Chapter- 01: Introduction

1.1 Background

In present period consumers are more concerned to find safe and healthy foods that have quality and convenience (Guerrero *et al*., 2013; Hocquette *et al*., 2012).The busy life schedule has opened the way for the fast food industry in most parts of the world. The traditional way of cooking is over and the fast food are visible everywhere. Fast food or ready to eat food are the food, which are easy to make and can be eaten in an easy manner. Fast food is food that can be quickly prepared and served (Guerrero *et al*., 2013).The term fast food was recognized in a dictionary by Merriam in 1951.The consumption is common among those in a search of eating tasty ready to eat food (Mensah *et al*., 2002).The consumption and uses of fast foods have currently become a vital part of convenient food preparation patterns all over the world including Bangladesh.

The most popular fast foods in Bangladesh are Sandwich, Burger, Pizza, Chicken fries, French fries etc. The consumption of these ready-to-eat foods has been reported to be associated with serious health problems (Adams and Moss, 2010; FDA, 2000). Changes in life-style and food habits have been bringing about this evolving shift from traditional foods. Nowadays people consciously or subconsciously make the decision to invest their time more in actions other than food preparation (Bas *et al*., 2006).

Besides, consumers prefer to have certain readily available foods for which they do not possess the skill and or equipment to prepare. Frying oil is used again and again to prepare these fast foods which is very unhealthy for our body. Many researches have been done on the harmful effects of consuming degraded frying oil (Paul *et. al.*, 1997). It is very clear in present that frying oil are harmful to human health (Clark *et. al.*, (1991).

The disease causing agents spread by fast food is not only incapacitate large groups of people but also sometimes result in serious disability or even death. However, food habits adopted by populations may mitigate or increase the hazards (WHO, 2008). The above mentioned hazards can be minimized to a great extent simply by monitoring the physiological parameters and microbiological quality of food and creating awareness among the people about the fundamental principles of sanitation

and hygienic quality of food (Slanetz *et al.,* 2012). Food-borne diseases and problems relating to the sanitary and microbiological quality of foods continue to be of major interest and concern in Bangladesh and other countries of the world. New problems have been created due to recent development of the processing, handling of foods, changes of food habits and availability of convenience foods.

1.2 Significance of the studies

Current consumer's oriented publicity on freshness of fast foods available for sale in stores has created general impression that food is closely related to acceptability. The question of dating and labeling or retail packages has been considered essential features of acceptable quality assurance and quality control programs (Altekruse *et al*., 2007). However, in Bangladesh such programs are still undeveloped, as little information is available on the physiological parameters and microbiological aspects and keeping quality of commercially processed burger and sandwich are under the prevailing conditions. The elements that are used to prepare burger and sandwich e.g meat, raw vegetables, egg etc. are mostly not hygienic and well maintained (Altekruse *et al*., 2009). Therefore, the present study is designed to determine the microbial load of commercial fast food such as burger and sandwich with the quality of oil used during frying meat, eggs sold at retail outlets of fast food shops in Chattogram city, Bangladesh.

Governments throughout the world are attempting to improve the safety of the food supply but the occurrence of food borne disease remains a significant health issue in both developed and developing countries (WHO, 2011). The global incidence of food borne disease is difficult to estimate, but it has been reported that in 2015 alone 1.8 million people died from diarrheal diseases. A great proportion of these cases can be attributed to contamination of food and drinking water (WHO, 2011). In countries where fast food vending is prevalent, there is commonly a lack of information on the incidence of food borne diseases related to fast food. (WHO, 2011). Due to lack of proper knowledge and guidance on fast food vending, vendors prepare their foods in explicitly unhygienic and sanitary conditions. Consumers who depend on such food are more interested in its convenience and usually pay little attention to its safety, quality, and hygiene. Their lack of knowledge on the epidemiological importance and public awareness of fast food which hampers

precise scientific approach of the food safety problem. Fast food poses a great threat to the human body. It may cause food borne illness as well as many life threatening diseases. Food borne disease is referred to any illness resulting from the consumption of contaminated food, pathogenic bacteria that contaminate food as well as chemical or natural toxins (Angelillo *et al*., 2011).

However, there were limited studies on specific hazards posed by cooked oil and microorganisms of public health concern in fast food. Therefore, the present study was attempt to assess the quality of frying oil which is used for frying meat, egg, fish, vegetables to prepare fast food like burger, sandwich etc. and its health hazard in human body.

1.3 Aims and objectives of the study

- To determine acid value, saponification value, iodine value, peroxide value of frying oil used in preparing fast food.
- To characterize the microbial load and diversity of selected food borne microbes in fast food.

Chapter-02: Review of Literature

2.1 Importance of fast food in city life

In developing countries a large proportion of ready to eat foods are sold on the street (Mensah *et al.,* 2002). According to the Food and Agriculture Organization, 2.5 billion people worldwide eat fast food every day (FAO, 2007). Fast foods have already become a common feature of urban life (Mehta and Swinburn, 2012). The increasing poverty and time constraints to survive in developing countries indicate that the fast food phenomenon will only increase (Mehta and Swinburn, 2012) with the increasing pace of globalization and tourism. The safety of fast food has become one of the major concerns of public health, and a focus for governments and scientist to raise public awareness of food (FAO, 2007)

2.2 Nutritional benefits of fast food

The fast food industry plays an important role in developing countries in meeting the food demands of the urban dwellers (Latham, 2007). Fast foods play significant nutritional role for consumers, particularly for middle and low-income sectors of the population, who depend on fast foods for their main food intake (Mensah *et al*., 2002). FAO reports that fast foods provide nutritionally balanced diets, sufficient in quantity and presenting options for variety and choice for consumers, particularly from middle and low-income sectors of the population, who depend heavily on them (FAO, 2007). The contribution to the daily food intake of urban dwellers is scarcely quantified in energy and nutrients (Mehta and Swinburn, 2012). The foods have been shown to contribute a substantial proportion of the daily requirement of energy and protein (25%-50%) for adolescents attending schools (Oguntona and Kanye, 1995) and in Bangladesh, urban construction workers in Dhaka (Korir *et al*., 1998) and Calcutta street traders (Chakravarty and Canet, 1996). Their nutritional value however depends on the ingredients used and how they are prepared stored and sold.

2.3 Economic benefits of fast food

The fast food industry offers a significant amount of employment, often to persons with little education and training (Latham, 2007). Fast food provides a substantial amount of income for most vendors, with most of them earning an income above the official minimum wage while some of them earn twice or more of this amount (Mwangi, 2002).

Fast food operations sometimes involve the entire family in the procurement of raw materials, preparation and cooking of the meals (Mensah *et al*., 2002). The role of women in the sector is significant, as they control a large share of market activity and commodity trading (Mensah *et al*., 2002). Fast food vendors benefit from a positive cash flow, often evade taxation, and can determine their own working hours. In selling snacks, complete meals, and refreshments at relatively low prices, they provide an essential service to workers, shoppers, travelers, and people on low incomes. However, the people who depend on such foods are often more interested in its convenience than in questions of its safety, quality and hygiene (Mensah *et al*., 2002; Muinde and Kuria, 2005).

2.4 Oil used in fast food

Vegetable fat and oil are triglyceride which consist of a glycerol that attached to three molecule of fatty acid by an ester bond. Oils can be categorized in different ways: the type of plant it was extracted from, the level of refinement, the method of extraction etc. (Okorie and Nwachukwu, 2014). Majorly, vegetable oils are usually named by their biological source such as palm oil, palm kernel oil, soybean oil, olive oil etc. Vegetable oils are regarded as an important component of the human diet. It is essential because it supplies nutrients, improves flavor, aids in the absorption of vitamins, and provides source of energy for our bodies (Okorie and Nwachukwu, 2014), as well as fatty acids (Vidrih *et al.*, 2010).

