



IMPACT OF REFINING ON PHYSIOCHEMICAL, HEAVY METAL AND MINERAL PROFILES IN VEGETABLE OILS

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Roll No.: 0116/03

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Session: June- December, 2017

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

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JUNE 2018

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

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Dedication

*I dedicate this small piece of work
to my beloved parents*

Acknowledgements

At first, I would like to express my deepest sense to —The Almighty Allah, who enables me to finish off the research work and dissertation successfully for the degree of Master of Science (MS) in Food Chemistry and Quality Assurance under the Department of Applied Chemistry and Chemical Technology, Chittagong Veterinary and Animal Sciences University (CVASU).

With great pleasure, I would like to express my deepest sense of gratitude and immense indebtedness to my respected teacher and research supervisor **Monsur Ahmad**, Assistant Professor, Department of Applied Chemistry and Chemical Technology, Chittagong Veterinary and Animal Sciences University for her scholarly guidance, valuable suggestions, constructive and constant inspiration throughout the entire period of the study in preparations of this manuscript.

I would like to pay my sincere regards and thanks to my respected teacher **Md. Fahad Bin Quader**, Assistant Professor and Head, Department of Applied Chemistry and Chemical Technology, Chittagong Veterinary and Animal Sciences University and chairman of thesis selection committee for her kind approval of my thesis.

I feel proud in expressing my regard and immense indebtedness to **Professor DR. Md. Rayhan Faruque**, Dean, Faculty of Food Science and Technology for kind co-operation, valuable suggestions and constructive criticism throughout the research period and for the thesis work.

I have the highest regards to all participants who helped me for carrying out this research work properly and authority of **BCSIR**, Chittagong, Bangladesh for their kind assistance.

I am deeply indebted to my Parents and family members without whose moral support, blessings and affection this would not have been a success. It would be impossible to list out all those who have helped me in one way or another in the successful completion of this work. I, once again express my heartfelt thanks to all those who helped me in completing this venture in time.

The author

June 2018

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List of abbreviations

Abbreviation	Elaboration
AAS	Atomic Absorption Spectrophotometer
AOAC	Association of Official Analytical Chemists
BBS	Bangladesh Statistics Bureau
BDS	Bangladesh standard
kg	Kilogram
CAC	Codex Alimentarius Commission
°C	Degreecentigrade
HCl	Hydrochloricacid
%	Percentage
ppm	Parts per million
H ₂ O ₂	Hydrogenperoxide
HNO ₃	Nitric acid
mg/L	Milligram/liter
ml	Milliliter
WHO	WorldHealthOrganization
US EPA	United State Environmental Protection Agency
BSTI	Bangladesh Standards and Testing Institute
meq	Mill equivalent
FA	Fatty Acid
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration

Abstract

The present study was carried out to examine the changes in physiochemical properties, heavy metal and mineral concentrations after refining of soybean and palm oil. The results showed that specific gravity have decreased after refining process. The value of specific gravity ranged from 0.919-0.929 for crude soybean oil and 0.913-0.923 for refined soybean oil; 0.958-0.95 for crude palm oil and 0.938-0.95 for refined palm oil. Correspondingly, the chemical properties such as acid value, peroxide value, and saponification value were decreased and iodine value was increased. Peroxide values were 9.243 meq O₂ /kg oil to 12.87 meq O₂ /kg oil and 11.26 meq O₂ /kg oil to 8.11 meq O₂ /kg oil for crude soybean and palm oil respectively and decreased to 1.112 meq O₂ /kg oil to 3.597 meq O₂ /kg oil and 4.41 meq O₂ /kg oil to 2.47 meq O₂ /kg oil for refined soybean and palm oil. Comparatively low iodine value was noticed and the values varied from 117.96 to 120.66 g I₂/100 g of oil for refined soybean oil. Iodine value for palm oil was found in acceptable range (66.75- 59.52 g I₂/100 g). Saponification value was found in acceptable range 194.12-170.033 mg KOH/g and 195.27-189.76 mg KOH/g for refined soybean and palm oil respectively. Acid value was similar with standard value for both soybean and palm oil (0.647-0.271 mg KOH /g oil and 0.967- 0.76 mg KOH /g for refined soybean and palm oil respectively). Iron, copper, lead and cadmium were analyzed by using AAS. Refining of the samples decreased the concentration of heavy metals (Pb and Cd) and mineral (Fe& Cu). Concentrations of all trace metals were within the limits of the accepted values by regulatory bodies. Concentration of Fe was 1.24- 2.8 mg/L and 1.79-3.15 mg/L for refined soybean and palm oil samples respectively. Concentration of Pb and Cd were below detection limit in refined soybean and palm oil. Concentration of Cu was 0.193 -0.413 mg/L and 0.1 -0.357 mg/L for refine soybean and palm oil. Hence these oils were safe for human consumption.

Keywords: *Crude oil, refined oil, trace metals, heavy metals, vegetable oil.*

Chapter-I

Introduction

Edible oils are very important food stuff for the world. The human body uses oils and fats in the diet for three purposes, such as being an energy source, being a structural component, and making powerful biological regulators. Oils and fats also play an important role in metabolic reactions in the human body (Khan et al., 2007). Vegetable oils and fats have wide application in foods where they are used in frying, salad dressing, shortening of pastry, margarine, cooking, and ice cream manufacture. In the world, vegetable oils and fat are found to be about 80–85% of edible oils and fat consumed by the public (Fupi and Morck, 1982).

The term crude oil is assigned to oil that is not processed after being extracted from the animal or plant material. In order to become suitable for human consumption, crude oils are generally refined to remove the majority of these unwanted components to produce a stable product with desired color and a pleasant taste, with the least possible damages on desirable components and with the least possible loss of oil (Suliman et al., 2013).

Refined oils are widely used by food industry due to their nutritional properties and their influence on the smell and taste of food products (Zulkurnain et al., 2012). During the industrial production of vegetable oils and fats by crushing or solvent extraction, various kinds of minor constituents such as dirt, moisture, gums, waxes, color pigments, flavoring substances, phosphates, carbohydrates, protein, pesticide residues, odoriferous materials, trace metals, free fatty acids and the possible residual content of the solvent used for the extraction process are incorporated into triglycerides and may cause the oil to be darkened, foamed, smoked, precipitated, develop off-flavour, decrease thermal and oxidative stability, and increase oil loss (Ghazani et al., 2013).

The traditional refining process of crude vegetable oils generally includes the steps of degumming, neutralization, bleaching and deodorization. However, during these processes, a large amount of the micronutrients and antioxidants such as, polyphenols, tocopherols, sterols, carotenoids are lost, which thus reduce substantially the nutritional value and quality of vegetable oils (Szydłowska-Czerniak, 2013).

Using figures for 2000/01, world average oil yields of different vegetable oils are: soybean (18.3%); rapeseed (38.6%); sunflower (40.9%); groundnut (40.3%); cottonseed

(15.1%); coconut (62.4%); palmkernel (44.6%); sesame (42.4%); linseed (33.5%); average for all oilseeds (25.8%). In addition, yields from palm fruit (45–50%), olive (25–30%) and corn (about 5%) are as indicated (Gunstone, 2000).

The oil palm (*Elaeis guineensis jacquin*) originated from South Africa. There are two different types of oils extracted from palm fruit. One type of oil is obtained from mesocarp part of palm fruit known as crude palm oil (CPO) and other obtained from the kernel of the fruit palm and known as palm kernel oil (PKO) (Gourichon, 2013). The differences are in color (raw palm kernel oil lacks carotenoids and is not red), and in saturated fat content: palm mesocarp oil is 49 percent saturated, while palm kernel oil is 81 percent saturated fats (Gunstone, 2000). Palm oil may be fractionated into a liquid fraction (olein) and a more solid fraction (stearin). Palm stearin, the harder fraction of palm oil, contains the more saturated fatty acids and TAGs (SiewWai, 2000). Recently, palm oil has become the second most consumed oil all over the world with a competitive price compared to other edible oils (Siddique et al., 2010). In much of Asia, palm oil is popular because of its relatively low price than soybean oil, its main competitor, reinforced by the modest freight costs from South East Asian suppliers. Also, the temperatures in the tropical/sub-tropical regions are warm enough to allow refined bleached deodorized (RBD) palm olein to be used as household oil, without fear of clouding.

