Comparative analysis and adulteration detection of commercial mustard oil

A Thesis Submitted

By

Kaberi Datta

Examination Roll No: MS 070723

Session: 2006- 2007

Submitted to

**Biotechnology and Genetic Engineering Discipline, Life Science School,
Khulna University, Khulna-9208, Bangladesh
in partial fulfillment of
the requirements for the degree of**

'Master of Science (MS) in Biotechnology and Genetic Engineering'

Biotechnology and Genetic Engineering Discipline
Life Science School, Khulna University
Khulna-9208, Bangladesh

July, 2009

Comparative analysis and adulteration detection of commercial mustard oil

A Thesis submitted

By

Kaberi Datta

Examination Roll No: MS 070723

Session: 2006-2007

Approved as to the style and content by

22/07/09

Supervisor

Dr. Kazi Mohammed Didarul Islam

Assistant Professor
Biotechnology and Genetic Engineering Discipline
Khulna University, Khulna-9208
Bangladesh

17/11/09

Co-supervisor

Dr. Khondoker Moazzem Hossain

Professor
Biotechnology and Genetic Engineering Discipline
Khulna University, Khulna – 9208
Bangladesh

17.11.09

Prof. Md. Raihan Ali

Chairman
Examination committee,
Biotechnology and Genetic Engineering Discipline
Khulna University
Khulna 9208, Bangladesh

July, 2009

Dedicated

TO MY PARENTS

WHO INTRODUCED ME TO THE TRUTHFULNESS OF LIFE

Comparative analysis and adulteration detection of commercial mustard oil

Declaration

This thesis reports the original research work of the author, except as otherwise stated. The material presented has not been submitted either in whole or in part for a degree from this or any other university. The findings of the research has not been/will not be published anywhere without the concurrence of the concerned supervisor/co-supervisor.

Signature : Kaberi

Student No. MS070723

Date : 17.11.09

ACKNOWLEDGEMENT

All praises to God, Who has given me the ability to perform this thesis work with sound condition.

I wish to acknowledge my profound indebtedness and deepest sense of gratitude to my honorable supervisor, Dr. Kazi Mohammed Didarul Islam, Assistant Professor, Biotechnology & Genetic Engineering Discipline, Khulna University, Khulna for his scholastic guidance, constructive suggestion, keen interest, strong inspiration, valuable criticism throughout the research work and preparing of the thesis paper.

I want to express my sincere and grateful thanks to my respected co-examiner Sawdagar Shamim Faruque, Assistant Director, Chemistry Division, Bangladesh Standards and Testing Institution (BSTI), Khalishpur, Khulna, Bangladesh for his constant inspiration, encouragement, innovative suggestions and advice during my thesis work at quality control laboratory of BSTI.

Cordial thanks go to Sheikh Masum Billah, Examiner, BSTI, Khalishpur, Khulna for his guidance and help to conduct each steps of this thesis work. I am also grateful to Md. Alamgir Hossain, Analyst, BSTI, Khulna.

The author wishes to express my gratitude to my respectable teacher Prof. Dr. Khondoker Moazzem Hossain, Head, Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna, for his communication to conduct my thesis work at the BSTI.

Appreciation is accorded to my friends, Reza and Jhumu, for their help, and also thanks to my all roommates especially, Rima for their co-operation during my thesis work.

Finally, I am grateful to my parents for their immeasurable blessing, continuous inspiration and for bringing me up to this level and supporting me all the time.

The Author

ABSTRACT

Mustard oil is one of the most important edible vegetable oil in the market. It is the major sources of beneficial unsaturated fatty acid which is important to human health. Due to the high price in the market, it is usual to mix adulterant with it. This study was carried out at Bangladesh Standard Testing Institute (BSTI), Khulna to evaluate the proper condition of commercial mustard oil available in the market. To perform this study, the quality analysis was done by physical and chemical assays. From the physical tests (moisture content, specific gravity, refractive index and color), it was observed that the analyzed values of Radhuni, Teer, Fresh and Local 3 among the experimental samples were within the acceptable range. In chemical tests (acid value, iodine value, saponification value, peroxide value and iron content), particularly Radhuni, Teer, Fresh and Local 3 samples fulfilled the required criteria among the investigated samples. The adulterant tests (synthetic allyl isothiocyanate, argemone oil, mineral oil) were carried out which reflected that synthetic allyl isothiocyanate and mineral oil were not added to any sample. As an adulterant, argemone oil was present in Dolfin, Local 1, Local 2 and Local 4. Through the analysis of physical and chemical parameters, four individual samples (Radhuni, Teer, Fresh and Local 3) out of eight were found to satisfy the standard set out by BSTI.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter one

Introduction

CHAPTER ONE

Introduction

Mustard oil is of vegetable origin and made from mustard seeds. The word *mustard* comes from the Middle English *mustarde,* meaning condiment. Commercial mustard and rapeseed are obtained from the *Brassica* genus belonging to the family *Cruciferae*. There are more than forty different varieties of mustard plants, but three produce the seeds that are used to make the mustard condiment. It is an annual crop. It can be produced from black mustard (*Brassica nigra*), brown Indian mustard (*Brassica juncea*), and white mustard (*Brassica hirta* or *Sinapsis alba*) (Weiss, 1983). Oil of *Brassica campestris* is known as rapeseed oil, and oil of *Brassica hirta*, is known as mustard oil.

Mustard oil is the third largest edible oil produced in the world after Soy oil and Palm oil. It is one of the most important oilseeds accounting for 30% of the oilseeds produced in Bangladesh. Our country is the 12th largest producer of rapeseed over the last five years from 1998-99 to 2002-03, with production averaging 255 thousand metric tons per year (PS&D Database, FAS/USDA; 2003). The total harvested area in the country was 816 thousand acres in 1990. The harvested area has decreased 6 percent from 816 thousand acres in the early 1990s to 813 thousand acres at the beginning of this decade. In spite of this, rapeseed production and yield have increased over the last decade by 9 percent in the country (FAO, 2005).

Our domestic production of mustard oil is 76,890 tones in 2001-02, 77,000 tones in 2002-03, 69,488 tones in 2003-04, 63,154 tones in 2004-05, 60,543 tones in 2005-06, and 79,200 tones in 2006-07. This production cannot meet our demand, because our total consumption level is 169 thousand metric tons oil that would be extracted from 445 thousand metric tons seed per year. On an average, our country has traditionally been importing rapeseed 189 thousand metric tons rapeseed per year. This imported seed produces 72 thousand metric tons oil that would be 38% oil extraction rate (Bangladesh Bureau of Statistics, Oil world, 2007). Local output of oils and fats is decreasing gradually. This is partly due to limited availability of land for oilseed crops; low application of technology; and farmers' interest in more profitable crops, among other factors.

Worldwide production of rapeseed (including canola) rose to 46.4 million metric tons in 2005. Major highest producing countries are China (13.0 million metric tons), Canada (8.4 million metric tons), India (6.4 million metric tons), Germany (4.7 million metric tons), France (4.4 million metric tons), UK (1.9 million metric tons) and Australia (1.1 million metric tons) (FAO, 2005).

Mustard oil is a good source of protein containing (28%-36%) and mostly composed of fatty acid. It is a good source of vitamin A, vitamin E, vitamin C, vitamin K and beta-carotene. It also contains vitamin B6, folic acid, magnesium, calcium, iron, niacin, and an excellent source of phytochemicals (Cheong and Seng, 1998).

Table 1.1 Nutrient composition of mustard oil per 10 ml

Nutrient composition	Range
Protein	1.88 g
Dietary Fiber	1.08 g
Omega 3 Fatty Acids	0.20 g
Calcium	38.92 mg
Magnesium	22.28 mg
Selenium	9.96 mg
Vitamin b3 (niacin)	0.60 mg
Zinc	0.44 mg
VitaminE	32 mg

Source: Agriculture Marketing Information Network (2004)

The phytochemicals are isothiocynate, phenolics, phytins and diathiol thiaones. Phenolics are tocopherols, phospholipids and amino acids that retard oxidation of oil. The oil contains phytins (2.3%) that has cariostatic property (Prakash *et al*, 2000). Mustard oil has anticancer properties because containing various components of oil, such as curcumin, antioxidants, and isothiocyantes (Ganesh, 2006). Antioxidants include vitamin E (tocopherol), selenium, magnesium, and monounsaturated fatty acid. Dietary antioxidants prevent low density lipoprotein (LDL) oxidation (Jha *et al*, 1995). This antioxident inhibits the growth of certain yeasts and molds, enabling mustard to function as a natural preservative. It increases oxidative

stability of the oil (Wagner *et al*, 2004). Mustard oil does not contain antigrowth factors or antithyroid compound that found in soybeans (Prakash *et al*, 2000).

Mustard oil has about 60% monounsaturated fatty acids of which 42% erucic acid and 12% oleic acid; additionally it has 21% polyunsaturates of which 6% is the omega-3 alpha-linolenic acid and 15% omega-6 linoleic acid (USDA Food Data, 2000). Traditional rapeseed oil contains higher amounts of erucic acid and glucosinolates, both of which were deemed undesirable for human consumption by the United States Food and Drug Administration (FDA, 1990).

Canola seed that is a variety of rape seed from which low erucic acid rapeseed oil and low glucosinolate meal are obtained. Canola is a trademarked quality description of a group of cultivars of rapeseed variants that is a heterogeneous mixture of several distinct *Brassicae* species including *B. Napus, B. Campestris, B. Juncea, B. Sarson* and others. It is a genetically engineered oilseed, developed in Canada in the 1960's and 1970's from the traditional rapeseed (Downey, 1964). It is also known as "LEAR" oil (for Low Erucic Acid Rapeseed). Canola was initially bred in Canada by Keith Downey and Baldur Stefansson in the 1970s. Currently, canola oil contains 0.5 to 1% erucic acid, which is well below the 2 percent limit set by the Food and Drug Administration (http://www.mayoclinic.com/health/).

Mustard oil is the so-called heart friendly oil, because it contains high amount of mono-unsaturated fatty acids and a good ratio of polyunsaturated and mono-unsaturated fatty acids. It contains the least amount of saturated fatty acids, making it safe for heart patients. Thus, the use of this oil can reduce the risk of coronary heart diseases (Manchanda, 2006).

Mustard oil contains omega 3 fatty acids (9-15%), which is also known as essential fatty acid. It is one of the richest sources of essential fatty acid (Ganesh, 2006). This fatty acid is indispensable to our health as our body cannot synthesize it by itself. It is the best source to repair nerve cells and reduces the risk of chemically induced cancer. It aids the proper development of the brain. It also sharpens our memory and vision in babies (Prakash *et al*, 2000). It has a beneficial role in prevention of hypertension, and inflammatory and immune disorders such as rheumatoid arthritis (Schmidt, 1994). It helps to lower cholesterol and

prevent clotted arteries that may result in strokes, heart attacks and thromboses (Kris-Etherton *et al*, 2003).

Mustard oil is used for various therapeutic applications. It is used to improve blood circulation and muscular development. It is considered helpful with the pain and discomfort associated with arthritis (Khosla, 2000). It stimulates discharges and other systems, increases appetite, keeps insects away, warming, increases perspiration helps cure rheumatism, boosts health and immunity, Crohn's disease, asthma, cramps and adult diabetes (Mukherjee, 2002). Mustard oil containing isothiocyanate stimulates enzymes that convert estrogen to a more benign type and may block steroid hormones that promote breast cancers (Wattenberg and Loub, 1978; Wattenberg *et al*, 1986).

Mustard oil has antimicrobial activity due to containing glucosinolates. It has anti-bacterial (Brabban and Edwards, 1995; Delaquis and Mazza, 1995; Holley, 2003), anti-fungal (Mayton *et al*, 1996; Olivier *et al*, 1999; Singh *et al*, 2008) and anti cancer (Nugon-Baudon and Rabot, 1994) properties.

Mustard oil is used as best cooking oil because of low saturated fatty acids (5.3%), high monosaturated fatty acids (64.3%) and alpha linolenic acid (10%). It has strong use for cooking purpose as it contains thirty percent calcium, phenolics, phytins and protein (Manchanda, 2006).

Mustard oil contains high amount of erucic acid that have significant value in industrial applications (Sonntag, 1991, 1995; Leonard, 1994; Taylor et al., 1994; Eskin *et al*, 1996). Therefore, it offers many industrial uses such as rubber additives, lubricating agent, fat liquoring of lather in tanneries, textile chemicals, detergent additives, plasticizers, candles, polishes and water binding agent. It has been used as raw materials in the preparation of soap products. This oil is also used as fuel oil (Ohlson, 1972).

The quality of the edible mustard oil can be considered from consumer acceptance and a food technological view. The characteristics of the oil can be physical, such as density, color as well as chemical like hydrolysis, oxidation, flavor and polymorphism (Smouse, 1994). For studying oil quality and stability, primary analysis performed on oil is the determination of

2006). A complete fatty acid analysis requires an initial separation based on chain length or unsaturation (Youngs *et al*, 1951).

In our country, mustard oil has a standard limit for each testing parameter that determined by Bangladesh Standard Testing Institute (BSTI). This limit distinguishes the quality of oil from adulterant one. In order to detect the purity of the oil, physical analysis like moisture content, specific gravity, color and refractive index as well as chemical analysis such as acid value, iodine value, saponification value, allyl isothiocyanate content and iron value was done on commercially available local market oil. Standard acid value is 3.00 (max.) that determines saturated fatty acid in mustard oil. In case of high content of free fatty acids, the oil turns into sour as well as shows discoloration. Iodine value is 98 to 108 that indicate the number of unsaturated fatty acids of the oil. When this range is changed, it indicates that the oil is adulterated with another one. Allyl isothiocyanate test is one of the most important test, because its higher presence in the oil indicates that synthetic allyl isothiocyanate is added to the oil that gives a toxic effect to human health. Natural allyl isothiocyanate range in the oil is 0.3 to 0.6%. Therefore, in order to detect the proper quality of mustard oil, physical and chemical parameters analysis is essential for human consumption.

