning and or thickening of the emulsion.   Viscosity maybe determined by ingredients interactions and particle size of the dispersed phase.

Particle size diameters on average were significantly higher for the control versus the experimental marinades. The surface weighted (sauter) average $D_{[3,2]}$ diameter of the emulsion marinade was 5.5 μm compared with 11.7 μm for the control (Figure 3.3). Volumetric weighted averages $D_{[4,3]}$ indicated a more dramatic difference from 54.2 μm for the emulsion marinades to 98.35 μm for control. Particle size by volume(figure 3.2) revealed two distinct distributions. In the emulsion marinade a central large peak is seen around the 14 μm diameter followed by smaller peak trailing at 208 μm diameter. The secondary trailing peak at 208 could be indicative of either shear-inducted coalescence or rapid protein-protein aggregation formed in mixing both of which are known conditions of dairy emulsions(Dickinson 2001). In the control marinade the particle size distribution exhibited a unimodal peak centered on 100-120 μm range indicating a greater proportion of larger size particles. These results are further supported by the D(0.5) difference of 16 μm for the enriched marinade and 77 μm for the control. Whey powders have been documented to have a wide particle size distribution ranging from 10 to 1200 μm as supplied from the plant, as a result of processing procedures at the manufacturing plant(Banavara 2003). Particle size was reduced by the high shear used in marinade preparation.

Figure 3.4 details cream formation and creaming indices over time. Values represent percentage of total volume occupied by the serum layer over time. At room temperature a cream layer appeared within the first 24 hrs indicating that the emulsion is not stable for extended periods. Further separation was noticed gradually until the completion of the study. Oil-in-water

emulsions stabilized with proteins are particularly prone to flocculation and coalescence as the primary mechanism of destabilization(Agboola and others 1998). Because of the absence of oil, little if any separation occurred in the control over the test period. Most importantly in both marinades, no sedimentation was noticed up to the termination of the test after 10 days suggesting that all other marinade constituents were uniformly dispersed within the system. Ingredient solubility and successful ingredient incorporation into a marinade has been a well established problem for processors, particularly the occurrence of marinade ingredient sedimentation in the brine tank. Our emulsion marinades which were subjected to high shear during preparation precluded the occurrence of sedimentation. A well dispersed oil in the marinade will ensure that all meat pieces receive the same amount of the ω-3 fatty acids added to fortify the meat.

A closer examination of δ potentials was performed to understand the emulsion stability. Figure 3.5 shows the effect of each ion addition to the marinade and resultant δ (mV). Upon addition of the phosphate and salt minor changes of only to -1.82 mV resulted as the neutral salts and phosphates did not fully form a repulsive double layer. The effects of the addition of these components on the δ-potential of the dispersion medium were not statistically significant. After addition of the protein concentrate, surface charge builds to -26.17 mV as a full plane of shear is established. As oil is added to the system, a final charge increase to -27.54 mV is achieved. After the oil is added, a charged interfacial layer is formed that is known to stabilize emulsions by thwarting coalescence (Dickinson and Matsumura 1991). The ultimate value (mV) of a stable systems is hypothesized for systems based up pH, solid content, or form of surfactant. Riddick (1968) defined a stable emulsion having a δ-potential of greater than -40 mV. For most systems however, a transitional region of flocculation or emulsification occurs from -15 mV to -30 mV,

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where by at -30 mV the forces are sufficient to achieve moderate stability (Tan 1997). These ranges are absolute of sign and respective to the alkaline range of our dispersed system. For a acidic environments, a positive charge magnitude of a equal or greater mV would be required for emulsion stability(Kulmyrzaev and others 2000). For our marinade system, pH values are slightly alkaline form 7.62-65, no significant difference, and the system is agreeably stable with the measured δ-potential.

Results of all tests suggest that a moderately stable emulsion marinade is more favorable compared to a traditional white marinade containing added binders or proteins. The viscosity is slightly greater, which may possibly aid in marinade retention. The particle size on average is smaller, which may aid in better dispersion throughout the muscle during injection. Creaming occurred but was minimal until day 2, when the first decline in the serum fraction was noticed. This length of time for marinade separation will be inconsequential during inject marination since all marination steps would have been accomplished within a 2 hour period. Finally, the δ-potential in the emulsion marinade was in the range of values for a normal balanced repulsive system. Therefore, the marinade will be able to remain stable when held up to a period of 16 hours.

3.4.2 Infusion parameters

Uptake among control and experimental (Table 3.1) marinades was close to the target of 120% with an average of 118.8 % for all marinades Individually, the experimental emulsion marinades had slightly higher retention after vacuum tumbling at 119.5%, but the value was not significantly different than the 117.8% value obtained with the control. Raw muscle pH values were in the range that is outside what would cause PSE or DFD therefore the meat used in these tests would be ideal for maximal water holding capacity and retention(Barbut and others

2005). The transition of pH from an average of 5.99 in the unmarinated to an average pH of 6.22 after marination indicates effective absorption of the marinade by the muscle. Finally overall cook yield data did not show a statistically significant difference between the control and the emulsion marinade.

Lipid profiles of the raw fully marinated breast meat were determined to assess the delivery of the omega-3 fatty acids into the muscle by the marination process. Lipid levels in the muscle (Table 3.2) reveal that the enriched marinade significantly altered the lipid profile of the raw chicken to include a higher percentage of ω-3 fatty acids(ALA, EPA, DPA, and DHA). Exact levels are detailed in table 3.3 with the calculated amounts in milligrams for either standard breast size or portion size. For the experimental whole breast, a total ω-3 enrichment of 281 mg was achieved in the muscle with 244 mg being from long chain fatty acids.   The enrichment is statistically significant (P <0.0001) compared with the control at 12 mg. Some minor marine lipids were seen in the control, however the levels were small and most of the ω3 was ALA . Chicken muscle is naturally high in linolenic acid primarily due to small deposits in the adipose tissue of fatty acids from feed, which contains a variety of lipids (Mourot and Hermier 2001). Currently, ω3 daily intakes in the US are estimated at 1.6 g/p/d with α-linolenic acid at 1.4 g/p/d and long chain EPA and DHA at max 0.2 g/p/d(Kris-Etherton and others 2000). An review by Ruxton and others(2005) surveys health benefits and intake considerations and these authors advised raising average intake   from 200 mg to 450 mg-900 mg levels of the long chain (LC) fatty acids. With these in mind, consumption of a fortified chicken breast meat under normal US intakes of chicken meat, would provide the recommended levels that would impart the health beneficial effects of the LC fatty acids.

For labeling, the Food and Drug Administration stipulates that intake should not exceed 3g of EPA and DHA per person per day. A qualified health claim is approved for suggesting reduction of risk of coronary heart disease with the listing of the EPA and DHA content, which would be applicable to the product (FDA 2004). Additionally, a nutrient content claim is approved for products containing 130 mg EPA or DHA in the reference amount customarily consumed(RACC, 21 CFR 101.13). For meat and poultry products, the RACC is 106 g raw and 85 g cooked meat. The RACC for raw products have values for EPA and DHA to be very close but the total amount of EPA and DHA when consuming the RACC   do not meet the labeling guideline for the health claim. However, if the entire fortified breast meat is consumed the amount specified in the guideline would be satisfied.

