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Omega-3 Fatty Acid Fortification of Marinara Sauce

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Omega-3 Fatty Acid Fortification of Marinara Sauce

A thesis presented
in partial fulfillment
of the requirement for the
Department of Rehabilitative Sciences
Nutrition Honors-in-Discipline Program
at East Tennessee State University

4/18/2021

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4/18/2021

W. Andrew Clark, PhD, Mentor — Date

4/17/2021

Mary Andreae, MS, RD, Reader — Date

ABSTRACT

Omega-3 Fatty Acid Fortification of Marinara Sauce

by

Hannah P. Collie

In westernized culture, there is a deficit of healthy fats in the average person's diet. Decreased intake of omega-3 fatty acids has been correlated to many different conditions such as cardiovascular disease, cancer, and chronic inflammatory issues. The "Mediterranean Diet" has been proposed as an ideal way to combat these issues. This diet promotes fish as a protein source and as a way to increase intake of essential polyunsaturated fatty acids. Due to location and dietary trends, fish is less often the main component of Northeast Tennessee's diet. This study investigated ways to fortify a more commonly consumed food in western culture, marinara sauce, with flaxseed oil, walnut oil, or anchovies. These fortified marinara sauces were compared to a commercial sauce, Paul Newman's Marinara. We hypothesized that adding omega-3 rich ingredients to a base marinara sauce recipe will significantly vary the fatty acid profile and increase the amount of omega-3 fatty acids and decrease the omega-6 to omega-3 fatty acid ratio. Marinara sauces were prepared and analyzed using proximate analysis methods to evaluate each variant sauce for macronutrient distribution. Preparation included cooking, freeze-drying, and grinding the variants into a fine powder. The tests that we performed included: bomb calorimetry, Kjeldahl protein analysis, Soxhlet fat analysis, ash/mineral analysis, FRAP Assay of antioxidant content, and gas chromatography to characterize fatty acid profiles. The focus of our proximate analysis was investigating the fatty acid composition to answer our main research question. Other tests conducted allowed us to understand better the nutrient composition of the

variants for possible future studies. The three variants showed a better omega-6 to omega-3 fatty acid ratio than the Paul Newman's sauce. Samples with the highest to lowest omega-6 to omega-3 ratio were Paul Newman's Marinara, Anchovies, Walnut Oil, Flaxseed Oil, respectively. Gas chromatography showed different concentrations of omega-3 fatty acids as (% area under the curve): flaxseed oil (32.42%), walnut oil (7.637%), anchovies (3.018%), and Paul Newman's Marinara (1.599%). All three variant sauces, flaxseed oil, walnut oil, and anchovy, compared to the commercial Paul Newman's sauce, had better omega-3 fatty acid content and lower omega-6 to omega-3 ratio. In conclusion, simple additions of omega-3 ingredients to marinara sauce could decrease the omega-6 to omega-3 ratio in the diet.

Acknowledgments

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CHAPTER 1

REVIEW OF LITERATURE

The Role of Omega-3 Fatty Acids in the Mediterranean Diet and Lifestyle

In the United States, the number of individuals overweight and obese, diagnosed with type 2 diabetes mellitus, and those with instances of cardiovascular disease has risen. "In 2010, approximately 84 million men and women 20 years and older in the United States (35% of the population) were affected by some form of CVD. Reports from National Health and Nutritional Examination Survey indicate that "The prevalence of metabolic syndrome (MetS) is approximately 38% among US adults with a persistent increase during the last three decades."[9] Due to adaptive eating patterns and changes in family dynamics, eating at fast-food restaurants and processed convenience food, among many other factors, has been attributed to the prevalence of metabolic syndrome. Nutrition research and sciences have invested time exploring the components and benefits of what has become known as the Mediterranean diet. Factors that characterize the eating patterns of those residing in Mediterranean areas include "…higher consumption of fruits, vegetables, and whole grains and a lower intake of calories, saturated fat, sodium, refined grains and added sugars."[9] The lower intake of saturated fat is also paired with the moderate intake of important unsaturated fats such as omega-3 fatty acids: EPA and DHA. "It is now recognized that monounsaturated fatty acids (MUFAs) found in extra virgin olive oil (EVOO) and n-3 polyunsaturated fatty acids (PUFA), mainly from fish, contributed to the beneficial effects of the MD observed in Crete's population."[9] Regional access to fresh fish and markets makes achieving this goal somewhat easier in the Mediterranean area than in some United States areas. However, it has also been found that the consumption of some nuts, seeds, and oils can also attribute to healthier fat consumption. A study in the American Journal of

Lifestyle and Medicine titled *The Mediterranean Diet, and Your Health* outlines key protective health benefits to implementing the Mediterranean diet in counteracting chronic disease. Much of this effect has to do with the balance of phytochemicals from increased consumption of colorful fruits and vegetables and a decreased consumption of red meats and processed sugars.[11] Largely, the differences in fat also do add to the well-regarded nature of this diet's impact. Examples of popular fat intake outlined by the Mediterranean Diet include olive oil and fatty fish and decreased saturated fat. The "American Style" Mediterranean diet has less to do with cultural ease and tradition and more to do with the recognizing of chronic disease epidemics and the desire to implement new, healthier dietary patterns in to one's lifestyle.[11] Altogether, a good starting point for many Americans would be to realize the importance of different fats, specifically omega-3 PUFAs, in their diet. "Both forms of omega-3 fatty acids (plant and marine) have shown to be strongly protective against the risk of MI (myocardial infarction)."[11] Together MCFA and PUFA can all attribute beneficial elements to a person's diet and health.