Adulteration has been a problem in the oil and fat trade for long time (Williams, 1966). Generally, expensive oil is intensively adulterated with the cheaper one (Shukla *et al*, 2005). Adulterant mixing in locally available market oil is a common problem in Bangladesh. Thus, our study was conducted on commercial mustard oil to analyze the quality of edible mustard oil available in the market.

Objective of this study

- Comparative analysis of available mustard oil in local market
- Detection of adulteration in locally available market oil

Chapter two

Literature review

CHAPTER TWO

Literature review

Around the world, mustard oil is common edible oil. This oil is extracted from seeds of a number of species belonging to the genus *Brassica*. It is therefore, difficult to identify the species from which the oil is derived. It is largely consumed in our country for edible purposes; small quantities are also used for pharmaceutical purposes. All manufacturers check the mustard at each point in the process. Government food processing regulations set parameters for cleanliness of standard oil preparation. In our research, we analyzed various parameters of quality control of mustard oil. For this purpose, we reviewed available information which supports the objectives of this research. Most of the literature is focused on the followings:

2.1 Processing of Mustard

Muster oil can be made in two different ways. By pressing the mustard seeds we get a vegetable oil. The other way is by grinding the seeds. It is then mixed with water and extracted the oil by distillation. The mustard seed itself is not spicy: When mixed with water or other cold liquid such as wine, vinegar, beer, a chemical reaction occurs between an enzyme and a glucoside from the seeds, resulting in the production of the oil, allyl isothiocyanate. Liquid oil and solid portion is then separated in filters. The solid portion, known as oil cake, is sold as cattle feed. Edible oil is packed either in tins, jars or food grade plastic pouches. The oil contents depend upon quality of seeds but the average recovery of oil from seeds is in the range of 34% to 38% (Vaughan and Hemingway, 1959). The nutritional value of the processed oils is lowered by processing, as nature designed foods so that unsaturated fatty acids and vitamin E complex occurs together in the same food. Processing destroys this vitamin (Gur and Harwood, 1991).

2.2 Fatty acid composition of mustard oil and its health effect

Mustard is comprised primarily of protein and fat. The fatty acid composition of the oil determines its nutritional quality. Oleic acid belonging to mono unsaturated fatty acid is nutritionally desired, which gives stability to the oil, along with two essential fatty acids, linoleic and linolenic. However, it also contains high amounts of erucic acid, which is nutritionally undesired. So, low erucic acid Brassica oil has a nutritionally desirable fatty acid profile, with low saturated fatty acids and significant levels of linolenic acid, an omega-3 fatty acid (Eskin *et al*, 1996).

Table 2.1 Fatty acid composition of mustard oil

Fatty acid composition	Range
Saturated fatty acid (SFA)	5.3%
Poly saturated ideal fatty acid (PSIFA)	4.7%
Mono unsaturated fatty acid (MUFA)	64.3%
Poly unsaturated fatty acid (PUFA)	24.8%
Cholesterol	0.0%

(Source: SAARC oils and fats in June & July, 2007)

2.2.1 Saturated fatty acid (SFA)

SFAs are those fatty acids, which have no double bonds in the carbon chain. It is 5.3% in mustard oil while soybean oil is 13.1%. This oil contains such SFA namely palmitic acid, stearic acid etc.

2.2.1.1 Health impact of SFA

SFAs are not good for health. Palmitic acid is the prime offender among all SFAs. Free fatty acids are considered as bad for health because these can increase LDL cholesterol that carries about 60-80% of the plasma cholesterol, which is called bad cholesterol (Gurr, 1992). High levels of blood cholesterol, in particular LDL cholesterol, constitute a major risk factor in coronary heart diseases (CHD) (Barr *et al*, 1992). The dietary consumption of saturated fats has, in recent years, been regularly cited as a major factor in the pathogenesis of

atherosclerosis and its associated sequelae, namely, ischemic heart diseases and peripheral vascular diseases, increasing serum cholesterol, thrombogenic and hyperlipidemic. These fatty acids also accumulate in the body cells and destroy myelin which coats and insulates nerve fibers. This results in loss of neurosensory functions and ultimately causes Adrenoleukodystrophy (ALD) (Peter, 1993). Therefore, high consumption of saturated oil is considered to be evil and its use is discouraged (Enas, 1996). But, a lack of adequate saturated fats leads to the failure of lung working mechanism (Enig, 2000).

2.2.2 Mono unsaturated fatty acid (MUFA)

Fatty acids with only one double bond are called MUFAs. The percentage of MUFA is higher in mustard oil, which is a desirable quality. Oleic acid and erucic acid are predominate MUFA in mustard oil.

2.2.2.1 Beneficial role of MUFA

MUFAs are beneficial for heart. These are therefore, metabolically more favorable than saturated or poly unsaturated fatty acids. MUFA may protect and lower bad cholesterol, low density lipoprotein (LDL) and increases high density lipoprotein (HDL), what is called good cholesterol. It is generally accepted that HDL protects against CHD and removes deposited cholesterol from artery walls along with excess cholesterol from fat-loaded cells (Caggiula and Mustad, 1997). Clinical studies have reported that the substitution of MUFA in place of SFA resulted in a reduction of total cholesterol and LDL cholesterol, perhaps without reduction in HDL cholesterol. Oleic acid has been shown to have a protective role during oxidative modification of lipoprotein in *in vitro* studies. Oxidized LDL accelerates cell production leading to arterial injury (Butanone *et al*, 1992) of whose ultimate fate is atherosclerosis. Oxidative changes of LDL increases atherogenicity (Parathasarathy and Rankin, 1992). Diets high in MUFA (oleic acid C18:1) make LDL resistant to oxidation, restore LDL-receptor activity, and markedly lower LDL levels (Enas *et al*, 2003). So, it is reported that dietary fats play an important role in the development of atherosclerosis by modulating serum cholesterol concentration (Grundy and Denke, 1990; Khosla and Sundram, 1996). Dietary MUFA such as oleic acid is equally as effective in lowering plasma cholesterol level as dietary PUFA (viz., linoleic acid) (Mattson and Grundy, 1985; Kris-Etherton and Yu,

1997). Valsta *et al*, (1992) showed that rapeseed oil lowered the harmful LDL cholesterol by 23% versus 17% for sunflower. Surprisingly, these oils enhanced platelet aggregation to collagen.

2.2.2.2 Harmful effect of MUFA

Erucic acid may be a harmful compound regarding its negative effect on health of heart. Feeding a high erucic acid rapeseed oil (HEAR) led to an even greater platelet reduction and increased platelet size (Kramer *et al*, 1998). Erucic acid is implicated with cancer and rancidity and glucosinolates are goitrogenic. Mustard oil is considered as good for heart but its erucic acid content is a concern, because erucic acid does not metabolize well in the body and weakens the heart muscles. So, a diet with high erucic acid rapeseed oil was associated with myocardial damage characterized by fatty deposits around the heart and kidneys and muscle lesions in the heart (Taylor *et al*, 1994; Eskin *et al*, 1996). With the development of new oil canola, mustard oil has been shown a protective effect when harmful part removed from it. So, canola oil with zero erucic acid oil, found 70 per cent reduction in heart attack deaths in people who already had a cardiac arrest (Reddy, 2004).

2.2.3 Poly unsaturated fatty acid (PUFA)

Fatty acids containing more than one double bond are called polyunsaturated fatty acids (PUFA). PUFAs like linolenic acid, linoleic acid and eicosanoic acid, which are belongs to the omega fatty acid series that is called Essential Fatty Acids (EFAs). There are 2 series of PUFA that are deemed EFAs namely Omega-3 (n-3) and Omega-6 (n-6). Omega-3 fatty acids are derived from linolenic acid and Omega-6 from linoleic acid (Grundy, 1986). EFAs are necessary fats that human cannot synthesize, and must be obtained through diet. PUFA content is lower (linoleic and linolenic acid: 13-17% and 5-18% respectively) in mustard oil (Ahmed *et al*, 2000). So, it contains significant amounts of polyunsaturated fatty acids (PUFA). Such polyunsaturated oils have been seen as desirable from the health point of view (Wijesundera *et al*, 2008).

2.2.3.1 Beneficial effect of PUFA

PUFA has recently found to be very good for health. It has a positive role in vascular diseases, inflammation, and allergic diseases and in platelet aggregation. Mustard oil has an ideal ratio of Omega-3 and Omega-6 fatty acids, which are essential for human health. Omega (n-3 and n-6) fatty acids are potent inhibitors of cancer. These may also lessen the severity of some autoimmune diseases. PUFA reduce plasma triglycerides and increase blood-clotting time. The omega (n-6 and n-3) fatty acids when substituted for saturated fatty acid lower serum, total cholesterol and LDL cholesterol (Lichtenstein *et al*, 1993). In addition to this, these have antioxidant activity that fends off artery damage from LDL cholesterol, thus benefiting the arteries. It is, therefore, used as a biological marker for healthy heart (Freese *et al*, 1997). So, it is reported that Omega-3 fatty acid is anti-thrombogenic where as Omega-6 fatty acid is anti-atherogenic (Failor *et al*, 1988).

2.2.3.2 Harmful effect of PUFA

High dietary PUFA causes bad impact on human health. The replacement of saturated fats by vegetable oils containing high levels of PUFA may also render the individuals susceptible to cardiovascular diseases (Lawson, 1997). High PUFA is shown to depress the response of lymphocytes to mitogens and antigens. However, a very high n-6 PUFA to n-3 PUFA ratio may increase the thrombogenicity through increased production of arachidonic acid and thromboxane A2. This is because linoleic and linolenic acids use the same set of enzymes for desaturation and chain elongation (Li *et al*, 1999). It results in a high degree of unsaturation in membrane phospholipids, which in turn may increase lipid peroxidation, cholesterol oxidation, free radical accumulation and membrane damage (Enig, 2000). The undesirable effect of PUFA is increased susceptibility for peroxidation. Lipid peroxidation is an oxidative deterioration of PUFA with the formation of highly toxic products such as peroxides and aldehydes (Lauridson *et al*, 1999). It is suggested that dietary ingestion of thermally or autoxidatively stressed PUFA rich culinary oils is more harmful compared with those similarly treated oils rich in saturated fatty acids or MUFA (Prabhu, 2000).

2.3 Analysis of testing parameters of mustard oil

On commercial oils and fats for various brands, Physical and chemical tests help to evaluate the suitability of oils for a specific purpose. For checking purity, chemical tests were developed long ago for their characterization. Every test has certain value in case of particular oils, but this value would be changed by environmental condition, mechanical problem and operation managing. So, physical and chemical tests are done to identify mixture of any other oil (Grifen, 1927).

2.3.1 Physical properties of the oil

The physical properties are moisture content (Grifen, 1927), specific gravity (Hildich, 1949), refractive index (Hildich, 1949) and color (Gardner, 1953) of the oil were determined using the standard methods.

2.3.1.1 Moisture content

Moisture content is the percentage of water that is present in the oil. A small amount of water is added to the processing of seed that indicates moisture content. In case of high moisture content, it may be sensitive to contamination by ferrous and rust particles (http://www.tis-gdv.de/tis_e/sitemap/sitemap.htm).

2.3.1.2 Specific gravity

Relative density is a simple ratio comparing the weight of oil to the weight of water. It is closely related to specific gravity and is usually quoted at 15.5°C (60°F). The test temperature must be quoted. The density may be used to convert mass to volume, giving invaluable information for packing operations. It may be measured using a hydrometer or by using a pyknometer (weighing bottle) (http://www.Seatons.com/). The specific gravity of practically all fats and oils lies between 0.90 and 0.95 (Hildich, 1949). The observed specific gravity of brassica seed oil at 25^0 C is 0.909 (Biswas *et al*, 2001).

2.3.1.3 Refractive Index

Refractive index of oil has been reported to increase on autoxidation. A rapid refractometric method has been used for the determination of light stability of oil. The pattern of changes in the refractive indices of fats and oils on autoxidation is related to rancid odor development (Arya *et al*, 2007). The refractive index of Brassica seed oil at 25^0C is 1.470 (Biswas *et al*, 2001). The rate of autoxidation and development of off-flavors depend on glyceride composition (percentage of linoleic acid), peroxidants, antioxidants, and handling and storage condition employed (Salunkhe *et al*, 1986).

2.3.1.4 Color

The Lovibond method employs a complex series of red, yellow, blue and neutral slides which are combined to give an exact match. The color of the sample is compared to standard glass slides. The color of an oil has a direct bearing on the color of the end product and is therefore of great importance to the customer (http://www.Seatons.com/).

2.3.2 Chemical properties of the oil

The chemical properties are acid value (Williams, 1966), iodine value (Williams, 1966), saponification value (Hildich, 1949), peroxide value (Jacobs, 1959) and iron value (Vogel, 1961) of the oil were determined by standard methods.

2.3.2.1 Acid value

Acid value (AV) is an important indicator of vegetable oil quality. AV is expressed as the amount of KOH (in milligrams) necessary to neutralize free fatty acids contained in 1 g of oil (ISO 660, 1983; Firestone, 1996).