3.4.3 Shelf life performance

Thiobarbituric acid reactive substances (TBARS)were measured after cooking to track development of secondary lipid oxidation by products, specifically malonaldehyde. A reaction between TBA and malonaldehyde is the major color producer but other aldehydes also forms the red chromogen. The chromogen may be measured spectrophotometrically at 530-32nm (Ulu 2004). A necessary step in most procedures is a heating step to accelerate formation of the chromogen, however, excessive heating (Salih and others 1987) has been linked to formation of a yellow pigment at 450 nm.   It has also been noted that lipid oxidation products such as furfural, alkanals, alkenals, alkadienals have been linked to a yellow chromophore(Grau and others 2000). Therefore, over the entire experiment absorbance at both 530 and 450 nm were measured.   Figure 3.6 shows the development of TBA values represented as mg malonaldehyde/kg sample as well as the absorbance values.   Each sampling point was an average of triplicates and values ranged from 0.11 mg on day 0 for the experimental to 5.0 mg on

day 7 for the control.   The control peaked at day 7 and declined at 10, indicating a conversion

from primary to secondary oxidization after day 3. Similar type values were reported by Rhee

and others(1996) who did a survey of various cooked refrigerated meats. Chicken showed the

most rapid rancidity development reaching TBA values in excess of 12-14 mg in the thigh and

upwards of 7 mg in breast meat at day 6. Oxygen transmission rate for the packaging material

used by these workers was slightly higher at 6510 cc/m^2 compared with our packaging.

More recently, Bou and others (2004) found that when fish oil was incorporated into chicken

feed, TBA values of meat were 1.8 mg at 15 days and 9.1 mg at 5 months storage at -20°C.

Bou and others(2004) also determined sensory acceptability scores on a 9 point scale(1= very

bad; 9 = very good). The twenty seven member panel scored the 5 month stored enriched

chicken sample above mentioned at 4.4 and a commercial sample at 4.7 for acceptability.

Therefore a good probability exists that our chicken samples, even at maximum value 4.6 mg

MDA/kg on day 10, may still be acceptable to consumers. The sensory properties of the fortified

and control chicken breast meat evaluated in our experiments will be reported in a separate

article.

Absorbance measured at the two wavelengths over time proved to be only significantly

different between samples at 532 nm. At 450 nm as seen in Figure 3.6, values fluctuated from

0.068-0.087, only a 0.019 range compared with 0.118 range at 532 nm. Additionally, the

absorbance at 450 nm did not positively correlate with the TBA values calculated from 532 nm

standard curve. Standard development for a curve at 450 nm was also irregular. For our

samples, the minor secondary yellow pigment development in an aqueous extract proved to have

negligible interference with the primary red chromogen. Accordingly, a distillate separation was

not required in extraction. In past, interference has been the justification for performing analysis

on distillates, which reduce the interference but increase oxidation of the sample(Salih and others 1987). Conclusive data for our experiment may only be drawn for the absorbance at 532, previous discussed.

Volatile production correlated well TBA values(Figure 3.7). Significant levels of ethanal, propanal, and hexanal were detected in samples from day 0 to day 10. Hexanal has been referenced as a primary aldehyde produced in the development of rancidity(Byrne and others 2002; Beltran and others 2003). Hexanal is a product of linolenic oxidation and the 13-hydroperoxide, exhibits a grassy odor at a threshold concentration of 4.5 µg/kg water (Sanches-Silva and others 2004). The peak area of the headspace volatile, hexanal, in the control was minute in day 0 and increased to 9.8 on day 7. Hexanal in the fortified sample developed gradually to peak at day 10. These values coincide with the trends in the TBA values with time. Strong correlation in hexanal and total volatiles with TBA has been noted before by Ahn and others(1998). They reported correlation between multiple muscle groups(*L. dorsi*, *R. femoris*, and *L. psoas*) in pork at 0.94. Our correlation coefficient between hexanal and TBA was 0.91 and 0.99, for the experimental and control samples respectively.

Additionally, ethanal and propanal were generated in significant amounts in both control and fortified samples.   Lee and others(2004) found both significant levels of ethanal and propanal in chicken meat from studies on glycosyl-ascorbic as an antioxidant . Our chromtograms showed an unknown peak at 9.680 minutes which did not correspond to any of the standards used. The detection was minor for this unknown compound, which could be any form of a short or long chain aldehyde, ketones, alcohol, hydrocarbon, or acid. Furthermore, the peak area of this unknown compound did not increase over time therefore it may not have a bearing on the level of rancidity. Total headspace volatiles developed only slightly more in the control

versus the experimental. Total peak areas at the conclusion of the study were only 42.03 for control compared with 38.39 for the enriched sample. For rancidity development, a normal shelf life is expected as no significant increase was seen in volatiles by use of ω3 fatty acids in the marinade.