Omega-3 Fatty Acids and Overall Health

The inclusion of fats in the diet affects one's daily functioning, long-term health, and overall metabolism. Recent nutrition literature and studies have focused on the pathways and benefits of omega-3 polyunsaturated fatty acids. "They are responsible for numerous cellular functions, such as signaling, cell membrane fluidity, and structural maintenance. They also regulate the nervous system, blood pressure, hematic clotting, glucose tolerance, and inflammatory processes which may be useful in all inflammatory conditions."[1]

The structure of omega-3 polyunsaturated fatty acids consists of a polyunsaturated fat's main backbone with "more than one carbon-carbon double bond."[1] The types of omega-3 fatty acids include α-linoleic acid (ALA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). "EPA and DHA are found in cold water fishes…"[1]In a westernized diet, fish consumption lacks many individuals' dietary intake. It can be hypothesized that the lack of fish consumption defines the omega-3 fatty acid deficiency and higher rates of heart disease and chronic inflammatory disease states. Some sources of EPA and DHA include, "…sardines, salmon, tuna, halibut, and other seafood such as algae and krill…"[1] ALA can be found in "…flax seeds, canola oil, soybeans, pumpkin seeds, perilla seed oil, walnuts, and their derivative oils."[1] Another component affecting a person's intake of omega-3 fatty acids through their diet could be a lifestyle factor such as vegetarianism or veganism. If one practice such diets, they could lack omega-3s because of the limited dietary sources from ALA rather than having the fish options from EPA and DHA.

Among the evidence that consumption of omega-3 fatty acids in the diet is necessary for optimal health is their role in reducing oxidative stress metabolism. With the function of aging and other biological mechanisms, oxidative stress in the human body is inevitable and can lead to cellular damage from free radicals if not addressed. “Free radicals can be generated as products of hemolytic, heterolytic, or redox reactions, which produce either charged or uncharged radical species.”[12] There are various ways that free radicals are generated in the body. Some of these include inflammation, exercise, specific metabolic pathways, smoking, radiation, drugs, and pollution. "Cancer and atherosclerosis, two major causes of death, are salient "free radical" diseases. Cancer initiation and promotion is associated with chromosomal defects and oncogene activation. It is possible that endogenous free radical reactions, like those initiated by ionizing radiation, may result in tumor formation.”[12] These particles become dangerous by stealing electrons from DNA and cell membranes, resulting in oxidative stress and inflammation. Ultimately the function of oxidative stress is paired to many ailments that are common experiences such as cancer, cardiovascular disease, and general aging. Omega-3 fatty acids are essential in maintaining cell membrane and cell membrane functions. As an example, athletes' increase in metabolic function and physical activity puts them at a higher risk of having free radicals in the body. One study found among athletes, omega-3 fatty acids were particularly helpful in skeletal muscle "anabolism and catabolism.”[1] “Positive findings in muscle recovery and subsequently, training adaptation, were reported in other similar studies. N-3 PUFAs attenuate the loss of muscle strength and range of motion. Blood markers of inflammation such as TNF-α and markers of muscle damage, such as myoglobin, creatine kinase, and skeletal muscle slow troponin I.”[1] The same study found a beneficial correlation between n-3 PUFA and energy. "…n-3 PUFAs has been found to decrease submaximal and peak heart rate as well as body oxygen consumption during exercise, resting heart rate variability, submaximal and resting heart rate, systemic vascular resistance, and diastolic blood pressure.”[1] For athletes and non-athletes alike, consuming the recommended amount of omega-3 fatty acids is imperative for overall health and well-being. In westernized diets, where development has led to a sharp increase in the over-consumption of both saturated and trans fats, it is clear that omega-3 polyunsaturated fatty acids offer more benefit to human metabolism and anti-inflammatory mechanisms.

Metabolism of Omega-3 Fatty Acids

The metabolism of omega-3 fatty acids and their role in managing hypertriglyceridemia gives insight into its main pathways and structural components. Hypertriglyceridemia is a condition where triglyceride levels are above 150 mg/dL and have negative cardiovascular health risks such as inflammation. "With increased TG, there are elevations of TG-rich lipoproteins (TRL) (very-low-density lipoproteins (VLDL) plus chylomicrons), and their remnants, which have been shown to contribute to the progression of atherosclerosis and CVD via several direct and indirect mechanisms. These mechanisms include the direct contribution to intimal cholesterol deposition and the activation and enhancement of proinflammatory, pro-apoptotic, and pro-coagulant pathways."[5]These pathways include biochemical nutrient pathways of the liver, specifically hepatic lipogenesis in relation to hypertriglyceridemia. "Results from pre-clinical and clinical studies suggest that omega-3 fatty acids decrease serum TG concentrations by reducing TG synthesis, reducing the incorporation of TG into VLDL, reduction TG secretion, and enhancing TG clearance from VLDL particles. Desaturase and elongase enzymes α-linolenic acid can transform to docosahexaenoic acid."[5] Increasing dietary intake of omega-3 fatty acids is important because a higher concentration of omega-6 fatty acids conversion of those are favored over conversion of omega-3 fatty acids. Due to these biosynthesis conversions in the body, PUFAs arachidonic acid (N6), docosahexaenoic acid (N3), and eicosapentaenoic acids (N3) are "precursors of mediators of a wide physiological importance. Eicosanoids are products of the arachidonic and eicosapentaenoic acid metabolism; their names derive from the Greek world-eikosi – twenty, as their precursor contains 20 carbon atoms. Arachidonic acid affected by cyclooxygenase undergoes conversion into prostaglandin E_2 (PGE_2), which is an inflammatory mediator, prostacyclin I_2 (PGI_2), responsible for blood vessel dilation and thromboxane A_2