AV determines the standard limit of saturated fatty acid. So, the acid value of oil may be used as a measure of quality. However, the acid value of the oil must not be too high, as this denotes an excessively high content of free fatty acids, which causes the oil to turn sour. Then, saturated fatty acid like palmitic acid turns into unsaturated form such as erucic acid which has a toxic effect on health. This may occur in case of long time preservation in the container. Discoloration may also occur (http://www.tis-gdv.de/tis_e/sitemap/sitemap.htm).

In commercial mustard oil, AV was determined 3.00 (max.) by BSTI (Personal communication). Sultana *et al*, (1998) showed in their research that processed sarsoon oil containing 39% erucic acid has an acid value of 2.80. They also found that canola oil containing 7% erucic acid has an acid value of 3.90. Niewiadomski (1990) reported acid value of 1.25 – 2.30 of high erucic acid rapeseed oil which was lesser than 2.80 (Sultana *et al*, 1998) of sarsoon oil (high erucic acid). Sarsoon oil was processed prior to feeding to reduce erucic acid (Mattil *et al*, 1964). Biswas *et al*, (2001) found that the acid values of brassica, linseed and sesame seed oils are 1.06, 1.42 and 9.80, respectively. The percentages of free fatty acid (as oleic) was calculated from acid value and were found to be 0.53%, 0.71% and 4.92%, respectively. As high percentage of free fatty is a determinant or indication of unsuitability of oil for edible purpose (Carroll and Noble, 1957), it seems that brassica and linseed oil may be suitable for edible purpose but sesame seed oil is not (Biswas *et al*, 2001). High acid values indicative of high free fatty acids obtained from sesame oil showed rancidity. Long storage of the oil seeds before or after processing may have been responsible for rancidity (Okpuzor *et al*, 2009). Nowsheen (1994) observed in her study that high acid value of canola oil might be due to improper handling or storage.

2.3.2.2 Iodine Value

Iodine value (IV) determines the number of double bonds that indicates the degree unsaturation of the oil or fat. It is one of the most valuable parameter for differentiating or identifying oils and readily serves to indicate the group to which the oil belongs. The unsaturated acids (oleic, linolenic) absorb halogens to form mainly addition products. In order to determine the degree of unsaturation, iodine is introduced to the oil. The iodine will attach itself over a double bond to make a single bond in where an iodine atom is now attached to each carbon atom in that double bond (Welter, 2007).

Higher iodine numbers do not refer to the amount of iodine in the oil, but rather the amount of iodine needed to "saturate" the oil, or break all the double bonds. The higher the iodine value the more double bonds are present, which consequently reflects the reactivity of the oil (http: //www.Seatons.com/). The higher the IV the more unsaturated the oil, the faster it will oxidize and the more it will polymerize. So, the unsaturated fatty acids of the oils are polymerized (Addison, 2007). With high IV of oil, it shows that oils are more unsaturated with high

unsaturated fatty acid containing oil. In case of low IV of oil, it is more saturated with high saturated fatty acid containing oil (Welter, 2007).

The iodine value corresponds to the average number of double bonds in the oil (Anonymous, 1987). Standard iodine value is 96 - 107 (black mustard oil) or 92 - 108 (white mustard oil) (http://www.tis-gdv.de/tis_e/sitemap/sitemap.htm). Youngs *et al*, has reported that original iodine value of rapeseed oil is 104.1. Iodine value was measured by the Wijs method with one-half hour reaction time (Youngs *et al*, 1951). Iodine value of 81.4 for rapeseed oil containing 43.3% erucic acid and 112 for canola oil (low erucic acid) has been reported (Weiss, 1970). Ackman (1977) reported that higher iodine value in canola than sarsoon oil indicates its higher degree of unsaturation. Sultana *et al*, (1998) showed in their research that processed sarsoon oil containing 39% erucic acid has an iodine value of 104.00. They also found that canola oil containing 7% erucic acid has iodine value of 109.00. The iodine value of brassica, linseed and sesame seed oils are 96.50, 190.00 and 105.00, respectively. The high iodine value of oil indicates the abundance of unsaturated fatty acids in oil (Biswas *et al*, 2001).

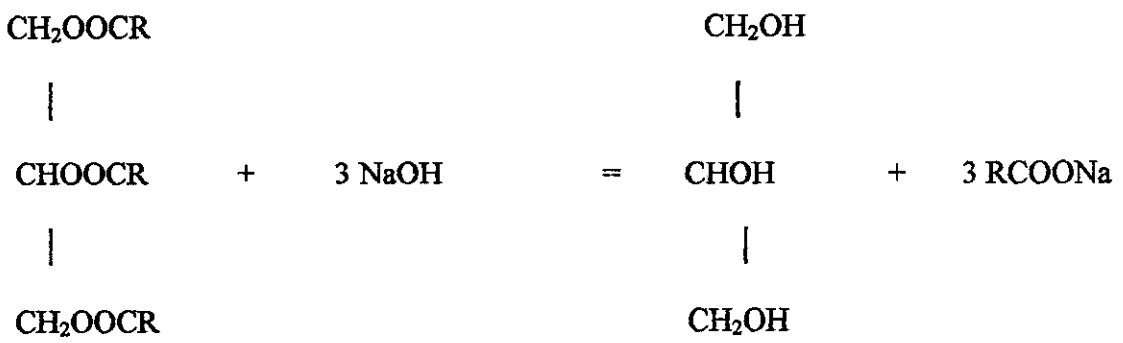
2.3.2.3 Saponification value

Saponification value (or "saponification number", also referred to as "sap" in short, which consists of the sodium salts of the fatty acids) represents the number of milligrams of potassium hydroxide or sodium hydroxide required to saponify 1g of fat under the conditions specified. It is a measure of the average molecular weight (or chain length) of all the fatty acids present. As most of the mass of a fat/trimester is in the 3 fatty acids, it allows for comparison of the average fatty acid chain length. The hydrolysis of a fat or oil is given the particular name, saponification (http://www.Seatons.com/).

If more moles of base are required to saponify N grams of fat then there are more moles of the fat the chain lengths are relatively small, given the following relation:

Number of moles = mass of oil/relative atomic mass

Saponification equation



Oleic Acid, R = $CH_3(CH_2)_7(CH)_2(CH_2)_7$

Plamitic Acid, R = $CH_3(CH_2)_{14}$

Stearic Acid, R = $CH_3(CH_2)_{16}$

Youngs *et al,* (1951) has reported that original saponification value of rapeseed oil is 174.3. Biswas *et al,* (2001) has showed that saponification value of brassica, linseed and sesame seed oil are 173.00, 191.00 and 196.00, respectively. These results indicate that brassica seed oil contains more long chain fatty acids than other two oils (Biswas *et al,* 2001). So, the saponification value is used primarily as an identification aid to detect adulteration (http://www.Seatons.com/).

2.3.2.4 Peroxide value

The number of peroxides present in a vegetable oil is an index of its primary oxidative level and consequently of its tendency to go rancid. Unsaturated free fatty acids react with oxygen and form peroxides, which determine a series of chain reactions that generate the production of volatile substances that bring the typical rancid smell. Those reactions are accelerated by high temperature and by light and oxygen exposure. The chemical analysis used to determine the peroxide value of vegetable oils is an index of its status of preservation. In fact, the lower is the number of peroxides; the better is vegetable oil quality and its status of preservation (http://www.Seatons.com/).

Peroxide value is correlated with the organoleptic evaluation of rancidity. It is reported that this value has its inherent limitations. Additionally, it does not precisely indicate the exact

termination of the induction period (Arya *et al*, 2007). Oils and fats spoil by readily becoming rancid. Rancidity is promoted by light, atmospheric oxygen and moisture that leads to change in odor and taste. Thus, the tanks and barrels must be filled as full as possible, taking into consideration the coefficient of cubic expansion.

The classical experiment of Geier (2004) has illustrated that the September 15 sample of oil tested from the same batch as the July 18 oil, in which the peroxide value was significantly higher after only 2 additional months in the bottle. The researcher observed that the maximum allowable peroxide value is 1.0 meq/kg, much lower than the 3.01 (July 18) and 4.43 (Sept. 15) meq/kg. So, he concluded that high peroxide value is mostly due to the age of the seeds. The value is correlated to spoilage that has already taken place in the oil, and thus time is the enemy (Geier, 2004). The peroxide value is higher of canola oil which indicates higher proportions of double bonds (Niewiadomski, 1990). Sultana *et al*, (1998) showed in their research that processed sarsoon oil containing 39% erucic acid has peroxide value of 3.60. They also found that canola oil containing 7% erucic acid has peroxide value of 5.10.

2.3.2.5 Iron value

Iron level is quite detrimental to the color and flavors stability of the oils. It leads to pro-oxidative effects resulting in poor color and poor flavor stability in mustard oil. Relatively high concentrations of iron often occur because of the inevitable contact of oils with iron in the course of extraction, storage and transport.

In commercial mustard oil, the maximum iron content is 5.00 mg/kg determined by BSTI (Personal communication). Biswas *et al*, (2001) has observed that iron content of brassica seed oil, linseed oil and sesame seed oil are 8.44 mg, 13.55 mg and 9.00 mg, respectively.

Table 2.2 BSTI Standards for mustard oil

Parameter	Serial no.	Characteristics	Limits
Physical	1.	Moisture content, percent by weight	0.10 to 0.21% (mix.)
	2.	Colour in a 5¼ inch cell on lovibond scale max (Y+ 5R)	30
	3.	Refractive index at 40 ^{0}C	1.4650 – 1.4670
	4.	Relative density (20^0 C/Water 20^0 C)	0.910 to 0.921
Chemical	1.	Acid Value	3.00 (max.), 0.6 mg/KOH per gram
	2.	Iodine value (Wijs)	98 to 108 0.6 mg/KOH per gram
	3.	Saponification value	169 to 176
	4.	Peroxide value, expressed as milliequivalents (meq.) oxygen per kg.	10 (mix.)
	5.	Iron value	5.00 mg/kg (mix.)
	6.	Allyisothiocyanate value (%)	0.3 to 0.6% (mix.)

2.4 Adulteration in mustard oil

Adulteration is a problem in mustard oil due to its high demand in the market. It is sometimes deliberate and occasionally, it is accidental. Indeed, accidental contamination is hard to avoid in modern bulk handling installations, where oils of different qualities must be pumped through common valves and pipelines. However, generally expensive oil is intensively adulterated with cheaper one (Shukla *et al*, 2005). Food safety is an essential public health issue for all consumers. Adulteration of mustard oil makes the product quality low to cheat the consumer is quite common. This can lead to health hazards and to financial losses for consumers, families, communities, and countries.

2.4.1 Adulteration with argemone oil

Argemone oil *(Argemone mexicana)* is occasionally found as a contaminant in edible oils, particularly in mustard oil. *Argemone mexicana* (Poppy Weeds) seeds that closely resemble

mustard seeds are often mixed as an adulterant with it, while extracting oil for edible purposes. Such oils are tremendous health hazard like epidemic dropsy. Mustard oil contaminated with argemone oil contains a toxic alkaloid, sanguinarine. It interferes with the oxidation of pyruvic acid which accumulates in blood (Govt. of India, Annual report, 2001-2002). This oil causes dreadful maladies like dropsy, necrosis, high tension glaucoma, diarrhea, vomiting and anemia (Shukla *et al*, 2005).

Babu *et al*, (2006) studied on safety level of contaminated mustard oil with argemone oil. They reported that epidemic dropsy is a disease caused by the consumption of mustard oil contaminated with argemone oil (AO). During 1998 dropsy in New Delhi, which is so far the largest with more than 3000 victims and over 60 deaths, it was enquired at various scientific and regulatory meetings about the maximum tolerated dose of AO. Hence, the study was aimed to investigate the safety levels of AO in rats. Animals were given AO in diet at a dose of 0.001%, 0.01%, 0.1%, 0.5% and 1% daily for 90 days and the two control groups received the standard diet with and without 1% mustard oil. A decrease in body weight gain (28–31%) was observed in 0.5% and 1% AO groups; while significant increases in relative lungs and liver weight was noticed in respective doses of 0.01% and 0.1% AO groups as well as in higher dosage animals. Reduction in RBC count and hemoglobin content ($p < 0.05$) was noticed in 0.01% and 0.1% AO exposed animals. This effect was more pronounced in higher AO doses. Serum marker enzymes including alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were found to be significantly elevated in 0.01–1% AO groups. Further, a decrease in albumin/globulin ratio (42–78%) was observed in the serum of 0.01% to higher AO dose groups. The levels of serum triglycerides and VLDL cholesterol were found to be enhanced ($p < 0.05$) in AO treated (0.01–1.0%) animals. Histopathological changes in lung were observed at 0.01% dose of AO while liver, kidney and heart produced changes at 0.1% AO and above doses. None of the parameters were found to be affected in 0.001% AO treated animals. These results suggest that the no observed adverse effect level (NOAEL) dose of AO is 0.001% in rats and considering a factor of 100 for humans for highly toxic compound, the safe limit of 0.00001% (100 ppb or 100 ng AO/g oil) AO can be implicated which shall contain only 0.55% of sanguinarine equivalent to 0.6 ng sanguinarine per gram oil. However, the minimum detectable limit of AO is 5 ppm (equivalent to 5 µg sanguinarine per gram oil)

with the present existing HPLC method, thereby suggesting that mustard oil should be absolutely free from AO contamination.

2.4.2 Adulteration with synthetic allyl isothiocyanate

The commercial value of mustard oil is quite often evaluated by the common man on the basis of the degree of sharpness of smell and pungency exhibited by the oil. The smell and pungency arise from the presence of allyl isothiocyanate (Shukla *et al*, 2002). Standard limit of allyl isothiocyanate is 0.30 – 0.60% (Brand and Jacquot, 2002).

