SUPPLEMENTATION OF OMEGA-3 FATTY ACID TO BAKERY PRODUCTS (BISCUIT AND CAKE) WITH FISH (*Pangasius hypophthalmus*) OIL

Jiko Sen

Examination Roll No: 0117/05

Registration No: 00408

Session: 2017-2018

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science (MS) in Food Chemistry and Quality Assurance

Department of Applied Chemistry and Chemical Technology Faculty of Food Science & Technology

Chattogram Veterinary and Animal Sciences University, Chattogram -4225, Bangladesh

June, 2019

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This is to certify that we have examined the above Master's thesis and have found that the thesis is complete and satisfactory in all respects and that all revisions required by the thesis examination committee have been made.

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June, 2019

Dedication

DEDICATED

TO

MY BELOVED MOTHER

ACKNOWLEDGEMENTS

First and foremost I thank the almighty God for the blessing and opportunity to pursue higher education and to accomplish the thesis for the degree of Masters of Science (MS) in Food Chemistry and Quality Assurance successfully.

I pay heartily gratitude to Professor **Dr. Goutam Buddha Das**, Vice-Chancellor, Chittagong Veterinary and Animal Sciences University (CVASU) for giving special opportunity and providing such research facilities.

Mere indebted thanks may not suffice the privilege to express my profound sense of gratitude to my supervisor **Md. Ashraful Islam** (Assistant Professor, Department of Applied Chemistry and Chemical Technology, Faculty of food science and Technology, CVASU) for unequivocal motivation, benevolent guidance, affectionate encouragement and constant supervision during the entire period of investigation and preparation of manuscript.

I feel much pleasure to convey my profound thanks **Md. Fahad Bin Quader,** Head and Assistant Professor, Department of Applied Chemistry and Chemical Technology, CVASU for his valuable advice, scholastic guidance, inspiration and suggestions during my study period.

I am grateful to Professor **Dr. Jannatara Khatun**, Dean, Faculty of Food Science and Technology, CVASU for her kind cooperation and providing necessary facilities to carry out this research.

I would like to give special thanks to the authority of Bangladesh Council of Scientific and Industrial Research (**BCSIR**) and Modern Food Testing Laboratory (**MFTL**) for providing laboratory facilities. I sincerely thank to the University Grants Commission for giving me a research grant to accomplish my research work.

Finally, I would like to pay my profound gratefulness and cordial love to my friends, juniors, family members and all well-wishers for their helping hand, understanding, endless patience and encouragement during my whole study period.

The Author

June, 2019

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LIST OF ABBREVIATION

Abbreviation	Elaboration
ALA	Alpha-Linolenic Acid
CHD	Coronary Heart Disease
CVD	Cardiovascular disease
DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
FAO	Food and Agricultural Organization
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
PUFA	Polyunsaturated Fatty Acid
SCD	Sudden Cardiac Death
SFA	Saturated Fatty Acid
TFA	Trans Fatty Acid
TG	Triglycerides
Ω-3	Omega-3

ABSTRACT

Bakery industry is an important segment within the food and beverage industries in Bangladesh. The major product markets within these industries are biscuits and cakes. This study was conducted to supplement omega-3 fatty acid to cakes and biscuits by fish oil replacing harmful TFA containing margarine. Oil of *Pangasius hypophthalmus* has good content of omega-3 fatty acid and its sufficient content of SFA made it suitable for using in bakery products. Dough was made with wheat flour (8% gluten for making cake and 10% for biscuit), baking powder, Milk, eggs, sugar and margarine; 100%, 80% and 60% or 50% fish oil (1:0, 4:1, 3:2 or 1:1:: Fish oil: Margarine respectively) were mixed with dough separately in each sample replacing margarine, then shaped it properly and baked for proper temperature an time. Fatty acid analysis of these finished products revealed that Omega-3 fatty acid increased significantly in the products containing fish oil. Highest value (4.19% and 4.03% respectively) of omega-3 fatty acid has been found in the biscuits and cakes containing 100% fish oil. Good content of omega-3 fatty acid (3.90% and 3.96% respectively) was also found in the biscuits and cakes containing 80% fish oil. All the proximate compositions were statistically varied ($P<0.05$) and found no significant different with the control products to most of the treated products. The sensory parameters indicated that the products containing 100% fish oil were preferred less than the products containing 80% fish oil and the control products. Considering the content of omega-3 fatty acid and panel"s preference, biscuits and cakes which were prepared using 80% fish oil would be the best choice for consumers.

Keywords: Fish oil, *Pangasius hypothalamus,* Omega-3 fatty acids, Trans fatty acids, Biscuits, Cakes, Proximate composition, Sensory parameter.

Chapter I: Introduction

1.1 General Feature

Presently individuals from all round the world are more worry on sound way of life. Subsequently, it can impact the eating conduct & food choice of consumers. These days sensory attributes of food for example taste, texture, flavor, appearance is principle choice of consumers. Notwithstanding sensory characteristics, nutritional estimation of ingested food can influence the food selection of purchasers. Consumers are additionally now giving more significance to healthy and nutritious quality food. Consumer mindfulness for the functional characteristics of the food products is expanding which is affecting their acquiring choice. The market for functional food products is expanding 10% every year (Piteira et al., 2006). Fats get negative criticism in our general public. In the 1960s, physicians started to advance low-fat diets as an approach to decrease coronary illness and cholesterol levels (La Berge, 2008). Now, scientists perceive the valuable well being impacts of certain fats. The important thing is to stress utilization of specific structures sound fat while maintaining a strategic distance from undesirable fats in eating regimens.

Fat allude to particles that have a specific chemical structure. The number of double bonds between segments of the molecule determines the kind of fat. Monounsaturated and polyunsaturated fats contain more double bonds, which influences their general structure. Not exclusively do different fats have different structural design, yet they additionally influence human well being in an unexpected way. For instance, trans fats and saturated fats are related with more elevated amounts of “bad” LDL cholesterol. This increases the risk of cardiovascular disease. Interestingly, monounsaturated and polyunsaturated are viewed as healthy fats. Boosting these fats in your diet increment levels of “good” HDL cholesterol and brings down cardiovascular risk.

Omega-3 fatty acids are basic fats, implying that your body cannot synthesis them on its own. Thus, one must get these fats from the eating regiment to stay solid. Fish oil is a great dietary sources, rich in essential fatty acids, particularly Polyunsaturated Fatty Acid (PUFA) as Eicosapentaenoic acid (EPA) and Docosahexaenoic acid

(DHA) (Kim *et al*., 2006, NLM, 2014). Around one-third of the fatty acids present in fish oil are omega-3 long-chain polyunsaturated fatty acid (LC-PUFAs), and it is unsaturated fat that has been connected to potential health benefits (Rizliya and Mendis, 2014). There was proof for the many numerous healthful wellbeing impacts associated with the utilization of the unique long-chain polyunsaturated ω-3 fatty acids (n PUFA) nearly found in fish and other marine living beings (Tocher, 2015).

Cardiovascular disease (CVD) is the leading cause of death all around. The dietary elements assume a significant job in assurance of CVD risk and in the pathogenesis of CVD. Dietary supplementation of fish and fish oils which are the rich sources of ω-3 fatty acids demonstrated the healthful impacts to the patients with dyslipidemia, atherosclerosis, hypertension, stoutness and inflammatory diseases **(**Aarsetoey H *et al*., 2012).

Consumption of Omega-3 polyunsaturated fatty acids (PUFA) diminishes overall mortality, which is brought about by myocardial infarction and abrupt passing in patients with Coronary Heart Disease (CHD). Omega-3 Supplements whether from dietary source or fish oil, supplements ought to be expanded particularly in the individuals who are in danger of CHD (Lee JH *et al*., 2008). Fish oil supplementation was recommended in the patients with risk factors for SCD such as left ventricular dysfunction, left ventricular hypertrophy, prior cardiac infarction or high grade ventricular dysrhythmias (Anand RG *et al*., 2008).

Pangasius hypophthalmus, also known as freshwater Tra catfish, is the primary business cultivated Pangasius species in Bangladesh. It is available and cost effective. The fat level of *Pangasius hypophthalmus* is 24.4% for the flesh and 20.6% for the viscera (Mai, 1998). The level of PUFA is 17.69% and MUFA is 34.69% in the fat Tra catfish (Ho et al, 2009). Concerning nutritional aspect, Tra catfish fillet is a potential well spring of omega-3 fatty acids and great fat nourishment (Ho et al., 2009). So, this fish oil can be utilized in bakery products to enrich the products with PUFA.

Bakery products are well known products, consumed by a wide scope of populaces, because of their ready to eat nature, varied taste, long time span of usability and generally moderate expense. High rivalries in the market and expanded interest for sound, natural and functional items lead to alter their nutritive composition by improvement. The interest of the food industry in the advancement of new items is constantly expanding and ending up all the more testing, because of purchasers mindfulness about more beneficial foods for body (Dias *et al*., 2015). Cake and biscuit are significant items in bakery industry and are very much connected with consumers everywhere throughout the world. But they are high in saturated and trans fats. Utilization of these bakery products containing high saturated, trans fat and less measure of essential amino acids prompts cardiovascular diseases. Various methodologies have been created for the incorporation of bioactives in food to make functional products, equipped for giving health benefit that goes beyond nutrition (Aboul fazli *et al.*, 2016; Duchateau and Klaffke, 2009). Fish contains significant amounts of all essential amino acids, especially lysine which is moderately poor in cereals (FAO, 2005). Thus, it is appropriate to utilize fish oil as an ingredient for bakery products for supplementation of Omega-3 PUFA.

The nature of biscuits and cake was determined on the premise on physico-chemical characteristics namely: Moisture content, Ash content, Fat content, Protein content, and Crude Fiber content and sensory analysis including sensory attributes namely colour, flavour, texture, taste and overall acceptability.

1.2 Aims and Objectives

General Objective:

The aim of my study was to formulate Omega-3 fatty acid supplemented cakes and biscuits using *Pangasius hypophthalmus* oil.

Specific Objectives:

1. To assess the unsaturated fat substance of *Pangasius hypophthalmus* oil.
2. To evaluate the nutritional value of Omega-3 fatty acid supplemented cake and biscuit with fish oil.
3. To survey sensory properties of composite cakes and biscuits.
4. To make nutritional comparison between experimental samples and commercial samples.

Chapter II: Review of Literature

A conceptual framework for the study dependent on the thoughts and ideas accumulated from survey work of existing writing of both hypothetical and observational nature will encourage arranging the investigation in a far reaching way. It likewise realizes the past research work completed in the region and acts as a torch for new research.

2.1 Functional food and its importance

Diet has a substantial role in sustaining human health as it meets the need of the body for essential nutrients and energy for normal physiology. Moreover, alongside providing satiety, food provides pleasant tastes and flavors. However, it is postulated that diet plays an important role in the body"s health maintenance through influencing risks of diseases. Functional foods provide the body with some beneficial support beyond their role in providing the body with essential nutrients (Granato et al., 2010). All foods are functional food to some extent because all foods provide taste, aroma, and nutritive value. However, foods are now being examined intensively for added physiologic benefits, which may reduce chronic diseases risk or otherwise optimize health. It is these research efforts that have lead to the global interest in the growing food category now recognized as "Functional Foods". In 1994, the National Academy of Sciences „Food and Nutrition Board" defined functional foods as "any modified food or food ingredient that may provide health benefit beyond the traditional nutrients it contains". Fatty fish contains omega-3 fatty acids as bioactive component which provide certain health benefits such as reduces TG, reduces heart disease, cardiac deaths and fatal and non fatal myocardial infarction. Probably the most intensively investigated class of physiologically active components derived from animal products are omega-3 fatty acids, predominantly found in fatty fish such as salmon, tuna, mackerel, sardines and herring (kris Erthen et al.,2004)

2.2 Bakery Products

Bakery products are significant part of a balanced diet and can be used as functional foods by providing specific nutrients. A wide assortment of such items can be found on supermarket shelves today. This includes unsweetened goods (bread, rolls, buns, crumpets, muffins and bagels), sweet merchandise (cake, biscuit, pancakes, doughnuts, waffles and cookies), and filled goods (fruit and meat pies, sausage rolls, pastries, sandwiches, cream cakes, pizza and quiche) and so on (Smith, 1972). The consumer demand has expanded for food item quality with taste, safety, convenience and nutrition. Thus, nutrition has risen as an added dimension in sustenance item chain development (Shahidi, 2002). Consumers are likewise now concerned about sound and nutritious quality food. Consumer‟s purchasing decisions is affected by their awareness for the functional characteristics of the food products. The market for functional food products is expanding 10% every year (Piteira et al., 2006).