(TXA_2) activating blood platelet aggregation and vasospasm. These prostanoids and leukotrienes derived from AA, EPA, and DHA represent how intake of beneficial polyunsaturated fatty acids can be linked to anti-inflammatory properties."[15] These small molecules function in the human body to be assembled and sent out in response to a signal in the body detecting damage and inflammation. They then travel to the site and begin meditating.

Omega-6 to Omega-3 Ratio

Omega-6 and omega-3 polyunsaturated fatty acids both provide health benefits. The ratio between the two has become unbalanced and is associated with increases in the overweight and obese. These two types of fatty acids are considered essential, meaning that they must be provided in the diet and cannot be made by the human body. Humans have a "lack of endogenous enzymes for omega-3 desaturation."[2] Because of trends in the westernized diet, the ratio that polyunsaturated fatty acids are being consumed is closer to levels of 20:1 (omega-6/omega-3) rather than a healthier ratio of 1:1. This ratio poses a threat to healthy human metabolism is because "… an unbalanced omega-6/omega-3 ratio in favor of omega-6 PUFAs is highly prothrombotic and proinflammatory, which contributes to the prevalence of atherosclerosis, obesity, and diabetes. Regular consumption of diets rich in omega-3 PUFAs has been associated with a low incidence of these diseases, particularly in Icelandic populations, Inuit indigenous people, and Native Americans in Alaska."[2] These locations are known for implementing fish into their diets in much higher proportions than people living in Westernized cultures. The benefits of these fish oils have lowered their omega-6/omega-3 ratios and have been proven to help their overall health and prevention of nutrition-related disease.

As mentioned, the distinguishing factor separating omega-6 and omega-3 fatty acids in the composition is due to the location of the first double bond on the molecule from the methyl end. "Omega-6 fatty acids are represented by linoleic acid (LA) (18:2ω-6) and omega-3 fatty acids by alpha-linolenic acid (ALA) (18-3ω-3)."[13] While still being a beneficial polyunsaturated fatty acid, the difference in structure could also account for the predisposition of proinflammatory characteristics that have especially been seen in individuals with higher ratios. In a study regarding omega-6/omega-3 ratio, authors DiNicolantonio and OKeefe provide evidence of the risks of having a higher than recommended omega-6/omega-3 ratio.[6] In regards to this, they found that "Compared with saturated fat plus trans-fat, a meta-analysis of RCTs found an increased risk of all-cause mortality, coronary heart disease mortality and cardiovascular events with omega-6 industrial seed oils."[6] Therefore, it's been researched and shown that out-of-balance consumption of omega-6/omega-3 fatty acids have negative consequences on health, whereas a balanced ratio that includes satisfactory amounts of omega-3 fatty acids can be beneficial by reducing harmful inflammatory effects.

CHAPTER 2

MATERIALS AND METHODS

Stovetop Preparation

The three variant fortified sauces were prepared to be compared to a constant commercial sauce on an electric stove. The commercial sauce used in the experiment was Paul Newman's - Newman's Own 24 oz jarred Marinara. This product contains no added sugar from the nutrition label, is made with extra virgin olive oil, and has no artificial flavors, colors, or preservatives added. Aside from this information, the preparation methods of this commercial product are unknown. For the variant sauces, the preparation methods stayed constant, with the changing variable of each having a different source of omega-3 fatty acid. The ingredients were: 13 g extra virgin olive oil, 784 g plum tomatoes, 1.5 g oregano, 0.8 g parsley, 1.2 g basil, 1 bay leaf, 6 g salt, 1 g pepper, and 7 g tomato paste. 13 g of a variant ingredient was added to this base recipe, flaxseed oil, walnut oil, and anchovies. The Dutch oven was preheated to medium-high heat. Once preheated, extra virgin olive oil and the variant ingredient were added and combined. Then, plum tomatoes were added to the pot and mashed with a utensil into a coarse mixture. To this, oregano, parsley, basil, bay leaf, salt, and pepper were added and stirred in with a mixing spoon. Tomato paste was added directly after and stirred in. The sauce was held then at medium-low heat for 20 minutes and covered to cook. After 20 minutes, the sauce was combined using an immersion blender while still hot. Once cooled, the sauce was placed into a 32 oz Styrofoam cup and a 0-degree Celsius freezer.

Freeze Drying

The frozen sauce was placed into a pre-weighed 600 mL LABCONCO freeze-dry flask and weighed prior to being placed on the LABCONCO FreeZone 2.5 freeze dryer with stainless steel adapters. Samples were allowed to freeze dry for 34 hours at 0.077 mBar and -50 degrees Celsius. When the flask was removed, it was weighed to determine the weight of water lost in the process. Once this number was determined, percent dry matter was determined with the formula: Percent dry weight = (dry weight of sauce/ frozen liquid weight of sauce) x 100.