Soybean is the dominant oil produced in the world, due to its favorable agronomic characteristics, its high-quality protein, and its valuable edible oil. It contributes over a half of all oilseeds produced worldwide. The US ranks first in soybean production (8.24 million tons), followed by Brazil, Argentina, China and EU-15 (Mielke, 2001).

Soybean oil has a high content of linoleic acid, and a lower level of linolenic acid. These are both essential fatty acids for humans and therefore of dietary importance (List et al., 1977).

Trace elements are of significant benefit to man when consumed in the right proportion and lack of the trace element in diet may constitute health problem that may be devastating. Heavy metals on the other hand are in general characterized as been toxic or poisonous. Since trace elements provide nutritional value, they are sometimes referred to

as micronutrients. The source of metal contamination can be come from many sources, in basic it can be divide into two types endogenous and exogenous (Pehlivan et al., 2008). Endogenous factor can be due to the metabolism of the plant itself which is depend on the soil, fertilizer and water where the plant growth (Rehana et al., 2009).

The principal source of oils contamination with heavy metals is their migration from arable soil into oil plants. Moreover, oils may be enriched with heavy metals during technological processes (Juszczak, 2008).The presence of heavy metals in vegetable oils may cause negative changes to their taste and smell. Catalytic oxidation of oils with molecular oxygen through metal ions (especially copper) leads to the so called rancidification of oils (Domka et al., 2010).

Analysis of Iron (Fe), Copper (Cu), Nickel (Ni), Zinc (Zn), Lead (Pb), Cadmium (Cd) and Chromium (Cr) in edible oil is most important because the metal are known to have a catalyst effect which accelerate the auto-oxidation mechanism that lead to oxidation or rancidity of the vegetable oil, especially Iron, Copper, and Nickel (Mareila et al., 2006). Other metal lead, Cadmium and chromium is of highly interest due to it toxicity effect.

Therefore, the present study was undertaken to determine the changes in the level of heavy metals and minerals in crude and refined edible oil after refining processes. Different physicochemical characteristics also were determined to identify the effect of refining.

1.1 Objectives of the study

The objectives of the study were:

1. To determine the physicochemical properties of soybean and palm oils (both crude and refined).
2. To determine the concentrations of heavy metals such as cadmium and lead in crude and refined oil samples of both soybean and palm oils.
3. To determine the concentrations of minerals such as iron and copper in crude and refined oil samples of both soybean and palm oils.

1.2 Anticipated outcome

The anticipated outcomes of this study were:

1. Estimated the physicochemical properties of crude and refined soybean and palm oil.
2. Estimated the concentration of heavy metals (Pb, Cr) in crude and refined soybean and palm oil.
3. Estimated the concentration of minerals (Fe and Cu) in crude and refined soybean and palm oil.

Chapter-II

Review of Literature

Vegetable fat and oil are triglyceride that is consisting 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 (refined palm olein) (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.

2.1 Palm oil

The oil palm is the most efficient oil-producing plant, with about 4.5 tonnes of oil per hectare per year (Robbelen, 1990). Palm oil is edible oil referred to by the FAO/WHO and CAC as being derived from the fleshy mesocarp of the oil palm fruit and Palm kernel oil is derived from the kernel of the fruit of the oil palm (Alimentarius, 2013).

Crude palm oil is not directly used for edible applications but it is refined in order to remove undesirable components such as free fatty acids (FFAs), partial acylglycerides, metals, colored compounds, odorous substances (Verhe et al., 2000).

Refining of crude oil is carried out by either physical refining or chemical process. The physical refining of palm oil consists of three main processing steps: degumming and bleaching steps (in the pre-treatment step) and deodorisation, for the purification of crude palm oil into edible oil. Dry acid degumming is usually practiced, whereby concentrated phosphoric acid is used in combination with adsorbents to remove gums and impurities. The pre-treatment step is the most crucial step for sufficient removal of impurities and undesired components that would adversely affect the quality of the end product when subjected to deodorization. In physical refining FFAs are removed by distillation, while in chemical refining FFAs are removed by chemical neutralization (alkali neutralization). Refining process involves degumming, neutralization, bleaching and deodorization (Zulkurnain et al., 2012).

According to Tang et al., (1995), 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. 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)

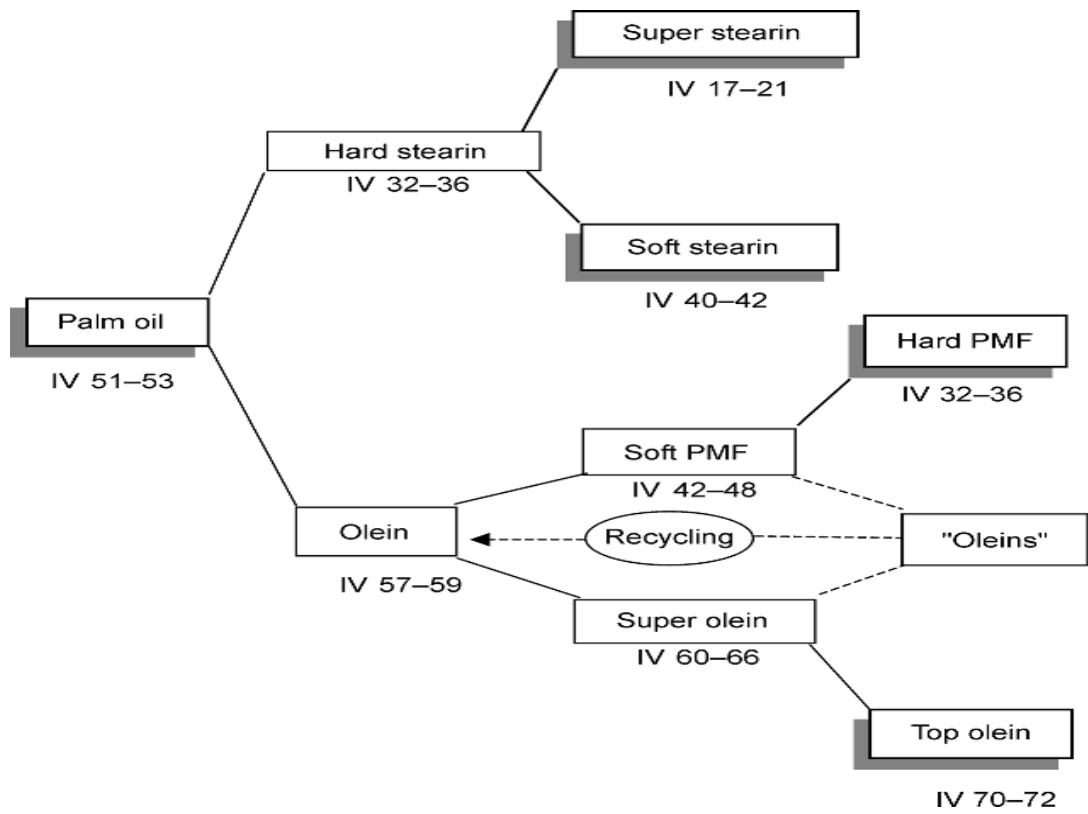


Figure 2.1: Dry multiple fractionation of palm oil (adapted with permission from Deffense, 1995).