Mustard oil has often been labeled and sold as (natural) mustard oil or adulterate mustard oil with synthetic chemicals like allyl isothiocyanate. It is well known that allyl isothiocyanate is a major component of the mustard oil. It can be synthesized at a much lower price than its cost when extracted from mustard seeds. Therefore, the adulteration of mustard oil by adding synthetic allyl isothiocyanate is very profitable (Jammy *et al*, 2003). The product obtained in this fashion is sometimes known as synthetic mustard oil. Commercial oils, adulterated by such synthetics, can often fool the less sophisticated nose, or satisfy those oil customers buying to a price, where authenticity is sometimes not a primary consideration.

The mustard oil shortage in the market creates quite ofenly a state of black marketing which is followed by supply adulterated mustard oil in market. The synthetic mustard oil is manufactured by colouring certain low priced vegetable oils with an oil soluble yellow dye followed by the addition of requisite amount of synthetic allyl isothiocyanate. The finished oil looks and smells exactly like natural mustard oil, but price wise its manufacturing is quite cheaper and profitable to the unscrupulous manufacturers and retailers (Shukla *et al*, 2005).

2.4.2.1 Natural allyl isothiocyanate verses synthetic allyl isothiocyanate

Allyl ($-CH_2=CHCH_2$), an unsaturated bond, imparts a characteristic odor in some compounds. Thiocyanate is a salt or ester of thiocyanic acid (HSCN). Allyl isothiocyanate is the organosulfur compound with the formula CH_2CHCH_2NCS (Romanowski and kienk, 2005). The main ingredient of mustard oil is allyl isothiocyanate. It produced from glucosinolate containing mustard seed. These glucosinolates are sulfur-containing molecules produced from amino acids by the secondary metabolism (Nugon-Baudon and Rabot, 1994). Mustard seeds contain an enzyme called myrosinase and a glucosinolate called sinigrin. These substances are

stored in separate cells under normal conditions, but react when the seeds are subjected to pressure or heat. In presence of water, myrosinase hydrolyses glucosinolates to form isothiocyanate, which is a chemical compound responsible for smell, strong flavour and pungency. Allyl isothiocyanate (in case of black mustard) has strong and pungent flavor, and normal isothiocyanate (in case of white mustard) is also called p-hydroxy benzyl isothiocyanate (PHBIT), has a pungent taste but is nearly odorless. Powdered mustard has essentially no aroma until it combines to moisture. Isothiocyanate give pungency to mustard oil (http://en.wikipedia.org/wiki/Allyl_isothiocyanate).

Synthetic allyl isothiocyanate is produced commercially by the reaction of allyl chloride and potassium thiocyanate (Romanowski and kienk, 2005).

$$CH_2{=}CHCH_2Cl + KSCN \rightarrow CH_2{=}CHCH_2NCS + KCl$$

It is colorless or pale-yellow, very refractive liquid with a very pungent, irritating odor and acrid taste (Budavari, 1996). It was produced in Germany, Japan, Mexico and the United States (Chemical Information Services, 1995).

Synthetic allyl isothiocyanate is used as a flavouring agent, in medicine as a rubefacient (counterirritant), as a fumigant, in ointments, in mustard plasters, as an adjuvant, as a fungicide, as a repellent for cats and dogs and as a preservative in animal feed (National Toxicology Program, 1991). It is also used as an intermediate to create a desired chemical compound through a series of chemical reactions, including in the field of fungicide, bactericide, wood preservative, pharmaceutical and plastic and rubber (http://en.wikipedia.org/wiki/Allyl_isothiocyanate).

2.4.2.2 Health impact of natural isothiocyanate

Naturally occurring allyl isothiocyanate is so much important on human health. It can interfere with the biotransformation of endogenous intermediates such as estrogen and androgen epoxides to detoxification system (Timms *et al*, 1987). Glucosinolate derivatives isothiocyanate inhibits mammary tumor (Wattenberg and Loub, 1978) and lung tumor (Wattenberg *et al*, 1986; Yano *et al*, 2000). So, it is known to have anti-tumor and anti-oxidant properties by inducing the activity of phase II detoxification enzymes in the urinary

bladder (Nugon-Baudon and Rabot, 1994). Isothiocyanates which has an antibacterial activity against the micro-organisms like *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Escherichia coli* and *Salmonella typhi* (Brabban and Edwards, 1995; Delaquis and Mazza, 1995; Holley, 2003; Singh *et al*, 2008). It has also effective antifungal activity against the food-borne fungi *Aspergillus terreus, Aspergillus flavus, Penicillium purpurogenum, Pencillium citrinum, Penicillium madriti* and *Fusarium graminarium* and *Neurosproa* yeast (Olivier *et al*, 1999; Singh *et al*, 2008).

2.4.2.3 Potential acute health effects of synthetic allyl isothiocyanate

Synthetic allyl isothiocyanate irritates the mucous membranes and induces eczematous or vesicular skin reactions (Gaul, 1964). It is extremely hazardous in case of eye contact (irritant). Inflammation of the eye is characterized by redness, watering, and itching. It is also very hazardous in case of skin contact (irritant, corrosive, permeator), of ingestion, of inhalation. Skin inflammation is characterized by itching, scaling, reddening, or occasionally, blistering. This substance is toxic to kidneys, lungs, liver, and mucous membranes. This highly toxic material may produce general deterioration of health by an accumulation in one or many human organs. Repeated or prolonged exposure to this substance can produce target organs damage. So, severe over-exposure can result in death (http://www.sciencelab.com/).

Shukla *et al*, (2002) has conducted a simple non-instrumental technique to differentiate a natural mustard oil sample from a synthetically made artificial mustard oil. Ally isothiocyanate, either in pure form or blended with some other oil, when refluxed with an aqueous solution of sodium azide (NaN_3) produces 1-allyl-2-tetrazoline-5-thione (Hatt-5), which upon reaction with a neutral or very slightly acidic solution of bismuth nitrate, $Bi(NO_3)_3$, produces a characteristic deep yellow precipitate of a co-ordination complex, ($Bi(att\text{-}5)_2\ NO_3$). In place of bismuth nitrate, bismuth chloride solution can also be used. The Allyl isothiocyanate content of the natural mustard oil, owing to some unknown reason, does not react with sodium azide, hence the final reaction with bismuth nitrate gives no colour reaction. However, synthetic or artificially-made mustard oil samples (to which allyl isothiocyanate has been deliberately mixed with any other oil) easily give a colour reaction with bismuth nitrate following heating with sodium azide solution. A synthetic mustard oil containing as little as 0.1% allyl isothiocyanate can be easily identified by this technique.

LIST OF ABBREVIATIONS

LEAR – Low erucic acid rapeseed

HEAR – High erucic acid rapeseed

LDL – Low density lipoprotein

HDL – High density lipoprotein

SFA – Saturated fatty acid

MUFA – Mono unsaturated fatty acid

PUFA – Poly unsaturated fatty acid

EFA – Essential fatty acid

AV – Acid value

IV – Iodine value

PHBIT - p-hydroxy benzyl isothiocyanate

AO – Argemone oil

KOH – Potassium hydroxide

NaOH – Sodium hydroxide

KI – Potassium iodide

$Na_2S_2O_3$ – Sodium thiosulphate

NH_4HCN – Amonium thiocyanate

$CHCl_3$ - Chloroform

Meq/kg - milliequivalents (meq.) oxygen per kg

Chapter three
Materials and methods

CHAPTER THREE

Materials and Methods

This study was conducted in the "Bangladesh Standard Testing Institute" (BSTI) at Khalispur, Khulna. To perform this study, eight commercial mustard oils available in the market were chosen. Among them, four samples were taken from well-known brand e.g., Radhuni, Dolphin, Teer and Fresh. The rest of four was taken from open market.

3.1 Sampling

The sample was taken in the beaker that was previously cleaned and dried in the electric oven. A cleaned glass rod was taken in the beaker that was used to transfer sample for testing. The following precautions were taken for sampling -

- All sampling instrument, if made of copper, brass or bronze, were nickel plated
- All sampling apparatus were cleaned and dried when used. Sampling instruments were cleaned with hot soapy water or other detergent
- Samples had never taken in an exposed place. The samples, the material being sampled, the sampling instruments and the containers for samples were protected from adventitious contamination. The test samples were placed in suitable clean and dry containers
- Samples were stored in such manner so that they were protected from light, temperature fluctuations and other abnormal conditions
- Sample containers were so filled that the air space above the liquid level was 5 to 10 percent of the capacity of the containers

3.1.1 Preparation of standard 0.1 N NaOH

4.001 g NaOH was taken for each liter of solution. Then 50 ml of the solution was taken into the pipette and titrated against 0.1N HCl, using 2 drops of methyl orange indicator. A second 50 ml of the solution was also titrated with phenolphthalein indicator. The factor for each indicator should be written on the bottle. The factor is obtained by multiplying the factor of 0.1N HCl by the number of ml of the latter used in titration and dividing by 50.

3.1.2 Preparation of standard 0.5 N HCl

18.233 g HCl was added to one liter of the solution or about 43 ml of concentrated HCl. The solution is standardized against pure Na_2CO_3.The Na_2CO_3 was best prepared by heating sodium bicarbonate of the highest purity by the following method -

A platinum dish was filled with pure powdered $NaHCO_3$ and placed in an air bath already heated to 200°C and raised the temperature to 270-280°C.This temperature is remained at 30 minutes and then cooled in a desiccator and before getting quite cold, transferred to a warm, dry, stoppered tube or bottle out of which it was weighed rapidly when wanted. For standardization of 0.5 N acid, 1.1 g of Na_2CO_3 was weighed out accurately.

Prepared Na_2CO_3 was dissolved in about 100 ml of water and 2 drops of methyl orange was added. Then the mixture was titrated with the acid to the end point where the color was found to be from yellow to pinkish orange. For very accurate work the end point had been matched against the color of 100 ml of distilled water saturated with CO_2 and containing 2 drops of methyl orange solution.

3.1.3 Preparation of standard 0.1 N HCl

3.637 gm HCl was taken in one liter of H_2O or about 8.45 ml of concentrated HCl. To standardize this preparation the same method was used as for 0.5 N HCl. About 1 g of Na_2CO_3 was dissolved in 500 ml of water in an accurate volumetric flask and 100 ml of this diluted solution was taken in the pipette and titrated against the 0.1 N HCl.

3.1.4 Preparation of starch solution

Five gm of soluble starch was taken in cold water, poured into 1000 ml of boiling water. Then this mixture was boiled for 5 minutes, cooled and filtered and 2 ml of $CHCl_3$ was added as preservative. This solution was freshly prepared every time.

3.1.5 Preparation of 10% KI solution

This solution was prepared by dissolving 10 g of potassium iodide free from potassium iodate in 90 ml of water.

3.1.6 Preparation of alcoholic KOH solution

Thirty five to forty gm of KOH was dissolved in 20 ml distilled water and added sufficient aldehyde-free ethyl alcohol (95% by volume) to make up to 1000 ml. It is allowed to stand overnight, decanted the clear liquid and kept in a bottle closed tight with cork or rubber stopper.

3.2 Parameters studied

The following physical, chemical and adulterant tests were carried for each mustard oil that collected from market.

3.2.1 Physical tests

a) Moisture content

b) Specific gravity

c) Refractive index

d) Color

3.2.2 Chemical tests

a) Acid value and free fatty acids

b) Iodine value (WIJS)

c) Saponification value

d) Peroxide value

e) Iron (Fe) value

3.2.3 Adulterant tests

a) Synthetic allyl isothiocyanate test

b) Argemone oil

c) Mineral oil test

3.3 Physical tests

3.3.1 Determination of moisture content (Air oven method)

Moisture content includes the moisture and any other material contained in the oil which is volatile under the conditions of the prescribed test.

Apparatus

- Moisture dish
- Desiccator
- Electric oven

Procedure

Ten gm of oil was taken into a moisture dish that had been dried previously, cooled in the disiccator and then weighed. The dish was placed in electric oven for approximately 3 hours at 105 ± 1°C. The dish was removed from the oven cooled in the desiccator to room temperature and weighed. This procedure was repeated but the dish was kept in the oven for one hour each time until the difference between two successive weighing does exceed 1 mg. The heated oil was preserved for the determination of insoluble impurities.

Calculation

Moisture content, percent by weight: $\frac{w \times 100}{W}$

Where, w = loss in weight in gm of the material upon drying

W = weight in gm of the material taken for the test

3.3.2 Determination of specific gravity

The specific gravity of vegetable oil was expressed as the ratio of the weight in air of a given volume of the sample at 30°C to that of an equal volume of water at same temperature.

Apparatus

- Westphal balance
- Spedomanometer or pycnometer
- Air oven

Procedure

Spedomanometer was cleaned and dried in the oven. Then it was cooled and filled with distilled water in such a manner as to prevent the entrapment of air bubbles. The stopper was inserted into the bottle so that the entire bulb completely covered with water. The Westphal balance was calibrated and spedomanometer with water was weighted. After removing water, the bottle was filled with oil, holding the bottle on its side in such a manner as to prevent the entrapment of air bubbles. The stopper was inserted carefully so that any oil has not come out through the capillary opening. The outer side of bottle was cleaned well and weighted it on the westphal balance.

Calculation

Specific gravity: $\frac{W}{w}$

Where, W- Weight in gm of the spedomanometer with oil

w- Weight in gm of the spedomanometer with water.

3.3.3 Determination of refractive index

Refractive index at a given temperature is the ratio of the velocity of light in the oil .It expresses the ratio between the sine of the angle of incidence to the sine of the angle of refraction when a ray of light of known wavelength passes from air into the oil.