Bomb Calorimetry

A bomb combustion capsule was tared on a top-loading pan balance, and 1.2 grams of freeze-dried and ground marinara sauce was placed in the capsule. The combustion capsule was then placed into the bomb vessel's ring holder with an ignition thread tied to complete the circuit between the vessel and the sauce sample. The bomb vessel was assembled with a closed valve, and the vessel was charged with 10 ATM of oxygen. Two liters of deionized distilled water were placed in the corresponding bomb bucket to the vessel. The vessel was placed into the bucket with ignition wires attached. The weight of the freeze-dried sauce sample was entered into the Parr 6200 Calorimeter computer along with bomb ID and sample name and then combusted. Bomb values were reported as calories per gram and adjusted for nitric acid formation during the bombing process. The vessel was removed from the bucket, and pressure was released. The inside of the vessel and capsule were rinsed with deionized distilled water into a 250-milliliter beaker and a few drops of methyl red indicator were added to the beaker. The solution was titrated using a sodium carbonate solution (0.0709 M) until color change was sustained to indicate and measure nitric acid formed during the combustion process. The formula used to determine total digestible calories: Total calories = total calories/gram of dry matter – mL of sodium carbonate needed to titrate the nitric acid formed.

Kjeldahl Procedure

The Kjeldahl procedure analyzed freeze-dried and ground marinara sauces to determine crude protein content. Kjeldahl flasks (100 mL) held 100 mg of freeze-dried sauce, 1.9 g of K_2SO_4 (Fisher Scientific), 80 mg of HgO (Fisher Scientific), 2 mL of H_2SO_4 (Fisher Scientific) and 2 Fisher porous boiling chips. The Kjeldahl flask was placed on a LABCONCO digestion unit with the neck of the flask placed into the glass manifold. The hood's air circulation was turned on, and the heater under the flasks was set to level 3. The sample was refluxed on the heater for 8 to 12 hours and then cooled. After the samples had cooled, 20 mL of deionized distilled water was added to the Kjeldahl flask. The flasks were reheated to boiling and then filtered using P5 grade Fisher brand qualitative grad plain paper circles into 125 mL Erlenmeyer flasks. The distillation process began with placing an Erlenmeyer flask with 5 mL of 4% boric acid solution with a few drops of Kjeldahl indicator under the condenser column on the LABCONCO rapid distillation unit to collect ammonia from the digested Kjeldahl samples. The filtered samples were poured into the top of the distillation unit. Once the sample was added to the distillation chamber, it was rinsed with deionized distilled water and 20 mL of $NaOH/Na_2O_3S_2$ (sodium thiosulfate solution) was poured into the top of the distillation unit and entered into the distillation chamber. The reaction between the digested sample and sodium thiosulfate converted the nitrogen ammonia. The mixture was distilled until the total volume of boric acid and ammonium solution collected reached 20-30 mL. The solution was titrated with 0.1 N dilute HCl until the indicator color was sustained. The mL of HCl used to titrate the solution, and grams of the sample were entered into the following equations to calculate percent protein: Nitrogen per Kilogram= [(mL HCl – mL blank) normality x 14.01] / weight of the dried sample (grams). Percent Protein= Nitrogen per kilogram x 6.38 / 10.

Soxhlet Extraction

Freeze-dried samples of marinara sauce were prepared for Soxhlet extraction. The freeze-dried sauce (2.5 g) was combined with 2.5 g of $NaSO_4$ (Fisher Scientific) in mortar. Using a mortar and pestle, the sample was mixed with $NaSO_4$ until it was a homogenous mixture. Cellulose extraction thimbles were labeled and weighed. Once the tare weights were recorded, 2 g of the homogenous mixture was measured into each thimble. The Soxhlet apparatus was assembled starting with the 250-mL round bottom flask filled with 200 mL of Petroleum Ether (PET) (Fisher Scientific) with 2 Fisher porous boiling chips and placed on the mantel heater. The Soxhlet extraction vessel with thimbles containing the homogenous sauce sample were attached to the top of the round bottom flask. Condenser was attached to the top of the extraction vessel and cold water was circulated. Mantle heaters were turned to level 3, and the sample refluxed for 8-12 hours. The heat and cool water were turned off after refluxing and the apparatus was allowed to cool. The PET liquid was drained from the thimbles, and the thimbles were placed in a 60-degree Celsius oven for 24 hours to ensure the samples are dry, and then reweighed to determine the amount of sample remaining in the cellulose thimble. The formula used to determine the fat percentage is as follows: Percent Fat = 100 – [(weight of thimble after Soxhlet – thimble weight – 1 g of $NaSO_4$) x 100]. The PET solution (containing the lipid soluble nutrients) was dried under Nitrogen in a 60^oC water bath. After the PET was evaporated, the ether extract solution was resuspended in 4 mL of hexane and stored at -30^oC prior to GC analysis

Ashing

The sides of crucibles were heavily marked with the sample ID using a graphite pencil. Crucibles were weighed, and 5 g of sample were added to each crucible. Crucibles were placed in the Thermolyne Ashing Oven and heated at 700 degrees Celsius for 5 hours. Crucibles were weighed the following ashing to determine the percent inorganic material with the formula: Percent inorganics = weight of inorganics / 5 grams x 100.