Characteristics	Palm Kernel Oil	Palm Kernel Stearin	Palm Kernel Olein
Apparent density, g/ml, at 40°C	1.4500 to 1.4518	0.9040 - 0.9059	0.9039 – 0.9056
Refractive index, n _D 40°C	0.904 to 0.905	1.4499 - 1.4501	1.4514 - 1.4522
Saponification value, mg KOH/g oil	243 to 249	245 – 255	231 – 244
Unsaponifiable matter, % by mass	0.30 to 0.39	0.22 - 0.60	0.26 - 0.72
Slip Melting Point, °C	27.3 to 28.0	31.3–33.1	21.8 – 26.0
Iodine Value (Wijs)	16.5 to 18.75	5.8 – 8.0	20.6 – 26.0
Fatty Acid Composition (wt % as methyl ester) by GLC			
C6:0	0.2 - 0.4	0 - 0.1	0.2 - 0.4
C8:0	3.2 – 4.7	1.5 - 2.6	3.6 to 5.0
C10:0	2.9 to 3.5	2.5 – 3.0	3.2 to 4.5
C12:0	45.5 to 49.8	54.8 - 58.2	42.0 - 46.5
C14:0	15.4 to 17.2	21.1 - 24.1	12.3 – 15.5
C16:0	7.9 to 9.2	7.2 – 8.6	7.4 – 10.6
C18:0	1.9 to 2.8	1.3 - 2.4	1.8 – 3.0
C18:1	13.7 to 16.9	4.6 – 7.2	14.6 - 21.3
C18:2	2.1 to 2.9	0.6 - 1.3	2.6 - 3.8
C20:0		0 - 0.3	0 - 0.3
Others	0.0 to 0.12		
Solid Fat Content (by Pulse NMR at temperature, °C)			
5	68.0 to 76.8	91.6 – 94.9	55.4 – 71.2
10	61.6-71.2	87.5 – 93.2	43.0 – 67.0
15	50.7-60.0	83.6 – 92.2	25.1 – 51.7
20	34.2-45.5	77.0 – 86.0	8.5 – 32.7
25	10.2-21.5	55.0 – 76.0	0 – 12.0
30	Nil	22.0 – 44.0	0
35	-	0	-

Figure 2.2: Characteristic of Palm kernel oil, palm kernel olein, palm kernel stearin
Source: Adapted from Gunstone, 2000)

2.2 Soybean Oil

Soybean oil is the oil produced in largest quantity and is second only to palm oil in traded oil. There is also a large trade in soybeans but no comparable trade in palm fruits, which are extracted as soon as possible close to the point of collection.

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 temperatures. 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 2.2: 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 tones, 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.

2.3 Physicochemical properties of palm and soybean oil

Most of the information concerning the physical properties of soybean and other vegetable oils was reported many years ago. However, there are recent developments in establishing mathematical models to predict changes in physical properties with FA composition and temperature.

According to Formo (1979), for vegetable oils, it has been shown that density decreases linearly with increase in temperature.

$$\rho = b + mT$$

Where ρ is the density, T is the temperature, and b and m are constants. The effect of temperature on the viscosity of various vegetable oils and Fatty acid (FA) was investigated by Nouredini et al., (1992). The relationship was expressed as-

$$\ln\mu = A + B/(T + C)$$

In which μ is viscosity in centipoises, A , B , and C are constants and T is temperature in Kelvin.

The viscosity of fatty systems was also predicted by Rabelo et al., (2000) using the same temperature–viscosity relationship. Wang and Briggs (2002) studied the viscosity of soybean oils with modified FA composition.

The refractive index (RI) is a parameter that relates to molecular weight, FA chain length, degree of unsaturation, and degree of conjugation. Perkins (1995) developed a mathematical relationship between RI and IV as

$$n_D^{25} = 1.45765 + 0.0001164 IV$$

The melting points (m.p.) of TAGs are related to the fatty acids present. For fatty acids, melting point depends on chain length and the number and position of double bonds. It increases with increasing chain length and decreases with increasing cis unsaturation.

Specific heat (C_p , in J/g K) of vegetable oil is influenced by temperature (Formo, 1979) as described in the following equation:

$$C_p = 1.9330 + 0.0026 T$$

According to Sipos and Szuhaj (1996), the solubility characteristics of vegetable oils in various solvents can be estimated from their dielectric constants or solubility parameter.

The results of Bailey et al., (1935) may serve as a good reference of the composition of soybeans such as moisture 8.0%, ash 4.6%, fat 18.0%, fiber 3.5%, protein 40.0%, pentosan 4.4%, sugars 7.0%, starch-like substances by diastase 5.6%, K_2O 2.3%, CaO 0.5%, MgO 0.5%.

Prodhan et al., (2011) studied parameters iodine value, saponification value, acid value and Reichert-Meissl number and unsaponifiable matter of different brands of soybean oil available in Bangladesh. The acid value, saponification value, Reichert- Meissl number, unsaponifiable matters were determined by the standard methods. Hanus method was followed to determine the iodine value of the oil. Saponification values of Mustafa, Muskan, Pusti, Teer, Fresh and Rupchanda were (202, 208, 224, 213, 210 and 237 respectively). Acid values of Mustafa, Muskan, Pusti, Teer, Fresh and Rupchanda

soyabean oils were (0.374, 0.748, 0.561, 0.374, 0.374 and 0.423 respectively). Iodine values of Mustafa, Muskan, Pusti, Teer, Fresh and Rupchanda soyabean oils were (85.45, 105.70, 89.64, 90, 105.26 and 110 respectively). Reichert-Meissl number of the Mustafa, Muskan, Pusti, Teer, Fresh and Rupchanda soyabean oils were (2.42, 2.8, 2.805, 2.97, 2.805 and 2.7 respectively). Unsaponifiable matter of the Mustafa, Muskan, Pusti, Teer, Fresh and Rupchanda soybean oils were 0.05, 0.06, 0.07, 0.04, 0.06 and 0.05 percent respectively.

Considering the significance of above parameters of soybean oil, Rupchanda posses the highest position in nutritional quality and better than other soybean oils for human health. According to Koushki et al., (2015), palm oil contains 50% saturated fatty acids. The saturated fatty acid to unsaturated fatty acid ratio of palm oil is close to unity and it contains a high amount of the antioxidants, β -carotene, and vitamin E. Palm oil contains a high proportion of palmitic acid as well as considerable quantities of oleic and linoleic acids.

The major composition of the kernel is oil (49%), followed by carbohydrate (26.1%), protein and crude fiber (8% each) (Ibrahim, 2013).

Triacylglycerols (TAG) are the primary neutral lipids in soybean oil. Due to the high concentration of unsaturated fatty acid in soybean oil, nearly all the TAG molecules contain at least two unsaturated fatty acids, and di- and tri saturates are essentially absent. Physical & chemical properties of crude soybean oil were specific gravity at 25° C 0.926, Refractive index at 25°C 1.4660, Acid number 0.88, Acid value (as oleic acid %) 0.44%, Saponification number 190.5, Ester number 189.62, Iodine value 124.56, Peroxide value 12.81, Unsaponifiable matter 1.76%. Physical & chemical properties of dewaxed crude soy bean oils were specific gravity 0.92-5, Refractive index at 25°C 1.4659, Acid number 0.98, Acid value (as oleic acid %) 0.49%, Saponification number 191.0, Ester number 190.02, Iodine value 124.7-5, Peroxide value 11.84, Unsaponifiable matter 0.51% 1.72% (List et al., 1977)..