Traditionally, the main source of vegetable oil in Bangladesh was home-grown and home processed mustard-seed. The market has changed dramatically in recent years with rapid growth in low-priced imports of soya and especially palm oil (Choudhury and Costa, 2012; FAO, 2013). Bangladesh's total vegetable oil market is 1.3 million MT of which about 55 % is consumed as food. Now mustard-seed-based oil comprises only 15 % of the total market, while soya constitutes about 35 %, palm 45 % and all other sources account for the residual 5% (FAO, 2013).The effect of the refining process on the properties of edible palm and soybean oils was studied by numerous researchers.

2.4.1 Palm oil

Palm oil is widely used in producing fast food in our country. According to Lin, (2011) palm olein, being the liquid fraction of palm oil, is clear at a room temperature of 28◦C.

Further fractionation of the olein produces a more unsaturated fraction, often called super-olein or double fractionated olein. These have higher levels of oleic and linoleic acids, ranging from 43–49% and 10–15% respectively, resulting in iodine values of 60–67. The content of unsaturated acids in superolein is about 59% compared to only 53% in the single fractionated olein. Palm stearin, the more saturated fraction of palm oil, is more variable in composition and thus in physical characteristics produced by partial crystallization.

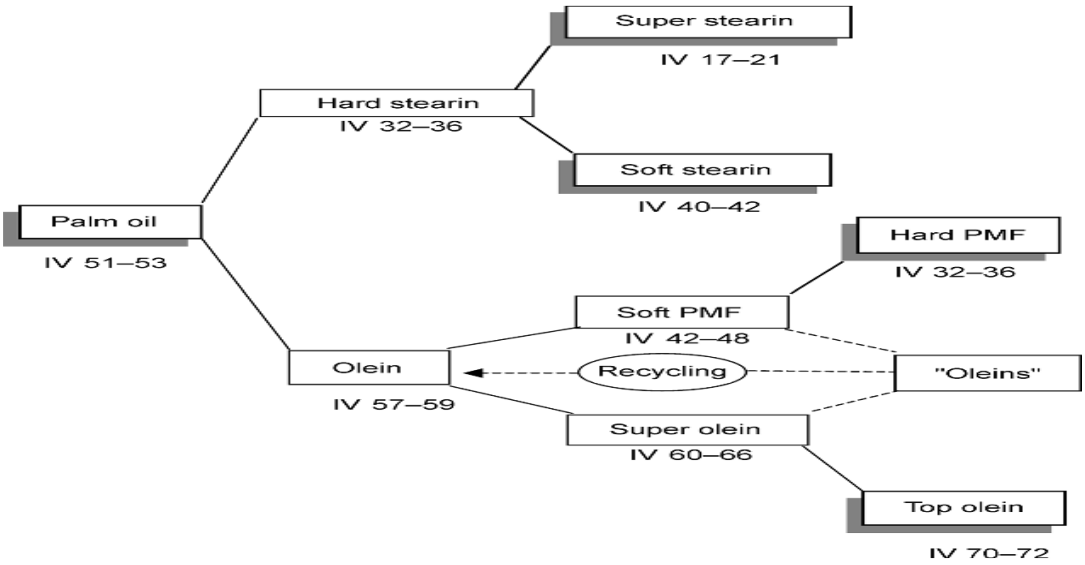

Figure2.1: Dry multiple fractionation of palm oil (adapted with permission from Deffense, 1995).

The palmitic acid content of the stearins varies from 49–68% and oleic content of 24–34% (Siew et al. 1990). Crude palm oil is rich in minor components such as carotenoids, tocopherols, tocotrienols, sterols, phospholipids, triterpene alcohols, squalene, aliphatic alcohols and aliphatic hydrocarbons (Goh *et al.,* 1985).

2.4.2 Soybean Oil

Soybean oil is produced in largest quantity and is the second traded oil. According to the investigations of (Cartter and Hopper, 1942), the oil content of the soybean seed was most specifically a varietal characteristic and the iodine number of the oil was about equally influenced by variety and climate. Temperature levels significantly influenced the calcium content of the seed produced by a given variety. Invariably high calcium content resulted when the soybeans were grown at high temperature. Total ash, phosphorus, and potassium content of the seed appeared to be influenced more by soil type and fertility than by variety or variation in climate.

Table 2.1: Average composition for crude and refined soybean oil.

Components	Crude oil	Refined oil
Triacylglycerols (%)	95–97	>99
Phospholipids (%)	1.5–2.5	0.003–0.045
Unsaponifiable matter (%)	1.6	0.3
Phytosterols	0.33	0.13
Tocopherols	0.15–0.21	0.11–0.18
Hydrocarbons	0.014	0.01
Free fatty acids (%)	0.3–0.7	<0.05
Iron(ppm)	1–3	0.1–0.3
Copper (ppm)	0.03–0.05	0.02–0.06

Source: Pryde 1980.

According to FAS/USDA (2015) report, import and domestic consumption of soybean oil in Bangladesh was 442000 and 530000 ton respectively in 2013/2014 while world production was about 45 million tones for the same period. The percentage of imported crude degummed soybean oil (CDSO) and crude soybean oil with respect to domestic consumption was about 80% during 2013/14 and the figure is consistently increasing.

2.5 Physical properties of palm and soybean oil

The physical properties of oils and fats are mainly referred to the melting and crystallization behavior with regards to the TAGs compositions. Melting and crystallization behavior of TAGs are very dependent on two factors; chemical structures and polymorphic behavior (Birker and Padley, 2014). The knowledge of palm oil physical properties is one of the key points for the development of palm oil fractionation technology, especially the crystallization selectivity (Soares *et al*., 2009). Crystallization selectivity is referred to the degree of compatibility of the different TAGs in the solid state. Most of the studies for fractionation are mainly focus on the effects of cooling conditions that affecting the crystallization selectivity ((Soares *et al*., 2009). Solid fat content (SFC), slip melting point (SMP), cloud point (CP), melting and crystallization properties by differential scanning calorimeter, crystals polymorphism studies by X-rays diffraction etc. are common methods used to determine the physical

properties of palm oil products of handling practices in fast foods in city area on human health remains unclear.

2.6 Chemical properties of palm and soybean oil

Soybean/Palm oil consists of mostly glyceridic materials with some non-glyceridic materials in trace amount (Chong, 1994). TAG is the most abundant glyceridic component in oil which comprises of triesters of high aliphatic acids or fatty acids, while monoacylglycerol (MAG) and diacylglycerol (DAG) are the minor glyceridic components in palm/soybean oil. TAGs are esters formed from glycerol acylation of three fatty chains, while acylation with one or two fatty chains formed partial acylglycerols. The hydrocarbon chains in the ester group, R could be varied in terms of carbon number and the chemical structure (bend structures for unsaturated fatty acids) (Chang, 2004). The physicochemical properties of the oil could be due to the types of fatty acid presence, and the manner in which fatty acids combine to form various TAG molecules (Naudet, 2006). In general, the hydrophobic nature of oil is due to the long fatty acid chains in the glyceridic materials.

2.7 Fatty acids composition of palm and soybean oil

For palm/soybean oil, the fatty acids composition falls within a very narrow range from twelve to twenty carbon number, with a balanced fatty acids composition between saturation and unsaturation (Berger, 2001). Table 1.1 shows the common name, systematic name, shorthand name of fatty acids presence in palm/soybean oil and its fatty acid composition. In most vegetable oils, the *sn*-2 position fatty acids of TAGs are preferentially occupied by unsaturated fatty acids such as oleic acid and linoleic acid. Saturated fatty acid (SFA) (e.g. palmitic acid) is found in the *sn*-2 position of animal fats TAGs for instance lard, tallow etc (Naudet, 2006)**.** Although palm oil contains high quantity of SFA, the *sn*-2 position fatty acids in the TAGs is preferably occupied by unsaturated fatty acids (mainly oleic acids) (Naudet, 2006**;** Kellens *et al*., 2007).