3.4.4 Conclusions

Marinated chicken enriched with an emulsion containing ω3 fatty acids contained a significantly higher   level ($P < 0.05$) of long chain polyunsaturated fatty acids in the meat compared to those treated with a standard marinade.   Marinade was physically stable to creaming and particle settling over the range of time marinades are prepared and used. Marinade stability was supported by measurements of viscosity, creaming index, particle size, and zeta potential. ω-3 fortification of meat through marinades resulted in functional levels actually retained within the tissue and current recommendations for levels of ω3 fatty acids into the diet can easily be met. Storage time for onset of spoilage and lipidolytic indicators of rancidity did not reveal stability issues when compared to a control.
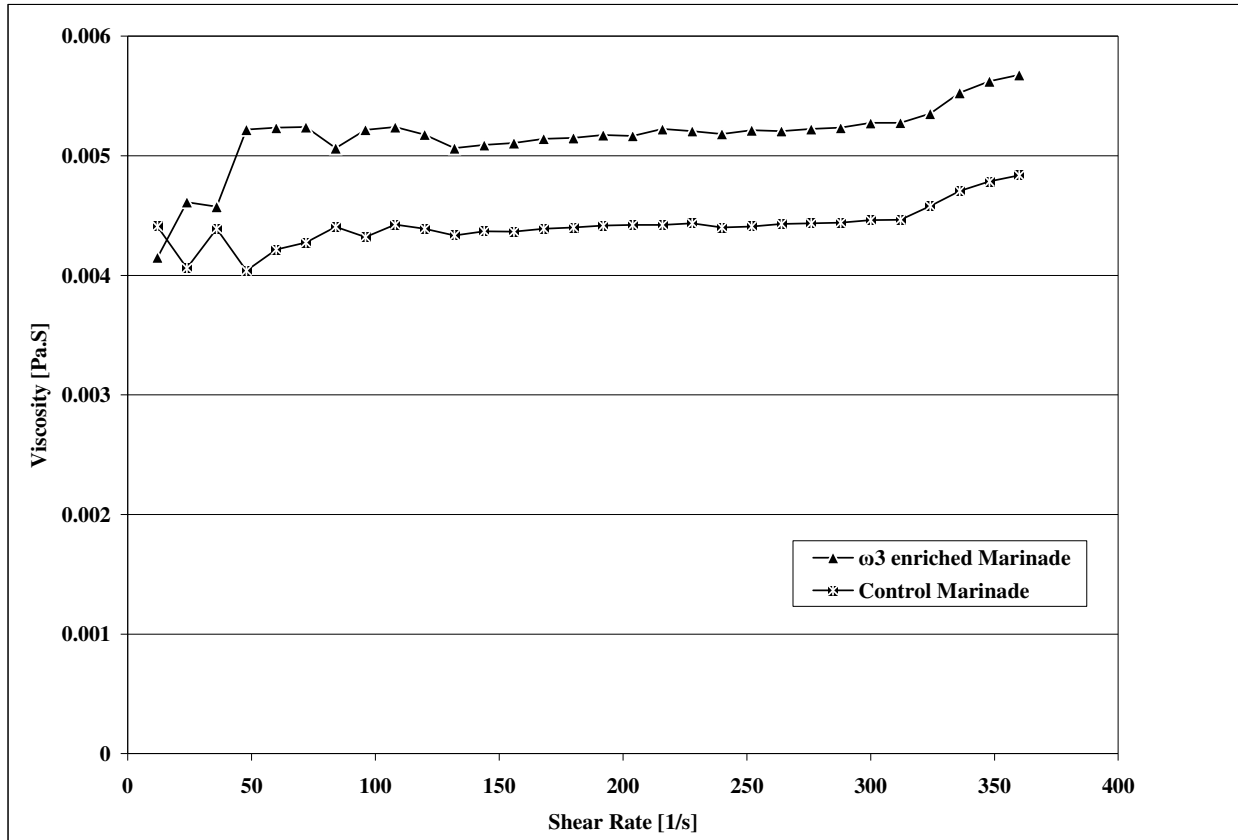
Figure 3.1: Effect of shear rate on viscosity on the test and control marinades. Curves significantly differ by (*P* < 0.05).
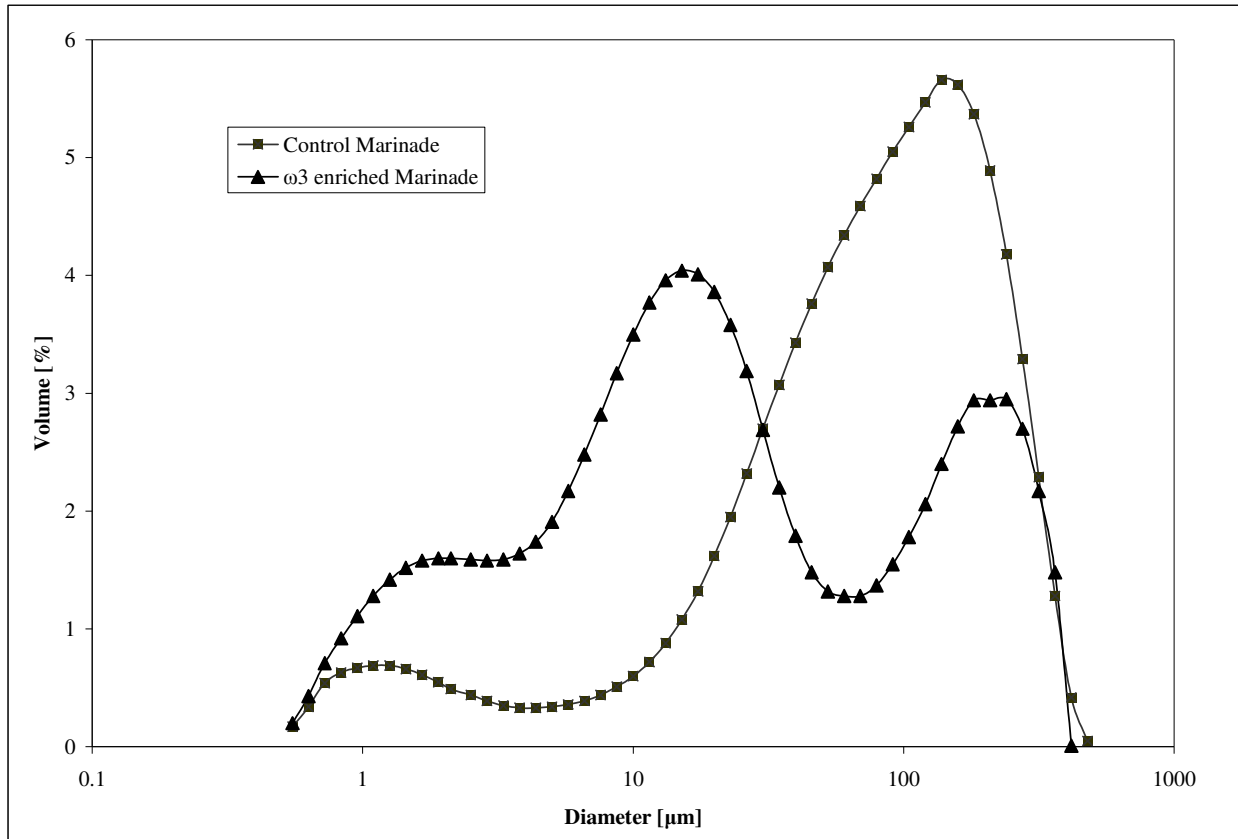
Figure 3.2: Particle size distributions, as measured by integrated light scattering for either the experimental marinade [6% Oil, 6% WPC] or control [6% WPC].
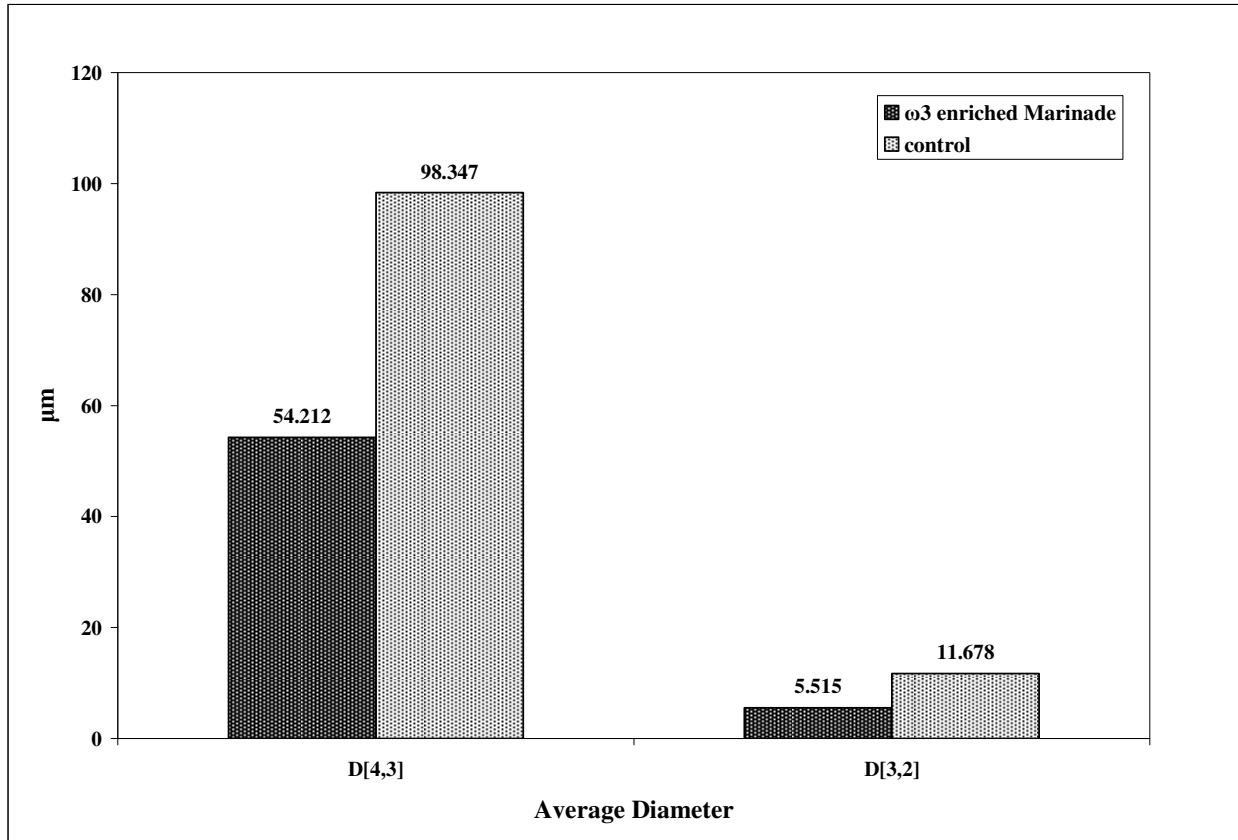
Figure 3.3: Average particle diameters for marinades reported in the volumetric mean D[4,3] or sauter mean D[3,2] values. Mean values significantly differ ($P < 0.05$).
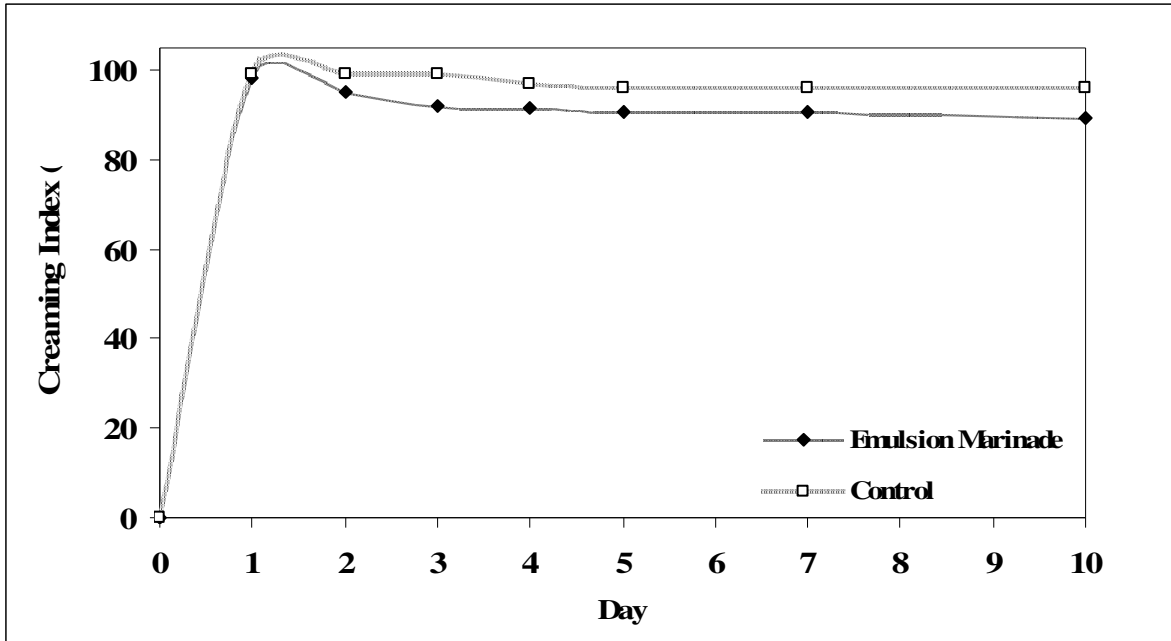
Figure 3.4: Effect of 6% wt WPC in stabilizing the emulsion with 6% wt FO. Values for creaming differ (*P* < 0.05).
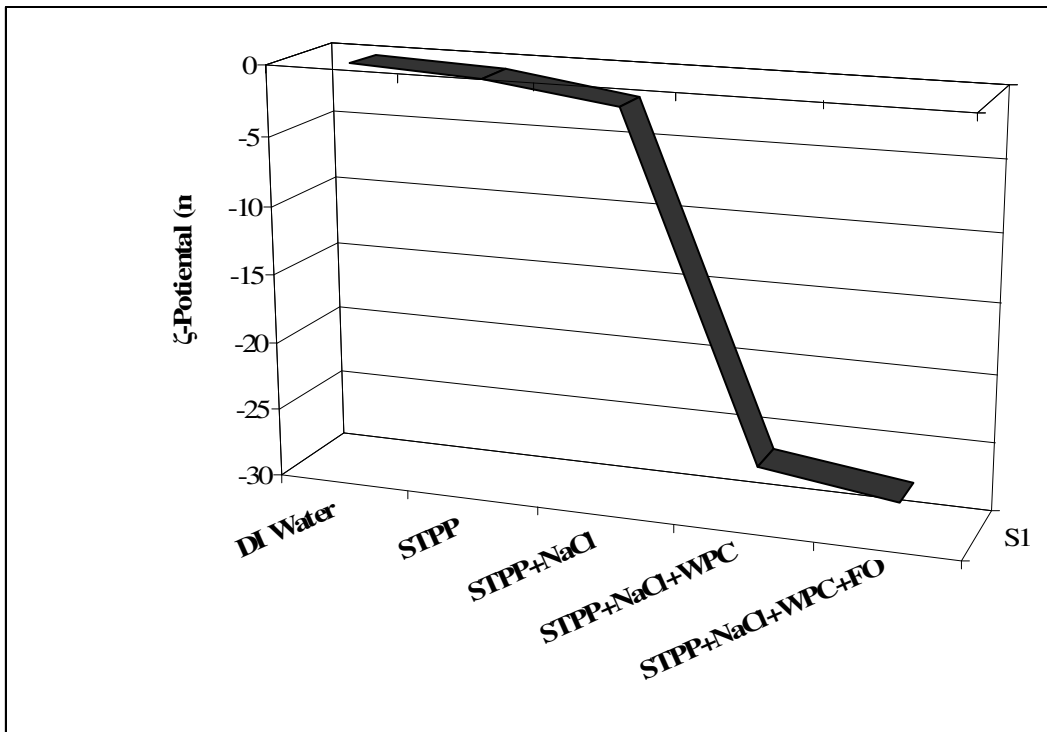