Fatty Acid Analysis

Two mL of the Ether extract/hexane solution (from Soxhlet Extraction) was combined with 2 mL of BF_3 (Fisher Scientific Boron trifluoride methanol) with a Teflon in a screw-top glass tube. Samples were vortexed before being placed in an Isotemp heating block (Fisher Scientific) at 100^{o} C for 1 hour to form fatty acid methyl esters. After heating, samples were cooled, and 1.5 mL of deionized distilled water was added to the vial, vortexed, and placed in a Sorvall Biofuge Primo Centrifuge (Thermo Scientific) and spun at 4,000 rpm for 5 minutes. The supernatant was removed from the sample and placed in a clean screw-top glass tube, placed back on the Isotemp heating block at 60 degrees Celsius, and dried under nitrogen gas. The sample was resuspended with 275 μL of hexane, and transferred to the gas chromatograph auto sample vial with a 300 μL glass insert. An internal standard of 5 μL of C17 was added to the vial. Methyl esters of fatty acids derived from the sauces were analyzed for fatty acids through flame ionization gas chromatography (Shimadzu GC-2010; Shimadzu Corporation, Kyoto Japan) using a capillary column (Zebron ZB-WAX, 30 m length, 0.25 mm i.d., 0.25 μL film thickness; Phenomenex Torrance, CA, USA). Column conditions included the carrier gas (helium) flow rate of 30 mL/min and a temperature program of a constant temperature ramp (2^{o}C/min) at an initial temperature of 160^{o}C, held for 5 minutes; 170^{o}C held for 8 minutes; 180^{o}C held for 10 minutes; 190ºC held for 15 minutes; 200ºC held for 15 minutes, and final oven temperature of 210ºC held for 20 minutes. Additional instrument conditions included: total run time of 100 minutes; autosampler injection volume of 1 μL; flame ionization detector temperature 255ºC; injector port temperature 250°C; hydrogen flow rate of 40 mL/min; and an airflow rate of 400 mL/min. Individual peaks were identified and compared with known standards. Fatty acids were quantified as a percent of total fat by using the total area's percent under the peak. Fatty acid peaks were averaged between the duplicate samples and compared to individual fatty acids using standards.

FRAP Assay

The spectrophotometer is turned on and set at 593 nm. The FRAP reagent was prepared the same day according to the procedure of Benzie and Strain.[19] Next, 2 g of freeze-dried samples are placed in a 250-mL beaker with 30 mL of deionized distilled water and heated on a magnetic stir plate at 100ºC for 30 minutes, and mixed using a magnet at low speed. A foil cap is placed on each beaker while heating. After 30 minutes of heating and agitation, the material was placed in a Falcon tube and centrifuged for 5 minutes at 4000 X G. One-hundred uL of supernatant of the marinara sauce variants were mixed with 900 ul FRAP reagent, vortexed, placed in the spectrophotometer and allowed to react for 4 minutes prior to analysis. Five replicates of each variant were run to ensure sample homogeneity.

Bowes and Church Micronutrient Cross-Reference

Cross-referencing the standardized micronutrient values was done through *Bowes and Church's Food Values of Portions Commonly Used*[16] was used for this purpose. The nineteenth edition was referenced to find the standard micronutrient values for the ingredients that were added to the variants. The portions recorded as grams in the text were expanded to represent the weights of the ingredients used. Each micronutrient value for the individual ingredients was added to show each micronutrient's total in each variant sauce.

CHAPTER 3

RESULTS: Tables and Charts

Soxhlet Fat Analysis

Table 1: Soxhlet Fat Analysis (% fat)	
Variant	% crude fat
Flaxseed Oil	32.30%
Walnut Oil	33.24%
Anchovy	26.35%
Paul Newman’s Marinara	14.95%

Table 1 shows the average percent fat data from each variant tested from Soxhlet Fat Analysis.

Kjeldahl Protein Analysis

Table 2: Kjeldahl Protein Analysis (% crude protein)	
Variant	% crude protein
Flaxseed Oil	9.97%
Walnut Oil	8.69%
Anchovy	16.79%
Paul Newman’s Marinara	14.07

Table 2 represents data collected from Kjeldahl protein analysis. The values listed represent an average of % crude protein for the samples.

Bomb Calorimetry

Table 3: Bomb Calorimetry	
Variant	Calories/gram
Flaxseed Oil	4837.5 calories
Walnut Oil	4785.36 calories
Anchovy	4471.79 calories
Paul Newman's Marinara	3980.58 calories

Table 3 shows the average number of calories in samples tested of each variant.

Ashing

Table 4: Ashing	
Variant	% Ash (non-organic matter)
Flaxseed Oil	15.36%
Walnut Oil	19.25%
Anchovy	10.87%
Paul Newman's Marinara	10.16%

Table 4 represents an average percent of inorganic material in the samples of each variant

FRAP Assay

Table 5: FRAP Assay	
Variant	Concentration (um/l)
Flaxseed Oil	4.75
Walnut Oil	4.32
Anchovy	3.39
Paul Newman's Marinara	2.85

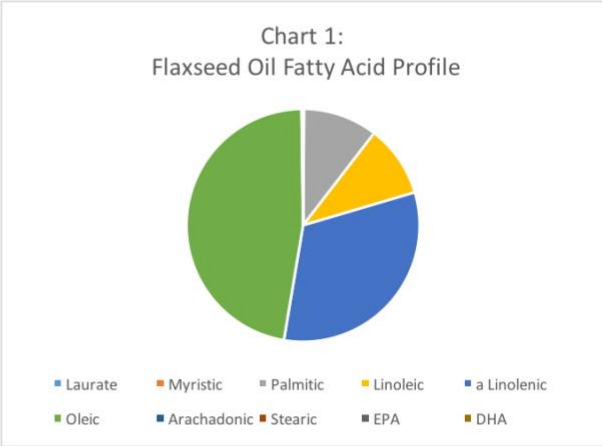
Table 5 shows percent antioxidant content of the variants tested.