There are numerous bakery products in Bangladesh and every one of them are prevalent in our nation. The bakery products such as biscuits and cake have turned out to be extremely well known in Bangladesh among all financial segment of populace. Those bakery products are produced using different flour particularly wheat flour.

2.2.1 Biscuit

A biscuit is a flour-based baked food product. Biscuits today can be savory or sweet, however most are small at around 5 cm (2.0 in) in diameter, and flat. Wheat flour containing low flour content (8-9.5%) is appropriate for making biscuit (Borla et al.,2004).Sweet biscuits are usually eaten as a snack food, and are, in general, made with wheat flour or oats, and sweetened with sugar or honey. Varieties may contain chocolate, fruit, jam, nuts, ginger, or even be used to sandwich other fillings. Savoury biscuits or crackers (such as cream crackers, water biscuits, oatcakes, or crisp breads) are typically plainer and generally eaten with cheese following a meal. Numerous savoury biscuits likewise contain additional ingredients for flavor or texture, such as poppy seeds, onion or onion seeds. The nutritive value, palatability, compactness and convenience of biscuits make it a ideal food (Kulkarni et al., 1997). Having low moisture content than cakes and bread, biscuits are commonly more secure from microbiological spoilage and have long time span of usability (Akubor, 2003).Biscuits have consistently been one of the most prevalent and appealing food products because of its predominant nutritional, sensorial and textural characteristics, ready to eat convenience just as cost intensity (Pratima et al.,2000).

2.2.1.1 Types of biscuit

It is progressively easy to isolate the "types" of biscuits by means of the method in which they are made, so below is a chart demonstrating the four fundamental techniques and examples of the types of biscuits which can be made utilizing these strategies.

Rubbed In Biscuits

- These types are principally founded on pastries and the name alludes to the rubbing in of the fat into the flour as would be done when making pastry such as short crust. This is done by placing the flour in a large mixing bowl, then adding butter which has been cut into pieces then rubbing the two together between your fingertips until the mixture takes after breadcrumbs.

- Depending on the sort of biscuit being made, other dry ingredients are then stirred in before the wet ingredients such as egg or milk, which will tie the batter together, prepared to be rolled out and molded/cut.

- The key factor when making these types of biscuits (as with all pastry) is not to over-do the rubbing in otherwise the gluten in the flour will be over-worked which will make the biscuits tough instead of crumbly.

Creamed Biscuits

- The name refers to the addition of the fat to sugar as would be done when making cakes. This is done by placing softened butter in a large mixing bowl, then adding the sugar, and beating the two together with a wooden spoon or electric whisk, until the mixture is very much mixed, light and fluffy.
- Depending on the type of biscuit being made, wet ingredients are then blended in such as eggs or milk, before the flour or other dry ingredients are included. These doughs are often very soft, so small spoonfuls are dropped onto baking sheets, well spaced apart.

Whisked Biscuits

- The name refers to the manner in which the egg content is treated.

- Egg whites - these are whisked until firm.

- Egg yolks/whole eggs - these are whisked together with the sugar content until thickened and lightened.
- These types of biscuits range from straight-forward whisked egg whites with added sugar in the form of light as air meringues to more substantial biscuits such as coconut macaroons.

Melted Biscuits

- The name refers to the manner in which ingredients are prepared. In most cases, the recipes include liquid sweeteners such as nectar, golden (corn) syrup or molasses. All sweeteners (including sugar) are set in an enormous saucepan together with the fat content, which is then gently melted and stirred until well combined. Often the other ingredients are added to the melted ingredients so most of the mixing is done in the saucepan rather than in a mixing bowl.
- The mixture can be soft enough to easily drop from a spoon such as brandy snaps, or thick enough to press into a tin such as flapjacks. Once again, as these recipes vary enormously, it is not possible to give a generic basic recipe here.

2.2.2 Cake

Cake is type of sweet substance that is generally baked. In their oldest forms, cakes were modifications of breads, yet cakes currently spread a wide range of preparations that can be simple or elaborate, and that share features with other desserts such as pastries, meringues, custards, and pies. Preparation of cake involves raising the temperature of highly aerated emulsions (cake batters). Batter is made with sugar, flour, eggs, fat, baking powder and salt. Other commonly used ingredients include emulsifiers and milk powder (D. Indrani, 2003). Cake batter is considered as a complex system in which air is mechanically dispersed in a continuous liquid phase containing dissolved or suspended dry ingredients (S. Sahin et al., 2008). The fuse of air cells during blending creates wet foam which is converted into solid foam (cake) after baking. Among other non fundamental cake batter ingredients, emulsifiers were accounted for increasing the storage time and the los modulus of cake batters and decrease batter density (R. Jyotsna et al., 2004). The advancement of cake structures begins from mixing, followed by starch gelatinization, protein denaturation, generation of carbon dioxide and other interactions between the ingredients during baking. Subsequently, physical and structural changes that happen during cake batter preparation may influence the primary ingredients performance or the quality of the final product (S. Sahin et al., 2008). According to bakerpedia, Wheat flour containing low flour content (6-8%) is appropriate for making cake.

2.3 Margarine in Bakery Products

Margarine is a fat utilized broadly as an ingredient or a spread that looks like butter, but is a common butter substitute. It is produced using the emulsion of vegetable and/or animal oil. Like other fats, margarine improves mouth feel and flavor in baked goods.

Margarine types for commercial baking include:

- Butter cream margarine
- Cake margarine
- Croissant margarine
- Puff pastry margarine
- Hard puff margarine

As a fat, margarine gives baked goods with texture, mouth feel, and other tangible qualities. Its other roles include extending a product‟s timeframe of realistic usability (Cheong et al., 2011).

Consuming saturated fats (all animal-derived fats) are the strongest method of raising serum cholesterol. So as to keep away from butter which is extremely high in saturated fat we normally go to margarines which are made of vegetable oils which have no significant amounts of saturated fat or cholesterol. The issue with margarines is that inappropriate ones can have a similar impact as saturated fat, contingent upon how hard they are. So as to make the vegetable oils hard enough to spread on bread, hydrogen must be added. The more hydrogenation, the more saturated and the more atherogenic they are. The stick margarines are the hardest. Hence, soft tub margarines are best.

Sadly, the more hydrogenation, the more it produces atherogenic fatty acids called trans fatty acids (Zock et al., 1997). Trans fatty acids have shown adverse effects on blood cholesterol. They may be worse than saturated fats in that they not only equal saturated fats in increasing the bad cholesterol known as LDL, but also decrease the good cholesterol known as HDL. Saturated fats do not lower HDL (Lichtenstein et al., 1996).

2.4 Omega -3 fatty acid

The principle four classes of fats found in our diets are: saturated, trans, polyunsaturated, and monounsaturated. The two major classes of PUFAs are the omega-3 and omega-6 fatty acids. Like all fatty acids, PUFAs comprise of long chains of carbon atoms with a carboxyl group at one end of the chain and a methyl group at the other. PUFAs are distinguished from saturated and monounsaturated fatty acids by the presence of at least two double bonds between carbons within the fatty acid chain. Omega-3 fatty acids have a carbon–carbon double bond located three carbons from the methyl end of the chain. Several different omega-3s exist, yet most of the scientific research centers around three: alpha-linolenic acid (ALA), Eicosapentaenoic acid (EPA), and Docosahexaenoic acid (DHA). ALA contains 18 carbon atoms, whereas EPA and DHA are considered "long-chain" (LC) omega-3s because EPA contains 20 carbons and DHA contains 22.Among the long chain omega-3 fatty acids (LC-PUFA), Docosahexaenoic acid (DHA) is the principal PUFA constituent of brain neurons, retinal cells, and primary structural component of skin, sperm, and testicles. Aside from being a significant structural component of cellular membranes, it performs varieties of functions in a number of cellular processes like transport of neurotransmitters and amino acids and modulates the functioning of ion channels and responses of retinal pigments (P. M. Kidd, 2007). DHA has been demonstrated to be especially significant for fetal brain development, optimal development of motor skills and visual acuity in infants, lipid metabolism in children and adults, and cognitive support in the elderly (P. M. Kidd, 2007).

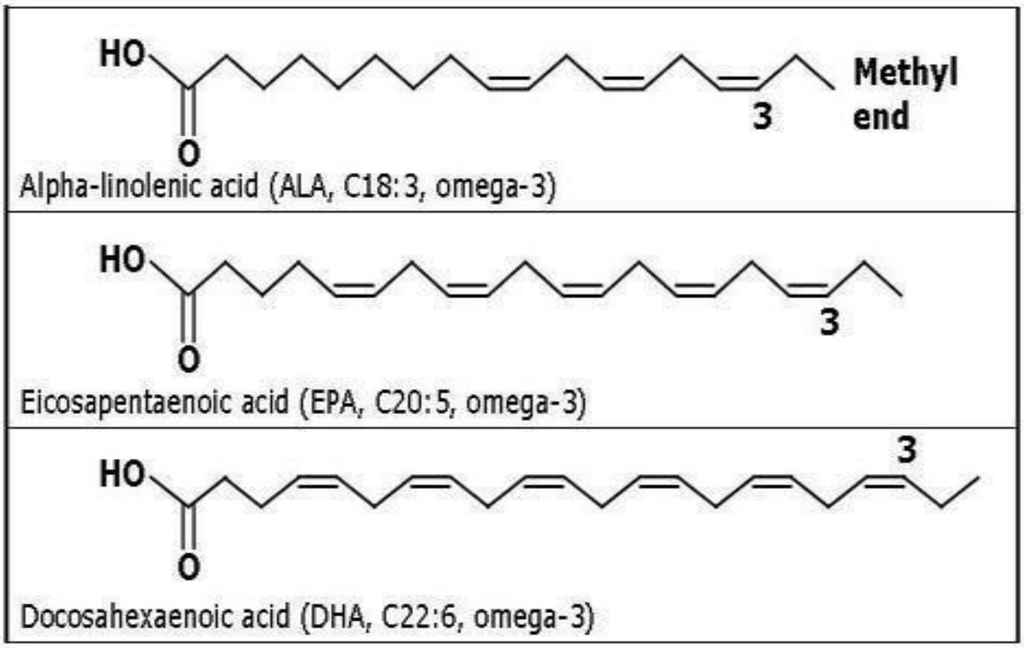

Fig1.1: Chemical Structure of Omega-3 fatty acids

2.4.1 Requirement of Omega-3 fatty acid

The most ideal approach to get them is by eating fatty fish at least twice a week, except on the off chance that you don‟t eat fatty fish often, you ought to think about taking a supplement.