Apparatus

- Refractometer
- Light source

Procedure

The instrument was placed so that diffused day light or any form of artificial light could be used for illumination. The temperature was adjusted to the refratometer. For oils, it would be 15, 20 or 25° C, depending on circumstances. The prism of the refrctometer was opened by means of the screw head, cleaned and completely dried. Then, a few drops of sample were placed on the lower prism. It was closed and tighted firmly with the screw head and allowed to stand for 3-5 minutes to come to uniform temparature.The alidade on the side scale was moved backward or forward until the field of vision was divided into light and dark portions. The screw head of the

compensator was rotated until a sharp colorless line was obtained between the fields, then adjusted this line so that it was fallen on the point of intersection of the 2 cross-hairs. The instrument was adjusted and lighted to obtain the most distinct reading possible, and then determined the refractive index.

3.3.4 Determination of color

Apparatus

- Lovibond tintometer or colorimeter
- Glass cell
- Filter paper

Procedure

The sample should be absolutely clear and free from turbidity. The glass cell was cleaned of the desired size with carbon tetrachloride and allowed to dry. It was filled with the clear filtered sample and placed the cell in position in the tintometer. Along side of it such red, yellow, blue or neutral lovibond glass slides as were necessary to match the color shade of the oil, observing the colors of the oil and of the combination of the glass slides through an eye-piece.

Calculation

Color: aY + 5bR

Where, a- the sum of the various yellow (Y) slides used

b- the of total of the various red (R) slides used

3.4 Chemical tests

3.4.1 Determination of Acid value and free fatty acids

Reagent

- Ethyl alcohol- 95% (by volume) neutral to phenolphthalein indicator
- Phenolphthalein indicator solution- 1% (w/v) neutral solution in ethyl alcohol or rectified spirit
- Standard NaOH solution- approximately 0.1 N accurately standardized

Procedure

Two to three gm of sample was taken in a 200 ml conical flask. Fifty ml of 95% alcohol that was neutralized previously with 0.1N NaOH and phenolphthalein. The sample was dissolved in it and was heated in hot plate till total oil was dissolved in alcohol. Then the flask was agitated thoroughly and titrated against 0.1N NaOH using phenolphthalein indicator until the pink colour persisted.

Calculation

Acid value: $\frac{56.1 \times V \times N}{W}$

Where, V- Volume in ml of standard sodium hydroxide solution used

N – Normality of standard sodium hydroxide solution used

W- Weight in gm of the sample taken for test

3.4.2 Determination of iodine value

Iodine value is the number of grams of iodine absorbed per one hundred grams of oil when determined by using Wijs solution.

Reagent

- 10% KI solution
- Starch solution
- Standard sodium thiosulphate solution-0.1N
- Acetic acid solution
- WIJS (iodine monochloride) solution-0.1N.
- Chloroform solution

Procedure

Sample of 0.1 to 0.5 gm was taken in iodine bottle. Fifteen ml chloroform was added to it. 2.5 ml WIJS solution (0.1N) was also taken in the bottle and shaken well. Then it was kept in the dark place for 45 minutes. After that 10% KI solution was added to each bottle that means 10 ml solution for each bottle. This mixture was titrated against 0.1 N sodium thiosulfate solution, running in rapidly at first until the color began to

fade and then used starch as an indicator, titrating more slowly until the blue color disappeared. The bottle should be stoppered and shaken violently during titration to make sure that all excess of iodine was removed from the $CHCl_3$. Besides this a blank test without sample was run out following exactly the same procedure.

3.4.3 Determination of saponification value

Sponification value is the number of milligrams of potassium hydroxide required to saponify one of the oil.

Reagent

- Alcoholic KOH solution
- Phenolphthalein indicator solution- One percent in 95% ethyl alcohol (by volume)
- Standard HCl (0.5 N)

Procedure

The sample of 1 to 1.5 gm was taken in the conical flask. Twenty five ml alcoholic KOH solution was added to the flask. A blank test was run out on 25 ml of the 0.5 N alcoholic KOH by measuring the same pipette under the same conditions. The mixture was connected to air condenser. Then the flask was heated on a water bath or an electric hot plate for about few hours. The mixture was boiled gently or steadily until the sample was completely saponified. After the flask and condenser were cooled, they were washed down the inside of the condenser with ten ml of hot ethyl alcohol neutral to Phenolphthalein. Then 1 ml of Phenolphthalein indicator solution was added and titrated with standard hydrochloric acid. A blank test was also conducted at the same time.

Calculation

Saponification value: $\frac{56.1(B - S)\,N}{W}$

Where, B –Volume in ml of standard hydrochloric acid required for the blank.

S - Volume in ml of standard hydrochloric acid required for the sample

N – Normality of the standard hydrochloric acid, and

W- Weight in gm of the sample taken for the test.

3.4.4 Determination of peroxide value

Peroxide value is the number of millmoles of peroxide in 1 kilogram of oil.

Reagent

- Acetic acid solution
- Chloroform solution
- 10% KI solution
- Standard $Na_2S_2O_3$ (.005N)
- Starch indicator

Procedure

The sample of 5 to 6 gm was taken into the iodine bottle. Then, thirty ml acetic acid – chloroform solution was added in which the ratio of acetic acid and chloroform was 6: 4. Next, 10% KI solution was added and titrated against $Na_2S_2O_3$ (0.005 N), in which starch was used as an indicator.

Calculation

$$\text{Peroxide rancidity (mEq/kg)} = \frac{1000 \text{ X N X (B - S)}}{\text{W}}$$

Where, B = volume in ml of sodium thiosulphate solution required for the blank

S = volume in ml of sodium thiosulphate solution required for the sample

N = strength of sodium thiosulphate solution

3.4.5 Determination of iron value

Apparatus

- 200 ml biker
- Hot plate
- Separating funnel
- Beaurate

Reagent

- Concentrated HCl
- HNO_3
- NH_4HCN solution
- Fe solution

Procedure

About eight to tem gm of sample was taken in the 200 ml biker. Concentrated HCl and water were added whose ratio was 1: 1 that means 10 ml of concentrated HCl was taken in10 ml of distilled water. Then this mixture was heated in mild temperature, keeping a glass rod with bikar.The duration of maintaing appropriate temperature was 3-4 hour. At this time, the appropriate level of the mixture was maintained; adding with water. The mixture was cooled completely and at the same time 20-25 ml water with 2-3 drops of HCl was heated for 15-20 min. Then, this mixture was transferred into separating funnel. From this, liquid portion was collected and rest of the material was discarded. Taken liquid sample was done previous volume by adding water and added 2-3 drops of conc. HNO_3; then heated for 15-20 min and cooled it. After that, twenty five ml of water and twenty five ml of NH_4HCN were mixed and this mixture was used as indicator; the main sample solution was titrated against Fe solution. A blank test was prepared before titration. The color of blank test was matched with other sample solution test.

3.5 Adulterant test

3.5.1 Test for the presence of synthetic allyl isothiocyanate

Allyl isothiocyanate is produced commercially by the reaction of allyl chloride and potassium thiocyanate. So, it can be synthesized at a much lower price than its cost when extracted from mustard seeds. Therefore, the adulteration of mustard oil by adding synthetic allyl isothiocyanate is very profitable. The product obtained in this fashion is sometimes known as synthetic mustard oil.

Apparatus

- Separating funnel
- Hot plate
- Filter paper

Reagents

- Sodium azide solution
- Bismuth nitrate

Procedure

100 ml of the commercial oil sample suspected to be artificial or synthetic mustard oil was taken into the conical flask. 100 ml of sodium azide solution (2.0 g per 100 ml) was added to the flask, and reflux the mixture on the hot plate or by direct heating for about 3 hours. The flask was cooled and transferred the contents of the flask to a 250 ml capacity separating funnel, and allowed the aqueous and oily layer to separate. The lower aqueous layer was collected in a beaker, and removed the oily layer. The aqueous layer was washed twice 50 ml of diethyl ether each time in order to remove any residual oily content. Now this aqueous solution was filtrated and boiled it to concentrate up to around half its volume. Now 1 ml of bismuth nitrate solution was taken in a test tube and mixes with it 1 ml or more of the above concentrated solution. A deep yellow precipitate was formed that indicated the tested oil sample contained artificial or synthetic mustard oil.

3.5.2 Test for the presence of argemone oil

Argemone oil is occasionally found as a contaminant in edible oils, particularly in mustard oil. Argemone seeds that closely resemble to mustard seeds and so it can be easily mixed with mustard seed as an adulterant.

Reagent

- Hydrochloric acid
- Ferric chloride solution- 25% (w/v) in water to which sufficient hydrochloric acid has been added to prevent hydrolysis

Procedure

About 10 ml of oil was taken in a test tube and added 3 ml of hydrochloric acid. The tube was immersed in a boiling water bath for about 2 minutes and mixed thoroughly by shaking .This procedure was repeated twice and then placed the tube in the water bath for about 5 minutes or until the acid and oil layers have been separated completely. The oil layer was taken in the pipette as much as possible and slowly added 1 ml of the ferric chloride solution along the sides of the care being taken to prevent the mixing of the oil and acid layers by rotating the tube gently. The tube was immersed in the water bath and allowed it to stand for about 10 minutes. An orange red precipitate in the acid layer or at the interface indicates the presence of argemone oil.

3.5.3 Test for the presence of mineral oil

This method is used for rapid detection of mineral oil in vegetable oils. The method is sensitive when mineral oil is present to the extent of more than 4%. For quantitative analysis, the method for the determination of unsaponifiable matter should be used.

Reagent

- Alcoholic KOH solution - approximately 0.5 N

Procedure

About 2 g of the oil was taken in a 150 ml of conical flask which has been previously thoroughly to ensure absence of water 25 ml of alcoholic KOH solution was added and saponified the oil by keeping the flask, fitted with a glass bulb air condenser, on a water bath for about 45 minutes. It was done carefully to ensure that saponification was completed and also that there was no loss of alcohol during the process of saponification. The conical flasks were cooled and added 120 ml of distilled water. The development of turbidity indicates the presence of mineral oil.

Chapter four

Result

CHAPTER FOUR

Result

4.1 Physical Parameters

The physical tests were conducted at Bangladesh Standards and Testing Institution (BSTI), Area Office, C. M Division, 62, Old Jessore Road, Khalishpur, Khulna to determine the following parameters:

– Moisture content

– Specific gravity

– Refractive index

– Color

The data corresponding to the physical parameters of different brands and open market sample of mustard oil are shown in Table 4.1.

Table 4.1: Physical parameters of different brands of experimental mustard oil

(Mean ± SD)

Parameter	Brands of mustard oil								
	Radhuni	Dolfin	Teer	Fresh	Local 1	Local 2	Local 3	Local 4	Level of significance
Moisture content (%)	0.12 ± 0.02	0.21 ± 0.01	0.25 ± 0.02	0.29 ± 0.03	0.25 ± 0.04	0.32 ± 0.02	0.39 ± 0.04	0.30 ± 0.01	*
Specific gravity	0.916 ± 0.002	0.926 ± 0.003	0.921 ± 0.001	0.922 ± 0.001	0.926 ± 0.007	0.938 ± 0.002	0.917 ± 0.001	0.943 ± 0.003	*
Refractive index	1.4667 ± 0.0002	1.4664 ± 0.0004	1.4669 ± 0.0002	1.4657 ± 0.0002	1.4670 ± 0.0002	1.4675 ± 0.0003	1.4670 ± 0.0003	1.4669 ± 0.0002	*
Color	24.83 ± 1.04	35.00 ± 2.5	25.5 ± 6.08	23.67 ± 2.9	39.33 ± 3.54	34.83 ± 2.25	22.00 ± 0.5	31.00 ± 1.32	*

*= 1% Level of Significance

4.1.1 Moisture Content (%)

Average moisture content with their standard deviation obtained from Radhuni, Dolfin, Teer, Fresh, Local 1, Local 2, Local 3 and Local 4 mustard oil were 0.12 ± 0.02, 0.21 ± 0.01, 0.25 ± 0.02, 0.29 ± 0.03, 0.25 ± 0.04, 0.32 ± 0.02, 0.39 ± 0.04, 0.30 ± 0.01, respectively that were presented in Table 4.1 and Figure 1. The standard range of moisture content in mustard oil is 0.25% (max.) as recognized by BSTI. The obtaining result showed that Fresh, Local 2, Local 3 and Local 4 did exceed the standard limit. Statistical analysis showed that there was significant difference within the moisture content of different mustard oil samples (Appendix-1). Mean value for moisture content was the highest in case of Local 3 (0.39 ± 0.047) and the lowest in Radhuni (0.12 ± 0.02). Moisture content promotes rancidity and leads to changes in color (yellow to brown), odor and taste in mustard oil. Therefore, it may be concluded that all of the market mustard oil samples for moisture content were not within the standard limit due to the inadequate means of processing, packaging and storage.