Table 2.2 Common name, Systematic name, Short hand name of fatty acids in oil and its fatty acid compositions

Common name	Systematic name	Short hand	FAC
Lauric	Dodecanoic	12:0	0.1-0.4
Myristic	Tetradecanoic	14:0	1.0-1.4

Palmitic	Hexadecanoic	16:0	40.9-47.5
Palmitoleic	Cis-9-Hexadecenoic	16:1ω7	0-0.4
Stearic	Octadecanoic	18:0	3.8-4.8
Arachidic	Eicosanoic	20:0	36.4-41.2
Oleic	cis-9-Octadecenoic	18:1ω9	9.2-11.6
Linoleic	cis-9, cis-12, Octadecadienoic	18:2ω6	0-0.6
Linolenic	cis-9, cis-12, cis-15-Octadecatrienoic	18:3ω3	0-0.4

Source: Sean, 2002; Siew, 2002

2.8 The chemical functions of ester groups in oil molecules

Glycerides or acylglycerols are made up of esters that attached to the glycerol backbone. In natural oils and fats, ester groups account for 90% to 96% of the overall molar mass of TAGs (Naudet, 2006). The ester groups in TAG play an important role in the chemical and physical properties of the oil. For saturated TAG, the fatty acids have straight chains that do not contain any special chemical functional group. Only carboxylic group in TAG molecules can act as the functional group for chemical reactions. The carbonyl/ester group of the TAGs can take place in many chemical reactions by inducing a special reactivity at the α-carbon (Ucciani and Debal, 1996). The nucleophilic behavior of the carbonyl carbon and the acidity behavior of hydrogen at the α-carbon (Rousseau and Marangoni, 2002). The electro negative oxygen pulls away electrons pair from the carbonyl carbon that lead to partial positive charge on the carbon. This partial positive charge carbon can easily attack by nucleophiles. In addition, the sp2 orbital of carbonyl carbon with flat plane structure may permit easier access of nucleophiles to the carbonyl carbon. The electronegative behavior of oxygen that attached to the carbonyl carbon may also increase the acidity of the hydrogens that attached to the α-carbon (Rousseau and Marangoni, 2002).These ester groups in the TAGs are responsible for several chemical reactions during modification of oils and fats, including alcoholysis, interesterification, reduction (hydrogenolysis), hydrolysis and saponification (Ucciani and Debal, 1996).

2.9 Reusing of frying oil

During frying, oils are degraded from thermal oxidation to form volatile and non-volatile decomposition products (Melton *et al.*, 2004). The chemical changes in frying oil also result in changes in the quality of fried food. The fatty acid composition of the frying oil is an important factor affecting fried food flavor and its stability; therefore, it should be low level of polyunsaturated fatty acid such as linoleic or linolenic acids and high level of oleic acid with moderate amounts of saturated fatty acid (Kiatsrichart *et al.,* 2010; Mehta and Swinbum, 2012). As a result, the quality of frying oil is important because of absorbed oil of fried products during deep frying. Soybean oil has a good nutritional profile due to high level of unsaturated fatty acid but less oxidative stability (Steenson and Min, 2000). Various method to improve oxidative stability of soybean oil has been developed and studied, for example, partial hydrogenation, fatty acid modification and blending with more saturated or monosaturated oils to reduce the amount of polyunsaturated fatty acids (Cuesta *et al.,* 1993; Hunter and Applewhite, 1991). Partial hydrogenation decreases polyunsaturated fatty acid but increases saturated fatty acid and trans-fatty acid to produce more stable frying oil. However, trans fatty acid may have adverse effects on cardiac health (Ascherio *et al.*, 2015). Palm oil, is considered value domestic oil in many countries of Africa and Asia. Nowadays, palm oil becomes useful for cooking because of very low cost. The advantages of the palm oil are not only economic. The high content in mono-unsaturated acids drop rates of LDL – bad cholesterol – all while maintaining the HDL or good cholesterol. It is also an excellent source vitamins A and E for Africa and Asia population (Zagre and Tarini, 2001). This work is to undertake a comparative study on the deterioration and the thermal stability of the two oils (soybean oil and refined palm oil) sold on the markets during cracklings of fritters.

2.10 Microbial food safety of fast foods

A lack of knowledge among fast food vendors about the causes of food-borne disease is a major risk factor (FAO, 1998). Poor hygiene, inadequate access to potable water supply and garbage disposal, and unsanitary environmental conditions such as proximity to sewers and garbage dumps further exacerbate the public health risks associated with street foods (Heaton and Jones, 2008). Traditional processing methods that are used in preparation, in appropriate holding temperatures and poor personal

hygiene of food handlers are some of the main causes of contamination of street-vended food (Mensah *et al*., 2002; Barro *et al.*, 2006). Recent studies have indicated that ready to eat foods and food preparation surfaces may be reservoirs for microbial contamination (Mankee *et al.*, 2005; Christison *et al.*, 2008). Fast foods in some Asian countries have been tested for various microorganisms of public health concern, including fecal coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* species and *Bacillus cereus* (FAO/WHO 2005). *Escherichia coli* and *Staphylococcus aureus* were recovered in a significant proportion of the food, water, hand and surface swabs tested in Bangladesh (FAO/WHO, 2005).

Chapter-03: Materials and Methods

3.1 Location of the experiment

The experiment was conducted from January to June 2019 in the laboratory of Department of Applied Chemistry and Chemical Technology, Chattogram Veterinary and Animal Sciences University (CVASU) and Food Testing Laboratory, Chattogram.

3.2 Collection of (experimental) repeated frying oil sample for laboratory analysis

Both refined bleached and deodorized soybean oil and pam oil were used in preparing fast food. These samples were collected randomly from ten local restaurants in Chattogram Metropolitan Area (CP Chawkbazar Branch, CP Kotowali Branch, Sadia's Kitchen, Red Chilly, Khadok 1, Cafe Milano, Face Food Restaurant, some restaurants of Finley Square and Sanmar Ocean city). A total of 20 (twenty) samples of repeatedly used frying oils were collected two times in a day (morning and evening) after frying chicken, beef, french fries etc. Different parameters of frying oil were determined in food chemistry laboratory to know the saponification value, free fatty acids, acid value and iodine value. The experiment was done using twenty samples with three replicates in each sample.

3.3 Collection and transportation of fast food sample for microbial analysis

Seven samples of different brands of burger and sandwiches available at retail stores were selected for this study. These brands belonged to Sadia's Kitchen, Red Chilly, Khadok 1, Cafe Milano, some restaurants of Finley Square and Sanmar Ocean city. Each brand of samples for this study was either wrapped or unwrapped. All these types are of two categories; pre-microwave oven (fresh sample from retail outlets) and post-microwave oven treated prior to sale or offered to customers. These burgers and sandwiches were prepared almost entirely using open hand and the ingredients used for their preparation did not receive any heat treatment or other processes. The ingredients were chicken and beef, egg, raw vegetables, tomato sauce and mayonnaise. The food items collected for this study were carefully handled and transported to the laboratory in aseptic condition and the samples were kept cool until they were subjected to bacteriological analysis.

3.4 Determination of parameters of oil used in producing fast food

3.4.1 Determination of Saponification Value

The saponification value is the number of mg of potassium hydroxide required to saponify 1 gram of oil. The oil sample is Saponified by refluxing with a known excess of alcoholic potassium hydroxide solution. The alkali required for saponification is determined by titration of the excess potassium hydroxide with standard hydrochloric acid.

Procedure

Saponification values of oil samples were determined according to AOAC (2016) official methods.

Three grams of each oil sample was weighed and taken into a round bottomed flasks; then 25mL of alcoholic potassium hydroxide solution was added into the flasks through a pipette. The blank determination was conducted along with the sample. Then it was boiled continuously for 1h under a reflux condenser and swirling the contents of the flask at frequent intervals. The excess alkali was determined while the solution was stilled hot by titration with the 0.5N HCl solution using 0.5 mL of the chosen indicator. Blank determination was carried out with the same KOH solution at the same time under the same conditions. The saponification value was estimated using the following equation:

$$\text{Saponification value} = \frac{56.1\times(b-a)\times N}{W}$$

Where, W is weight of sample that equals 2 grams, b is blank titer value, $a$is sample titer value, and N is 0.5 normality of HCl.

3.4.2 Determination of Acid Value

Acid Value is the number of milligram of KOH needed to neutralize the FFA present in1g of oil/Acid Value of oil samples (calculated as oleic acid percentage) were determined by standard method described in AOAC (2016) for Oils and Fats.