Notwithstanding, it‟s important to ensure your supplement contains enough EPA and DHA. These are the most useful types of omega-3 fats and are found in fatty fish and algae. There is no official recommended daily allowance for omega-3s. Nonetheless, most health organizations concur that 250–500 mg of combined EPA and DHA is enough for adults to maintain overall health (FAO, 2008). This requirement may fluctuate in special health condition. The following health conditions have been shown to respond to omega-3 supplements.

Heart disease

One study followed 11,000 people who took an 850-mg dose of combined EPA and DHA consistently for 3.5 years. They encountered a 25% reduction in heart attacks and a 45% reduction in sudden death (Marchioli et al., 2002).The American Heart Association, among other organizations, recommends that individuals with coronary heart disease take 1,000 mg of combined EPA and DHA every day.

Depression and anxiety

Studies exhibit that high doses of omega-3, extending from 200–2,200 mg per day, can diminish the symptoms of depression and anxiety. In cases of mood and mental disorders, a supplement with higher measures of EPA than DHA might be ideal (Sublette et al., 2011)

Cancer

High consumption of fish and omega-3 fatty acids has been connected to a reduced risk of breast, prostate, and colon cancers (Terry et al., 2004)

Omega-3 for children and pregnant women

Research demonstrates that omega-3 fatty acids, particularly DHA, are vital before, during, and after pregnancy. An extra 200 mg of DHA is suggested for pregnant and nursing mothers. The recommended dose for infants and children is 50–100 mg of combined EPA and DHA per day (Koletzko et al., 2008).

2.5 Fish oil, a good source of Omega-3 fatty acid

Fish oil is oil derived from the tissues of oily fish. Fish oil is a very effective nutrient and contains significant omega 3 fatty acids that can be absorbed effectively.

Assisi et al. (2006) stated that Fish oil contains both Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA). There is evidence from different investigations supporting intake of recommended amounts of DHA and EPA in the form of dietary fish or fish oil supplements lowers triglycerides, reduces the risk of death, heart attack, dangerous abnormal heart rhythms, and strokes in people with known cardiovascular disease, slows the buildup of atherosclerotic plaques ("hardening of the arteries"), and lowers blood pressure slightly. However, high doses may have harmful effects, for example an increased risk of bleeding. Fish oil comes from cold water fish such as mackerel, tuna, salmon, cod and many other fresh water fish. It is recommended for a heart healthy diet.

2.5.1 Fish Oil Benefits

1) **According to American Heart Association, (2010) Fish Oil Benefits are**

- **Less Pain and Inflammation**

Fish oil has an extensively positive effect on your inflammatory response. Through several mechanisms, they regulate the body‟s inflammation cycle, which counteracts and mitigates painful conditions like arthritis, prostatitis, cystitis and anything else endingin "itis."

- **Cardiovascular Health**

Fish oil contains omega 3 fatty acids, which have been proven to work wonders for your heart and the miles and miles of arteries and veins that make up your cardiovascular system. They help to bring down cholesterol, triglycerides, LDLs and blood pressure, while simultaneously espanding good HDL cholesterol. This adds a very long time to your future.

- **Protection from Stroke and Heart Attack**

When plaque develops on arterial walls and then breaks loose, it causes what‟s known as a thrombosis, which is an extravagant method for saying clot. If a clot gets stuck in the brain, it causes a stroke and when it plugs an artery, it causes a heart attack. Research shows omega 3 fatty acids break up clots before they can bring on any harm.

- **Better Brain Function and Higher Intelligence**

Pregnant and nursing mothers can greatly affect the intelligence and joy of their babies by supplementing with fish oil. For adults, omega 3 improves memory, recall, reasoning and focus.

- **Less Depression and Psychosis**

Making you smarter is not all omega 3 does for your brain. Psychiatry department researchers at the University of Sheffield, alongside numerous other research studies, found that omega 3 fish oil supplements "alleviate" the symptoms of depression, bipolar and psychosis.

- **Optimizing Baby Growth**

For pregnant women, fish oil can be advantageous to the health and development of your fetus during pregnancy. This is because the substance of DHA and the benefits of omega 3 rich in pangasius fish. The two substances are valuable for:

- ✓ Maintain fetal health while in the womb
- ✓ Keeping fetal growth healthy
- ✓ Keep the fetus healthy

- **Lower Incidence of Childhood Disorders**

Studies demonstrate that children (and grownups) with ADD and ADHD experience an enormously improved quality of life and those with dyslexia, dyspraxia and compulsive disorders have gotten a new lease on life.

- **Reduction of Breast, Colon and Prostate Cancer**

Omega 3 fish oil has been shown to help prevent three of the most common forms of cancer – breast, colon and prostate. Science tells us that omega 3s accomplish these in three ways. They stop the alteration from a normal healthy cell to a cancerous mass, inhibiting unwanted cellular growth and causing apoptosis, or cellular death, of cancer cells.

2) Cardiovascular effects of fish oil

Cardiovascular disease (CVD) is the leading cause of death all around. The dietary factors play an siginificant role in determination of CVD risk and in the pathogenesis of CVD. To reduce the risk of CVD dietary variables are modified (Alissa et al., 2012). Long chain polyunsaturated fatty acids can"t be synthesized by humans. Because of this, the ω-3 fatty acids synthesized in plants and in marine microalgae. Fish and fish oil contain large amount of two ω-3 fatty acids, eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3) which show cardio protective effects (Weitz et al.,2010). Dietary supplementation of fish and fish oils which are the rich sources of ω-3 fatty acids demonstrated the useful impacts to the patients with dyslipidemia, atherosclerosis, hypertension, obesity and inflammatory diseases (Aarsetoey et al.,2012).

In 2010, Kromhout et al. showed in the Zutphen Study, a prospective cohort study in the Netherlands, that eating fatty fish once or twice per week was related with a lower risk of fatal CVD compared with men who did not eat fish.

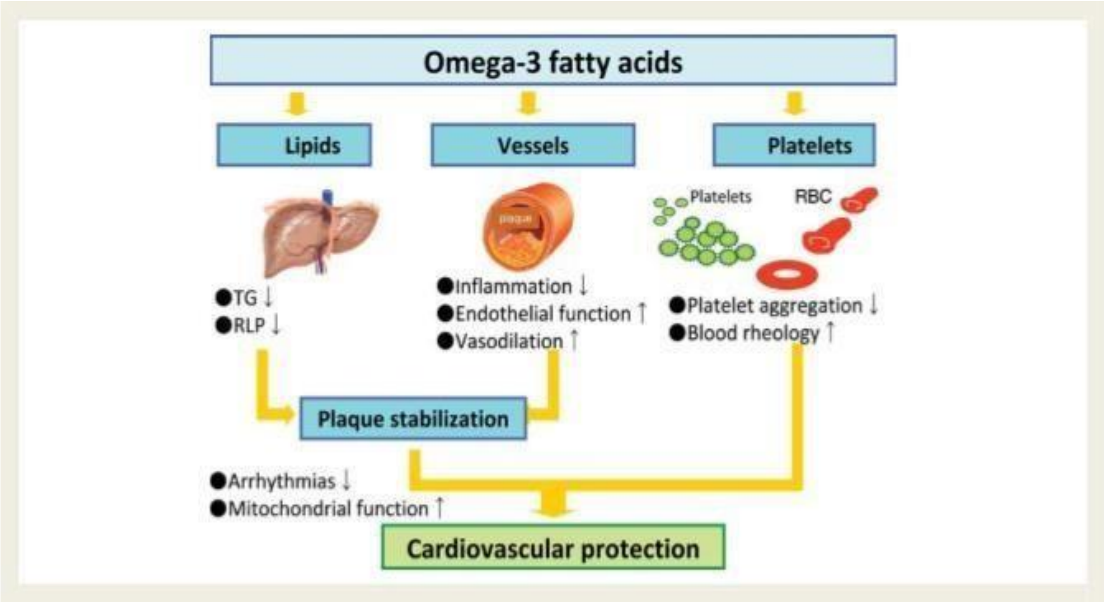

Fig 2.1: Effect of Omega-3 fatty acid in cardiovascular protection

3) Diabetes prevention of fish oil

In diabetic women, higher consumption of fish and long chain ω-3 fatty acids was related with lower incidence of CHD. Women who consumed fish less than one serving per month compared with fish consumption 1-3 times per month, the relative risk of CHD was 0.70, for once in a week 0.60, for 2-4 times per week 0.64 and for 5 or more times per week 0.36. Higher consumption of fish ≥5 times each week altogether brought down the occurrence of CHD in diabetic women (Hu et al., 2003). Plasma lipid modulatory effect was showed by EPA and DHA in CVD risk and diabetes (Minihane et al., 2013). Fish oil supplementation diminished the triglycerides, raised LDL cholesterol and has no statistically significant effect on glycemic control, total cholesterol, HbA1c, fasting plasma glucose and postprandial plasma glucose in Type 2 diabetes (Chen at el., 2015). Fish ω-3 fatty acids show beneficial effects for aversion of Type 2 diabetes in Asian populace which proposes that relationship between consumption of fish or fish oil and the development of Type 2 diabetes relies upon geographical variety (Yanani et al., 2015).

4) Inhibition of platelet aggregation

Fish oil containing omega-3 fatty acids decreases the risk of thrombosis by inhibiting platelet aggregation. Significantly, omega-3 fatty acids inhibit platelet TXA2 synthesis and acts as antagonists of the pro-aggregatory TXA2/PG H2 receptor in human platelets in vitro (swann et al., 1989). Supplementing a diet with omega-3 fatty acids down-regulate mRNA expression of platelet-derived growth factor-A and -B in mononuclear blood cells in humans (Kaminski et al., 1993).

5) Triglyceride-lowering effects

Omega-3 fatty acids play an important role to regulate genes that are critical for controlling lipid homeostasis. Omega-3 fatty acids decline VLDL assembly and discharge, bringing about reduced triacylglycerol production, through a decreased activity of sterol receptor element-binding protein-1c, which is the key switch in controlling lipogenesis (Sampath et al., 2005). In addition, omega-3 fatty acids could promote β-oxidation simultaneously in mitochondria and/or peroxisomes, possibly through the activation of peroxisome PPAR-a, leading to the reduction of fatty acids substrate for triglyceride synthesis (Oi K et al., 2004). The remainder lipoprotein (RLP), produced from the triacylglycerol-rich chylomicrons and VLDL, applies potent pro-atherogenic effects and is thus viewed as a significant risk factor of CVD (Oi K et al., 2004). The association of RLP has been recommended in the pathogenesis of sudden cardiac death and restenosis after coronary angioplasty. Although omega-3 fatty acids don"t majorly affect fasting total cholesterol and LDL cholesterol levels, EPA adequately diminishes RLP in hyperlipidaemic patients (Nakamura et al., 1999).

6) Improvement of endothelial function

Long-term treatment with fish oils augments endothelium dependent relaxation of normal porcine coronary arteries (Shimokawa et al, 1987), for which EPA, a major omega-3 fatty acids of fish oils, is responsible for the augmentation (Shimokawa et al, 1989). This augmenting effect of EPA was additionally noted in porcine coronary micro vessels (Shimokawa et al, 1988). Long-term treatment with fish oils improves endothelium-dependent relaxation of hypercholesterolaemic and atherosclerotic porcine coronary arteries (Shimokawa et al, 1988) and femoral veins (Komori et al, 1989). EPA augments endothelium-dependent relaxation by NO as well as that by endothelium-derived hyperpolarizing factor (Tagawa et al, 2002).