Figure 3.5: Effect of ingredient addition up zeta potential of marinade system. Deionized water and STPP mean difference is greater than 0.05 and is not significant. All other points are significant $P < 0.05$.
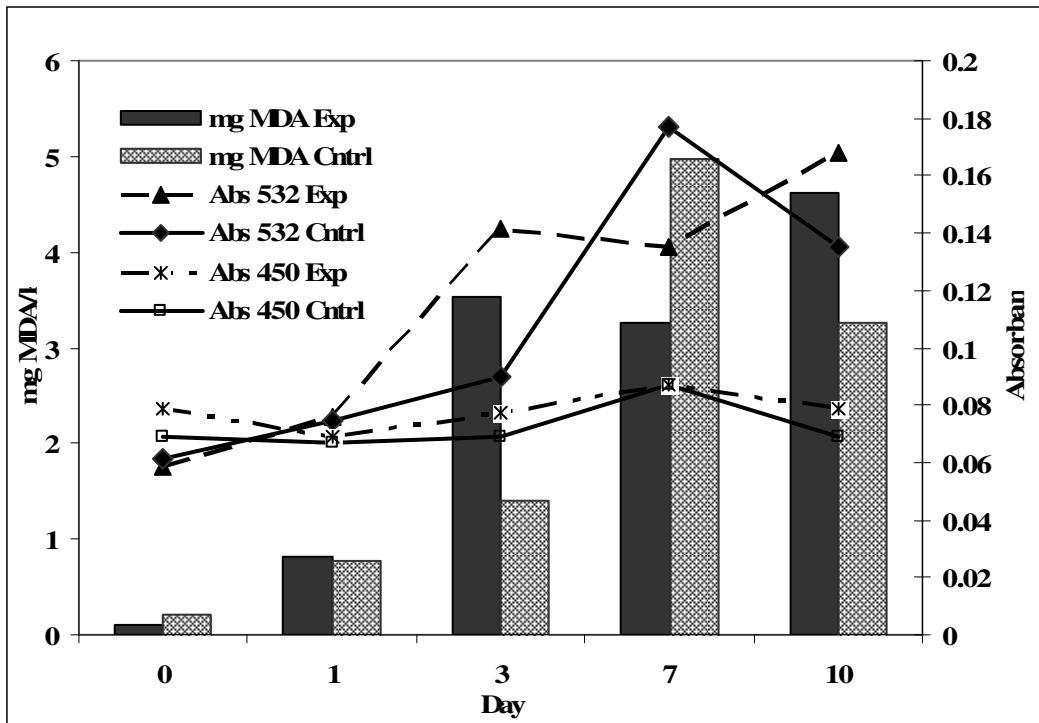
Figure 3.6: Development of TBA values in stored refrigerated samples over time. Absorbance values over time at 532 nm and 450 nm are indicated with lines. All TBA values differ in treatment and in day by at least *P* < 0.05. 532 and 450 nm absorbance values significantly differ by day and treatment *P* < 0.05. No significant interaction seen between 450 and 532 values.
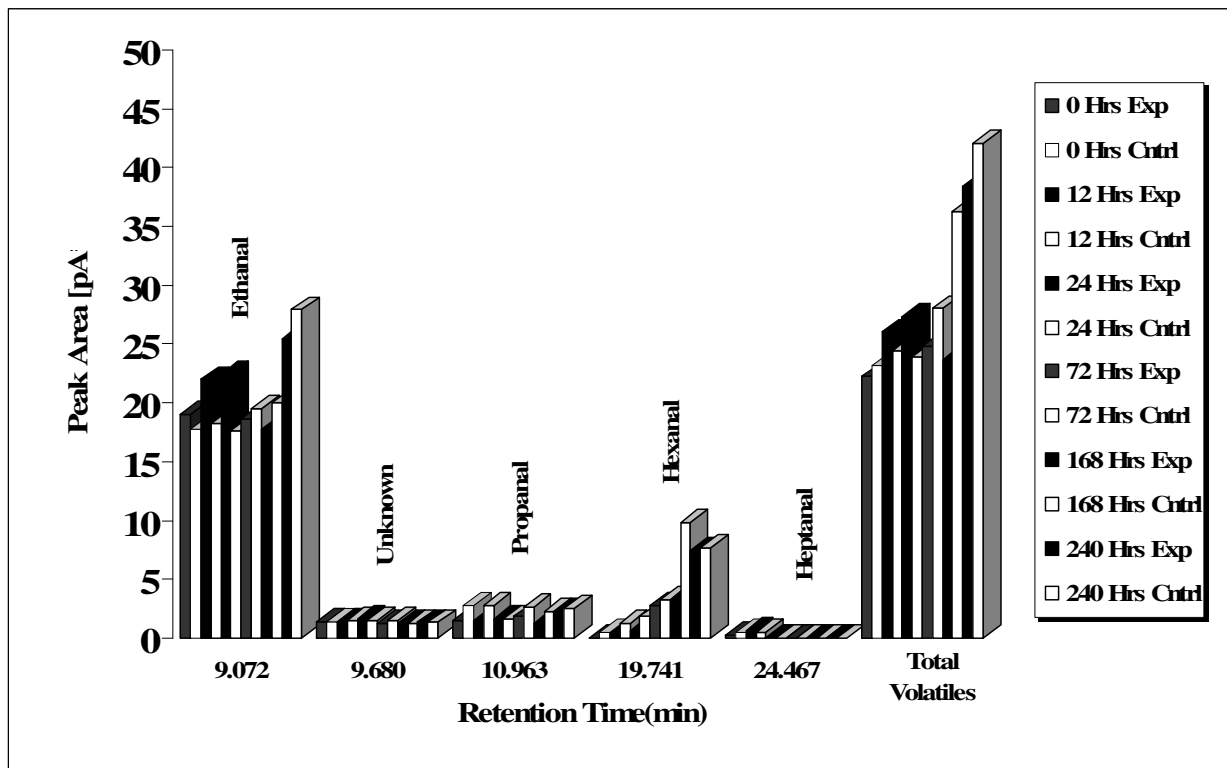
Table 3.7: Headspace volatiles sampled over time respective to the retention time of the standard. Statistically significant effects (P< .05) were seen in ethanal, propanal, and hexanal development both between in treatment and in day.