Procedure

All the frying oil samples were accurately weighed into a conical flask. Then 20-50 mL of 95% ethanol was added into the flask, and the mixture was neutralized with 0.1 N aqueous alkali using 0.5 mL of the 1% phenolphthalein indicator. Then the solution was boiled as hot as possible titration was carried out with 0.1 N aqueous alkali solutions. The solution was shaked vigorously during the titration. The first appearance of the red coloration that did not fade within 10 sec. was considered the end point and the volume

of the alkali required were recorded. The acid value was estimated using the following equation:

$$\text{Acid Value} = \frac{\text{N of alkali} \times \text{ml of alkali} \times 56.1}{\text{Wt. of sample (g)}}$$

3.4.3 Determination of Iodine Value

The iodine value of an oil/fat is the number of grams of iodine absorbed by 100g of the oil/fat, when determined by using Wijs solution. The iodine value is a measure of the unsaturation of the oil and is expressed in terms of the number of centigrams of iodine absorbed per gram of sample. Iodine value was determined according to AOAC (2016) Official Method 920.159. This determination was carried out in triplicate.

Procedure

All the frying oil samples were accurately weighed into a conical flask and then 25mL of carbon tetra chloride was added to each oil sample and content was mixed well. 25mL of Hanus reagent was added to the solution, swirled for proper mixing, and kept in the flask in dark for half an hour. After standing, 15mL of potassium iodide solution was added and then 100mL of distilled water was added into the mixture and 1mL starch indicator solution was added to the sample solution. Then, liberated iodine was titrated with 0.01N of sodium thiosulphate solution; then, at the end, blue color was formed and then disappeared after thorough shaking. The blank determination was carried in the same manner as test sample but without oil. The iodine value was estimated using the following formula:

$$\text{Iodine value} = \frac{(b - a) \times N \times 1.269 \times 100}{W}$$

Where,

b is blank titer value,

a is sample titer value,

N is normality of thiosulphate, and

W is weight of sample.

3.4.4 Determination of Peroxide Value

Detection of peroxide gives the initial evidence of rancidity in unsaturated fats and oils. Peroxide Value was determined according to AOAC Official Method 965.33.

Procedure

All the frying oil samples were accurately weighed into a conical flask and 50 ml of acetic acid in isooctane solution was added. The solution was swirled followed by the addition of freshly prepared saturated solution of KI (0.5 ml) and swirled again for 2 minutes. It was covered with aluminum foil and kept in the dark for 5 minutes. Thereafter, 30 ml of distilled water and 1 ml of 1% starch solution were added and the solution titrated with 0.01 N solution of sodium thiosulphate until the dark/black coloration disappeared. The blank titration was also conducted simultaneously.

$$\text{PV (meq/kg)} = \frac{N \times (Vs - v_b) \times 1000}{\text{Wt.of sample (g)}}$$

Where,

N = normality of sodium-thiosulfate,

V_s = sodium-thiosulfate consumed by sample (ml), and

V_b = sodium-thiosulfate consumed by blank (ml).

3.5 Preparation of samples for microbial analysis

Portions of all food samples were uniformly homogenized in food grinder. The grinder was pre washed with boiled water and then properly cleaned with 70% ethanol solution. Three of 50ml beaker was taken and 9 ml of normal saline, 9 ml of Selenite broth and 9 ml of alkaline peptone water was poured into each beaker. Quantity of 1gm grinded sample of each sandwich was taken aseptically with a sterile forceps and transferred carefully into each of the beaker. Thus 1:1 dilution of the samples was obtained. Then on using the vortex machine the mixture was mixed. Later different serial dilutions ranging from 10^{-2} to 10^{-6} were prepared according to the standard method (ISO, 1995). The enrichment media mixture (Alkaline peptone water and Selenite Broth) were put into 36^0C incubator for four hours. All the samples were studied in quantitative method.

3.6 Preparation of agar media plates and enrichment broth

Large and small agar plates were sterilized in hot air oven. Respective media recipe ingredients for agar medium were chosen and mixed in respective portion. For the agar medium the solvent that was used is distilled water. Then the agar media were mixed properly by applying heat and then were being autoclaved at 121^0C for 15 munities for

sterilization. The hot agar media was then poured into agar plates inside laminar airflow and cooled down to 20-25^{0}C. All the agar plates were kept at 4^{0}C for further use. Selenite Broth for enrichment of *Salmonella* and *Shigella* species were made.

3.7 Detection of the presence of total coliform count

For the determination of total coliform count 100 µl of each tenfold diluted sample was transferred to MacConkey agar plate. For each dilution six test plates containing MacConkey agar were used. The spread plate technique was done. All the agar plates were incubated at 37^{0}C temperature for 24 hours and 48 hours respectively. Pink or red and colorless colonies were observed on MacConkey agar media. The total coliform count was calculated according to ISO (1995). The results of the total coliform count were expressed as the number of organism or colony forming units per gram (CFU/gm) of sandwich sample.

3.8 Detection of the presence of *Salmonella* and *Shigella* Species

For the detection of the presence of Salmonella and Shigella spp 100 µl of enriched media and sample mixture from Selenite broth was transferred to one plate of xylose lysine deoxycholate (XLD) agar. It has a pH of approximately 7.4, leaving it with a bright pink or red appearance due to the indicator phenol red. The spread plate technique was done. The agar plate was incubated at 37^{0}C temperature for 24 hours and 48 hours respectively. Sugar fermentation lowers the pH and the phenol red indicator registers this by changing to yellow. Most gut bacteria, including *Salmonella*, can ferment the sugarx ylose to produce acid; *Shigella* colonies cannot do this and therefore remain red. After exhausting the xylose supply *Salmonella* colonies will decarboxylate lysine, increasing the pH once again to alkaline and mimicking the red *Shigella* colonies. Salmonellae metabolise thiosulfate to produce hydrogen sulfide, which leads to the formation of red colonies with black centers and allows them to be differentiated from the similarly coloured *Shigella* colonies. Other enterobacteria such as E. coli ferment the lactose and sucrose present in the medium to an extent that prevent pH reversion by decarboxylation and acidify the medium turning it yellow.

- *Salmonella* species: red colonies, some with black centers. The agar itself will turn red due to the presence of *Salmonella* type colonies.

- *Shigella* species: red colonies.

- Coliforms: yellow to orange colonies.

- *Pseudomonas aeruginosa*: pink, flat, rough colonies. This type of colony can be easily mistaken for Salmonella due to the color similarities.

The result of the total *Salmonella* and *Shigella* species count were expressed in colony formation, colony color and color changing capability of the media.

3.9 Detection of the presence of total fungal count

For the detection of total fungal count 100µl of each tenfold diluted sample was transferred to Sabouraud Dextrose Agar (SDA) agar plate. For each dilution six test plates containing SDA were used. The spread plate technique was done. All the agar plates were incubated at 25- 27^0 C temperature for 24 hours and 48 hours respectively. White, black, yellowish, branched colonies were observed on SDA media. The total fungal count was calculated according to ISO (1995). The results of the total fungal count were expressed as the number of organism or colony forming units per gram (CFU/gm) of sandwich sample.

3.10 Statistical Analysis

The obtained data were stored in Microsoft Excel 2013 and then significant differences were determined by one-way analysis of variance (ANOVA) and Duncan's multiple range test using R Statistical Software (version 3.4.1; R Foundation for Statistical Computing, Vienna, Austria). The significance level was set at the level of $p<0.05$.

Chapter-04: Results

The chief purpose of chemical properties of frying oil and microbiological examination of burger and sandwich are to give assurance that these fast food will be acceptable from the public health standpoint and that these fast food will be of satisfactory quality. The quality of frying oil in terms of chemical parameters such as acid value, saponification value, iodine value and peroxide value were analyzed.