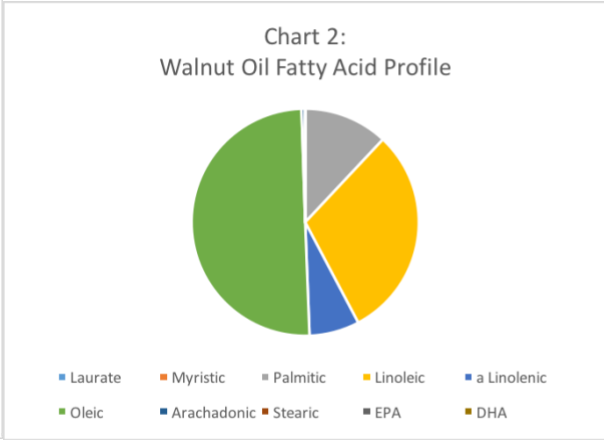
Gas Chromatography

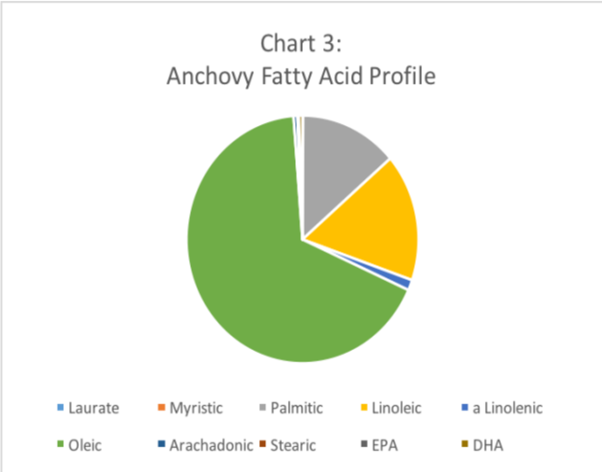
Table 6: Gas Chromatography										
Variant	Fatty Acid (% area under the curve)									
	Laurate 12:0	Myristic 14:0	Palmitic 16:0	Linoleic 18:2 (n-6)	α-Linolenic 18:3 (n-3)	Oleic 18:1 (n-9)	Arachadonic 20:4 (n-6)	Stearic 18:0	EPA 20:5 (n-3)	DHA 22:6 (n-3)
Flaxseed oil	0.05%	0.10%	10.31%	9.97%	32.22%	47.16%	0.20%	0.00%	0.00%	0.00%
Walnut oil	0.00%	0.04%	11.95%	30.27%	7.12%	50.08%	0.52%	0.00%	0.00%	0.00%
Anchovy	0.00%	0.11%	16.29%	20.00%	1.65%	60.47%	0.61%	0.10%	0.17%	0.59%
Paul Newman's Marinara	0.06%	0.26%	22.61%	20.86%	1.58%	50.62%	0.02%	0.00%	0.00%	0.00%

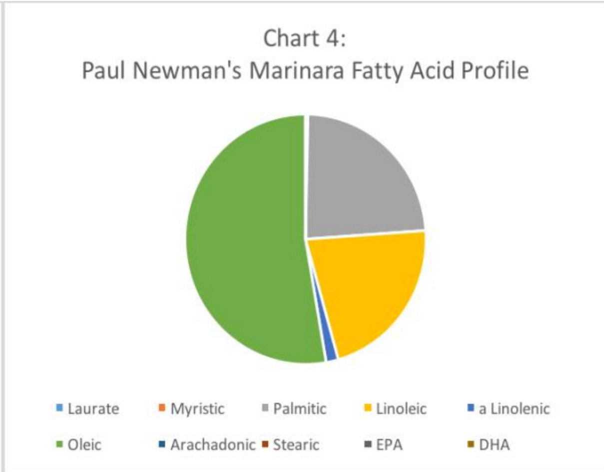
Table 6 shows each fatty acid found in the variants. The percentage of these fatty acids found represent an average of each variant sample tested.

Gas Chromatography cont.









These charts graphically depict the different percentages of fatty acids found in each individual variant sample.