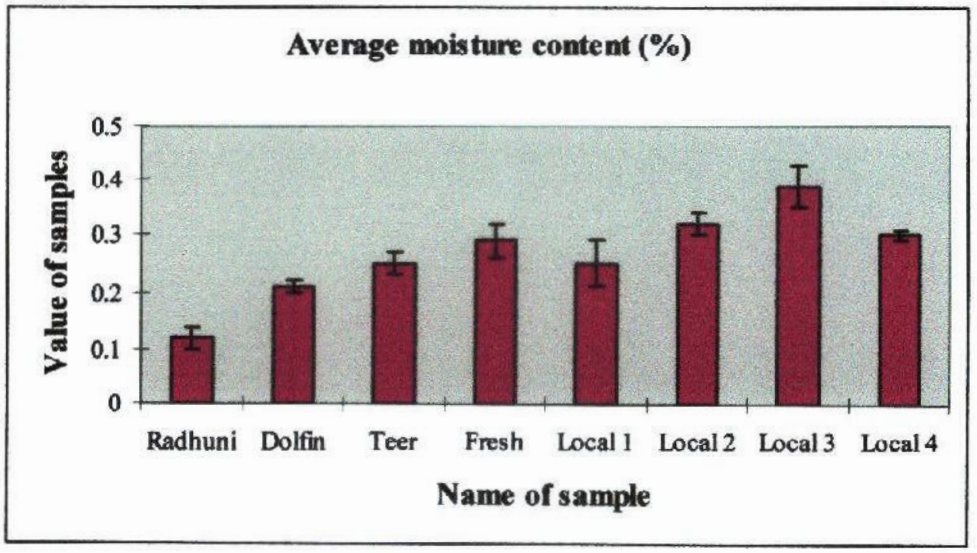


Figure 1: Average moisture content of different mustard oil samples

4.1.2 Specific gravity

The mean specific gravity for mustard oil samples of Radhuni, Dolfin, Teer, Fresh, Local 1, Local 2, Local 3 and Local 4 were 0.916 ± 0.002, 0.926 ± 0.003, 0.921 ± 0.001, 0.922 ± 0.001, 0.926 ± 0.007, 0.938 ± 0.002, 0.917 ± 0.001 and 0.943 ± 0.003, respectively and these values are presented in Table 4.1 and illustrated in Figure 2. Statistical analysis showed that there was a significant difference of specific gravity of different mustard oil samples collected from market (Appendix-2). The standard limit of specific gravity of mustard oil at 20^0 C is 0.910-0.921 according to BSTI. The obtaining result showed that Local 1 (0.926 ± 0.007), Local 2 (0.938 ± 0.002), Local 4 (0.943 ± 0.003) and Dolfin (0.926 ± 0.003) did exceed the standard range. Biswas *et al*, (2001) observed that specific gravity of Brassica seed oil at 25^0C was 0.909. It was observed that Local 4 (0.943 ± 0.003) and Local 2 (0.938 ± 0.002) had the highest specific gravity and Radhuni (0.916 ± 0.002) had the lowest specific gravity. From this study it was found that Dolfin, Local 1, Local 2 and Local 4 did not maintain standard specific gravity limit. It is assumed that these four samples of mustard oil may contain other oil or adulterants. Therefore, it is said that specific gravity range of all commercial mustard oil samples were not within the standard limit.

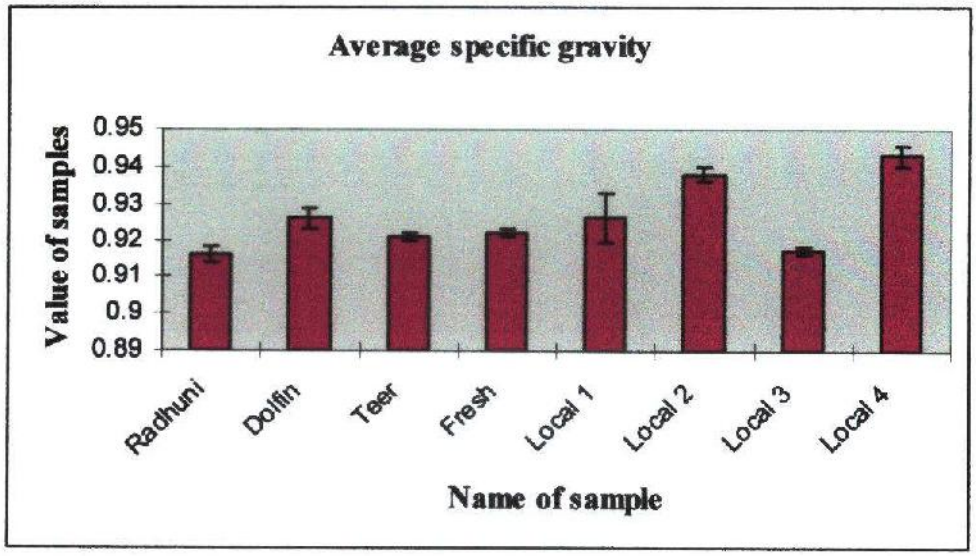


Figure 2: Average value of specific gravity for different mustard oil samples

4.1.3 Refractive index

Average refractive index with their standard deviations obtained from Radhuni, Dolfin, Teer, Fresh, Local 1, Local 2, Local 3 and Local 4 were 1.4667 ± 0.0002, 1.4664 ± 0.0004, 1.4669 ± 0.0002, 1.4657 ± 0.0002, 1.4670 ± 0.0002, 1.4675 ± 0.0003, 1.4670 ± 0.0003 and 1.4669 ± 0.0002, respectively that are presented in the Table 4.1 and figure 3. Statistically, there was a significant difference of refractive index among different samples of mustard oil (Appendix-3). The standard limit of refractive index in mustard oil is 1.4650-1.470 as recognized by BSTI. The result showed that refractive index of Local 2 (1.4675 ± 0.0001) was not within the standard range. It was observed that mean value of refractive index was the highest in Local 2 (1.4675 ± 0.0003) and the lowest in Fresh (1.4657 ± 0.0002). The refractive index of Local 2 was not within standard range and this variation was not so much important to indicate autoxidation. Biswas *et al*, (2001) found that refractive index of Brassica seed oil at 25^{0}C was 1.470. Arya *et al*, (2007) reported that high value of refractive index related to rancid odor development of the oil. So, it can be concluded that all of collected samples from the market were not deteriorated.

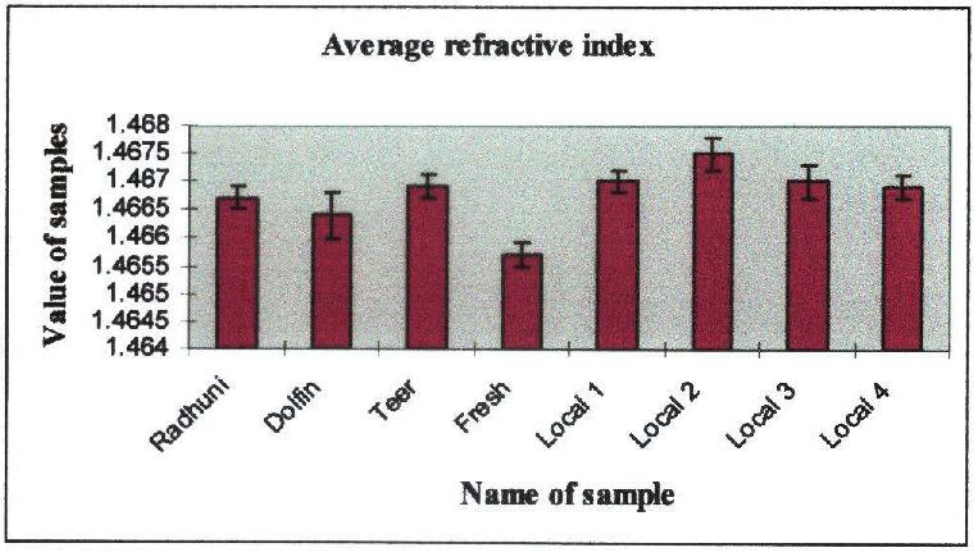


Figure 3: Average value of refractive index for different mustard oil samples

4.1.4 Color

The mean value with standard deviation of color for different brands and open market oil samples such as Radhuni, Dolfin, Teer, Fresh, Local 1, Local 2, Local 3 and Local 4 were 24.83 ± 1.04, 35.00 ± 2.5, 25.5 ± 6.08, 23.67 ± 2.9, 39.33 ± 3.54, 34.83 ± 2.25, 22.00 ± 0.5 and 31.00 ± 1.32, respectively. The standard limit of color of mustard oil is 30.00 (max.) as recognized by BSTI. The obtaining result of color for Dolfin (35.00 ± 2.5), Local 1 (39.33 ± 3.54), Local 2 (34.83 ± 2.25) was not within the standard limit. There was a significant difference of color of different brands and Local samples of mustard oil (Appendix-4). It was observed that average value of color was the highest in Local 1 (39.33 ± 3.54) and the lowest in Local 3 (22.00 ± 0.5). In case of high value of color, the samples are contaminated with iron due to mechanical processing and transporting. Thus, it can be concluded that all of the commercial mustard oil samples were not within the standard limit.

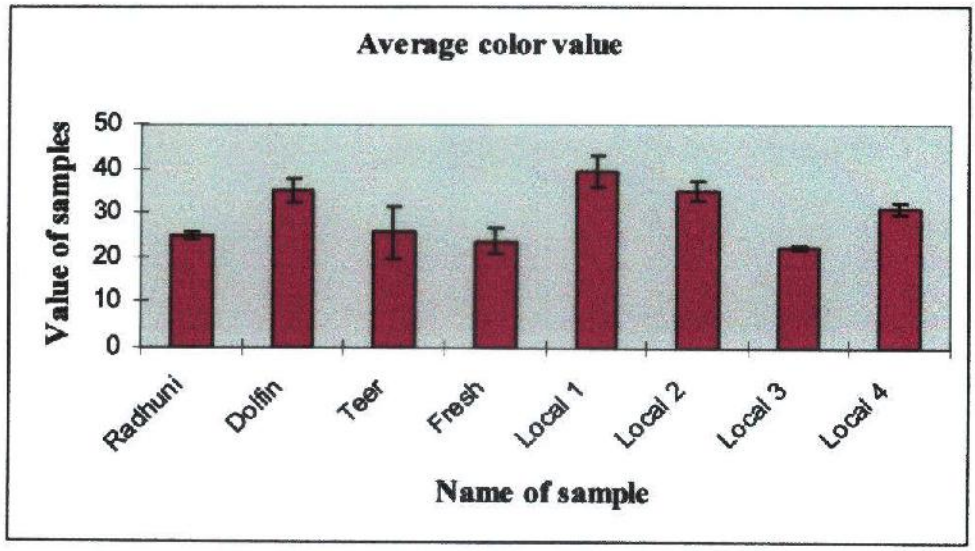


Figure 4: Average value of color for different mustard oil samples

4.2 Chemical Parameters

The chemical tests were conducted at Bangladesh Standards and Testing Institution (BSTI), Area Office, C. M Division, 62, Old Jessore Road, Khalishpur, Khulna to analyze the following parameters:

– Acid value

– Iodine value

– Saponification value

– Peroxide value

–Iron value

The data corresponding to the chemical parameters of different brands of mustard oil are shown in Table 4.2.

Table 4.2: Chemical parameters of different brands of experimental mustard oil (Mean ± SD)

Parameters	Brands of mustard oil								
	Radhuni	Dolfin	Teer	Fresh	Local 1	Local 2	Local 3	Local 4	Level of significance
Acid value	4.05 ± 0.15	3.63 ± 0.16	3.11 ± 0.13	2.5 ± 0.28	3.3 ± 0.09	4.2 ± 0.32	3.09 ± 0.08	4.45 ± 0.60	*
Iodine value	101.91 ± 2.4	73.92 ± 1.6	105.36 ± 0.83	101.25 ± 1.4	100.0 ± 1.3	122.53 ± 2.09	100.5 ± 2.1	115.47 ± 1.12	*
Saponification value	172.02 ± 2.07	180.5 ± 5.1	173.28 ± 1.5	172.8 ± 2.1	176.3 ± 1.7	184.11 ± 1.6	169.8 ± 6.1	186 ± 0.59	*
Peroxide value	0.0	0.0	0.0	0.0	0.0	4.5 ± 0.02	0.0	0.0	
Iron value	5.03 ± 2.07	6.63 ± 1.62	5.19 ± 0.34	5.07 ± 0.17	11.09 ± 2.7	9.37 ± 0.61	3.72 ± 0.11	4.96 ± 0.34	*

* = 1% Level of Significance

4.2.1 Acid Value

The average acid value of commercial mustard oil samples are presented in the Table 4.2 and Figure 5. The mean acid value for mustard oil obtained from Radhuni, Dolfin, Teer, Fresh, Local 1, Local 2, Local 3 and Local 4 were 4.05 ± 0.15, 3.63 ± 0.16, 3.11 ± 0.13, 2.5 ± 0.28, 3.3 ± 0.09, 4.2 ± 0.32, 3.09 ± 0.08, and 4.45 ± 0.60, respectively. The standard limit for this value is 3.00 mg/g (maximum) as recognized by BSTI. Statistically it was found that there were significant differences within the acid value of mustard oil samples (Appendix-5). It was observed that Local 4 showed the highest acid value (4.45 ± 0.60) and Fresh showed the lowest acid value (2.5 ± 0.28). From this study it was found that acid value of Radhuni (4.05 ± 0.15), Dolfin (3.63 ± 0.16), Local 1 (3.3 ± 0.09), Local 2 (4.2 ± 0.32) and Local 4 (4.45 ± 0.60) were not within the standard range. In the obtaining result, high acid value of these samples may be due to long shelf life. Okpuzor *et al*, (2009) reported that high acid value indicative of high free fatty acid showed rancidity. So, it can be concluded that all of the collected samples from the market were not within the standard limit.