4.1 Chemical properties of frying oil

4.1.1 Saponification value of frying oil

Alkali-reactive groups as well as the type of glycerides of fats and oils can be measured by the saponification value. The highest saponification value of frying soybean oil in the morning and evening period was found sample no. 9 (262.94mg KOH/g); (264mg KOH/g) respectively and lowest value in morning and evening in sample no.10 (203.43mg KOH/g); (212.12mg KOH/g). The highest saponification value indicates that the presence of low amount of glycerides with the lowest molecular weights (Muhammad *et al*., 2006). In the time of evening period the saponification value of frying oil was 264 mg KOH/g which was not the standard range of Codex Alimentarius Commission (190-209 mg KOH/g) (Table 4.5). Table. 4.1 shows that the collected samples of the used oil for frying were significantly differ ($P < 0.05$) in terms of saponification value.

Table 4.1: Saponification Value of frying oil collected in morning and evening

Sample ID	Category	Mean±SD	*p*-value
S1	Morning	207.50±0.28	<0.001*
	Evening	213.20±0.35	
S2	Morning	256.11±0.58	<0.001*
	Evening	262.11±0.42	
S3	Morning	260.98±0.54	0.13
	Evening	261.66±0.29	
S4	Morning	228.31±0.92	<0.001*
	Evening	233.64±0.23	
S5	Morning	231.28±0.21	<0.001*
	Evening	237.78±0.22	
S6	Morning	235.91±1.61	0.002*

	Evening	242.28±0.08	
S7	Morning	223.63±0.21	0.001*
	Evening	226.35±0.50	
S8	Morning	246.91±1.74	0.01*
	Evening	252.40±0.13	
S9	Morning	262.94±1.52	0.23
	Evening	264.20±0.07	
S10	Morning	203.43±0.26	<0.001*
	Evening	212.12±0.21	

Note: Means ± SD within the column bearing different superscripts are significantly different (P< 0.05). *Significant

4.1.2 Iodine Value of frying oil

Degree of unsaturation in oil and fat can be measured by the iodine value. Codex standard for iodine value is 50-55 g I_2 /100 g for oil. Here sample no. 6 had the highest iodine value (35.04 g I_2 /100 g); (34.40 g I_2/100 g) in morning and evening respectively indicating it was not good quality. Higher iodine value of oil also suggested that it was less stable in normal condition (Akinola *et al.*, 2010). The lowest iodine value was found in morning and evening in sample 4 at 16.41g I_2/ 100g; 15.40g I_2/ 100 g respectively which pointed out that frying oil was highly saturated.

Table 4.2: Iodine Value of frying oil collected in morning and evening

Sample ID	Category	Mean±SD	*p*-value
S1	Morning	17.52±0.16	0.07
	Evening	17.28±0.05	
S2	Morning	24.54±0.11	<0.001*
	Evening	23.42±0.09	
S3	Morning	19.57±0.12	<0.001*
	Evening	18.69±0.08	
S4	Morning	16.41±0.06	<0.001*
	Evening	15.40±0.08	
S5	Morning	29.48 ±0.05	0.01*
	Evening	28.25 ±0.46	
S6	Morning	35.04±0.07	0.01*
	Evening	34.40±0.26	

S7	Morning	18.76±0.25	0.002*
	Evening	17.74±0.03	
S8	Morning	19.09±0.06	<0.001*
	Evening	17.20±0.09	
S9	Morning	17.44±0.01	<0.001*
	Evening	16.23±0.09	
S10	Morning	20.48±0.06	<0.001*
	Evening	18.82±0.05	

Note: Means ± SD within the column bearing different superscripts are significantly different (P< 0.05). *Significant

4.1.3 Peroxide Value of frying oil

The rancidity level of fats and oils are measured by peroxide value. It give a concept about the oil quality. The result tabulated in Table 4.3 showed the range of peroxide value was between 14.25-30.53 meq O_2/kg for soybean oils. The standard range of Codex Alimentarius Commission of peroxidase value is up to 10. Here the result showed that the frying oil has no optimum quality to use further. The higher value of peroxide implied that all samples are more susceptible to rancidity (Brien, 2004).

Table 4.3: Peroxide Value of frying oil collected in morning and evening

Sample ID	Category	Mean±SD	*p*-value
S1	Morning	28.49±0.41	0.002*
	Evening	30.19±0.03	
S2	Morning	29.58±0.13	<0.001*
	Evening	30.53±0.08	
S3	Morning	21.945±0.06	<0.001*
	Evening	22.60±0.02	
S4	Morning	23.52±0.1	<0.001*
	Evening	26.72±0.14	
S5	Morning	17.17±0.23	<0.001*
	Evening	18.56±0.07	
S6	Morning	28.45±0.01	<0.001*
	Evening	30.04±0.15	
S7	Morning	14.25±0.36	0.02*
	Evening	15.09±0.20	

S8	Morning	23.14 ±0.14	0.13
	Evening	23.58 ±0.37	
S9	Morning	21.19 ±0.19	0.004*
	Evening	21.86 ±0.06	
S10	Morning	20.19 ±0.24	<0.001*
	Evening	22.12 ±0.09	

Note: Means ± SD within the column bearing different superscripts are significantly different (P< 0.05). *Significant

4.1.4 Acid Value of frying oil

The amount of free fatty acids present in fat or oil is pointed out with acid value. Higher acid value implies the lower quality of oil or fat and the oil or fat is going to be rancid. In this present study sample 4 had the highest value of acid (5.19 mg KOH/g); (5.67mg KOH/g) among all frying oils in morning and evening respectively. Sample 2 had the lowest value (1.60mg KOH/g) in morning whereas in evening it was (2.81mg KOH/g). Frying oil had the significantly (P < 0.05) higher acid value (5.19 mg KOH/g) which indicated the sample as a low quality oil (Atinafu and Bedemo, 2011). In evening sample number 4 had highest acid value which indicate that this frying oil is not suitable for normal use.

Table 4.4: Acid Value of frying oil collected in morning and evening

Sample ID	Category	Mean±SD	*p*-value
S1	Morning	4.04±0.06	0.001*
	Evening	5.25±0.23	
S2	Morning	1.60±0.01	<0.001*
	Evening	2.81±0.04	
S3	Morning	2.14±0.04	<0.001*
	Evening	2.85±0.11	
S4	Morning	5.19±0.03	<0.001*
	Evening	5.67±0.07	
S5	Morning	2.20±0.01	<0.001*
	Evening	4.12±0.18	
S6	Morning	1.83±0.005	0.01*
	Evening	2.47±0.23	
S7	Morning	3.80±0.03	0.004*

	Evening	4.31±0.15	
S8	Morning	2.88±0.01	0.001*
	Evening	3.55±0.14	
S9	Morning	4.14±0.03	0.02*
	Evening	4.54±0.18	
S10	Morning	2.14±0.02	<0.001*
	Evening	2.83±0.06	

Note: Means ± SD within the column bearing different superscripts are significantly different (P< 0.05). *Significant

4.1.5 Codex standards for soybean and palm oil.

Codex standards for soybean and palm oil is shown in Table 4.5

Table 4.5: Codex standards for soybean and palm oil.

Parameter	Soybean Oil	Palm oil
Saponification value (mg KOH/g)	189-195	190-209
Acid value (mg KOH/g)	0.6	0.6
Iodine value (g I_2/100g)	124-139	50.0-55.0
Peroxide value (meq O_2/kg)	up to 10	up to 10

Source: CAC, 1999

4.1.6 Quantitative microbial analysis of all the unwrapped fast food samples

From the table-4.6 has been also seen that sandwich from Red Chilly cafeteria and Cafe Milano shows the same amount of microbial growth. Vegetable sandwich from Cafe Milano shows the least microbial growth in all the agar media.