7) Plaque stabilization

As referenced above, through their anti-inflammatory effects, omega-3 fatty acids could not just counteract the plaque development yet in addition add to the plaque stabilization (Yasuda et al., 2010). The randomized clinical trial exhibited that omega-3 fatty acids supplementation considerably build tissue levels of EPA and DHA and diminishes macrophage infiltration and thickened fibrous cap in human carotid arteries (Thies et al., 2003). Exacerbated release of matrix metalloproteinase (MMP) by the activated endothelium and macrophages plays a pathological role in plaque progression and instabilization (Morishige et al., 2003).

2.6 Pangasius hypophthalmus

Pangasius hypophthalmus is a species of shark catfish (family Pangasiidae) local to the rivers of Southeast Asia and furthermore known as thai pangus. It has been developing as a financially significant species to South-East Asian aquaculture. This species has moved toward becoming as a significant aquaculture species in Indonesia, Malaysia, Cambodia, Bangladesh and China (FAO, 2010). It is especially significant for their quick development, all year production and high productivity. Originally, *P. hypophthalmus* is a catfish of the Mekong delta being recognized as the most significant and largest inland fishery in the world. This exotic species which was brought from Thailand in 1989 has been established as a cultured species in Bangladesh as recognized by FAO in 1990. As of late, High yield and low production cost makes thai pangas one of the most popular commercial culturablespecies , and thai pangus fry are now producing by many hatcheries all over the country to meet up the farmer"s demand. Contingent upon accessibility of fry the monoculture system of thai pangus has generally been spreaded all through the nation. Presently the fish farmers are also too much interested to culture thai pangus. Right now, Pangas cultivating is one of the quickest developing types of aquaculture in Bangladesh (Anka et al., 2013).

Much the same as different kinds of fish, pangasius fish have a delicious and delicate taste. Almost all parts of the fish can be consumed. Meat and the head can be handled into delightful cooking, while the bone can be a fish soup that is similarly delicious. As one sort of fish that much supported by the people of Bangladesh, pangasius of has a lot of advantages that are good for us especially benefits for health and fulfillment of nutritional intake in the body.

Few studies have been done on the chemical composition of this fish.

Ho BT. et al., 2009 stated that the crude fat content found in *Pangasius hypophthalmus* was 2.55% of wet weight. The SFA content was 42.63%, the percentage of PUFA was 17.69%, MUFA was 34.69%, Linoleic acid (C18:2n-6) 8.43%, DHA (C22:6n-3) was 4.74% and the EPA (C20:5n-3) content was 0.31% of the fatty acid in fat.

Men et al., 2005 estimated the crude fat content of *Pangasius hypophthalmus* and found 2.95g/100g of wet weight.

2.7 Fish oil in Bakery Products

Scarcely any examinations have been done to fortify bakery products with omega 3 fatty acid by utilizing fish oil.

Agni Kumar et al., 2015 Fortified cakes with fish oil encapsulate to improve the consumption of health-beneficial polyunsaturated fatty acids like EPA and DHA. Cortés, 2012 planned to develop a cookie with added omega-3. Three different commercial form of omega 3 were assessed as DHA and EPA (emulsion, powder and oil) at different omega 3 (ω3): omega 6 (ω6) ratios (1:5, 1:8, 1:10). Through this study was found that the emulsifiers properties of omega-3 help the incorporation of air into the system, just as to the softness of the cookies.

2.8 Gas Chromatography principle

The principle in gas chromatography involves separation of volatile components of the sample based on their partition co-efficient. This is ratio of solubility of substance in between gaseous mobile phase and stationary liquid phase. The components of the sample that are partitioned into gas come out first while others come later.

The stationary phase is a liquid layer supported over a stationary phase while the mobile phase is an inert and stable gas. Hence it was named Gas-Liquid chromatography (GLC). The development of GC as an analytical technique was pioneered by Martin and Synge 1941; they suggested the use of gas-liquid partition chromatograms for analytical purposes.

- Samples analyzed by GC must be volatile (have a significant vapor pressure below 250 °C)
- Most GC analysts are under 500 Da Molecular Weight for volatility purposes.
- Highly polar analysts may be less volatile than suspected when dissolved in a polar solvent or in the presence of other polar species due to intermolecular forces such as hydrogen bonding.

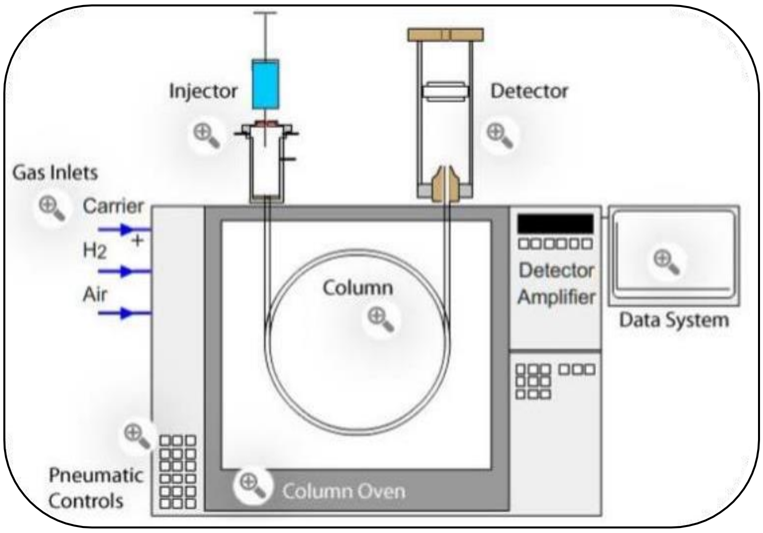

Fig 2.8.1: Gas Chromatography

2.8.1 Gas chromatography instrumentation

The gas chromatography apparatus can be listed as:

1. **The mobile phase gas in a cylinder:** The mobile phase is an inert gas (monoatomic element gases or non-reactive gases like nitrogen, helium & hydrogen). The carrier gas is kept in a metallic cylinder and outflow is controlled by a regulator. From the gas carrier cylinder, the gas is passed under a fixed rate through a pressure gauge which indicates the speed of flow of gas into the column. Most commonly used gas is helium.
2. **The injection system:** Here the sample is volatilized and the resulting gas entrained into the carrier stream entering the GC column.

Many inlet types exist including:

- Split / Splitless

- Programmed Thermal Vaporizing (PTV)
- Cool-on-column (COC) etc.

The COC injector introduces the sample into the column as a liquid to avoid thermal decomposition or improve quantitative accuracy.

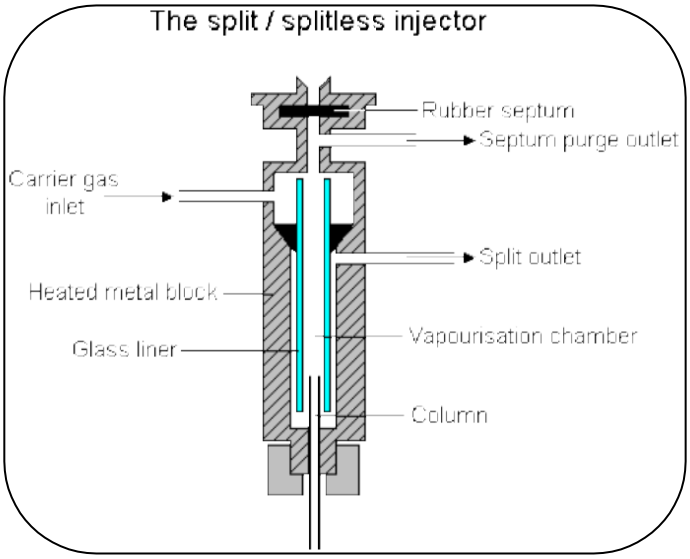

Fig 2.8.2: Split/ Splitless injector

3. The column

The gas chromatography column is usually long (few meters like 3 to 6 meters) and coiled for accommodation into a small thermal chamber. The column is mostly made of steel or glass.

The GC columns are of three types:

- ✓ Packed column. This is a column into which solid beads are packed. This column has advantages like efficient separation and precise readings.
- ✓ Tubular column. Here into a stainless steel hollow tube a thin layer of liquid is coated to act as a stationary phase. This column offers the least resistance to the flow of gas.
- ✓ Support coated tubular column**.** Here into stainless steel column, a thin solid layer is coated on to which a thin layer of liquid stationary phase is present.

4. **Column Oven**

Temperature in GC is controlled via a heated oven. The oven heats rapidly to give excellent thermal control. The oven is cooled using a fan and vent arrangement usually at the rear of the oven

5. **Detector**

The detector responds to a physicochemical property of the analyte, amplifies this response and generates an electronic signal for the data system to produce a chromatogram. GC detectors detect the isolated components and help in the identification and quantification of the sample. Four types of GC detectors like:

- **Thermal conductivity detector:** There are two columns which have a conducting wire in between. The gas is allowed to pass through the two columns of detectors. Since the temperature of both gases is the same, the thermal conduction is constant. When the sample is injected into the gas chromatography column, the effluent gas carries the sample components into the detector column. Since effluent gas is mixed with sample components there results in the difference in thermal conductivity from prior one recording. This difference in conductivity is specific for the component analyzed. This is recorded for further comparison and identification of the components and their quantity.

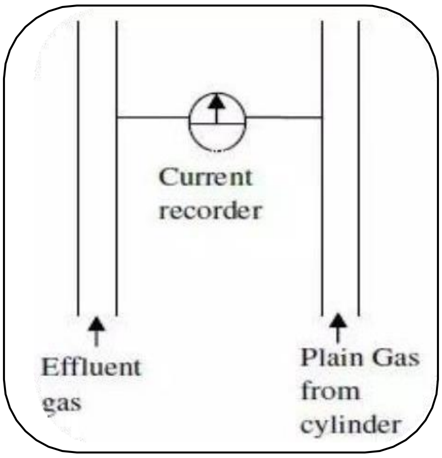

Fig 2.8.3: Thermal conductivity detector

- **Flame ionization detectors:** Here the sample components from effluent are ionized by subjecting to flame in a chamber. These ions raise upwards and are attracted towards anode or cathode based on the charge on them. When they impinge on the electrodes, the current is passed which is recorded.

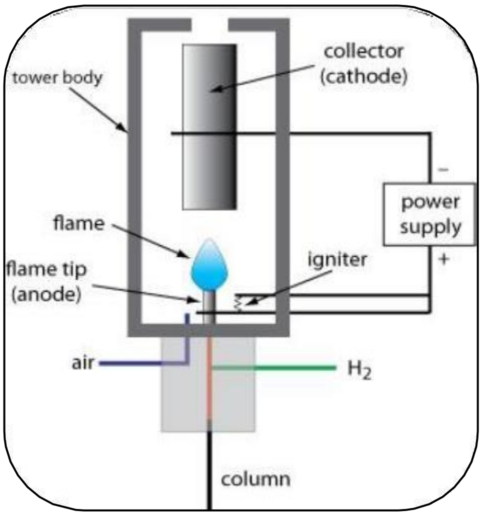

Fig 2.8.4: Flame ionization detector

- **Argon ionization detector:** These detectors are similar to flame ionization detectors with the only difference that argon ion gas is used to ionize the sample molecules. The argon ions are obtained by reacting argon gas with radioactive elements. Once argon ionizes they try to get back to the stable state by either taking or giving electron from the sample components thus making sample molecules to ions for detection.