Parameter	Experimental	Control
pH		
raw muscle	5.98^A	6.01^A
marinade	7.62	7.65
marinated muscle	6.20^B	6.23^B
cooked muscle	6.34	6.38
Retention*	(%)	(%)
injection(target 10%)	108.50^C	108.50^C
tumble(target 10%)	119.50	117.80
after cooking	104.61^D	104.15^D

Table 3.1 – Mean muscle pH values and pickup among breast meat marinated. Values in rows without a common subscript differ significantly ($P < 0.05$).

Item	Enriched Chicken			Control			*P*-value
Total fatty acids, g/100g of fresh tissue	0.82	±	0.015	0.68	±	0.03	0.057
C14:0	3.05	±	0.052	1.02	±	0.11	0.002
C16:0	17.94	±	1.406	21.06	±	1.14	0.477
C16:1*cis*-9	5.47	±	0.053	4.38	±	0.20	0.017
C18:0	5.91	±	0.667	7.80	±	0.22	0.063
C18:1*cis*-9	17.45	±	1.322	25.01	±	1.88	0.043
C18:2*cis*-9, 12	9.19	±	0.937	17.40	±	1.54	0.023
C18:3*cis*-9, 12, 15(ALA)	1.30	±	0.004	0.86	±	0.08	0.016
C20:4*cis*-5, 8, 11, 14	2.47	±	0.175	4.16	±	0.42	0.034
C20:5*cis*-5, 8, 11, 14, 17 (EPA)	3.65	±	0.140	0.00	±	0.00	0.001
C22:5*cis*-7, 10, 13, 16, 19 (DPA)	1.00	±	0.001	0.48	±	0.04	0.003
C22:6*cis*-4, 7, 10, 13, 16, 19 (DHA)	3.86	±	0.134	0.30	±	0.03	0.001
Unidentified	28.71	±	4.24	17.52	±	1.86	0.098
SFA*	37.72	±	0.59	36.23	±	0.80	0.167
MUFA*	32.16	±	0.01	35.70	±	0.90	0.031
PUFA*	30.12	±	0.61	28.07	±	0.11	0.043
PUFA:SFA ratio	0.80	±	0.040	0.78	±	0.014	0.403
n-6:n-3 ratio	1.19	±	0.15	13.26	±	0.70	0.003

*SFA = C14:0, C16:0, C18:0, and MUFA = C16:1, C18:1. Total SFA, MUFA, and PUFA were calculated and corrected for the unidentified fatty acids, so that the three fatty acids add to 100%.

Table 3.2: Least square means (±SE) for fatty acid composition (g/100 g of total fatty acids) in raw chicken treatments.

Item	Enriched Chicken		Control	
	Whole Breast	Raw-RACC	Whole Breast	Raw-RACC
Serving Size	350 g	106 g	350 g	106 g
C14:0	87.48 mg	26.50 mg	24.19 mg	7.33 mg
C16:0	514.85 mg	155.92 mg	501.20 mg	151.79 mg
C16:1*cis*-9	157.07 mg	47.57 mg	104.30 mg	31.59 mg
C18:0	169.73 mg	51.41 mg	185.70 mg	56.24 mg
C18:1*cis*-9	500.84 mg	151.68 mg	595.20 mg	180.26 mg
C18:2*cis*-9, 12	263.66 mg	79.85 mg	414.13 mg	125.42 mg
C18:3*cis*-9, 12, 15(ALA)	37.21 mg	11.27 mg	20.47 mg	6.20 mg
C20:4*cis*-5, 8, 11, 14	70.88 mg	21.47 mg	99.06 mg	30.00 mg
C20:5*cis*-5, 8, 11, 14, 17 (EPA)	104.69 mg	31.71 mg	0.00 mg	0.00 mg
C22:5*cis*-7, 10, 13, 16, 19 (DPA)	28.78 mg	8.72 mg	11.51 mg	3.49 mg
C22:6*cis*-4, 7, 10, 13, 16, 19 (DHA)	110.70 mg	33.53 mg	7.24 mg	2.19 mg
Long Chain ω3	244.18 mg	73.95 mg	18.76 mg	5.68 mg
Total ω3	281.39 mg	85.22 mg	39.22 mg	11.88 mg
Total ω6	334.54 mg	101.32 mg	513.19 mg	155.42 mg

Table 3.3: Calculated intake values based upon the average breast size 350 grams or the 106 g reference amount customarily consumed(RACC) for ready to cook poultry. Figures calculated on table 3.2 numbers.

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CHAPTER 4

CONSUMER ACCCEPTABILITY AND SENSORY ATTRIBUTES OF CHICKEN BREAST

MEAT ENRICHED WITH N-3 FATTY ACID APPLIED IN THE MARINADE [2]

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[2] Williams, BC, Shewfelt, RL, and Toledo, RT. To be Submitted to the Journal of Food Science

4.1 Abstract

Long chain Omega 3 fatty acids have been linked to a multitude of health benefits if incorporated into the human diet. Finding new avenues of introducing these healthy fatty acids is a pressing goal for the food industry. Boneless chicken breast meat was marinated with a stable emulsion that contained deodorized menhaden oil(Omega Pure) containing 20-26% omega 3 fatty acids whey protein concentrate, salt, and phosphate. Sensory testing was done to assure commercial acceptability of the product. The trained panel found (P < 0.05) no significant development of off flavors. A triangle test indicated a difference in the enriched and conventionally marinated chicken meat at P < 0.001. Demographic data showed that no significant gender, consumption frequency, or health education impacts the willingness to purchase the ω-3 fatty acid enriched chicken meat. In a blind acceptance test, the willingness to purchase the control chicken was greater than the enriched product.

4.2 Introduction

According to the United States Department of Agriculture's most recent statistical report, Georgia produced 6.3 billion pounds annually (2003) of chicken with a market value of over $2.1 billion dollars(USDA-NASS 2005). Chicken growth, production, and marketability have and will continue to be one of Georgia's largest economic bases. For food companies, the search for new and improved ways of adding value to chicken is a consistent goal. The nutraceutical and functional foods market has proven a novel avenue for food companies offer high value added products with a market potential of $12.835 billion US by 2009(Mint*e*l 2004). Thus, a new approach to value added chicken could involve nutraceuticals.

In prior research, a ω-3 enriched marinade successfully modified chicken breast lipid through infusion(Williams and others 2006). Enriched chicken breast meat contained a significantly higher amount (P < 0.0001) of long chain Eicosapentanenoic(EPA) C20:5ω3, Docosahexaenoic(DPA) C22:5ω3, and Docosahexaenoic (DHA) C22:6ω3 fatty acids than the control. A common drawback of incorporating highly unsaturated fatty acids in tissue has been development of fishy off flavors(Edwards and May 1965; Miller and Robisch 1969; Hargis and Van Elswyk 1993; López-Ferrer and others 2001; Mielnik and others 2002). Use of deodorized oils and antioxidant additives have assisted in preventing the off flavor development.

The oil selected was a refined menhanden oil, which has an added tocopherol blend to prevent oxidation of the oil. The objective of these sensory experiments is trifold. First via a difference test, determine if a difference exists as a result of ω-3 enrichment. Second using a trained panel, closely examine these differences along with other meat sensory descriptors. Finally by way of an acceptance test, quantify a willingness of purchase to see if the differences impact a consumer's decision to purchase the enriched chicken meat.