Table 4.6: Quantitative microbial analysis of all the unwrapped fast food samples

Sample	Types of ingredients	Colony forming unit/ml in qualitative specific agar media				
		NA CFU/ml	MAC CFU/ml	EMB CFU/ml	MSA CFU/ml	SDA CFU/ml
1	Chicken salad	3.7×10^6	NG	NG	1.2×10^6	1.7×10^6
2	Egg salad	2.1×10^6	NG	NG	2.0×10^4	10.5×10^6
3	Chicken salad	6.3×10^6	1.0×10^4	5.0×10^4	1.7×10^6	1.7×10^6

4	Beef	8.5×10^8	3.6×10^8	6.0×10^8	4.7×10^8	5.4×10^8
5	Egg salad	2.7×10^7	NG	NG	9.7×10^7	8.6×10^7
6	Chicken salad	6.6×10^5	4.0×10^5	1.4×10^5	3.6×10^5	8.6×10^5
7	Vegetable	2.3×10^4	NG	NG	1.3×10^4	2.1×10^4

NG= No Growth

Quantitative microbial analysis of all the unwrapped sandwich samples showed that the beef sandwich from the Khadok 1 shows the highest microbial count in all the agar media. Egg salad sandwich from Sadia's Kitchen shows the second highest growth though it didn't show any growth in McConkey and EMB agar plate.

Chapter-05: Discussion

5.1 Saponification value of frying oil

Saponification value of oil is the best indicator to know the alkali-reactive group and type of glycerides of fats and oils. Optimum level of saponification value usually 189-195 (mg KOH/G) oil is desired by the nutritionist. The highest saponification value of frying oil in the morning period was found in sample no. 9 (262.94mg KOH/g) and lowest value in morning sample no.10 was (203.43mg KOH/g). In evening the highest value was in sample 9 (264mg KOH/g) and lowest in sample 10 (212.12mg KOH/g). The highest saponification value indicates that the presence of low amount of glycerides with the lowest molecular weights (Onyeke *et al*., 2002). The standard range of Codex Alimentarius Commission (190209 mg KOH/g) is (189-195mg KOH/G). The collected frying oil sample had high saponification value that indicated the repetition of oil many times.

5.2 Peroxide value of frying oil

Changes in peroxide value (PV) for collected samples of used frying oil were significantly increased in frying process of fast food. Kabashi, 2000 was noted that the rate of formation of peroxides was faster in used soybean oil compared with other vegetable oils. The result tabulated in Table 4.3 showed the range of peroxide value was between 14.25-29.58 meq O_2/ kg for frying oils in morning and in evening it was between 15.09-30.53 meq O_2/kg, these results were similar when compared to (Serjouie, 2010) , (Kabashi, 2004) and (Shakak *et. al.*, 2015), who found that the (PV) after frying of chips was 8 mg O_2/kg, 8.9 to 21.8 mg O_2/kg and 12 mg O_2/kg respectively. The higher value of peroxide implied that all samples are more susceptible to rancidity (Brien, 2004). The PV of oil reflects the quality of the food ingredient that fried with such oil. The standard range of Codex Alimentarius Commission of peroxidase value is upto 10. Here the result showed that the frying oil has no optimum quality to use further.

5.3 Iodine value of frying oil

From table 4.2 it was observed that the highest iodine value in the morning was (35.04g I_2 /100g) and in evening the value was (34.40g I_2/100g). Codex standard for iodine value is 50-55 g I_2 /100g for palm oil. Changes in iodine value for the collected samples

of the used frying oil was significantly decreased. Higher iodine value of oil also suggested that it was less stable in normal condition (Akinola *et al.*, 2010). Decrease in IV of oil by frying process was reported by (Khattaab *et al.*, 1974) for groundnut, cotton seed and sesame oils. The lowest iodine value was found in sample 4 at 15.40g I_2/ 100 g which pointed out that frying oil was highly saturated.

5.4 Acid value of frying oil

Acid value helps to know the amount of free fatty acids present in fat or oil. Higher acid value implies the lower quality of oil or fat and the oil or fat is going to be rancid. Table 4.4 showed that the collected samples of soybean oil used in frying fast food had the highest acid value of (5.19 mg KOH/g) among all frying oils in morning and sample 2 had the lowest value (1.60 mg KOH/g). During evening period sample 4 had the highest value of acid (5.67 mg KOH/g) among all soybean oils and sample 2 had the lowest value (2.81mg KOH/g). Frying oil had the significantly ($P < 0.05$) higher acid value (5.19 mg KOH/g) which indicated the sample as a low quality oil (Atinafu and Bedemo, 2011). These results were more than those reported by Mohammed (2013) and Kabashi (2000) which were 0.29% and from 0.22% to 0.37% respectively.

5.5 Microbiology of fast food

Food is one of the basic needs of the human body. Here the fast food plays a very important role in urban human life. All these fast food may cause food borne diseases e.g. food poisoning, diarrhea, cholera, typhoid, infection in gastrointestinal tract etc. In this study a total of seven burger and sandwich samples from different fast food shop were tested for quantitative test. Quantitative microbial analysis of beef sandwich from Khadok 1 shows the highest number of bacterial colony count in all of the agar media. The number is around 6.0×108 CFU/ml in table 4.6. The egg salad sandwich from all the fast food shops shows moderate growth, around 3.7×106 CFU/ml on average in most of the agar media. It has been found that most of the egg salad sandwich shows no growth in McConkey agar and eosin methylene blue agar plate which shows there is no growth of *E. coli* and Gram negative pathogenic organism in the agar plate. On the other hand some egg sandwich shows the growth of *Staphylococcus* species which may cause food poisoning in the human body. The vegetable sandwich from Cafe Milano showed the lowest growth of microorganism in all the agar plates which is on average 2.3×104 CFU/ml. This sample was also free from most of the pathogenic bacteria but on the other hand it contains *Staphylococcus* species which may cause food

poisoning. In another study (Khan *et al*., 2002) observed high percentage of Coliform species from the raw vegetables (9.2×106 /100g) samples. In the present study *Klebshiella* and *Shigella* species were found but the origin of these in the sandwich sample and the pathogenicity was not determind. The *Saccharomyces sp* may come from the dough of the bread.

5.6 Effect of frying oil on public health hazard

The nutritional aspects of consuming deteriorated frying fats and oils is a growing concern in today's world. In Europe, many nations have already established laws, regulations, or recommendations against the use of deteriorated frying oils. Many researches have been done on the harmful effects of consuming degraded frying oil, currently (Paul et al., 1997). Oxidation products of frying oil can cause harmful effects. Peroxide formation is a major point of concern from a toxicological point of view because it destroys Vitamin E in the body membranes (Viola et al., 1988). Peroxide and acid value is important for the measurement of frying oil deterioration. Peroxide value of fresh oils are usually less than 10 meq/kg; when the peroxide value is between 30 and 40 meq/kg, a rancidity of oil is usually developed (Godswill, Awuchi Chinaza et al., 2008).The peroxide value (PV) is the most common parameter used to assess the oxidative state of oils. Freshly refined oils usually have a Peroxide value lower than1 meq/kg oil, and oil is considered to be rancid at a peroxide value above 10 meq/kg oil, (Gunstone, et al., 2008). Acid value of fresh oils are usually less than 0.6 mg KOH/g ; when the acid value is more than 2.5 mg KOH/g , acceptability of oil is lost (Paul et. al., 1997). According to results of this study, acid and peroxide values of frying oils are higher than safe levels in all fast food restaurants that used in morning and evening time (Sebastian et al., 2014) collected fresh, inuse, and discarded oil samples from restaurants in Toronto, Canada, and found a high rate of damaged oil as evidenced by high peroxide value (ranged from 3.3 to 48.1 meq/kg compared to 2.9 to 141 meq/kg in the current study), free fatty acid concentration, and p-anisidine. The same findings were reported from restaurants in Taiwan (Liao LM et al., 2005).

Chapter-06: Conclusion

The edible soybean oils are the most preferred and cheap available type of vegetable oils with their respective qualities and health benefits. The results of this study concluded, oils that were used in frying fast food degraded normal quality parameters in terms of saponification value, iodine vale, peroxide value and acid value. The quality of frying oil declined than normal in morning whereas in evening its chemical parameters become worst in the concept of Codex Alimentarius Commission. Repeated use of frying oil may not have proper amount of fatty acid and by destroying ester bonds in oil the quality deteriorate from normal level. Repeated use of oil for frying should be strictly prohibited.