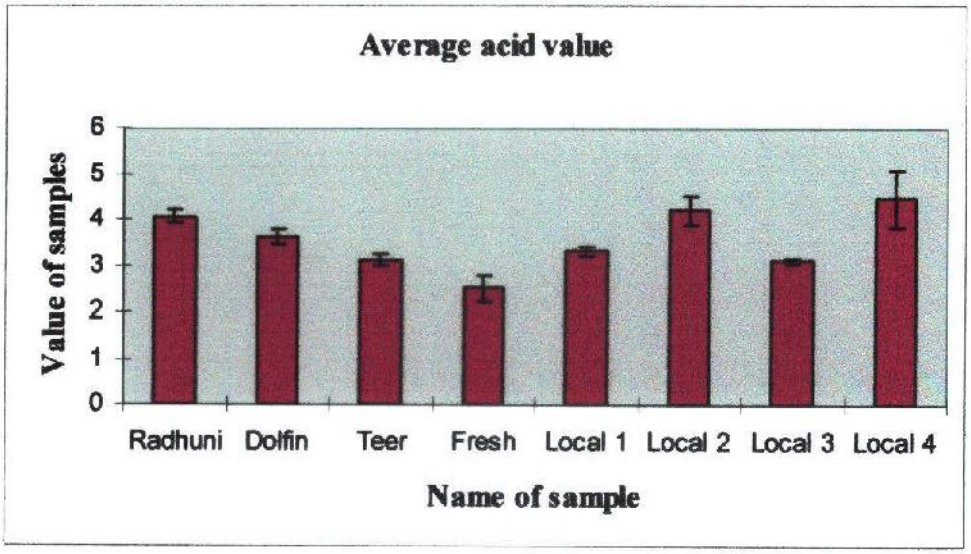


Figure 5: Average acid value of various mustard oil samples

4.2.2 Iodine Value

Mean iodine value of mustard oil obtained from Radhuni, Dolfin, Teer, Fresh, Local1, Local 2, Local 3 and Local 4 were 101.91 ± 2.4, 73.92 ± 1.6, 105.36 ±0.83, 101.25 ± 1.4, 100.0 ± 1.3, 122.53 ± 2.09, 100.5 ± 2.1 and 115.47 ± 1.12, respectively and these values are demonstrated in Table 4.2 and illustrated in Figure 6. Statistical differences are highly significant among those mean values (Appendix-6). The standard limit of iodine value for mustard oil is 98 to 108 as recognized by BSTI. From this study it was observed that iodine value obtained from Radhuni, Teer, Fresh, Local 1 and Local 3 did not exceed the standard limit. This result agreed with the work of Youngs *et al*, (1951) who reported that original iodine value of rapeseed oil was 104.1. Another research work of Biswas *et al*, (2001) showed that iodine value of Brassica seed oil was 96.5. Mean iodine value was the highest in case of Local 2 (122.53 ± 2.09) and the lowest in case of Dolfin (73.92 ± 1.6). The obtaining result also showed that iodine value of Local 2 (122.53 ± 2.09), Local 4 (115.47 ± 1.12) and Dolfin (73.92 ± 1.6) was not within the range of BSTI. So, these samples indicate the probability of adulterant mixing to those. In case of low iodine value of mustard oil, it indicates the oil is more saturated with high saturated fatty acid containing oil (Welter, 2007). High iodine value of oil indicates the abundance of unsaturated fatty acid in oil (Biswas *et al*, 2001) that shows the more unsaturated with other oil. Thus, it can be concluded that all of the commercial mustard oil samples were not within the standard range.

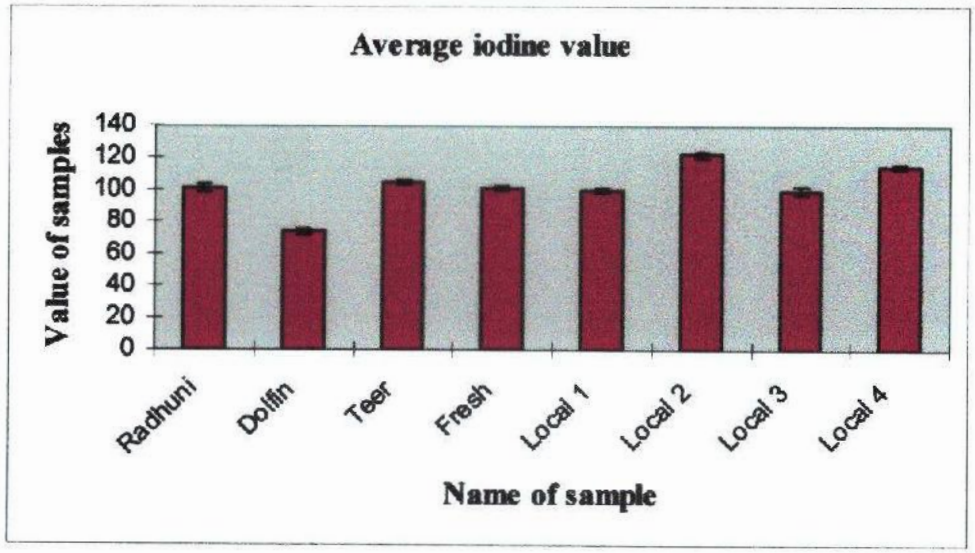


Figure 6: Average iodine value for different mustard oil samples

4.2.3 Saponification Value

Average saponification value with their standard deviation for mustard oil obtained from Radhuni, Dolfin, Teer, Fresh, Local 1, Local 2, Local 3 and Local 4 were 172.02 ± 2.07, 180.5 ± 5.1, 173.28 ± 1.5, 172.86 ± 2.1, 176.39 ± 1.7, 184.11 ± 1.6, 169.85 ± 6.1, and 186.0 ± 0.59, respectively that are presented in Table 4.2 and Figure 7. Statistical analysis showed that differences were highly significant in the saponification value among different mustard oil samples (Appendix-7). The standard limit of saponification value is 169 to 176 according to BSTI. The result showed that the saponification value of Radhuni (172.02 ± 2.07), Teer (173.28 ± 1.5), Fresh (172.86 ± 2.17), Local 1(176.39 ± 1.77) and Local 3 (169.85 ± 6.1) were within the standard limit. Youngs *et al*, (1951) has reported that saponification value of rapeseed oil was 174.3. Another study of Biswas *et al*, (2001) showed that saponification value of Brassica seed oil was 173 that supported the present result. It also showed that the saponification value of Dolfin (180.5 ± 5.1), Local 2 (184.11 ± 1.6) and Local 4 (186.01 ± 0.59) were not within the range. From this result it was observed that Local 4 (186.01 ± 0.59) had the highest saponification value and Local 3 (169.85 ± 6.1) had the lowest saponification value. Therefore, it can be concluded that all of the collected samples from the market were not within the standard limit.

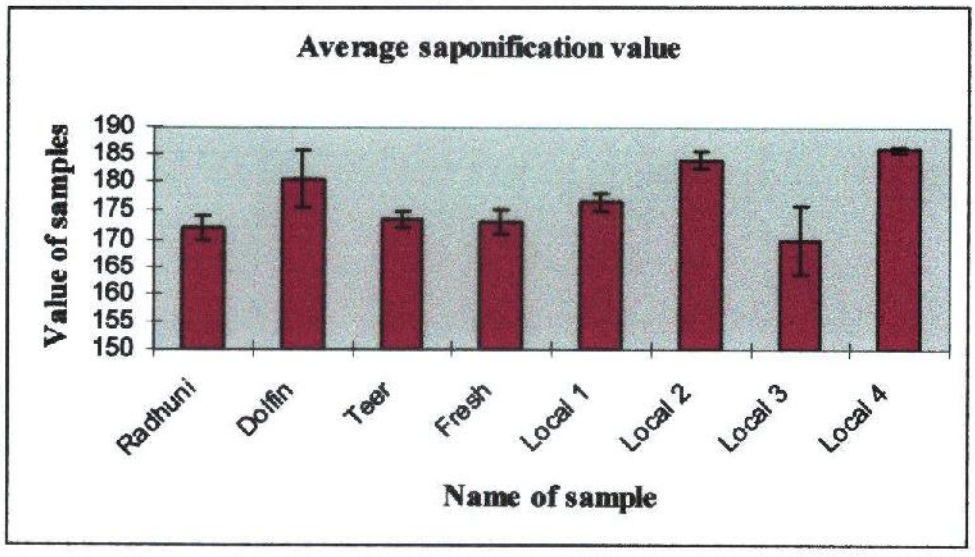


Figure 7: Average saponification value of different mustard oil samples

4.2.4 Peroxide value

The average peroxide value of different mustard oil samples were presented in the Table 4.2 and figure 8. From the table it is observed that the mean peroxide value for Radhuni, Dolfin, Teer, Fresh, Local 1, Local 2, Local 3 and Local 4 mustard oil were 0.0, 0.0, 0.0, 0.0, 0.0, 4.5 ± 0.02, 0.0 and 0.0 respectively. The standard range of peroxide value in mustard oil is 10.0 milliequivalents peroxide oxygen/kg as recognized by BSTI. The result showed that mustard oil sample of Local 2 (4.5 ± 0.02) did not exceed the standard range of peroxide value. Arya *et al*, (2007) have reported that peroxide value has its inherent limitations. This value is correlated with organoleptic evaluation of rancidity. The oxidation process is called rancidification and can make foods unpalatable. Peroxide value (POV) is highly correlated with the total concentration of major odorants. Fatty acid oxidation produces free radicals which have high reactivity with oxygen leading to rapid conversion to peroxides or hydro peroxides (Addis, 1986). This breakdown mechanism is responsible for the production of color defects, rancid taste, off flavors as well as the myriad of other reactions which reduce shelf life and nutritional value of oil containing foods (White, 1991; Rodriguez *et al*, 1997). So, it can be said that peroxide value of all of the samples did not exceed the range.

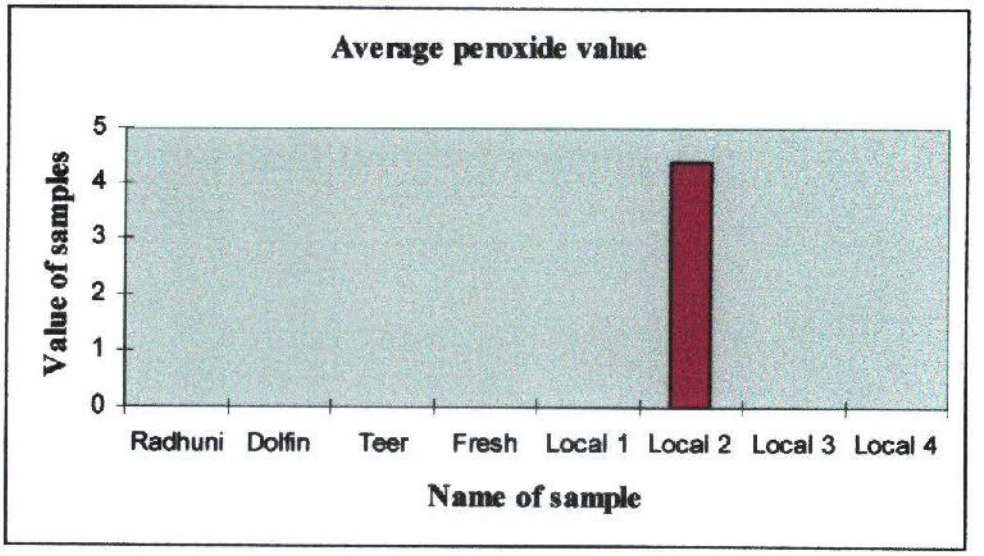


Figure 8: Average peroxide value of different mustard oil samples

4.2.5 Iron Content

Iron content with standard deviation of mustard oil obtained from Radhuni, Dolfin, Teer, Fresh, Local 1, Local 2, Local 3 and Local 4 were 5.03 ± 2.07, 6.63 ± 1.62, 5.19 ± 0.34, 5.07 ± 0.17, 11.09 ± 2.7, 9.37 ± 0.61, 3.72 ± 0.11 and 4.96 ± 0.34, respectively that are shown in Table 4.2 and Figure 9. It was found that there was significance different of iron content among different brands of mustard oil (Appendix-9). The standard range of iron content in mustard oil is maximum 5.00 mg/kg according to BSTI. The result showed that mustard oil of Dolfin (6.63 ± 1.62), Local 1 (11.09 ± 2.7) and Local 2 (9.37 ± 0.61) did exceed the standard range. Biswas *et al*, (2001) observed that iron content of brassica seed oil was 8.44 mg. From this result, it was observed that the mean value of iron content was highest in Local 1(11.09 ± 2.7) and lowest in Local 3 (3.72 ± 0.11) mustard oil. Relatively high concentrations of iron often occur because of the inevitable contact of oils with iron in the course of extraction, storage and transport. Thus, it can be concluded that iron value of all of the collected samples were not within the standard limit.