Hasan et al., (2016) studied qualitative evaluation of the edible oils that are used for cooking purpose in Bangladesh. Sixteen brands of different oil samples were purchased from local market of Jhenidah city of Bangladesh. The quality of edible oils was analyzed by evaluating physicochemical properties such as density, moisture content, boiling point, peroxide, acid, iodine and saponification values using standard methods. Highest

density observed in Deshi/Ghani mustard oil (0.9683 g/ml) and lowest in Mustafa soybean oil (0.901 g/ml). On the other hand, moisture content was observed in between 0.2% to 0.82%. The highest and lowest saponification value was obtained 211.4 mg KOH/g for Jui coconut oil and 158.55 mg KOH/g for Deshi/Ghani mustard oil, respectively. It was observed that Olive oil showed maximum (12.43 meq O₂/kg) and Teer soybean oil showed minimum (1.01 meq O₂/kg) peroxide values. Highest acid value was observed in Jui coconut oil (18.04 mg KOH/g) and lowest in Fresh soybean oil (0.36 mg KOH/g). On the contrary, highest iodine value was observed in Rupchanda soybean oil (109.96 g I₂/g oil) and lowest in Jui coconut oil (6.48 g I₂/g oil).

A study of oxidative stability of soybean oil at different stages of refining indicated that crude oil was the most stable and highly purified oil the least stable (Kwon et al., 1984). During refining quality of soybean oil are changed (Jung et al., 1989). These are given in the following table:

Table2.3: Effect of processing steps on quality of soybean oil

Sample name	Phosphorus (ppm)	Iron (ppm)	Free fatty acid (%)	Peroxide value (meq/kg)	Tocopherol (ppm)
Crude	510	2.9	0.30	2.4	1670
Degummed	120	0.8	not available	10.5	1579
Refined	5	0.6	0.23	8.8	1546
Bleached	1	0.3	0.08	16.5	1467
Deodorized	1	0.3	0.00	0.0	1138

Source: (Jung et al., 1989).

Asemave et al., (2012) founded that the Saponification value (SV) ranged from, 191.00 KOH/g of oil, Ground nut oil has the highest SV, and soya bean oil gave the least SV; iodine values (IV) are in the range of 124.4 – 51.20; 31.76 – 6.83 values were obtained for viscosity; acid value (Av) were obtained as 5.54 – 4.2; and specific gravity (SG), were found as 1.01276 – 0.86.

According to Tesfaye and Abebaw (2016), the result of physicochemical properties shows that acid value in Avena and Viking oils which is 0.091 ± 0.0042 mg KOH/g and 0.082 ± 0.0047 mg KOH/g, respectively, from palm oil variety and in Selam and Nur oils 0.276 ± 0.0056 mg KOH/g and 0.188 ± 0.0050 mg KOH/g, respectively, from noug oil variety, saponification value in Avena and Viking oils which is 190.27 ± 3.528 mg KOH/g and 189.80 ± 3.528 mg KOH/g, respectively, and in Selam and Nur oils 187.00 ± 5.668 mg KOH/g and 143.05 ± 8.527 mg KOH/g, respectively, iodine value in Viking and Avena oils which is 97.545 ± 0.6345 gm and 53.807 ± 1.0150 gm, respectively, and Nur and Selam oils 116.410 ± 0.3863 gm and 88.153 ± 0.5300 gm, respectively, and peroxide value in Viking and Avena oils which is 1.413 ± 0.0808 meq/kg and 1.23 ± 0.0305 meq/kg, respectively, from palm oil variety and in Selam and Nur oils 0.893 ± 0.0503 meq/kg and 1.460 ± 0.0600 meq/kg, respectively, from noug oil variety were obtained.

2.4 Analysis of heavy metal in edible oil:

Metal has become a common contamination to the food product, which it found the way into the edible oil through environment and processing contamination such as crushing, distillation, and hydrogenation. Many metals are beneficial to humans but their levels in foods are of significant interest because, above certain levels, they may become harmful. Consequently, it is important to ascertain the levels of both beneficial and toxic metals in foods, and such information is imperative in estimating dietary intakes of such metals. The Global Environment Monitoring System Food Contamination Monitoring and Assessment Programmed (GEMS/Food) of the World Health Organization (WHO) encourages studies of total diet as a means of evaluating food contamination of chemicals and by extension, human intake of such chemicals around the world (WHO, 2007).

In recent times, there have been an increasing number of publications on the trace elements contents of food and foodstuff, especially with the enhanced awareness on the role of trace elements in human health. Increasing numbers of publications have reported the deleterious effects that trace metals have on the flavor and oxidative stability of oils, since some metals could catalyze oxidation of fatty acid chains, exerting a deleterious influence on shelf life and nutritional value (Galeano et al., 2006).

The metal take up by the plant is very depend on the amount and type of metal that content in the soil, water or fertilizer that used in the plant (Rehana et al.,2009).

The source of cadmium and lead (Pb) can be due to combustion of fuel in refinery process, industrial emission, and from packaging material such as colorant and stabilizer in plastic (Giacomo et al., 2004).

Zinc is an essential mineral that required by human body, which is important for normal growth, wound healing, normal taste sensational, and appetite. Zinc usually is required at low concentration; however at high concentration of Zinc it can pose some side effect that had health deleterious impact. The daily recommended amount of zinc (Zn) intake for an adult male is at 15 mg and for adult female is at 12 mg (Durali et al., 2009).

In General various analytical and sample preparation methods had been propose for the analysis of metal in edible oil. Most of the analytical methods used are multi-metal analyses that can perform in a single run. The most frequent used technique is by Inductively Couple Plasma (ICP) either of Mass Spectrometry (MS) or Atomic Emission Spectroscopy (AES), other method also had been reported included Graphite furnace Atomic Absorption Spectrometric (GFAAS), or as well the Atomic Absorption Spectroscopy (AAS).Atomic Absorption (AAS) method is sometimes preferable by laboratory due to its low operation cost. The Edible Oil sample was prepared by microwave digestion with 65% nitric acid and Hydrogen Peroxide. The following metal Copper, Zinc, Iron, and cobalt was analyze with AAS, while Cadmium and lead was analyzer with GFAAS. Table showed the analysis result of the metal in edible oil namely Olive Oil, Sunflower oil, Hazelnut Oil, and Margarine Oil (Durali et al., 2009).

Table2.4: Determination of Zinc, Cadmium and Lead in Edible Oil by Atomic Absorption Spectrometry (AAS):

Sample	Zinc (mg/Kg)	Cadmium ($\mu\text{g}/\text{kg}$)	Lead (mg/Kg)
Olive Oil	1.03 ± 0.1	0.15 ± 0.02	0.03 ± 0.003
Hazelnut Oil	1.15 ± 0.1	4.57 ± 0.4	0.01 ± 0.01
Sunflower Oil	1.10 ± 0.1	3.76 ± 0.4	0.01 ± 0.01
Margarine	2.71 ± 0.2	3.66 ± 0.3	0.01 ± 0.01

According to Tesfaye and Abebaw (2016), levels of Cu in Viking and Avena oils that were 0.62 ± 0.022 mg and 0.28 ± 0.004 mg, respectively, and in Selam and Nur oils 0.86 ± 0.003 mg and 0.42 ± 0.005 mg, respectively, and the levels of Zn in Viking and Avena oils that were 1.58 ± 0.154 mg and 1.27 ± 0.255 mg, respectively, and in Selam and Nur oils 1.19 ± 0.160 mg and 1.47 ± 0.567 mg, respectively, were obtained.

According to Giacomo et al., (2004) Cadmium and lead contamination are hardly associated to edible oil processing, it is mostly are cause by the environment exposure rather than process related. It present can be causes by deposition or bioaccumulation from the soil or water due to pollution. Cadmium and Lead are known to be accumulating in biological system for long half-life. The source of cadmium and lead (Pb) can be due to combustion of fuel in refinery process, industrial emission, and from packaging material such as colorant and stabilizer in plastic.