In microbial study all agar media showed the highest number of bacterial colony count. Microbial contamination may also come from environment, meat, salad or the dough of the sandwich sample. Overall, results of this study revealed that in many fast food restaurants in Chattogram city, oils that are in use for frying contain high levels of toxic materials, which have harmful effect on human health.

Chapter-07: Limitations and recommendations

7.1 Limitations

In case of frying oil

1. Only edible in-use frying oil was analyzed but fresh and discarded frying oil parameters did not estimated.
2. Sample size was small.
3. Physical property like color, viscosity, specific gravity was not analyzed.
4. Only morning and evening sample had been tested.

In case of microbial analysis

1. Qualitative microbial analysis was not included here
2. More sample size was needed

7.2 Recommendations and future perspective

1. Repeated use of frying oil should be banned on the hazardous point of view
2. Repeated use of frying oil in evening period is more deteriorate than morning
3. Remaining parameters should be analyzed in future
4. Further studies should be performed to evaluate the nutritional value, heavy metals profile, antimicrobial activities of several branded oils.
5. Procurement of raw materials of the best possible microbiological quality.
6. Appropriate processing of fast foods should be maintained.
7. Microwave oven treatment prior to serving to consumer.
8. Quality packaging should maintain to keep foods fresh and get rid of health risk factors.

References

Adams MR, Moss MO. 2010. Food Microbiology. Royal Society of Chemistry, Cambridge. 13: 479.

Akinola FF, Oguntibeju OO, Adisa AW, Owojuyigbe OS. 2010. Physico-chemical properties of palm oil from different palm oil local factories in Nigeria. Journal of Food, Agriculture & Environment. 8 (3&4):264-9.

Alimentarius C. 2013. Standard for Named Vegetable Oils - Codex Stan 210-1999: 16.

Altekruse SF, Street DA, Fein SB, Levy AS. 2007. Consumer knowledge of food borne microbial hazards and food-handling practices. Journal of Food Protection. 59: 287–294.

Altekruse SF, Yang S, Timbo BB, Angulo FJ. 2009. A multi-state survey of consumer food-handling and food consumption practices. American Journal of Preventive Medicine. 16: 216–221.

Angelillo IF, Viggiani NM, Greco RM, Rito D. 2011. HACCP and food hygiene in hospital: knowledge, attitudes, and practices of food services staff in Calabri, Italy. Infection Control Hospital Epidemiology. 22:1-7.

AOAC. 2016. Official Methods of Analysis. 20th Edition, Association of Official Analytical Chemists. Washington, DC., USA.

Ascherio A, Hennekens JE, Buring C, Master MJ, Stampfer WC. 2015. Trans-fatty acid intake and risk of myocardial infraction. Circulation. 89: 94-101.

Azanza PV, Gatchalian CF, Ortega M. 2000. Food safety knowledge and practices of street food vendors in a Philippines university campus. International Journal of Food Science Nutrition. 51: 235-246.

Badrie N, Joseph A, Chen A. 2004. An observational study of food safety practices by street vendors and microbiological quality of street-purchased hamburger beef patties in Trinidad, West Indies. International Journal of Food Safety. 3: 25-31.

Barro N, Bello AR, Itsiembou Y, Savadogo A, Nikiema AP. 2007. Street vended foods improvement: contamination mechanism and application of food safety objective strategy: critical preview. Pakistan Journal of Nutrition. 6: 1-10.

Bas M, Ersun AS, Divan G. 2006. The evaluation of food hygiene knowledge, attitudes, and practices of food handlers in food businesses in Turkey. Food Control. l17: 317–322.

Berger KG. 2001. Palm oil. Structured and Modified Lipids. New York: Marcel Dekker Inc. 119-153.

Birker PJ and Padley FB. 2014. Physical properties of fats and oils. Recent Advances in Chemistry and Techonology of Fats and Oils. New York: Elsevier. 1-11.

Cartter JL, Hopper JL. 1942. Influence of variety, environment, and fertility on the chemical composition of soybean seed. US. Department of Agricultural Technology Bull. No.787.

Chakravarty I, Canet C, 1996. Street foods in Calcutta. Food Nutrition and Agriculture. 17(18):30-37.3.

Chang MK, Abraham G, John T. 2004. Production of cocoa butter-like fat from interesterification of vegetable oils. Journal of Oil Chemical Society. 67. 832-834.

Chong LD, Traynor-Kaplan A, Bokoch GM and Schwartz MA. 1994. The small GTP-binding protein Rho regulates a phosphatidylinositol 4-phosphate 5-kinase in mammalian cells. Cell biology. 79(3): 507-513.

Choudhury RA, Costa MC. 2012. Impact of Government Law on edible oil supply Chain in Bangladesh perspective. International Journal of Supply Chain Management. 1(1): 33–38.

Christison CA, Lindsay D, Von Holy A. 2008. Microbiological survey of ready-to-eat foods and associated preparation surfaces in retail delicatessens, Johannesburg, South Africa. Food Control. 19(7): 727-733.

Clark WL and Serbia GW. 1991. Safety aspects of frying fats and oils, Food Technol. 45(2): 84.

Cuesta C, Sanchez-Muniz S, Lopez-Varela R. 1993. Thermoxidative and hydrolytic changes in sunflower oil use in frying with a fast turnover of fresh oil. Journal of Oil Chemistry Society .70: 1069-107

FAO/WHO.2005. Informal food distribution sector in Africa (street foods): importance and challenges CAF 05/4

FAS/USDA. 2015. Foreign Agricultural Service/USDA office of Global Analysis. Oilseeds: World Markets and Trade.

FDA, 2000. Food Code Recommendation of the United States Public Health Service. Food and Drug Administration, Washington, DC., USA.

Food and Agriculture Organization of the United Nations (FAO). 2013. FAO STAT Database, Commodity balances. Rome. 13.

Ghobadi and Saeed 2018. "Acid and Peroxide Values and Total Polar Compounds of Frying Oils in Fast Food Restaurants of Shiraz, Southern Iran." International Journal of Nutrition Sciences 3(1): 25-30.

Godswill, Awuchi, Chinaza. 2018. "Effects of repeated deep frying on refractive index and peroxide value of selected vegetable oils.

Goh EM and Timms RE. 1985. Determination of mono-and diglycerides in palm oil, olein and stearin. Journal of the American Oil Chemists' Society. 62(4): 730-734.

Guerrero A, Valero MV, Campo MM, Sañudo C. 2013. Some factors that affect ruminant meat quality: from the farm to the fork. Review. Acta Scientiarum. Animal Science. 35(4): 335-347.

Gunstone FD. 2008. Oils and fats in the food industry (1st ed.). Wiley-Blackwell Chapter 8.

Heaton JC, Jones K. 2008. Microbial contamination of fruits and vegetables and the behaviour of enteropathogenic in the phyllosphere. The Applied Microbiology. 104(3): 613-26.

Hocquette JF, Botreau R, Picard B, Jacquet A, Pethick DW. 2012. Opportunities for predicting and manipulating beef quality. Meat Science. 92(3): 197-209.

I.S.I. Handbook of Food Analysis (Part XIII)-1984 Page 67/ IUPAC 2.201(1979) / I.S: 548 (Part 1) – 1964, Methods of Sampling and Test for Oils and Fats/ ISO 660:1996 Determination of acid value and acidity).

Kabashi E, Agar JN, Taylor DM, Minotti S, Durham HD. 2004. Focal dysfunction of the proteasome: a pathogenic factor in a mouse model of amyotrophic lateral sclerosis. Journal of neurochemistry. 89 (6):1325-35.

Kellens M, Gibon V, Hendrix M, Greyt WD. 2007. Palm oil fractionation. Eur. Journal of Lipid Science Technology. 109. 336-349.

Khan AA, Kim SJ, Paine DD, Cerniglia CE. 2002. Classification of a polycyclic aromatic hydrocarbon-metabolizing bacterium, Mycobacterium sp. strain PYR-1, as Mycobacterium vanbaalenii sp. nov. International Journal of Systematic and Evolutionary Microbiology. 52(6):1997-2002.