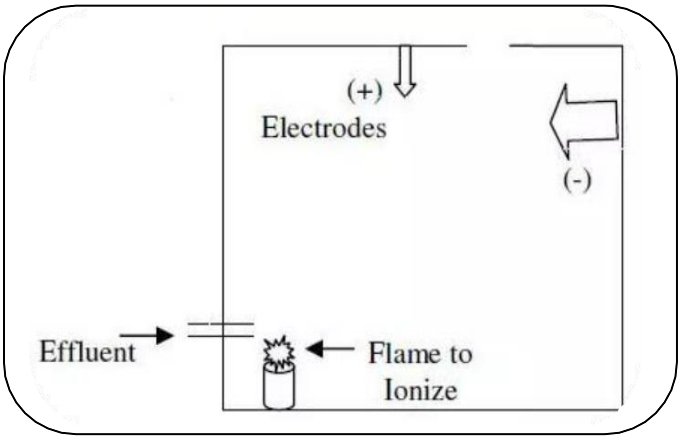

Fig 2.8.5: Argon ionization detector

CHAPTER III: MATERIALS AND METHODS

3.1 Collection and preparation of sample

The fish (*Pangasius hypophthalmus*) were bought fresh from the market. Prior to analysis, the internal organs were removed and the fish was washed to remove the residual blood. Fish fillet was obtained by cutting the fish lengthwise along the backbone to obtain maximum amount of flesh without including the backbone. The fillet was cut into small pieces.

3.1.1 Extraction of Lipid

Extraction of the fish lipids were done according to the method of Bligh and Dyer (Bligh et al., 1959) with some modification by Kinsella et al (Kinsella et al., 1977). Representative samples of fish tissue (50g) were homogenized in a blender for 2 minutes with a mixture of methanol (100 ml) and chloroform (50 ml). Then 50 ml of chloroform was added to the mixture. After blending for additional 30 seconds, distilled water (50 ml) was added. The homogenate was stirred with a glass rod and filtered through a whatman no.1 filter paper on a Buchner funnel under vacuum suction. 20 ml chloroform was used to rinse the remainder. The filtrate was allowed to settle to separate into the organic and aqueous layers. The chloroform layer containing the lipids was transferred into another beaker and. 3 g of anhydrous sodium sulphate was added to remove any remaining water. The mixture was filtered through a whatman no. 1 filter paper and chloroform was used to rinse the remainder. Finally, a known amount of BHT (of about 0.02 g) was added to the lipid solution as an antioxidant (Kinsella et al., 1977). The solution was then evaporated to a constant weight in a tarred 100 ml round-bottom flask with a rotary evaporator at $40^{o}C$.

3.2 Biscuit preparation

The basic formulation used for preparation of cracker biscuits has been adopted from the recipes used by hoseney and kent (Hoseney, 1986; Kent, 1984) and shown in table 3.1. All the ingredients were weighed accurately for each sample.

3.2.1 Procedure

Preheat the oven

At first oven was preheated to 20 C (Anis and Lubna, 2014).

Combine the dry ingredients

Sugar was grounded and flour, baking powder, salt and sugar were added in a large bowl. Whisk together until well blend.

Add wet ingredients

Egg (after beating), and milk together were poured into the bowl containing dry ingredients.

Mix the ingredients to form a dough

All ingredients were then mixed all until perfect dough was formed. After mixing the dough was transferred to a floured surface for kneading.

Rolling and shaping

The dough was rolled down and cut into the desired shape by a cookie cutter.

Bake the biscuits until lightly browned

The biscuits were placed in to the oven at 210˚C for about 25 min (Anis and Lubna, 2014)

Cooling

After removing from the baking pan the biscuits were allowed to cool for 15 mins.

Table 3.1: Formulation of Biscuit

Ingredients	**Sample 1 (S_0)**	**Sample 2 (S_1)**	**Sample 3 (S_2)**	**Sample 4 (S_3)**
Wheat Flour	100 g	100 g	100g	100 g
Margarine	32 g	0 g	6.4 g	16 g
Fish Oil	0g	32g	25.6g	16g

Sugar	45 g	45 g	45 g	45 g
Salt	0.7 g	0.7 g	0.7 g	0.7 g
Baking Powder	1.5 g	1.5 g	1.5 g	1.5 g
Egg	30 g	30 g	30 g	30 g
Milk	5ml	5ml	5ml	5ml
Vanilla Essence	1 g	1 g	1 g	1 g

Here, **S** = Control;

$\mathbf{S_1}$= Biscuits having 100% fish oil instead of margarine;

$\mathbf{S_2}$ = Biscuits having 80% fish oil;

$\mathbf{S_3}$ = Biscuits having 50% fish oil.

3.3 Cake preparation

The chocolate cake was prepared by a single bowl mixing method (Neville and Setsar, 1986). The formulation has been adopted from the recipes reported by Siti-Faridah and Noor-Aziah (Siti-Faridah and Noor-Aziah, 2012) and shown in table 3.2.

3.3.1 Procedure

Combining and mixing the ingredients:

The eggs and sugars were mixed for 2-5 minutes. The mixture was then mixed for 2 minutes to get lighter, creamier and floppy batter. After mixing, the sifted wheat flour and cocoa powder were added into a mixing bowl and mixed for 2 minutes. Margarine was heated until melted. The mixture was poured into bowl and mixed well.

Baking:

The batter was then transferred to greased non-stick cake pan and baked in an electric oven at 170°C for 35 minutes.

Cooling:

The cakes were then removed from the oven and allowed to cool for 2 hours and were later packed in a low-density polyethylene packages at room temperature for 24 hours prior to physical quality analysis.

Table 3.2: Formulation of Cake

Ingredients	**Sample 1 (C_0)**	**Sample 2 (C_1)**	**Sample 3 (C_2)**	**Sample 4 (C_3)**
Wheat Flour	100 g	100 g	100g	100 g
Margarine	40 g	0 g	32 g	24 g
Fish Oil	0g	40g	8g	16g
Sugar	113 g	113 g	113 g	113 g
Salt	0.5 g	0.5 g	0.5 g	0.5 g
Baking Powder	3 g	3 g	3 g	3 g
Egg	85 g	85 g	85 g	85 g
Milk	80ml	80ml	80ml	80ml
Cocoa Powder	20g	20g	20g	20g
Vanilla Essence	1 g	1 g	1 g	1 g

Here, $\mathbf{C_0}$ = Control;

$\mathbf{C_1}$= 100% fish oil instead of margarine;

$\mathbf{C_2}$ =80% fish oil;

$\mathbf{C_3}$ = 60% fish oil.

3.4 Proximate Analysis

3.4.1 Sample preparation

Firstly, Sample was prepared for the analysis by grinding it in the mortal pestle.

3.4.2 Determination of crude fat

This was determined by solvent extraction gravimetric method described by Kirk and Sawyer (1980). Five gram of sample was wrapped in a porous paper (whatman filter paper) and put in a thimble. The thimble was put in a soxhlet reflux flask and mounted into a weighted extraction flask containing 200 ml of petroleum ether. The upper of the reflux flask was connected to a water condenser.

The solvent (petroleum ether) was heated, boiled vaporized and condensed into the reflux flask filled. Soon the sample in the thimble was covered with the solvent until the reflux flask filled up and siphoned over, carrying its oil extract down to the boiling flask. This process was allowed to go on repeatedly for 4 h before the defatted sample was removed, the solvent recovered and the oil extract was left in the flask. The flask (containing the oil extract) was dried in the oven at 60°C for 30 min to remove any residual solvent. It was cooled in desiccator and weighed. The weight of oil (fat) extract was determined by difference and calculated as a percentage of the weight of sample analyzed thus:

$$\boldsymbol{Fat}(\%) = \frac{\text{weight of petridish with sample} - \text{weight of empty petridish}}{\text{weight of sample}} \times 100$$

3.4.3 Determination of crude fiber

Crude fiber was determined by the method AOAC (2000). (5.0 g) processed sample was boiled in 150 ml of 1.25% H_2SO_4 solution for 30 min under reflux. The boiled sample was washed in several portions of hot water using a two-fold cloth to trap the particles. It was returned to the flask and boiled again in 150 ml of 1.25% NaOH for another 30 min under same condition. After washing in several portion of hot water the sample was allowed to drain dry before being transferred quantitatively to a weighed crucible where it was dried in the oven at 105°C to a constant weight. It was thereafter taken to a muffle furnace where it was burnt, only ash was left of it. The weight of the fiber was determined by difference and calculated as a percentage of the weight of sample analyzed thus:

$$\textbf{Crude Fiber}(\%) = \frac{W_2 - W_3}{\text{Weight of sample}} \times 100$$

Where,

W_2 = weight of crucible + sample after washing and drying

W_3 = weight of crucible + sample

3.4.4 Determination of ash content

This was done by the furnace gravimetric method described by the james (1995) and AOAC (2000). Briefly 5g of the samples was measured in a previously weighted porcelain crucible. The sample was burnt to ashes in a muffle furnace at 55^0C. When it has become completely ashed, it was cooled in a desiccator and weighted and analyzed thus:

$$\textbf{Ash Content}(\%) = \frac{\text{weight of Crucible with ash Residue} - \text{Weight of empty crucible}}{\text{Weight of Sample}} \times 100$$

3.4.5 Determination of Moisture content

Moisture content was determined adopting AOAC (2005) method 14.004.

Procedure

5g sample was taken in a pre-weight crucible (provide with cover) which was previously heated to 130^{0}C. The sample was dried for 1 hour in an air oven at temperature 130^{0}C± 30^{0}C. The crucible was while still in oven then transferred to desiccator and weighed immediately after reaching at room temperature.

$$\textbf{Moisture}(\%) = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Sample wight}} \times 100$$

3.4.6 Determination of Protein

Protein content was determined using AOAC (2005) method 2.049. The method was as follows:

Reagent required

i) Concentrated sulphuric acid (nitrogen free) 20 ml.

ii) Digestion mixture

Potassium sulphate =100gm

Copper sulphate = 10 g

Selenium di-oxide = 2.5g well mixed in mortar and kept in a dry place.

iii) Boric acid solution = 2% solution in water

iv) Alkali solution = 400g sodium hydroxide in water and dilute to 1 litre.

v) Mixed indicator solution: Bromocresol= 0.1g and methyl red= 2g dissolved in 250 ml ethyl alcohol

vi) Standard HCl: 0.1 N

Procedure

5g digestion mixture was weighed accurately and transferred into a dry 300ml Kjeldahl flask. A suitable quantity of the sample (1g for each) was transferred into the flask. 20ml of sulphuric acid was added, heated continuously until frothing ceased and then simmered briskly. The solution became clear in 15-20 min., continued heating for 45 min. After cooling, 100ml water was added, and transferred quantitatively to a 1 liter round- bottom flask; the final volume was about 500 ml. Added gently sown the side enough sodium hydroxide solution to form a participate of cupric hydroxide and immediately connected the flask to steam-trap and condenser. Then 50ml of boric acid solution, 50ml distilled water and 5 drops of indicator solution were added to a 500ml conical receiving flask. Positioning the condenser distillation was carried out for 4 to 5 minutes or until about 250 ml of distillate was attained, the contents of the receiving flask were titrated with 0.1 N hydrochloric acid, the end point was marked by a brown color. A reagent blank as also determined and deducted from the titration. One milliliter of 0.1N hydrochloric acid is equivalent to 1 mg of nitrogen. A protein conversation factor was to calculate the percent protein from nitrogen determination. Percentage of nitrogen and protein calculated by the following equation:

$$\textbf{Nitrogen}(\%) = \frac{(Ts - Tb) \times \text{N of HCL} \times 14 \times \text{Vol. made up the digest}}{\text{Wt.of sample (gm)} \times \text{A liquor of the digest taken}} \times 100$$

=

Where,

T_s = Titre volume of the sample (ml)

T_b = Titre volume of the blank (ml)

Protein (%) = % Nitrogen × Protein factor.