4.3 Methods

4.3.1 Materials

Chicken was procured from a local chicken supplier Wayne Farms, LLC(Oakwood, GA). Individual breast meat pieces ranged from 250-350 grams. The whey protein concentrate used was Proliant's(Ames, IA) 8600 partially hydrolyzed WPC. The menhaden oil used was obtained from Omega Protein(Reedville, Virginia). Omega Pure™ refined deodorized menhaden oil with 2000 ppm added mixed tocopherols was used in all experiments. The phosphate used was Brifisol STP New by B.K. Giulini(Simi Valley, CA).

4.3.2 Sample Preparation

Raw fully marinated chicken treatments were vacuum sealed after marination and stored at -30°C for less than 1 month. Prior to the day of the respective panel, a cold thaw of the required quantity of sample was performed. The chicken breast meat was then cooked in an Alkar(Lodi, WI) smokehouse employing electric heat and steam (75%RH). The smokehouse schedule used was acceptable as no over cooking, excess browning, or extraneous flavors developed. Endpoint temperature was targeted at 73.8°C. Samples were hot diced in a Urschel dicer to a target dice of 1.9 cm x 1.9 cm x 1.9 cm(standard ¾ dice) and held hot in Cambro cart(Huntington Beach, CA) for transfer to sensory booths. Panelists were served samples in 4 oz soufflé cups with a randomized 3 digit code. An unsalted soda cracker along with an ambient temperature cup of water was provided for palate cleansing.

4.3.3 Trained Panel

The standard guidelines published by the American Meat Science Association were followed to evaluate the sensory attributes of the chicken(ASMA 1995). A panel of 10 consumers (4 male, 6 female) was trained on the attribute terms and definitions commonly used for meat evaluation. A warm-up exercise to familiarize each panelist was given prior to each panel. Each panelist evaluated on an adapted 8-point categorical scale with a ballot of six descriptors. The descriptors evaluated were initial juiciness, sustained juiciness, initial tenderness, sustained tenderness, chicken flavor, and off flavor.

4.3.4 Triangle Testing

A standard testing protocol ASTM E 1885-04 was followed as a guideline for the triangle test. Each assessor was prescreened so that only consumers of chicken products and those with no allergic responses to fish, whey, or wheat were selected. He or she received instructions prior to testing as to the protocol of the test. A total of 83 panelists who only evaluate one triad participated to maximize significance.

4.3.5 Willingness to Purchase Testing

A 5-point purchase intent scale was used to quantity a consumer's acceptance judged by intent to purchase. Panel size consisted of 75 assessors. Prior to evaluation, each panelist was asked demographic questions to profile his or her attitude towards purchase of nutraceutical products and in particular chicken. Specific demographic data recorded were generation, gender, consumption frequency, health awareness, and conscious impact of awareness upon purchase decision.

4.3.5 Statistical Analysis

Data analysis was done using SAS 9.1(SAS Institute, Cary, NC). Analysis of variance was performed on all attributes. In comparison of individual attribute means among treatment, a Tukey's W procedure was used at the 0.05 significance level.

4.4 Results and Discussion

In prior investigations(Williams and others 2006), applicable levels of ω-3 infusion were reported at 281 mg in the muscle with 244 mg being from long chain fatty acids. At these levels upon cooking and tasting, the trained panel found little if any differences between enriched samples and control. Samples were steam cooked and immediately served to panelists. Table 4-1 details assessors' scorings between the enriched and control sample. Juiciness in samples was not significantly different. Initial juiciness was described as the amount of free fluid released upon the first bite. The control was scored less at 5.7 compared to 6.3 for the experimental chicken, where 5 indicates "slightly juicy" and 6 indicates "moderately juicy." Likewise, for sustained juiciness rankings were again higher for the experimental at 6.3 compared to 5.3 for the control. Tenderness was defined as the easiness with which meat is divided into fine particles during mastication. The initial tenderness proved to the only significant difference with the experimental chicken receiving at score of 6.7, "very tender" and control with 5.9 "moderately tender." Tenderness throughout the mastication was scored at 6.3. Chicken flavor was scored as more developed in the experimental versus the control, but the difference was not significant. Off flavor in either sample was minor with the highest rating of 2.3, "Practically None", referring to the control. The enriched sample mean value was even lower at 1.6. Natural poultry flavors and the accompanying lipidolytic degradative impact have been defined by multiple descriptors(Lyon and Lyon 1996; Civille and Dus 1992). The decision was made to

group flavor and off flavor descriptors on the 8-point categorical scale . The development of a "fishy" or oxidized off flavor, as not being seen significantly in samples, may require more holding or abuse than the few minutes (<15 minutes) between cooking and tasting.   General concerns relating to usage of the 8 point categorical scale can be justified from a few historical uses. Adaptations of the structured/unstructured scale have repeatedly been made to improve testing accuracy. In the past, Barbut and others(1988) used an 7 point structured and more recently Hoz and others(2004) used a 10 cm unstructured scale. Industry standards such a the 9, 15, or 18 point scales may also be used. A review by Villanueva and others(2005) concluded that the hybrid hedonic and hedonic scales are generally easy for panelists to use. Therefore, the use of an 8 point hedonic scale would be acceptable in sensory evaluation of poultry meat if the correct descriptors are used for the attributes evaluated. For all germane purposes, the 8-point scale used in this work provided meaningful results with no evidence of bias in the data generated.

Triangle testing followed a standard method as referenced above(ASTM E 1885-04). Accordingly, the design and presentation followed the standard six sequences referenced for presentation of samples to the panelist. 13 complete series of the six sequences were completed allowing for more than adequate repetition.   Scorings reveal a different in samples at P<.0001. 56 of the 78 panelists correctly identified the odd sample in the triad while 22 failed to correctly identify the odd sample. The difference detected is not adequately elucidated by this evaluation.   Thus other descriptive or affective tests were also done to allow for better conclusions.

Profiling consumers' affective liking or attitude towards a product has always posed a challenge for food and marketing professionals. Simple hedonic scale of acceptance only offers

the ability to provide a ratio or interval scaling of attributes, whereby panelists may rate preference and provide a degree of preference for particular attributes of a product(Peryam and Girardont 1952). A noted drawback of the hedonic rating scale is extrapolation of the scale to make a general predictions of acceptance, most notably, the evaluator's failure to relate to the consumer's decision to a purchase. When making the decision to purchase, an individual must reconcile all preferences and decide if he or she truly likes the product enough to purchase it. Failure to hurdle this constraint is one cause product failure upon launch. Therefore proper assessment of consumer's buying intention may prove to be a more sensitive gauge of acceptance.