Copper, iron, chromium, aluminum, lead and cadmium were analyzed from the samples of palm oil, groundnut oil and soybean oil which were collected from Wurukum and modern (Wadata) markets of Makurdi town. Palm oil gave 11.370 mg kg⁻¹, 0.078 mg kg⁻¹, 2.3319 mg kg⁻¹, 0.1780 mg kg⁻¹, 1.9358 mg kg⁻¹, and 0.0220 mg kg⁻¹ for iron, copper, chromium, lead, aluminum and cadmium respectively. In the Groundnut oil, the concentrations (mg kg⁻¹) of iron, copper, chromium, lead, aluminum and cadmium were obtained as 8.5109 , 0.0633 , 2.7067 , 0.1631 , 1.7742 and 0.0207 respectively. For the Soybean oil sample, the levels (mg kg⁻¹) were 8.7519 , 0.0475 , 1.7559 , 0.1631 , 0.3837 ,

and 0.0200 for iron, copper, chromium, lead, aluminum and cadmium respectively (Asemave et al., 2012).

Szyczewski et al., (2016) analyzed the variation in concentrations of heavy metals-copper, zinc, lead, cadmium, iron and manganese in different fractions of oil during production. The concentration of heavy metals in the 1st production fractions of soybean oil were 0.45, 1.74, 0.97, 2.03, 0.23, 0.14 for Cu, Zn, Mn, Fe, Pb, and Cd, respectively. In the 5th fractions the values of Cu, Zn, Mn, Fe, Pb and Cd were 0.05, 0.69, 0.15, 0.18, 0.08, and 0.01 respectively.

Lead and cadmium are known to be acute and chronic poisoning which can cause failure to the kidney, liver, heart and immune system. In addition to that, exposure to such metal is also known to cause chromosome aberration, cancer, and birth defect. For these reasons many countries have set the permitted amount of lead (Pb) in oil at 0.1mg/Kg and Cadmium (Cd) at 0.05 mg/Kg (Durali et al., 2009).

As according to World Health Organization (WHO) the permitted doses of Cadmium (Cd) and lead (Pb) for an adult per week are set at 0.5 mg and 3.0 mg respectively (WHO, 2007).

Chapter-III

Materials and Methods

The experiment was conducted in the laboratory of the Department of Applied Chemistry and Quality Assurance laboratory, Chittagong Veterinary and Animal Sciences University (CVASU), Chittagong and Bangladesh Council of Scientific and Industrial Research (BCSIR) laboratory in Chittagong.

3.1 Sample Collection

Total 4 brands of crude and refined soybean and palm oil were collected from oil processing industries in Chittagong. Refined soybean and palm oil were collected after refining of those samples. For each brand we collected three samples in order to get clear concept about each product. All samples collected in a pre-cleaned plastic bottle and stored in a dark place at ambient temperature until analysis. All chemicals and reagent used in this study were analytical grade.

3.2 Equipment:

Digital analytical balance was used to weigh oil sample. Conical flasks, beakers, watch glass, and pipette were used in the measurement and preparation of sample and standard solutions. Volumetric flask was used to dilute sample solution and prepare standard solution. Atomic Absorption Spectrophotometer (AA320N, China) was used for determination of Fe, Cu, Cd and Pb content inedible oil.

3.3 Chemicals (Reagent)

All reagents are of analytical grade. Deionized water was used for preparation and dilutions of standard solutions. Nitric acid (HNO_3) and sulfuric acid (H_2SO_4) reagents were used for cleaning of glassware and for digestion of the samples. Representative edible oil samples were used for analysis. Chloroform, potassium iodide (KI), glacial acetic acid, and sodium thiosulphate were used for estimation of peroxide value. Hydrochloric acid (HCl), sodium hydroxide (NaOH), ethanol, and phenolphthalein

indicators were used for estimation of acid value and saponification value, respectively; carbon tetra chloride, potassium iodide, and Hanus reagent were used for estimation of iodine value.

3.4 Determination of the Physicochemical Properties

3.4.1 Determination of Specific Gravity

Specific gravity was determined by picnometrically according to AOAC (2016) Official Method 9201.212 at temperatures ranging from 20.0 to 50.0 °C.

Procedure

The weight of the empty pycnometer (specific gravity bottle) was taken and noted as (a) and the bottle subsequently filled with distilled water in such a manner as to prevent any entrapment of bubbles, stopper and reweighed and noted as (b). The pycnometer was dried, filled with oil sample also preventing bubbles and the stopper properly inserted and weight noted as (c).

$$\text{Specific gravity} = \frac{c-a}{b-a}$$

Where,

a = weight of the empty pycnometer

b= weight of the pycnometer with distilled water

c= weight of the pycnometer with oil sample

3.4.2 Determination of Acid Value

Acid Value is the Number of milligram of KOH needed to neutralize the FFA present in 1g of oil. Acid Value of oil samples (calculated as oleic acid percentage) was determined by standard method described in AOAC (2016) for Oils and Fats.

Procedure

The oil was stirred thoroughly and 5 grams and 10 grams of crude oil samples and refined oil samples respectively were accurately weighed into a conical flask. Added to it were 50 ml of absolute ethanol and 3 drops of phenolphthalein indicator solution. This was

heated (warmed) and swirled while titrating with 0.1 N of NaOH until a faint pink color which persisted for at least 30 seconds was observed.

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

3.4.3 Determination of Saponification Value

Saponification values of oil samples were determined according to AOAC (2016) Official Methods 920.160.

Procedure

Two grams of each oil sample was weighed in 250mL Erlenmeyer flasks; then 25mL of alcoholic potassium hydroxide solution was added into the flasks. The blank determination was conducted along with the sample. The samples flask and the blank flask were connected with air condensers and boiled gently in the water bath, steadily until the saponification was completed, indicated by absence of oily matter and the appearance of clear solution. Clarity was achieved in half hour boiling. After the flask and the condenser cooled, inside of the condensers was washed down with about 10mL of ethanol and then 1mL of phenolphthalein indicators was added to the solution. Excess potassium hydroxide was titrated with 0.5N hydrochloric acid until cloudy solution was formed. 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.

3.4.4 Determination of Peroxide Value

PV was determined according to AOAC Official Method 965.33.

Procedure

5 grams of the homogenous oil sample was 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.

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

Where,

N = normality of sodium-thiosulfate,

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

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

3.4.5 Determination of Iodine value

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

Five grams of oil samples was weighed in 250mL conical flasks 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.

3.5 Determination of trace metals:

Heavy metal content was determined according to AOAC (2016) Official Method 974.27. Analysis of Fe, Pb, Cd and Cu were carried out for all crude and refined soil samples by using Atomic Absorption Spectrophotometer (AAS).

3.5.1 Sample Digestion:

2g of each of the samples were weighed into a beaker on an electronic weighing balance. 5mL of each of concentrated nitric and sulfuric acids were added to the beaker already containing 2g of the oil. Into the beakers 2mL of hydrogen peroxide was added and then heated on a heating mantle until a clear solution was obtained. The content of the beaker were allowed to cool and then filtered. The resulting solution was made up to 50mL using distilled water and then transferred into a plastic bottle for metal analysis by AAS method (Orhio et al., 1998).

Instrumentation : Atomic Absorption Spectrophotometer (AAS) (Type: iCE 3300 AA system, Thermo Scientific, designed in UK) was used to determine Fe, Pb, Cd and Cu in oil sample. The analysis is carried out using respective hollow cathode lamps under standard instrumental conditions.