Omega-6 to Omega-3 ratio from gas chromatography

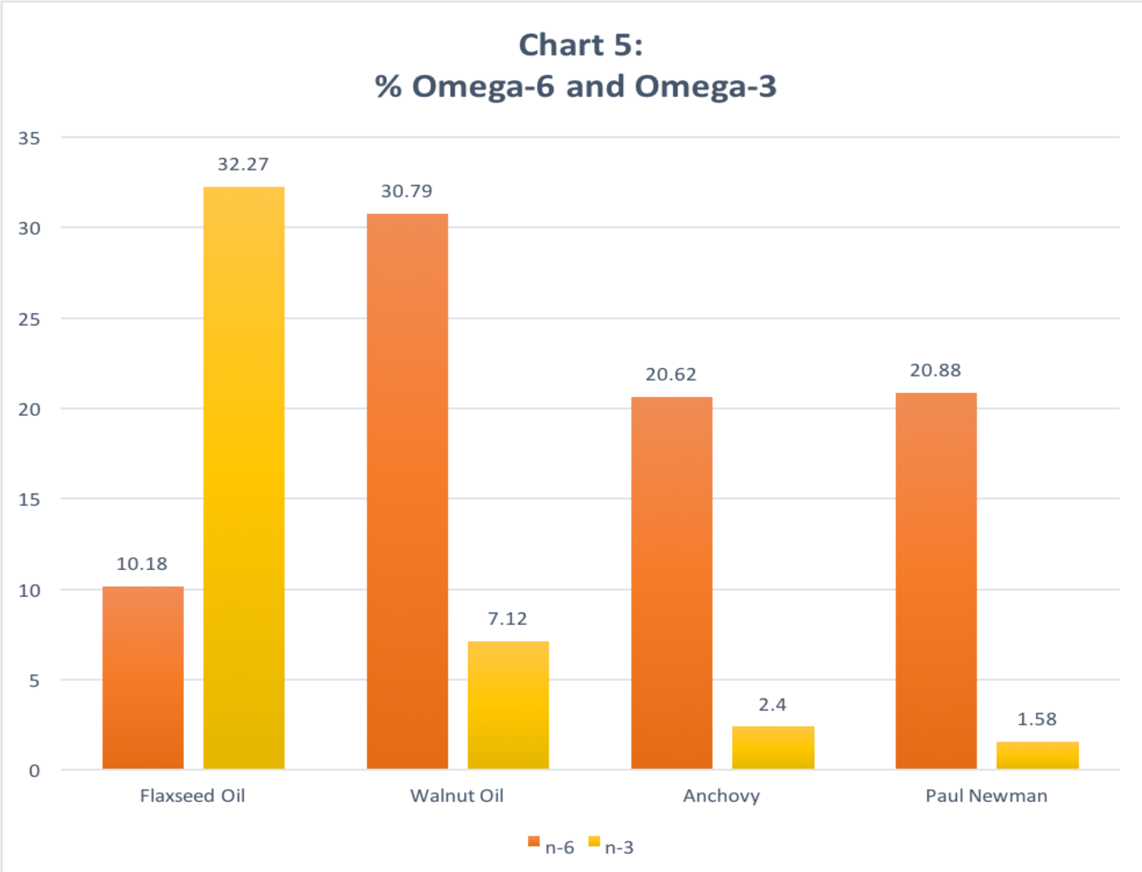


Chart 5 visually represents a comparison of the omega-6 to omega-3 ratio in each variant sauce tested. The orange indicates percent omega-6, and the yellow represents percent omega-3.

Omega-6 to Omega-3 ratio

Flaxseed Oil: 0.316:1

Walnut Oil: 4.33:1

Anchovy: 8.59:1

Paul Newman: 13.23:1

Bowes and Church Micronutrient Cross-Reference

Table 6: Bowes and Church Micronutrient Values										
Variant	Micronutrient									
	Na (mg)	Ca (mg)	Mg (mg)	Zn (mg)	Mn (mg)	K (mg)	P (mg)	Fe (mg)	Cu (mg)	Se (mg)
Flaxseed oil	1134.29	244.42	87.86	1.17	0.63	1543.73	152.06	7.89	0.57	1.01
Walnut oil	1134.29	244.42	87.86	1.17	0.63	1543.73	152.06	7.89	0.57	1.01
Anchovy	1611.39	274.32	96.96	1.49	0.64	1614.58	184.56	8.09	0.61	9.85

Table 6 shows values from Bowes and Church's nutritional reference. This is based upon the recipes created and the ingredients that were included in them, along with the amounts of each. Values shown represent the amount for the entire recipe, not a serving size.

CHAPTER 4

DISCUSSION

Soxhlet Fat Analysis

Soxhlet fat analysis determines the percent crude fat in a sample. Flaxseed oil and walnut oil were close in value of percent fat at 32.29% and 33.24% respectively. The anchovy variant and Paul Newman's Marinara were less, having percent fat 26.35% and 14.94% respectively. Although 13g of anchovies, flaxseed oil, and walnut oil were used for the variants, anchovies are a mixture of protein and fat while the others are plant-sourced and contain predominately fatty acids. This supports the data that anchovy would have less of a percent fat than the other variants tested. The Paul Newman's commercial sauce has olive oil listed in the ingredients on the nutrition label, which accounts for some of the percent fat seen in this data from the Soxhlet analysis.