The affective sensory results are displayed on page Table 4-2 by percentage scored in the top two categories of a 5 point scale. This form of analysis has been suggested by Moskowitz(1994) as the primary way to report the proportion of consumers who give a specific response. Results indicate that both health awareness and the healthiness of a food product are less than 50% of the scored values. Proportions scoring in the top categories showed 39.2% of 74 panelist "very" or "expertly" aware of health and 43.2% use healthiness of a food product "very" or "critically" important in purchase level decision. Finally, the percentage of panelist scoring a willingness to purchase as "probably" or "definitely" would buy were 60.8% for the enriched sample and 85.1% for the control. From these percentages, more panelists prefer the control to the enriched sample. An open concession of the experiment is that the product upon holding for the duration of the panel may allow for development of the "fishy" off flavors not detected in the trained panel. All samples were held hot as specified by the US food code, but formation of this flavor may show that at short holding of less than 15 minutes is suitable, where as longer may result in negative scores.

However still, both scorings indicated a willingness to purchase. A noted difference, males(76.9%) were able to pick up the off flavor notes of the samples more than females(52.1%). Consumption patterns of chicken type products were not significantly different between the two genders. Therefore, males could possibly be more sensitive to chicken flavors or off flavors.

Marketing and consumer behavior experts continually seek the predictor attributes that could link a demographic or personality trait to a purchase decision(Eertmans and others 2005). Additionally, as examined in Shewfelt(1999), quality as related in fresh fruits and vegetables but more generally noticed for all purchase decisions maybe dichotomized by either product-oriented quality or consumer-oriented quality. These two interact to generate a paradox where by product specific quality improvements/and or attributes intercede in the willingness of consumers to purchase a product. Extrapolated to the case of purchase of chicken products, specific nutraceutical enrichments may or may not be strongly appealing to consumer's health or health impact as a criterion in making a purchase decision.

The enriched chicken breast samples and the control did differ by 24.3%. The control did place higher and a panelist would "probably purchase" the sample; whereas, the experimental chicken received only a "Might/Might not purchase" rating. These rating may as reflect development of off flavors, which were beyond the capability of experimenter's control. These differences may also lead to the triangle test showing a significant difference. However, a commercial preparation or hot hold for a product of this type should not exceed the 15 minute hold time used for the trained panel, where no differences. It could additionally be postulated, that upon knowledge of the health benefits or labeling claims of the enriched chicken more panelists would be more willing to purchase the product. These standard sensory comparisons stipulate blind evaluation as to eliminate bias.

4.5 Conclusions

The trained panel detected (P < 0.05) no significant development of off flavors in chicken breast meat enriched with ω-3 faty acids . However a triangle test with a large number of panelists indicated a difference in the enriches and control samples at $P < 0.001$. Demographic data showed that the effects of gender, consumption frequency, or health education is not significant in influencing the willingness to purchase the ω-3 fatty acid enriched chicken. In a blind acceptance test in a large consumer panel, the willingness to purchase the control was greater than for the enriched sample. Therefore based upon the trained data, an ω-3 enriched chicken breast meat, upon a rapid cook and consumption, would be acceptable in commercial markets and would not present significant sensory barriers to acceptance.

	Descriptors					
Treatment	Initial Juiciness	Sustained Juiciness	Initial Tenderness	Sustained Tenderness	Chicken Flavor	Off Flavor
Enriched Chicken Breasts	6.3	6.3	6.7*	6.3	6	1.6
Control	5.7	5.3	5.9*	6.3	5.1	2.3

Table 4.1: Panelist scoring from trained panel based upon 8 pt categorical scale (1=extremely dry, 2=very dry, 3=moderately dry, 4=slightly dry, 5=slightly juicy, 6=moderately juicy, 7=very juicy, 8=extremely juice) for off flavor (1=none, 2=practically none, 3=traces, 4=slight, 5=moderate, 6 slightly abundant, 7=moderately abundant, 8=abundant). *indicated differences in panelists scores by P < .05

Gender	n	Health Awareness	Health as a Discriminator in Purchase Decision	Enriched Sample	Control Sample
M	26	42.3%	38.5%	76.9%	84.6%
F	48	37.5%	45.8%	52.1%	85.4%
Total	74	39.2%	43.2%	60.8%	85.1%

Table 4.2: Demographic groupings and willingness to purchase scores on a 5 point categorical scales. Scores represented as a percentage response in the 4 or 5 grouping. Health Awareness(1=Not aware, 2=Somewhat aware, 3=Moderately Aware, 4=Very Aware, 5=Expertly Aware), Health as a discriminator(1=No Importance, 2=Some Importance, 3=Moderate Importance, 4=Very Important, 5=Most Important), Willingness to purchase(1=Definitely Not Buy, 2=Probably Not Buy, 3=Might/Might Not Buy, 4=Probably Would Buy, 5=Definitely Would Buy).

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CHAPTER 5

CONCLUSIONS

The addition of menhaden oil naturally rich in ω-3 fatty acids into a marinade and later infusion into chicken breast proved a viable method of infusion. Marinade formulation targeted meat compositional levels of 1% menhaden oil, 1% salt, 1% whey protein concentrate, and 0.4% sodium tripolyphosphate. Ingredient mixing resulted in a oil emulsion marinade with a viscosity of 5.2 mPa s and 5.5 $D_{[3,2]}$ mean sauter particle size. The first hypothesis states that an stable emulsion may be formulated to ensure the normal injection process. The stability indicator of creaming revealed separation after 24 hours. Surface charges, measured through zeta potiental, indicate a moderately stable emulsion system. These results are sufficient for a commercial marination process, which effectively support hypothesis i.

Hypothesis ii relates to that premature development of rancidity in the chicken as a result of enrichment will not occur. Hexanal and thiobarbituric acid reactive substances did not develop significantly faster for the experimental when compared with the control. Peak values for TBA values were only 5.0 mg malonaldehyde/kg meat on day 7 for the control. Hexanal values increased to peak area values of 9.8 on day 7. Both indicators are not significantly different in treatment. Therefore, hypothesis ii is validated.

Muscle lipid levels for an average breast were 281 mg of ω-3 fatty acids. The long chain(EPA, DPA, and DHA), constituents consisted of 244 mg. These levels are significant

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when compared to the control at only 12 mg. If integrated into a typical US diet, one chicken breast would successfully provide a documented health beneficial level of ω3 fatty acids.

Sensory experiments to quantify difference, flavor, and acceptability proved hypothesis iii to be valid. Hypothesis iii states that although a detectable difference maybe found between control and experimental chicken consumers will still be willing to purchase the product. In a ASMA trained panel, the enriched chicken panel had no detectable off flavors scored at 1.6 with the control ranked with a higher degree of off flavor development.

In triangle testing, a detectable difference between samples was significantly found. The attribute of difference may not be determined from the test. A consumer panel additionally found both the control and experimental chicken suitable for purchase, based upon a 5 point willingness to purchase scale. The control ranked only slightly higher by 0.6 of a point.

These experiments have proved a new commercial operation to incorporate ω-3 fatty acids into a food system. All objectives defined as such have been answered; however the ultimate objective is whether with the marketability of the chicken will justify the expense of the treatment for a company to produce it.