3.4.7 Determination of Carbohydrate

Carbohydrate was determined by the following formula as per AOAC, 2000.

Carbohydrate (%) =
100–[crude protein (%)+crude fat(%) +crude fiber(%)+ash(%)+moisture(%)]

3.5 Sensory evaluation of finished products

The sensory characteristics of the cake and biscuit samples were evaluated. Samples were evaluated for color, flavor, texture and overall acceptability. A 1-9 point hedonic rating test was performed to assess the degree of acceptability of the samples containing fish oil. A set of 20 panelists was selected from teacher, students and employee of the Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University and briefed on procedure before evaluation. One slice from each sample of cake and one piece from each sample of biscuit were presented to 20 panelists. The taste panelists were asked to rate the sample for color, flavor, texture and overall acceptability on a 1- 9 point scale, where 1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither like nor dislike; 6 = like slightly; 7= like moderately; 8 = like very much; 9 = like extremely (Amerine et al., 2013).

3.6 Statistical analysis

All the data were compiled in MS Excel. Raw data related to Moisture, Protein, Fat, Carbohydrate, Ash, Appearance, Texture, Color, Flavor and Overall acceptability etc. were tested for normality by using normal probability plot and analyzed for one way ANOVA by using STATA (2017). The level of significance was set ≤ 0.05.

CHAPTER IV: RESULTS

In this section, fatty acid composition of fish oil and margarine, omega-3 fatty acid content of different prepared biscuits and cakes, proximate analysis of prepared biscuits and cakes have been shown.

4.1 Fatty acid composition of fish oil and Margarine

Total fatty acid percentages of experienced margarine and fish oil have been shown in table 4.1 and the comparison between them has been shown in figure 4.1. In this figure it is clear that Margarine contains more total SFA content and PUFA content than in fish oil. TFA content has been only found in margarine. But it contains no Omega-3 fatty acid (Total DHA and EPA) where fish oil contains good omega-3 content.

Table 4.1 Total fatty acids percentage in fish oil and margarine

Fatty Acid	Fish oil (F_1) (%)	Fish oil (F_2) (%)	Margarine (M_1) (%)	Margarine (M_2) (%)
Total SFA	34.80	38.71	47.32	51.34
Total MUFA	42.69	39.35	25.05	23.13
Total PUFA	17.88	18.60	20.90	18.56
DHA	5.19	4.37	0.0	0.0
EPA	0.41	0.62	0.0	0.0
TFA	0.0	0.0	3.57	4.58

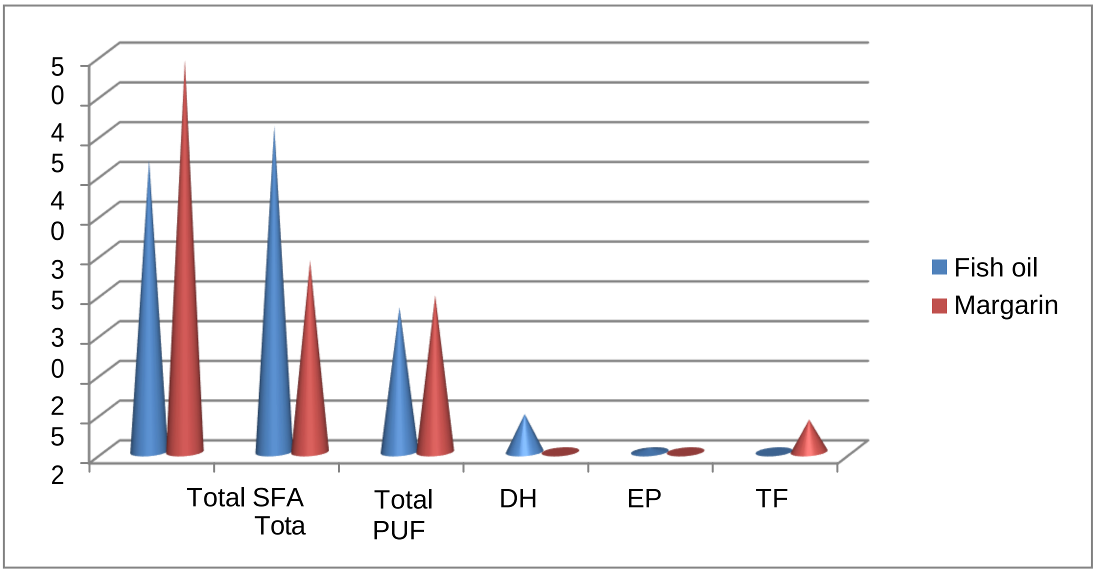

Figure 4.1: Total fatty acids percentage comparison between fish oil and margarine

4.2 Fatty acid composition of Biscuit and Cake sample

Fatty acid compositions of prepared biscuits and cake sample have been presented in table 4.2 and table 4.3 respectively. In these data, it is clear that biscuits and cakes prepared with margarine contain no Omega-3 fatty acid but Trans fatty acid, where biscuits and cakes containing fish oil contain omega-3 fatty acid but no trans fats. Figure 4.2 and figure 4.3 showing the comparison among the biscuit and cake samples respectively in which different percentages of fish oil have been used as ingredients. Omega-3 fatty acid has been found highest in the biscuit (4.19%) and cake (4.03%) sample where 100% fish oil has been used. But omega-3 fatty acid is not significantly less in the biscuit and cake samples containing 80% fish oil.

Table 4.2(a): Total fatty acids percentage in biscuit oil samples.

Fatty Acid	S_0 (%)	S_1 (%)	S_2 (%)	S_3 (%)
Total SFA	54.29	43.15	45.30	56.37
Total MUFA	26.06	38.08	35.63	28.71
Total PUFA	13.17	14.15	13.45	10.12
C22:6n-3	NIL	3.97	3.54	1.06
C20:5n-3	NIL	0.22	0.36	0.45
TFA	3.41	NIL	0.65	2.37

S_o = Control; **S_1**= 100% fish oil instead of margarine; **S_2** =80% fish oil; **S_3** = 50% fish oil

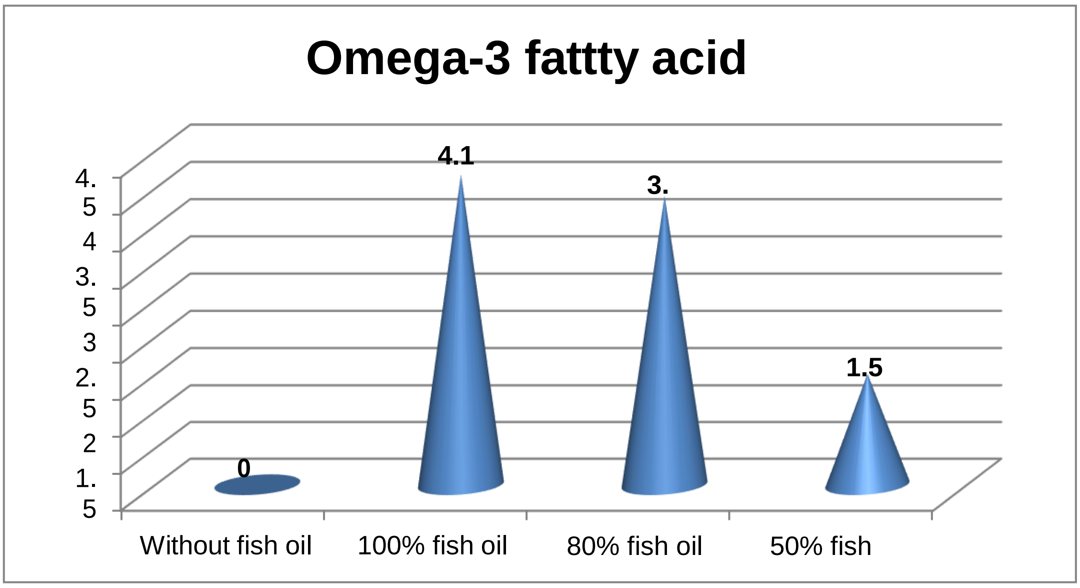

Figure 4.2(a): omega-3 fatty acid content of biscuit sample

Table 4.2(b): Total fatty acids percentage in cake oil samples

Fatty Acid	**C_0 (%)**	**C_1 (%)**	**C_2 (%)**	**C_3 (%)**
Total SFA	59.17	45.42	50.15	52.24
Total MUFA	23.00	34.28	29.87	27.72
Total PUFA	11.29	16.50	16.23	12.34
C22:6n-3	NIL	3.55	3.85	0.97
C20:5n-3	NIL	0.48	0.55	0.48
TFA	4.33	NIL	0.95	1.90

C_o = Control; **C_1**= 100% fish oil instead of margarine; **C_2** =80% fish oil; **C_3** = 60% fish oil

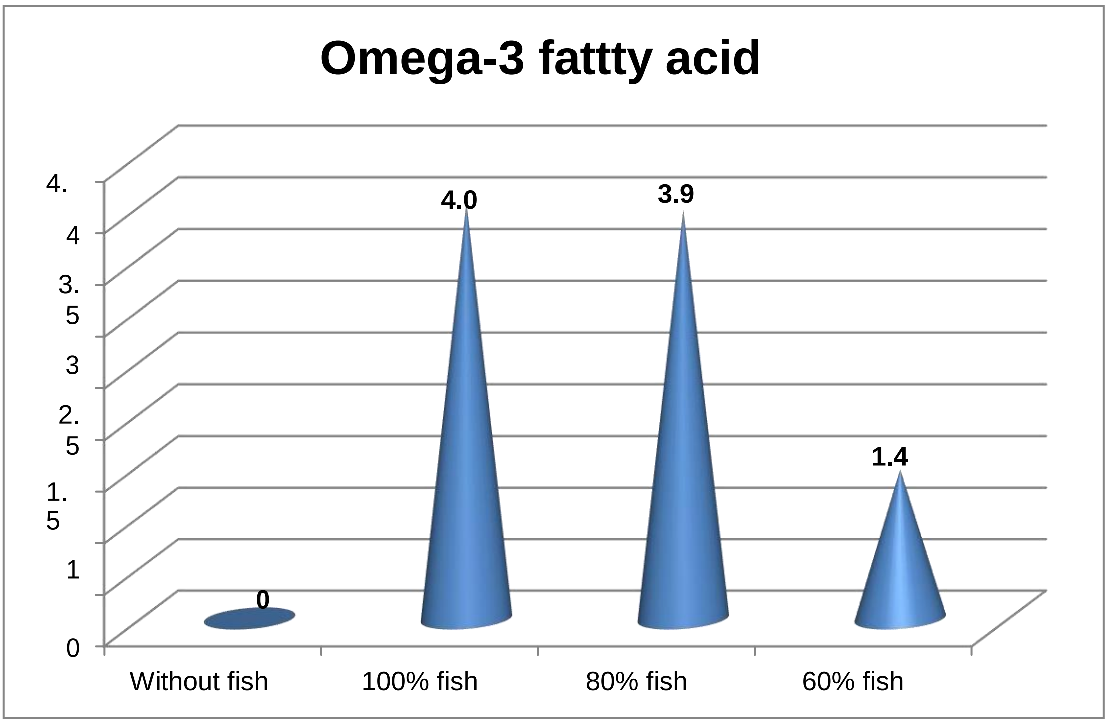

Figure 4.2(b): omega-3 fatty acid content of cake sample

4.3 Nutritional Compositions of prepared Biscuits

The results of proximate analysis on control biscuit sample and biscuit samples treated with varied percentages of fish oil have been presented in table 4.3. A one-way analysis of variance (ANOVA) was carried out for the biscuit samples. The study has revealed that Biscuits treated with fish oil have higher level of fat content than that of control biscuits prepared from 100% margarine and significant difference found among Control and the treated samples at $P < 0.05$. No significant difference has been found in the case of fiber and ash content. Significant difference found in the case of protein and carbohydrate content, although no significant difference found between S_1 (100% fish oil) and S_2 (80% fish oil) at $P < 0.05$.