Fatty Acid Profiles

The samples all contained a variety of omega-9, omega-6, omega-3 fatty acids, and some saturated fat. Overall, the variant with the highest percentage of omega-3 fatty acids was the flaxseed oil at 32.22% omega-3 fatty acid followed by walnut oil at 7.12%. The omega-3 fatty acids contributing to this percentage included α-linolenic acid, EPA, and DHA. The walnut oil variant contained a higher omega-6 percentage than any other variant tested coming from linoleic acid. Other variants contained linoleic acid as well but in smaller percentages: flaxseed oil (9.98%), anchovies (around 20%), and Paul Newman’s (20.86%). Each of the variants contained a large percentage on average of the omega-9 monounsaturated fatty acid from oleic acid: flaxseed oil (47.16%), walnut oil (50.08%), anchovy (60.47%), and Paul Newman's Marinara (50.62%). Which is probable coming from the 13g of olive oil added to the fortified variants and the olive oil listed in the ingredients for Paul Newman's Marinara. Interestingly, Paul Newman's Marinara had a similar average of oleic acid, but lacked important omega-3 fatty acids. Overall, Paul Newman's Marinara only contained 1.58% α-linolenic acid and 0% EPA or DHA. The anchovies were the only variant to contain omega-3 fatty acids from EPA and DHA, however, the percent found was low due to the small number of anchovies added to the sauce. Additionally, each of the variants had a lower average percentage of saturated fat than Paul Newman's Marinara (22.93%): flaxseed oil (10.45%), walnut oil (11.99%), anchovy (16.50%). The omega-6 to omega-3 ratio for Paul Newman's Marinara Sauce was 13.23:1. All of the variants managed to have a lower ratio than this: flaxseed oil- 0.32:1, walnut oil- 4.33:1, and anchovies- 8.59:1. Each variant successfully lowered the omega-6 to omega-3 fatty acid ratio, increased the percentage of omega-3 fatty acids, and lowered saturated fats in comparison to the Paul Newman's Marinara.

Kjeldahl Analysis

Flaxseed oil (9.97%) and walnut oil (8.69%) variants were close in crude protein. Because anchovies contain protein from being an animal source, protein found in the anchovy oil was higher than that of the other variants at 16.79% crude protein. Lastly, Paul Newman's Marinara Sauce had more protein than the flaxseed oil and walnut oil variants but less than the anchovy oil variant at 14.07%. It is possible that the tomatoes used in the Paul Newman's Marinara contributed to this difference in protein content than the variety of tomatoes used in the variants. This could be due to different types of tomatoes used or the stage of harvesting. Of the other ingredients, the differences between the variants can only be attributed to the ingredients that were added for fortification of omega-3 fatty acids.

Bomb Calorimetry

Flaxseed oil (4837.50 calories/g) and walnut oil (4785.36 calories/g) had similar caloric content indicating a similar macronutrient content. Because fat is more calories per gram than protein, this data also explains that the anchovy variant has more protein than the others and less fat because the caloric value for the anchovy variant was less at 4471.79 calories. This value is lower because the 13 g of anchovies added to the variant sauces is a mixture of protein and fat rather than being 100% fat which would lower the expected calorie content. Paul Newman's Marinara that is without any added fortification has the least number of calories at 3980.56. The amount of inorganic material in the Paul Newman's Marinara could have diluted it to have a lesser caloric value than the fortified sauces.

Ashing

Flaxseed oil variant had 16.36% inorganic material while walnut oil had more at 19.25% inorganic material. A study of walnut oil found it to be high in both iron and zinc from processing. This could explain the higher percentage of inorganic material found.[20] The anchovy was similar to Paul Newman's Marinara with 10.87% and 10.16% inorganic material, respectively. Bowes and Church's values support the large percentage of inorganic material in the variants because of the substantial mg of micronutrient material from the ingredients used in the variants. From evaluation of Bowes and Church's values, much of the inorganic material seems to be coming from the sodium and potassium in the canned tomatoes[16]. It is likely that Paul

Newman's Marinara also contains sodium and potassium that would contribute to the large percent of inorganic material.

Frap Assay

As expected, the unfortified Paul Newman's sauce offered the smallest antioxidant content at 2.85 um/l. Unlike other tests, the flaxseed oil variant was best in regards to the antioxidant content of 4.75 um/l. Flaxseed contains antioxidants lignans, tocopherols, and beta-carotene.[18] Lignans are able to have health benefits such as reducing the risk of cardiovascular disease[18]. Walnut oil was close to flaxseed oil with a value of 4.32 um/l. Walnut oil has beneficial antioxidants such as tocopherols, polyphenols, and phytosterols.[17] The anchovy variant had 3.39 um/l antioxidant content. Each variant increased in antioxidant content compared to the store-bought sauce because of the individual antioxidant components in the ingredients that were used for fortification.

CHAPTER 5

CONCLUSION

The variants were created to compare omega-3 fortified versions to a manufactured marinara sauce (Paul Newman's) to answer the research question "Could fortifying a base marinara sauce with omega-3 fatty acids decrease the omega-6 to omega-3 fatty acid ratio while increasing the overall omega-3 fatty acid content?" The study suggests that making a homemade sauce with omega-3 rich ingredients can decrease the omega-6 to omega-3 fatty acid ratio while increasing the omega-3 fatty acid content. The variants that were found to do this included walnut oil and anchovies. Surprisingly the flaxseed oil had a higher omega-6 to omega-3 fatty acid ratio and a lower overall omega-3 fatty acid content than the store-bought sauce. Regarding increasing antioxidant dietary intake, flaxseed oil was found to be the best choice for these parameters. Due to the Covid-19 pandemic, hedonic testing could not be completed due to social distancing and CDC guidelines. Future experiments should consider the inclusion of a hedonic test. The study results investigating fortified sauces support further research of omega-3 fatty acid fortification and the possible beneficial health implications this could have on dietary intake. Ultimately, our hypothesis that fortifying sauce with certain ingredients, walnut and anchovy, increases the overall omega-3 fatty acid content compared to Paul Newman's marinara sauce, so the null hypothesis was rejected.

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