3.5.2 Procedure of Atomic Absorption Spectrometry (AAS):

Samples were evaluated by Atomic Absorption Spectrophotometer as per protocol described by (Ahamed et al., 2010). Approximately 12 ml of processed samples were taken in a cup suitable to fit into the sample tray of the Atomic Absorption

Spectrophotometer (iCE 3300). On starting the machine around 2-3ml processed sample were automatically drawn for a single metal detection (Pb/Cd/Cu/Fe). The sample solution was aspirated by a pneumatic analytical nebulizer and transformed into an aerosol, which was then introduced into a spray chamber, where the sample was mixed with the flame gases and conditioned in a way that only the finest aerosol droplets (less than 10 μ m) entered into the flame. This conditioning process was responsible that only about 5% of the aspirated sample solution reached the flame. On top of the spray chamber was a burner head that produced a flame that was laterally long (usually 5–10 cm) and only a few mm deep. The radiation beam passed through this flame at its longest axis, and the flame gas flow-rates was adjusted to generate the highest concentration of free atoms. The burner height was also adjusted, so that the radiation beam passed through the zone of highest atom cloud density in the flame, resulting in the highest sensitivity.

The processes in a flame include the following stages:

- i. **Desolation (drying):** The solvent was evaporated and the dry sample nanoparticles were remained.
- ii. **Vaporization (transfer to the gaseous phase):** The solid particles were converted into gaseous molecules.
- iii. **Atomization:** The molecules were dissociated into free atoms.
- iv. **Ionization:** Depending on the ionization potential of the analytic atoms and the energy available in a particular flame, atoms in part converted to gaseous ions. In flame Atomic Absorption Spectrometry a steady-state signal was generated during the time when the sample was aspirated and read the results on the screen of the machine concentration of metals was expressed in average mg/L.

3.5.3 Standard Preparation for Calibration:

Stock standard solution containing 1000mg/L in 2% HNO₃ of the metals Pb, Cu, Fe, and Cd, was prepared from atomic absorption spectroscopic standard solution. The stock solutions were then used for preparation of calibration standards and spiking standards in the analysis of the samples (Weiner, 2007).

Working Curve:

A series of five standard solutions of known concentrations was prepared for each analyte to be analyzed in order to obtain the calibration curve, normally a linear regression equation:

$$y = ax + b,$$

Where y is the instrument response, x is the concentration of the chemical, a is the slope factor (the calibration sensitivity), and b is the intercept (the instrument response when analyte is absent, $x = 0$) (Weiner, 2007).

3.6 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: IV

Results

In this chapter result of the study are given under the following headings. The sample of crude soybean and palm oil marked out as CSO and CPO respectively. On the other hand refined oil of soybean and palm oil marked out as RSO and RPO respectively.

4.1 Physicochemical properties of soybean oil

The physical and chemical properties of crude and refined soybean oil were analyzed and data presented in Table 4.1.

The value of specific gravity ranged from 0.919-0.929 for crude soybean oil sample and 0.913-0.923 for refined soybean oil sample. Saponification values of CSO₁, CSO₂, CSO₃, and CSO₄ were 203.55, 172.93, 204.89 and 174.47 respectively. Peroxide values were 9.243 to 12.87 for crude oil sample and 1.112 to 3.597 for refined oil samples. Iodine value is higher for CSO₁ sample which is 120.66 g I₂ /100 g of oil. The minimum acid value concentration for crude oil and refined oil were 1.643 mg KOH /g oil and 0.271 mg KOH /g oil respectively.

Table 4.1: Physical and chemical properties of crude and refined soybean oil

Sample ID (n=3)	Parameters Specific gravity	Saponification value (mg /g oil)	Peroxide value (meqO ₂ /kg oil)	Iodine value (g I ₂ /100 g of oil)	Acid value (mg KOH /g oil)
CSO ₁	0.929±0.0 ^a	203.55±0.5021 ^a	9.243±0.038 ^a	113.90±0.190 ^b	1.643±0.32 ^{ab}
RSO ₁	0.923±0.0010 ^b	193.83±0.289 ^a	1.183±0.012 ^d	120.66±0.210 ^a	0.647±0.015 ^a
CSO ₂	0.925±0.00058 ^b	172.93±0.055 ^b	12.87±0.058 ^a	110.0±0.0057 ^c	1.81±0.01 ^a
RSO ₂	0.920±0.00058 ^{cd}	171.083±0.074 ^b	4.75±0.1 ^{bcd}	117.96±0.025 ^a	0.48±0.006 ^c
CSO ₃	0.919±0.00058	204.89±0.0005	8.867±0.058 ^a	110.90±0.10 ^c	1.896±0.087 ^a

	de	8 ^a	bc		
RSO₃	0.916±0.00058 _{ef}	194.12±0.0 ^{ab}	1.112±0.015 ^e	118.12±0.289 ^a	0.277±0.011 _{5^{bc}}
CSO₄	0.919±0.00153 _{cd}	174.47±0.164 ^b	11.463±0.06 _{3^{ab}}	110.02±0.029 ^c	1.97±0.012 ^a
RSO₄	0.913±0.001 ^f	170.033±0.058 _b	3.597±0.005 _{7^{cd}}	118.12±0.032 ^a	0.271±0.008 ^b _c

All values are means ± SD. a-e Means not sharing a common superscript letter with in a column are significantly different (P < 0.05)

4.2 Heavy metals (Pb, Cd) and minerals (Fe, Cu) concentrations of crude and refined soybean oil

The heavy metals concentrations of the individual crude and refined soybean oil studied were presented in Table 4.2. The summary of results and comparison of the Cu, Fe, Pb and Cd concentrations of the crude and refined oils were presented in table 4.2.

Table 4.2: Metals concentration of crude and refined soybean oil (mg/L)

Sample ID (n=3)	Parameter			
	Iron(mg/L)	Lead (mg/L)	Cadmium (mg/L)	Cupper
CSO1	5.25±0.10 ^a	0.012±0.001 ^{ab}	ND	0.041±0.0085 ^{abc}
RSO1	2.47±0.058 ^b	0.0±0.0 ^b	ND	0.037±0.0115 ^{bc}
CSO2	5.55±0.02 ^b	0.019±0.0015 ^a	ND	0.06±0.01 ^a
RSO2	2.8±0.1 ^b	0.0±0.0 ^b	ND	0.039±0.001 ^{abc}
CSO3	4.97±0.03 ^a	0.010±0.0 ^{ab}	ND	0.0483±0.00289 ^{ab}
RSO3	2.32±0.005 ^b	0.0±0.0 ^b	ND	0.01933±0.00115 ^c
CSO4	3.25±0.01 ^{a^b}	0.017±0.002 ^a	ND	0.055±0.005 ^c
RSO4	1.24±0.012 ^b	0.0±0.0 ^b	ND	0.0413±0.00231 ^{abc}

All values are means ± SD. a-c Means not sharing a common superscript letter with in a column are significantly different (P < 0.05).ND- Not Detected; Instrument Detection limit is 0.001mg/kg. ND- Not Detected; Instrument Detection limit is 0.001mg/kg.

4.3 Physicochemical properties of palm oil

The value of specific gravity ranged from 0.958-0.95 for crude palm oil sample and 0.938-0.95 for refined palm oil sample. Saponification values were 199.97, 197.85, 204.08 and 196.87 for CPO₁, CPO₂, CPO₃, and CPO₄ respectively. Peroxide values were 11.26 to 8.11 for crude oil sample and 4.41 to 2.47 for refined oil samples. Iodine value was higher for CPO₃ sample which is 66.75g I₂ /100 g of oil .The minimum acid value concentration for crude oil and refined oil were 1.43 mg KOH /g oil and 0.76 mg KOH /g oil respectively.