Khan NA, McCaskey TA. 1972. Incidence of salmonellae in commercially prepared sandwiches for the vending trade. Department of Animal and dairy Sciences, Auburn University Agricultural Experiment Station, Auburn, Alabama 36830.

Korir SC, Imungi JK, Muroki NM. 1998. Proximate chemical composition of street foods and their energy and protein contribution to the nutrition of manual workers in Nairobi. Ecology of food and nutrition. 37(2):123-133.

Latham MC. 2007. Human nutrition in the developing world (No. 29). Food & Agriculture Organization.

Liao LM, Huang SY, Lu YF. 2005. A survey study on frying oil of restaurants in the Taipei area. Food service Research. 16:60–80.

Lin SW. 2011. 2 Palm Oil. Vegetable oils in food technology: Composition, properties and uses. Journal of Food Science. 2(1): 25.

Mahale DP, Khade RG, Vaidya VK. 2008. Microbiological analysis of street vended fruit juices from Mumbai city, India. Journal of Food Safety. 10(9):31-34.

Mankee A, Chin AL, Indalsingh R, Khan R, Mohammed F, Rahman R, Sooknanan S, Adesiyun AA. 2005. Microbial quality of doubles sold in Trinidad. Food microbiology. 22(6): 601-607.

Mehta U. and B. Swinburn, 2012. A review of factors affecting fat absorption in hot chips. Food Science Nutrition. 41: 133-154.

Melton L, Jafar SD, Sykes MK. Trigiano. 2004. Review of stability measurements for frying oils and fried food flavor. Journal of Oil Chemistry Society. 71: 1301-1308.

Mensah P, Manu D, Owusu-Darko K, Ablordey. 2002. Street foods in Accra, Ghana: how safe are they? Bulletin of World Health Organization. 80: 546-554.

Muinde OK and Kuria E. 2005. Hygienic and sanitary practices of vendors of street foods in Nairobi, Kenya. African Journal of Food, Agriculture, Nutrition and Development. 5(1).

Mwangi I, Berkley J, Lowe B, Peshu N, Marsh K. 2002. Acute bacterial meningitis in children admitted to a rural Kenyan hospital: increasing antibiotic resistance and outcome. The Pediatric infectious disease journal. 21(11):1042-1048.

Naudet M. 2006. Main chemical constituents of fats. A Comprehensive Treatise (Volume 1). Paris: Lavoisier Publishing. 67-116.

Oguntona CR and Kanye O. 1995. Contribution of street foods to nutrient intakes by Nigerian adolescents. Nutrition and Health. 10(2):165-171.62-266.

Okorie SU, Nwachukwu CN. 2014. Comparative evaluation of quality characteristics of oils extracted from some selected legumes and cereal. IOSR Journal of Environmental Science, Toxicology and Food Technology. 8(7): 66-69.

Onyeke EN and GN, Acheru. 2002. Chemical composition of selected Nigerian oil seeds and physicochemical properties of the oil extracts. Food Chemistry. 77: 431-437.

Paul S, GMittal and Chinnan.1997. "Regulating the use of degraded oil/fat in deep-fat/oil food frying." Critical reviews in food science and nutrition 37 (7): 635-662.

Rousseau D. and Marangoni A. 2002. Chemical interesterification of food lipids: Theory and practice. Food Lipids Chemistry, Nutrition, and Biotechnology .New York: Marcel Dekker Incorporation .301-333.

Sebastian A, Ghazani SM, Marangoni AG. 2014. Quality and safety of frying oils used in restaurants. Food Research International. 64:420-30.

Siew W L.2002. Palm oil. Vegetables Oils in Food Technology Composition, Properties and Uses. United Kingdom: Blackwell Publishing. 59-97

Slanetz LW, Chichester CO, Gaufin AR, Ordal ZJ. 2012. Microbiological Quality of foods. Academic Press. New York.

Soares ASD, da Silva RC, da Silva, KC, Lourenço MB, Gioielli, LA.2009. Effects of chemical interesterification on physicochemical properties of blends of palm stearin and palm olein. Food research international. 42(9):1287-1294.

Steenson DF and Min DB 2000. Effects of b carotene and lycopene thermal degradation products on the oxidative stability of soybean oil. Journal of Oil Chemistry Society. 77(11): 1153-1160.

Steenson DF, Min DB. 2000. Effects of carotene and lycopene thermal degradation products on the oxidative stability of soybean oil. Journal of Oil Chemical Society. 77(11): 1153-1160.

Ucciani E and Debal A. 1996. Chemical properties of fats. Oils and Fats Manual (volume 1). Paris: Lavoisier Publishing. 325-443.

Verhe R, Verleyen T, Huyghebaert A, Dewettinck K, DeGreyt W. 2000. Tocopherol oxidation during deodorization, Proc. of the New Trends in Edible Oil Refining.

Vidrih R, Vidakovic S, Abramovic H. 2010 Biochemical parameters and oxidative resistance to thermal treatment of refined and unrefined edible oils. Czech Journal of Food Science. 28(5): 376-384.

Viola P and Bianchi A. 1988. Clinical aspects of the frying of foods, in Frying of Food, Principles, Changes, New Approaches, Varela, G., Bender, A. E., and Morton, I. D., Eds., Ellis Horwood, Chichester, England. 129.

Walczak E, Czaplinska W, Wilgosz M, Wojtatowicz M, Robak. 2007. RAPD with microsatellite as a tool for differentiation of Candida genus in yeast isolated in brewing. Food microbial. 24:305-312.

WHO, 2008. Microbiology aspects of food hygiene. Technical Report, Series No.399.World Health Organization (WHO), Geneva.

WHO.2011. Knowledge =prevention. The five keys to safe food.18thApril 2011. Food safety and zoonoses http://www.who.int/foodsafety/en/

Zagre NM and Tarini A. 2001. Non refined palm oil: beyond its role for fighting vitamin deficiency. Journal of Medical Nutrition. 37(2): 53-58.

Zulkurnain M, Lai OM, Latip RA, Nehdi IA, Ling TC, Tan CP. 2012. The effects of physical refining on the formation of 3-monochloropropane-1, 2-diol esters in relation to palm oil minor components. Food Chemistry. 135(2): 799–805.

APPENDIX-I

Phosphate buffered saline (PBS)

PBS was prepared by dissolving 8.0 g of NaCl, 0.2 g of KCl, 1.44 g of $Na_2 HPO_4$ and 2.0 g of $KH_2 PO_4$ in 800 ml of distilled water. The pH was adjusted to 7.4 with HCl. The final volume was adjusted to 1 liter by distilled water. The solution was sterilized by autoclaving and was stored at room temperature.

Media composition

The composition of the media used in the present study has been given below. Unless otherwise mentioned, all the media were autoclaved at 121^0 C for 15 min.

Nutrient Agar (Himedia, India)

Ingredients	**Amounts (g/L)**
Peptic digest of animal tissue	5.0
Beef extract	1.50
Sodium chloride	5.0
Yeast extract	1.50
Agar	15.0

Mac Conkey agar (Oxoid, England)

Ingredients	**Amount (g/L)**
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.075
Agar	12.0
Peptone	5.0
Sodium chloride	5.0

Eosine methylene blue agar (Oxoid, England)

Ingredients	Amount (g/L)
Peptone	10.0
Sucrose	5.0
Lactose	5.0
Di-potassium phosphate	2.0
Eosin Y	0.14
Methylene blue	0.065
Agar	13.50

Mannitol Salt agar (Oxoid, England)

Ingredients	Amount (g/L)
Peptone	10.0
Manitol	10.0
Lab-lemco powder	1.0
Sodium chloride	75.0
Phenol red	0.025
Agar	15.0

Brief Biography

Ahmed Al Rafsan passed the Secondary School Certificate Examination in 2008 and then Higher Secondary Certificate Examination in 2010. He also received Bachelor of Food Science and Technology (BFST) degree from Faculty of Food Science and Technology in 2015, Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram. Now, he is a candidate for the MS degree in Food Chemistry and Quality Assurance under the Department of Applied Chemistry and Chemical Technology of faculty of Food Science and Technology.

His research interests are in the areas of Product development, improving nutritional value of food, quality control of food.