Table 4.3: Proximate composition of margarine and fish oil treated biscuit samples

Quality parameters	Sample			
	S_0	S_1	S_2	S_3
Fat	12.70 ± 0.17^{c}	14.13 ± 0.15^{a}	13.9 ± 0.1^{a}	13.4 ± 0.17^{b}
Crude fiber	0.57 ± 0.58^{a}	0.467 ± 0.06^{a}	0.53 ± 0.058^{a}	0.53 ± 0.058^{a}
Moisture	5.70 ± 0.10^{a}	5.37 ± 0.12^{b}	5.47 ± 0.12^{ab}	5.33 ± 0.058^{cd}
Ash	1.07 ± 0.06^{a}	1.17 ± 0.58^{a}	1.20 ± 0.10^{a}	1.20 ± 0.00^{a}
Protein	11.00 ± 0.10^{c}	11.87 ± 0.12^{a}	11.7 ± 0.10^{a}	11.47 ± 0.06^{b}
Carbohydrate	68.97 ± 0.25^{a}	67.00 ± 0.17^{b}	67.20 ± 0.10^{b}	68.07 ± 0.12^{c}

S_o= Control; S_1=100% fish oil instead of margarine; S_2 =80% fish oil; S_3=50% fish oil.

Values in the table represent mean± SD and the presence of different superscript along a row indicates a significant difference and the same superscripts are not significantly different at $P < 0.05$.

4.4 Nutritional Compositions of prepared Cake

The results of proximate analysis on control cake sample and cake samples treated with varied percentages of fish oil have been presented in table 4.4. A one-way analysis of variance (ANOVA) was carried out for the cake samples. Although higher level of fat content has been found in sample C_1 (100% fish oil) than that of control sample, no significant difference found among the samples in terms of fat content at $P < 0.05$. No significant difference found in terms of crude fiber content and ash content among the samples, where significant difference found in terms of moisture, protein and carbohydrate content among the samples at $P < 0.05$.

Table 4.4: Proximate analysis of Cake

Quality parameters	Sample			
	C_0	C_1	C_2	C_3
Fat	15.00 ± 0.10^{a}	16.33 ± 0.15^{a}	15.63 ± 0.21^{a}	15.03 ± 0.15^{a}
Crude fiber	0.28 ± 0.03^{a}	0.25 ± 0.02^{a}	0.30 ± 0.02^{a}	0.24 ± 0.02^{ab}
Moisture	29.67 ± 0.15^{a}	29.00 ± 0.27^{b}	30.13 ± 0.31^{a}	28.07 ± 0.25^{c}
Ash	0.93 ± 0.06^{a}	0.77 ± 0.06^{ab}	0.80 ± 0.10^{a}	1.00 ± 0.10^{a}
Protein	14.07 ± 0.15^{a}	13.53 ± 0.21^{b}	13.97 ± 0.21^{ab}	14.57 ± 0.25^{a}
Carbohydrate	40.06 ± 0.23^{a}	40.11 ± 0.39^{ab}	39.17 ± 0.23^{b}	41.10 ± 0.70^{a}

C = Control; C_1= 100% fish oil instead of margarine; C_2 =80% fish oil; C_3 = 60% fish oil.

Values in the table represent mean± SD and the presence of different superscript along a row indicates a significant difference and the same superscripts are not significantly different at $P < 0.05$.

4.5 Hedonic Sensory score of prepared Biscuit and cake Samples

The sensory evaluation scores of biscuit and cake samples have been shown in table 4.5(a) and table 4.5(b) respectively. Statistical analyses were performed using the SPSS (version 20). Difference in sensory scores was detected using one way analysis of variances (ANOVA). A significance level of (P< 0.05) was used for the study.

Table 4.5(a): Hedonic Sensory score of prepared Biscuit sample

Parameters / Samples	Appearance	Texture	Aroma	Crunchiness	Taste	Overall Acceptance
S_0	8.47 ± 0.52^a	8.47 ± 0.52^a	8.93 ± 0.26^a	8.73 ± 0.45^a	9.0 ± 0.00^a	8.80 ± 0.41^a
S_1	8.33 ± 0.72^a	6.73 ± 0.70^c	6.47 ± 0.64^c	6.67 ± 0.62^c	6.60 ± 0.51^c	6.80 ± 0.56^c
S_2	8.53 ± 0.52^a	7.53 ± 0.52^b	7.93 ± 0.59^b	7.67 ± 0.49^b	8.13 ± 0.52^b	7.73 ± 0.46^b
S_3	8.60 ± 0.51^a	8.13 ± 0.52^a	7.93 ± 0.70^b	6.60 ± 0.51^b	8.00 ± 0.53^b	7.93 ± 0.59^b

S = Control; **S_1**= 100% fish oil instead of margarine; **S_2** =80% fish oil; **S_3** = 50% fish oil.

Values in the table represent mean± SD and the presence of different superscript along a column indicates a significant difference the same superscripts are not significantly different at $P < 0.05$.

After doing the ANOVA test for biscuit it has been interpreted that, there were no significant differences among the samples in terms of appearance. But sample S_3 ranks higher than sample S_2 and S_1 in case of overall acceptance. So, I should proceed with the sample S_3. But S_3 contains less amount of omega-3 fatty acid content (1.51%). That's why I have preceded with the sample S_2 which contains greater amount of omega-3 fatty acid (3.9%).

Table 4.5(b): Hedonic Sensory score of prepared Cake Sample

Parameters	Crust Characteristics		Crumb Characteristics		Taste	Appearance	Air Cell	Overall Acceptance
Samples	Color	Consistency	Texture	Aroma				
C_0	8.67±0.49[a]	7.87±0.64[a]	7.60±0.74[a]	8.73±0.46[a]	6.6±1.12[a]	7.87±0.6 4[a]	7.93±0.96[a]	7.4±0.51[a]
C_1	8.53±0.52[a]	7.13±0.92 [a]	7.2±0.94[a]	6.47±0.64[c]	6.2±1.13[a]	8.07±0.9 6[a]	8.07±0.70[a]	6.93±0.70[a]
C_2	8.47±0.52[a]	7.47±0.92[a]	7.2±0.56[a]	7.73±0.59[b]	7.07±0.70[a]	7.93±0.59[a]	7.73±0.96[a]	7.47±0.74[a]
C_3	8.53±0.52[a]	7.07±1.16[a]	7.27±1.01[a]	7.93±0.70[b]	6.73±0.96[a]	7.53±0.92[a]	8.0±0.76[a]	7.47±0.51[a]

C_o = Control; **C_1**= 100% fish oil instead of margarine; **C_2** =80% fish oil; **C_3** = 60% fish oil.

Values in the table represent mean± SD and the presence of different superscript along a column indicates a significant difference the same superscripts are not significantly different at $P < 0.05$.

After doing the ANOVA test for biscuit it has been interpreted that, there were no significant differences among the samples in terms of appearance in terms of Crust Characteristics, Taste, Appearance, Air cell and Overall acceptances. Significant difference found in term of aroma only. But sample C_2 rank higher than sample C_1 and same as sample C_3. So, I have proceeded sample C_2 because of its good acceptance and good level of omega-3 fatty acid.

Chapter V: Discussions

The current study has been completed in Chattogram Veterinary and Animal Sciences University and evaluated the fatty acid content specially omega-3 fatty acid in enriched bakery products with fish oil. All the important findings of this study with its limitation and recommendations have been discussed in this section.

The fatty acid analysis of Margarine and fish oil has revealed that fish oil of *Pangasius hypophthalmus* contains more omega-3 fatty acid, where margarine contains no omega-3 fatty acid. The fish oil also contains less SFA than margarine. The obtained result from fatty acid analysis of fish oil was almost similar to that results reported by Ho BT. et al. (2009), although the MUFA content was slightly greater (41.02%) than the article report (34.69%). The difference observed in these compositions of fish oil may be due to varietal differences, agro-ecological condition, feeding composition, methods of analysis etc.

The fatty acids analysis of prepared bakery products evidenced that the developed products with fish oil have greater content of omega-3 fatty acid than the products with only margarine. The omega-3 fatty acid content has gradually increased with increasing the percentage of fish oil. Omega-3 fatty acid content has been found highest (4.19% and 4.03% respectively) in the biscuit and cake samples in which 100% fish oil has been used but it was not much greater than the sample using 80% fish oil. It has also been found that, Percentage of omega-3 fatty acid content was lost in the biscuit and cake sample‟s oil, which was greater amount in fish oil (5.3%). This lost might occur due to the high temperature during preparation of biscuits and cake or due to oxidation (Santhanam AK., 2015). TFA content was gradually decreased with increasing the percentage of fish oil and ceased to zero in the sample prepared with 100% fish oil.

The results of sensory evaluation have showed that the use of fish oil in bakery products is acceptable. The values of overall acceptability have shown that highest mean value has been found for B_0 in biscuit sample and for C_2 and C_3 in cake sample. The value of overall acceptance of sample B_2, B_3 have been found near to the value of B_0. There were no significant differences ($P<0.05$) found in overall acceptability of cake but significant difference found in case of biscuits. Consumers preferred the fish oil containing cakes more than the control cakes. But, the fish oil containing biscuits were preferred less than the control biscuits. This is because fish flavor reduced the acceptability of the biscuit samples. Cocoa powder was used as an ingredient in cake samples which might cover the flavor of fish oil.

The current study shows that, the incorporation of fish oil is more satisfactory in cake than in biscuit. According to the consumer preferences and omega-3 fatty acid content it can be said that the cake sample containing 80% fish oil (fish oil: margarine:: 4:1) would be the best choice for using fish oil in bakery products.

Chapter VI: Conclusion

Some health transitions have happened worldwide during the last few years because of the life style change. This presumably is the cause of so many nontransmitted epidemic diseases. Obesity, diabetes, cardiovascular diseases and hypertension have steadily increased which undoubtedly lead to the life style and quality depreciation. It could also cause a huge number of early stage deaths. Therefore, in the community, health professional and the academic society there is a serious concern about this problem. It is more convenient to modify the existing products with locally available raw materials to add some additional attributes without changing sensory parameters. There are different ways that have been attempted to address this issue. For instance, creating food rich in omega-3 fatty acid with less saturated fatty acids is one of them. Most health organizations concur that 250–500 mg of omega-3 fatty acid is enough for adults to maintain overall health (FAO, 2008). Stephen NM., 2010 indicated that cooking and microwave heating are the better processing methods to retain the health beneficial omega-3 fatty acids in comparison to frying and canning. This work recommends oil of *Pangasius hypophthalmus* as a potential substitute for supplementation of omega-3 fatty acid to baked products. From the above parameters and data observed it may be concluded that oil of *Pangasius hypophthalmus* can be satisfactorily added to baked products to supplement omega-3 fatty acid. From nutritional point of view and organoleptic qualities, 80% fish oil (4:1 ratio of fish oil and margarine) incorporation found to be best among all the treatments. *Pangasius hypophthalmus* is available in all season and have a good percentage of fat. Considering the nutritional value and consumer preference, bakery products from fish oil will be superior to products from margarine.

Chapter VII

Recommendations and future perspective

Currently, bakery products are prepared using margarine or butter which are high in saturated fatty acid and trans fatty acid but contains no omega-3 fatty acid. This study indicated that oil of *Pangasius hypophthalmus* has the ability to add omega-3 fatty acid in bakery products.