Table 4.3: Physical and chemical properties of crude and refined palm oil

Sample ID (n=3)	Parameter Specific gravity	Saponification value (mg /g oil)	Peroxide value (meqO/kg oil)	Iodine value (g I ₂ /100 g of oil)	Acid value (mg KOH /g oil)
CPO1	0.958±0.0012 ^a	199.97±0.025 ^{ab}	8.11±0.010 ^{ab}	56.10±0.100 ^d	1.94±0.32 ^{ab}
RPO1	0.95±0.00058 ^{abc}	191.11±0.102 ^c	4.41±0.010 ^{bc}	63.24±0.015 ^{abc}	0.833±0.015 ^c
CPO2	0.952±0.001 ^{abc}	197.85±0.010 ^{abc}	10.66±0.015 ^a	58.62±0.015 ^{cd}	1.95±0.015 ^{ab}
RPO2	0.948±0.00058 ^{bc}	189.76±0.010 ^c	3.24±0.010 ^{bc}	65.57±0.153 ^a	0.967±0.015 ^{bc}
CPO3	0.95±0.0 ^{abc}	204.083±0.072 ^a	11.26±0.006 ^a	60.03±0.058 ^{bcd}	1.42±0.010 ^{abc}
RPO3	0.938±0.001 ^d	195.27±0.355 ^{bc}	2.48±0.021 ^c	66.75±0.010 ^a	0.880±0.010 ^c
CPO4	0.954±0.0015 ^{ab}	196.87±0.021 ^{abc}	9.12±0.012 ^{ab}	54.80±0.004 ^d	2.07±0.057 ^a
RPO4	0.942±0.00058 ^{cd}	190.84±0.040 ^c	2.47±0.010 ^c	59.52±0.051 ^{bcd}	0.76±0.010

All values are means ± SD. a-d Means not sharing a common superscript letter with in a column are significantly different (P < 0.05).

4.4 Heavy metals (Pb, Cd) and minerals (Fe, Cu) concentrations of crude and refined palm oil

The metal contents of the individual crude and refined palm oil studied were presented in Table 4.4. The summary of results and comparison of the Cu, Fe, Pb and Cd contents of the crude and refined oils were presented in table 4.4.

Table 4.4: Metals concentration of crude and refined palm oil (mg/L)

Sample ID (n=3)	Parameter Iron(mg/L)	Lead (mg/L)	Cadmium (mg/L)	Copper (mg/L)
CPO1	7.46±0.015 ^a	0.059±0.001 ^b	ND	0.117±0.006 ^a
RPO1	3.15±0.010 ^{bcde}	0.010±0.0 ^b	ND	0.10±0.0 ^a
CPO2	6.47±0.057 ^{abcd}	0.0503±0.00058 ^b	ND	0.149±0.001 ^a
RPO2	1.79±0.012 ^c	0.010±0.0 ^b	ND	0.100±0.0 ^a
CPO3	7.0±0.0 ^{abc}	0.068±0.020 ^a	ND	0.357±0.461 ^a
RPO3	2.49±0.012 ^{de}	0.019±0.00058 ^b	ND	0.109±0.00 ^a
CPO4	7.25±0.010 ^{ab}	0.059±0.0015 ^b	ND	0.179±0.0015 ^a
RPO4	2.95±0.025 ^{cde}	0.020±0.0 ^b	ND	0.140±0.10 ^a

All values are means ± SD. a-e Means not sharing a common superscript letter with in a column are significantly different (P < 0.05).ND- Not Detected; Instrument Detection limit is 0.001mg/kg

Chapter: V

Discussion

5.1 Evaluation of Physicochemical properties of the crude and refined oils:

Specific gravity is the heaviness of a substance compared to that of water and it is expressed without units. There was a slightly decrease in the specific gravity of soybean and palm oils during the refining process (Table 4.1 & 4.3). According to Aluyor et al., 2009, a slight decrease in specific gravity induced by chemical refining may be because of the removal of some polar compounds, gums and some coloring components from the oil by alkali refining, which affected the weight of the oil after refining. The specific gravity (SG) of all fats and oils virtually observe between 0.90-0.95 (Islam et al., 2008) which is similar with the finding of this study. The SG value obtained for soybean oil samples was varied from 0.929 to 0.913 and 0.958 to 0.938 for palm oil samples. The SG values of refined oil samples indicate the purity of all samples and it can be said that the oil samples are well refined.

The acid value (AV) is an important determinant of oil quality (Gharby et al., 2015). It is used to measure the extent to which glycerides in the oil has been decomposed by the lipase and other physical factor such as light and heat (Hilditch, 1949). The acid value determination is particularly important for industrial purposes since acid value can modify the organoleptic or physicochemical properties of oil. Acid value can act as pro-oxidants in vegetable oils and result in an undesirable taste and flavor (Ghazani et al., 2013). The acid value of the crude soybean oil (1.67-1.94 mg KOH/g oil) was almost similar to that reported by Reza et al., (2009) in crude soybean oil (2.09 mg KOH/g oil). The acid value of the crude palm oil was (1.42-2.07 mg KOH/g oil) which was lower than that reported by Onyema and Ibe, (2016). The AV obtained for soybean oil samples after refining was varied from 0.647 to 0.271 and 0.967 to 0.76 for palm oil samples almost similar to that reported by Onyema and Ibe, (2016). As shown in Table 4.1, the quantities of free fatty acids of crude oil samples are significantly higher than those of refined oil samples. The highest AV value in refined soybean oil was found in RSO₁ (0.647 mg KOH/g) and in refined palm oil is (0.967 mg KOH/g). According to standard,

maximum value in refined oil is 0.6 mg KOH/g (BDS, 1979; CAC, 2001). In our study the AV was found within the standard value for all refined soybean oil samples but AV of all refined palm oil samples were higher than the maximum limit. Low acid value is an indication of freshness and suitability for oil or fat for edible purpose while high acid value indicate the rancidity which is caused by hydrolysis of ester link and oxidation of double bonds of triglycerides.

Peroxide value (PV) is used as a measure of the extent to which rancidity reactions have occurred during storage and could be used as an indication of the quality and stability of fats and oils. PV reflected the formation of hydroperoxides that caused by the primary oxidation. It may break down into nonvolatile secondary product, which decreases the quality of oil. The amount of oxidation products in the soybean and palm oils changed after refining process (Table 4.1 & 4.3). According to Kreps et al., (2014), the refining process removes 79.2% of hydroperoxides from crude oil. Nzikou et al.,(2009) and Anwar et al.,(2007) reported peroxide values of 1.67 and 1.27 meq/kg of soybean oil, respectively; these values are similar with RSO₁ and RSO₃ while lower than RSO₂ and RSO₄. The values for all oil samples were within CAC, (2001) standards (up to 10 mill equivalents of active oxygen/kg oil).

The saponification value (SV) describes the average molecular weight of the fatty acids in the lipids (Zahir et al., 2014). Slight decrease in saponification values could be noticed after the refining process for palm oil, while soybean oil exhibited a little change with regards to the refining of the oil. These results are in agreement with those of Onyema and Ibe, (2016) who noted that the saponification value decreased with the progression of the oils from crude to refined state. The decrease is understandable with the purification of the oil as the percentage free fatty acids and other fatty components with high molecular weight in the oil being reduced during refining (Chawla, 2003).

The range of standard SV value is 189-195 mg KOH/g oil and 190-209 mg KOH/g oil for refined soybean and palm oil respectively (BDS, 1979; CAC, 2001). Highest SV value was found 194.12 mg KOH/g and 195.27 mg KOH/g for refined soybean and palm oil respectively.

The determination of iodine is useful as a measure of the degree of unsaturation of the oils. The changes of iodine value (IV) of the soybean and palm oils are presented in (Table 4.1 & 4.3). The iodine value obtained for soybean and palm oils has increased during the refining process; this was an indication that the degree of unsaturation increases progressively as a result of refining. These findings are not supported by previous reports of Dalia et al., (2015), who reported that the iodine content was stable throughout the refining. Soybean oil has comparatively high iodine value than palm oil which means more double bonds. The range of IV value for most of the commercial soybean oil is from 124 to 136 while the range of palm oil is from 48 to 60 (Jamieson, 1943).

According to, BDS (1979), IV value requirement for soybean oil is 120- 143 and for palm oil is 50-59 and CAC (2001), IV value requirement for soybean oil is 118- 141 and for palm oil is 50-55. However the mean value for soybean oil was lower than that of standard range which suggest that the oil samples may be blended with other less unsaturated and economically cheap vegetable oil. The IV value for palm oil was similar with standard value.

5.2 Elemental concentration of crude and refined soybean and palm oil:

The presence of heavy metals in crude and refined oils was studied (using AAS) and the results are shown in table 4.2 & 4.4 for soybean and palm oil respectively.

From the results, it was observed that Fe has the highest concentration in the entire oil samples. Highest Fe value was found 5.55 mg/L and 7.46 mg/L for crude soybean and palm oil respectively. On the other hand, maximum Fe value was found 2.8 mg/L and 3.15 mg/L for crude soybean and palm oil respectively. Slight decrease in Fe content could be noticed after the refining process for palm and soybean oil. Although, the concentration of Fe is highest amongst the metals in these oil sample. The Fe value obtained for the refined oil samples were lower than that found by Innocent and Ugochukwu (2015). It is essential nutrient of blood and skeleton; digestion of large amounts can lead to its accumulation in the body leading to tissue damage and hyperhaemoglobularia (Ronbintech, 2002).

Lead (Pb) has no favorable role in human metabolism and creates progressive toxicity (Zhu et al., 2011). The maximum concentration of lead in crude and refined palm oil, are 0.0680, and 0.020 mg/l respectively. Although highest concentration of lead in crude soybean oil sample is 0.019 mg/l but refined sample are free from Pb. Lead level of 10µg/dL or above is a cause for concern. Lead has harmful health effects even at lower levels and there is no known safe exposure level. Lead level of 10µg/dL or above is a cause for concern. Lead has harmful health effects even at lower levels and there is no known safe exposure level (Garard, 1970). In addition, exposure to amount of lead above 0.01 mgL⁻¹ is detrimental to health, as it may result in possible neurological damage to fetuses, abortion and other complication in children under three years old (Ali, 2005).

Cadmium is known as a principal toxic element, since it inhibits many life processes (Kowalewska et al., 2005; Vetter, 1993). It can be taken up directly from water, and to some extent, from air and through food, and it has a tendency to accumulate in both plants and animals (Pehlivan et al., 2008). Cadmium concentrations were below the detection limit in all of oil samples (Table 4.2 & 4.4).

The maximum concentration of Cu in palm oil and soya bean oil were determined to be 0.140, and 0.0413 mg l⁻¹ respectively. Its concentration was determined to be highest in palm oil and lowest in soya bean oil. Though Cu is vital for maintain good health however very high intake can be reason for reason for undesirable health problems such as liver and kidney damage (Ikem and Egiebor, 2005). In general, the recommended value for intake of copper by WHO is 1.3mg/kg per day as the maximum. In addition rancidity of oil may accelerate with the presence of excess Cu which act as a catalyst (Jamieson, 1943). In the literature Cu levels in edible oil sample were described in the range of 0.02-0.33 µg/g (Garrido et al., 1994; Zhu et al., 2011).

Chapter: VI

Conclusion

In the present study, the impact of refining process on the physicochemical properties and content of heavy metals and minerals were studied. Crude and refined sample of the same oils were analyzed. Evidently, refining processes have more significant changes to chemical properties and heavy metal concentration of the oils investigated. Chemical properties are the ones that were largely affected in the course of the refining processes. With reference to acid value and percentage free fatty acid (%FFA) content, there was a decrease in its content from crude oil to refined oil. This was no surprise as the acid value (free fatty acid) was targeted specifically in refining via deacidification or neutralisation in order to reduce its toxicity to human (Aluyor et al., 2009). Peroxide value follows the same trend. It is also seen that with the reduction in acid value (%FFA) between crude and refined oil, peroxide value decreased. Hence, acid value and peroxide value has a direct variation with each other. In other words, decrease in acid value (free fatty acid) reduces its decomposition to volatile compounds (aldehydes and ketones) which causes rancidity in oil (Sharma et al., 2002); thus, stability and shelf life of the oil was increased because the peroxide value, which measures the level of deterioration in the oil, has decreased.

However, low iodine value of the soybean oil samples indicate mixing with other cheap oil products and high acid value of palm oil samples indicate that the oil were not well refined.

The presence of heavy metals in vegetable oils may cause negative changes to their taste and smell. Catalytic oxidation of oils with molecular oxygen through metal ions (especially copper) leads to the so-called rancidification of oils. A phenomenon termed “flavor reversion” of oils occurs in the presence of heavy metals and oxygen (Zioła-Frankowska et al., 2011). Treatment process regarding refining and packaging procedures may be responsible for heavy metal contamination in oil. Oil producer should take further care in refining in order to improve the quality of oil following the standard specifications.

The concentration of Fe, Pb, Cd and Cu in the oil samples were within the standard limit.
Hence these oils are safe for consumption.

Chapter-VII

Recommendations and Future perspectives

Refining is a necessary process to make a vegetable oil suitable for human consumption. Human body uses oils and fats in the diet for three purposes, as energy sources, as a structural component and to make powerful biological regulators. So, maintaining quality of edible oil is a great concern. Metals present in edible vegetable oil, could be from the soil, pollution or even during manufacturing process. Some elements such as Cu, Zn, Fe, can act as nutrients and are important for health while others such as Ni, Pb, Cd, Ag, Hg may be harmful especially when not consumed in the right proportion.

The level of these metals (especially those that are harmful) may be reduced by more careful handling practices. The dangers and threatening effects of these metals can be prevented as follows:

- Appropriate regulatory measures and establishment of facilities for environmental sound collection and disposal of hazardous waste containing heavy metals with high concentration.
- Production in both industries and at local level should be done using stainless steel equipment.
- Refining process should be followed properly.
- Awareness campaign must be created on the production of oil, storage and probably the rate of consumption of the respective vegetable oils.
- Subsequent research work should be carried out by relevant ministries to identify and control the concentration of metals present in the edible oil.

However, there were some limitations in this study such as some heavy metals- Ni, Cr, As etc. need to analyze. Cholesterol level, phenolic compounds, antioxidant capacities and fatty acid composition during refining should be determined.

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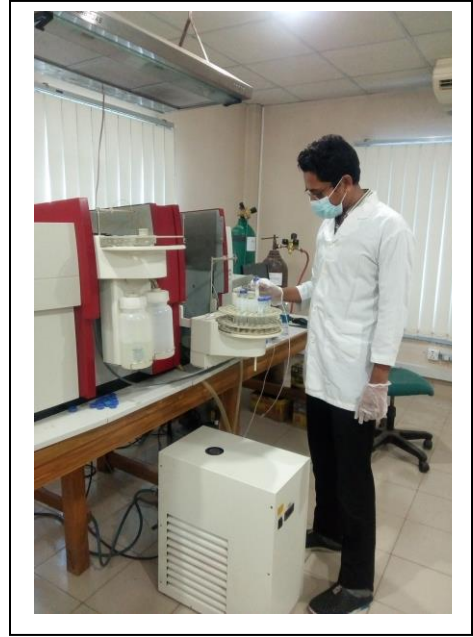
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Appendix A: Photo gallery



Brief Biography

Mohammad Ariful Islam 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, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong. Now, he is a candidate for the MS degree Food Chemistry and Quality Assurance under the Department of Applied Chemistry and Chemical Technology of same faculty.

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