There are other area can be investigated more as a completion of this study. Omega-3 fatty acid is prone to be degraded quickly in presence of light and oxygen and lose their sensory and nutritional values during storage. So, using suitable packaging methods and some antioxidents is another possibility to be researched.

In this study, *Pangasius hypophthalmus* was used for collecting fish oil. Other fish species especially sea fish oil which is rich in PUFA could also be used in future research to see the suitability in using bakery products.

The air cell quality of cake was not so good because of using hand mixing and little knowledge on pouring technique. So, improvement in dough mixing and pouring technique may increase the air cell quality. As in this study we didn"t use any improver for enhancing cake and biscuit quality, so taste and texture may improve with addition of commercially available improver compound.

Overall acceptance of bakery food products is usually influenced by its making method, ingredients and how they are baked. So, the modern instruments, standard procedure and quality control assessment should be ensured for consumer acceptance.

Industrial fish extraction method and proper use of the byproducts (fish muscles) need to be adapted to reduce the production cost of these modified bakery products.

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Appendix A: Rating Score for different hedonic attributes of Biscuits

Hedonic attributes		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean	STDV
	S_0	8	8	9	9	9	8	8	8	8	8	8	9	9	9	9	8.47	0.516
Color	S_1	8	7	9	9	9	9	8	7	9	8	8	8	9	9	8	8.33	0.724
	S_2	8	9	9	8	8	9	9	9	9	8	9	8	8	9	8	8.53	0.516
	S_3	9	9	9	8	9	9	8	9	8	9	9	8	9	8	8	8.6	0.507
	S_0	9	9	9	8	8	9	8	8	9	8	8	9	8	8	9	8.47	0.516
Texture	S_1	7	8	7	6	7	6	7	7	7	7	7	8	7	6	6	6.87	0.64
	S_2	8	7	7	8	7	8	8	7	8	8	7	7	7	8	8	7.53	0.516
	S_3	9	8	8	8	8	9	7	8	9	8	8	8	8	8	8	8.13	0.516
	S_0	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	8.93	0.258
Flavor	S_1	7	7	6	7	7	6	7	5	6	6	7	6	7	7	6	6.47	0.64
	S_2	8	9	9	8	8	8	8	7	8	7	8	8	7	8	8	7.93	0.594
	S_3	9	8	8	9	8	7	8	8	7	7	8	8	8	9	7	7.93	0.704
	S_0	9	9	9	9	9	8	9	9	8	9	9	8	9	9	8	8.73	0.458
Crunchiness	S_1	6	7	6	7	7	7	7	7	7	7	7	5	6	7	7	6.67	0.617
	S_2	7	8	7	7	8	8	8	8	7	8	8	8	8	7	8	7.67	0.488
	S_3	8	8	7	7	7	8	8	8	8	7	7	7	8	8	8	7.6	0.507
	S_0	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	0
Taste	S_1	7	7	6	6	7	7	7	6	7	7	6	7	7	6	6	6.6	0.507
	S_2	8	8	9	8	8	8	8	7	8	8	8	8	9	9	8	8.13	0.516
	S_3	8	9	9	7	8	8	7	8	8	8	8	8	8	8	8	8	0.535
	S_0	9	9	9	9	9	8	9	8	9	9	8	9	9	9	9	8.8	0.414
Overall	S_1	7	7	8	7	7	6	7	6	7	7	6	6	7	7	7	6.8	0.561
	S_2	8	8	8	7	7	7	8	7	8	8	8	8	8	8	8	7.73	0.458
	S_3	9	8	8	8	8	8	7	7	8	9	7	8	8	8	8	7.93	0.594

Here,

S = Control; **S_1**= 100% fish oil instead of margarine; **S_2** =80% fish oil; **S_3** = 50% fish oil.

<u>Hedonic Scale Used</u>: Like extremely = 9, Like very much = 8, Like moderately = 7, Like slightly = 6, Neither like nor dislike = 5, Dislike slightly = 4, Dislike moderately = 3, Dislike very much = 2, Dislike extremely = 1

Appendix B: Rating Score for different sensory attributes of cakes

Hedonic attributes		Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean	Std
		S_0	9	8	9	9	9	8	8	9	8	9	8	9	9	9	9	8.667	0.488
Crust	Color	S_1	9	8	9	9	8	9	8	8	9	8	8	9	9	9	8	8.533	0.516
Characteristi		S_2	8	9	8	8	8	9	9	9	9	8	9	8	8	9	8	8.467	0.516
		S_3	9	8	9	8	9	9	8	9	8	9	9	8	9	8	8	8.533	0.516
		S_0	8	7	7	8	8	8	9	8	7	9	7	8	8	8	8	7.867	0.64
	Consistancy	S_1	8	8	7	7	8	6	8	7	7	6	5	7	8	7	8	7.133	0.915
		S_2	8	9	7	6	7	8	8	8	9	6	7	8	7	7	7	7.467	0.915
		S_3	9	9	7	5	6	7	6	7	5	7	8	9	6	5	7	6.867	1.407
		S_0	8	8	8	7	8	7	5	7	7	8	8	7	8	8	9	7.533	0.915
	Texture	S_1	7	8	7	6	8	5	7	7	8	7	7	8	7	7	9	7.2	0.941
		S_2	7	7	7	8	7	7	8	7	7	8	6	7	7	7	8	7.2	0.561
Crumb		S_3	6	7	8	5	8	5	7	8	7	8	8	8	8	8	8	7.267	1.1
Characteristic	Flavor	S_0	9	9	8	9	9	9	9	9	9	8	8	9	9	8	9	8.733	0.458
		S_1	7	6	6	7	7	6	7	5	6	6	7	7	7	7	6	6.467	0.64
		S_2	9	7	7	8	8	8	8	7	8	7	8	8	7	8	8	7.733	0.594
		S_3	9	8	8	9	8	7	8	8	7	7	8	8	8	9	7	7.933	0.704
		S_0	8	9	8	9	9	8	9	8	8	9	7	7	7	6	7	7.933	0.961
Air cell		S_1	8	8	8	9	9	8	9	9	7	8	8	8	8	7	7	8.067	0.704
		S_2	8	8	7	7	7	8	7	7	9	9	9	9	7	6	8	7.733	0.961
		S_3	8	7	8	8	7	7	8	8	8	8	9	9	9	9	7	8	0.756
		S_0	8	8	8	6	6	7	6	7	8	7	5	6	7	5	5	6.6	1.121
Taste		S_1	7	7	7	7	7	8	5	5	4	5	7	6	5	6	7	6.2	1.146
		S_2	7	6	7	7	7	6	8	6	8	8	7	7	8	7	7	7.067	0.704
		S_3	7	7	5	6	8	8	8	7	7	6	7	7	7	5	6	6.733	0.961
		S_0	8	9	8	8	8	8	8	8	7	8	9	7	7	7	8	7.867	0.64
Appearance		S_1	8	9	8	7	8	9	9	9	7	8	9	6	7	8	9	8.067	0.961
		S_2	8	7	8	8	8	8	8	7	8	7	8	8	8	9	9	7.933	0.594
		S_3	8	8	7	8	7	8	7	7	8	9	9	8	6	7	6	7.533	0.915
		S_0	8	7	8	7	7	8	8	7	7	7	7	7	8	7	8	7.4	0.507
Overall Acceptance		S_1	8	7	8	7	7	7	6	6	6	7	6	7	7	7	8	6.933	0.704
		S_2	8	8	7	8	7	7	8	6	8	6	8	8	7	8	8	7.467	0.743
		S_3	7	8	8	8	7	7	8	7	7	7	7	7	8	8	8	7.467	0.516

Here,

S = Control; **S_1**= 100% fish oil instead of margarine; **S_2** =80% fish oil; **S_3** = 50% fish oil.

<u>Hedonic Scale Used</u>: Like extremely = 9, Like very much = 8, Like moderately = 7, Like slightly = 6, Neither like nor dislike = 5, Dislike slightly = 4, Dislikemoderately = 3, Dislike very much = 2, Dislike extremely = 1

Appendix C: Panelist work for Sensory evaluation

Sensory panelist doesn't do it-

- Eat, drink or smoke within 90 minutes prior to test
- Use gum, mints etc. flavored items within 30 minutes
- Wear perfume, cologne and fragrance item during test
- Talk and comment during evaluation
- Taste if you have a cold
- Share the product with others
- Taste if you have a lot of prior knowledge about the manufacturing which you maydislike

Sensory panelist should-

- Be attractively dressed and well groomed.
- Be tactful and concerned about the exhibitors and their feelings.
- Have a pleasant manner; smile; be prompt.
- Avoid consulting with spectators.
- Hide personal likes and dislikes.
- Be familiar with the products being judged.
- Take the time to get a general picture of the entries.
- Recognize quality standards.
- Don‟t give top placing if entries are notworthy.
- Make quick and firm decision.
- Offer compliments and constructive criticism.
- Be fragrance neutral.
- Participate regularly.
- Take time and focus during test.
- Follow the method and instruction precisely.
- Be confident in initial judgment.
- Take sensory evaluation seriously.
- Rest and cleanse your palate for next sample.
- Offer reasons for decisions, encourage the exhibitor to continue, to learnand to improve.
- Don‟t rule out unfamiliar ways of doing things if the results obtained are satisfactory. Judge the result that you see, rather than what "might" have been done.

Remember

- No food entry is so poorly done that it is not worthy of an encouraging comment
- No food entry is so well done that some improvement may not be made

Appendix D: Collection of fish oil

Fig: Fish collection and filleting

Fig: Fish fillet

Fig: Fish oil extraction

Fig: Extracted fish oil

Appendix E: Biscuits preparation

Weighing ingredients

Dough making

Shaping

Baking

Prepared Biscuits

Appendix F: Cakes preparation

Dry and wet ingredients

Ingredients mixing

Prepared dough

Baking

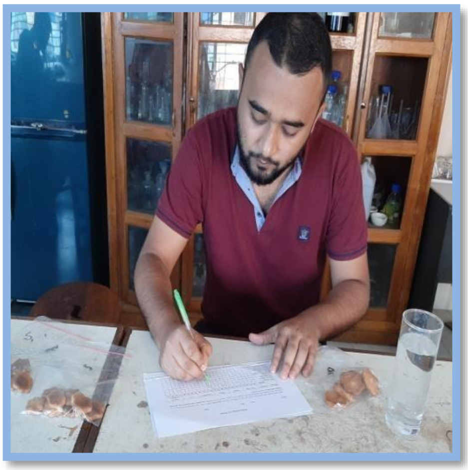
Prepared Cake

Appendix G: Prepared sample and GC instruments

Fig: Sample Preparation and GC analysis

Appendix H: Proximate analysis and Sensory evaluation

Different steps of Proximate Analysis



Sensory Evaluation by panelists

Brief Biography

Jiko Sen has passed the Secondary School Certificate (SSC) Examinations in 2008 with Grade Point Average (GPA) 5.00 followed by Higher Secondary Certificate (HSC) Examination in 2010 with GPA 4.50. He received the B.Sc. (Hon‟s) in Food Science and Technology in 2015 (held in 2016) from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, he is a candidate for the degree of MS in Food Chemistry and Quality Assurance under the Department of Applied Chemistry and Chemical Technology, CVASU.

He has immense interest to work in Nutritional supplementation and fortification of food like improving the fatty acids profile in bakery products, fast foods, processed food products etc and also in the area of public health perspective like heavy metal detection in egg, meat, meat products, water, milk, vegetables. He has also much interest to improve the nutritional status of malnourished children of under-developed and developing countries through working in the area of public health.