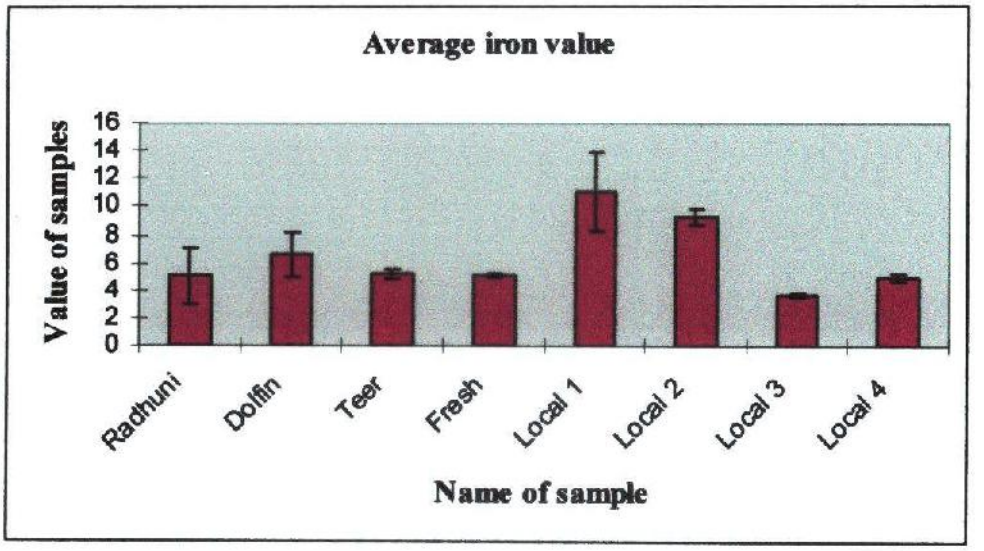


Figure 9: Average iron content of different mustard oil samples

4.3 Adulterant test for mustard oil

4.3.1 Test for synthetically made artificial mustard oil

The results of different mustard oil sample collected from market in regard to synthetic allyl isothiocyanate presence are presented in Table 4.3. Shukla *et al*, (2002) observed a simple and reliable, non-instrumental technique which could differentiate natural (unadulterated) and synthetically made mustard oils. This technique was followed to detect the presence of synthetic allyl isothiocyanate in commercial mustard oil. In order to detect such a fraud, the detailed analysis of mustard oils obtained from market was done for adulterate analysis of mustard oils.

Table 4.3: Test for the presence of synthetic allyl isothiocyanate in different commercial mustard oil samples

Mustard oil sample	Test color results
Radhuni	Yellowish color
Dolfin	No color
Teer	No color
Fresh	Yellowish color
Local 1	Yellowish color
Local 2	Yellowish color
Local 3	Not analyzed
Local 4	Not analyzed

The obtaining results presented in the Table 4.3 showed that synthetic allyl isothiocyanate was not found in the tested samples. Since synthetic allyl isothiocyanate is added to low priced vegetable oil which is colored by oil soluble yellow dye in order to prepare artificial

mustard oil, this test result shows a deep yellow precipitate. This test gives positive reaction with artificial or adulterated oil containing down to 0.1% of added allyl isothiocyanate (Shukla *et al*, 2005). In this study, except Local 3 and Local 4 all of samples are analyzed. Although Local 3 sample was collected from open market, its quality was fulfill with BSTI standard that is indicative of unadulterated oil. Its source of collection was reliable and its preparation method was followed to classical processing. So, it is proved that it was not artificially prepared. That is why, it could not be analyzed. Another sample Local 4 was not analyzed due to limitation of collected sample. From this qualitative test result, it was found that Radhuni gave yellowish color, and Teer gave no color that showed in the picture 10. Appearance of no color in Dolfin and yellowish color in Fresh, Local 1 and Local 2 was showed in the picture 11. So, from this result it can be concluded that all of the collected samples except Local 4 contain natural allyl isothiocyanate and these samples were not artificially prepared.

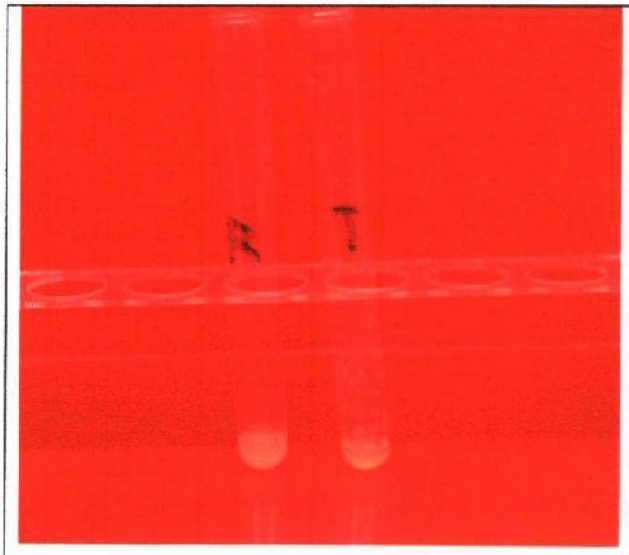


Figure 10: Appearance of yellowish color in Radhuni and no color in Teer

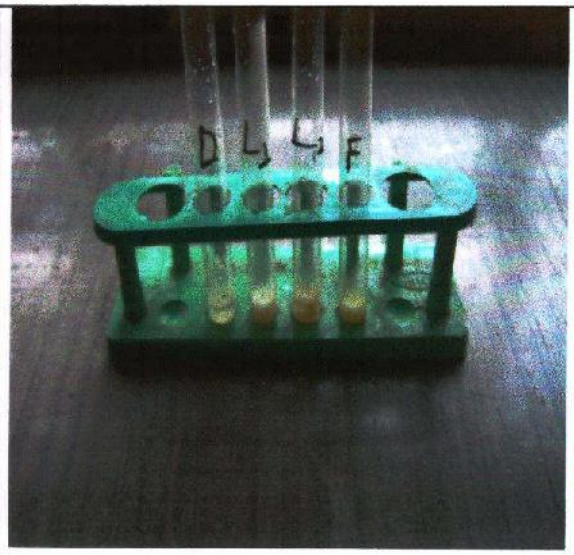


Figure 11: Appearance of no color in Dolfin, yellowish color in Fresh, Local 1 and Local 2

4.3.2 Test for argemone oil in mustard oil as adulterant

The results of different brands and open market mustard oil sample in regard to argemone oil presence are presented in Table 4.4. This test is done to follow the method that determined by BSTI.

Table 4.4: Test for the presence of argemone oil in different mustard oil samples collected from market

Mustard oil sample	Test color results
Radhuni	No color
Dolfin	Orange red precipitate
Teer	No color
Fresh	No color
Local 1	Orange red precipitate
Local 2	Orange red precipitate
Local 3	No color
Local 4	Orange red precipitate

The results are presented in the Table 4.4 that show the qualitative test of argemone oil in the commercial mustard oil collected form the market. This test gives an orange red precipitate in the acid layer or at the interface indicates the presence of argemone oil. In this study, brands of Radhuni, Teer and Fresh, and Local sample 3 does not give any color. So, it indicates the absence of argemone oil in those samples. But, Dolfin and other three open market samples like Local 1, Local 2 and Local 4 give orange red precipitate that indicate the presence of argemone oil. The consumption of mustard oil containing argemone oil causes health hazards that occurs epidemic dropsy in human. Therefore, it can be concluded that all of the collected samples were not free from argemone oil (adulterant).

4.3.3 Test for mineral oil in mustard oil as adulterant

The results of individual mustard oil sample in regard to mineral oil presence are presented in Table 4.5. This test is done to follow the method that determined by BSTI. For quantitative analysis, this method for the determination of unsaponifiable matter is also used.

Table 4.5: Test for the presence of mineral oil in different brands of mustard oil samples

Mustard oil sample	Test color results
Radhuni	No color
Dolfin	No color
Teer	No color
Fresh	No color
Local 1	No color
Local 2	No color
Local 3	No color
Local 4	No color

The results presented in the Table 4.5 that show the qualitative test in different commercial mustard oil samples. This test develops turbidity that confirms the presence of mineral oil. In this study, all of the samples give no color. So, it can be said that no of the sample contain mineral oil. Generally, mineral oil found in the mustard oil due to the poor transportation procedure. The consumption of mustard oil containing mineral oil causes health hazards of human. So, it can be concluded that all of the collected samples were free from mineral oil.

Chapter five

Discussion

CHAPTER FIVE

Discussion

We have taken eight different commercial mustard oil samples from Khulna local market to analyze their quality and to detect the adulterant mixed with it. There are different well-known brands and open market commercial mustard oil in our market and many of them are suspected to fail in maintaining BSTI standard. This occurs generally because of the shortage of mustard oil production and its high price. Despite of this, due to increasing awareness on good health implications of mustard oil in our diets, peoples are now prefer to purchase this cholesterol free vegetable oil. In our study, we have investigated the quality of saleable mustard oil using various parameters are analyzed and compared the values with BSTI standard. There are two types of characteristics like physical and chemical criteria are analyzed.

The physical characteristics are moisture percentage, specific gravity refractive index and color. The average moisture content of Radhuni, Dolfin, Teer, Fresh, Local 1, Local 2, Local 3 and Local 4 mustard oil are 0.12, 0.21, 0.25, 0.29, 0.25, 0.32, 0.39, 0.30, respectively in which BSTI maintains 0.10 to 0.25% standard range. Among these result, Fresh, Local 2, Local 3 and Local 4 crosses the BSTI standard. This may be due to the inadequate means of processing, packaging and storage.

The mean specific gravity for Radhuni, Dolfin, Teer, Fresh, Local 1, Local 2, Local 3 and Local 4 are 0.916, 0.926, 0.921, 0.922, 0.926, 0.938, 0.917 and 0.943, respectively. The standard limit of specific gravity of mustard oil at 20^0 C is 0.910-0.921 according to BSTI. The result of Radhuni, Teer, Fresh, and Local 3 show within the standard limit that support the study of Biswas *et al* (2001) in which, they observed that specific gravity of Brassica seed oil at 25^0C was 0.909. But Dolfin, Local 1, Local 2, and Local 4 go over the standard range that indicate the mixing of other oil or adulterant.

Average refractive index of Radhuni, Dolfin, Teer, Fresh, Local 1, Local 2, Local 3 and Local 4 are 1.4667, 1.4664, 1.4669, 1.4657, 1.4670, 1.4675, 1.4670 and 1.4669, respectively. The standard limit of refractive index in mustard oil is 1.4650-1.470 as recognized by BSTI. The values of all of the samples except Local 2 maintain the BSTI standard. These results

also support the study of Biswas *et al*, (2001) in which, they found that refractive index of Brassica seed oil at 25^{0}C was 1.470.The result of Local 2 is not present within the standard range. Although the refractive index of Local 2 crosses the range, this value is not a significant indicator of autoxidation. Arya *et al* (2007) has reported that high value of refractive index related to autoxidation that develops rancid odor in the oil. Common edible oils exposed to florescent light test show that refractive index range is 1.4776. An increase order (0.001 ± 0.0003) of that range shows the stage of development of perceptible rancid odor.

The mean value of color for Radhuni, Dolfin, Teer, Fresh, Local 1, Local 2, Local 3 and Local 4 are 24.83, 35.00, 25.5, 23.67, 39.33, 34.83, 22.00 and 31.00, respectively. The standard limit of color of mustard oil is 30.00 (max.) as recognized by BSTI. The result of color for Dolfin, Local 1 and Local 2 does not show within the standard limit. In case of high value of color, the samples are contaminated with iron due to mechanical processing and transporting.

The chemical characteristics are acid value, iodine value, saponification value, peroxide value and iron value. The mean acid value for Radhuni, Dolfin, Teer, Fresh, Local 1, Local 2, Local 3 and Local 4 are 4.05, 3.63, 3.11, 2.5, 3.3, 4.2, 3.09, and 4.45, respectively. The standard limit for this value is 3.00 mg/g (maximum) as recognized by BSTI. The value of Teer, Fresh and Local 3 does not cross the limit that support the research work of Sultana *et al* (1998) in which, they showed that processed sarsoon oil containing 39% erucic acid has an acid value of 2.80. Another report on acid value is 1.25 – 2.30 of high erucic acid rapeseed oil that observed by Niewiadomski (1990). Biswas *et al* (2001) has found that the acid value of brassica seed oil is 1.06. The result of acid value of Radhuni, Dolfin, Local 1, Local 2 and Local 4 go over the standard range. Okpuzor *et al* (2009) reported that high acid value indicative of high free fatty acid showed rancidity that may be due to long storage of the oil seeds before or after processing. So, it can be said that high acid value of these samples may be due to long shelf life. Mustard oil has a shelf life of up to one year.

The Mean iodine value of Radhuni, Dolfin, Teer, Fresh, Local1, Local 2, Local 3 and Local 4 are 101.91, 73.92, 105.36, 101.25, 100.0, 122.53, 100.5 and 115.47, respectively. Standard iodine value determined by BSTI is 98 to 108. In our study, iodine value of Radhuni, Teer,

Fresh, Local 1 and Local 3 were found within the standard limit. The result of these samples agreed with the work of Youngs *et al* (1951) who has reported that iodine value of rapeseed oil is 104.1. Sultana *et al* (1998) has confirmed in their research work that processed sarsoon oil has an iodine value of 104.00. Another research work of Biswas *et al* (2001) has showed that iodine value of Brassica seed oil is 96.5. In the obtaining result, iodine value of Dolfin, Local 2 and Local 4 goes beyond the range of BSTI. In case of low iodine value of mustard oil, it is more saturated with other oil containing high saturated fatty acid (Welter, 2007). So from this logic, it can be said that Dolfin may be adulterated with saturated fatty acid containing oil. Biswas *et al* (2001) has stated in their study that the high iodine value of oil indicates the abundance of unsaturated fatty acids in oil. Therefore, high iodine value containing oil like Local 2 and Local 4 may be adulterated with high unsaturated fatty acid containing oil. So, it can be said that these samples indicate the probability of adulterant mixing with other oil.

Average saponification value of Radhuni, Dolfin, Teer, Fresh, Local 1, Local 2, Local 3 and Local 4 are 172.02, 180.5, 173.28, 172.86, 176.39, 184.11, 169.85, and 186.0, respectively. Standard saponification value established by BSTI is 169 to 176. In our study, saponification value of Radhuni, Teer, Fresh, Local 1 and Local 3 sustain the standard limit. The report on saponification value of rapeseed oil is 174.3 that presented by the research work of Youngs *et al* (1951). Another study of Biswas *et al* (2001) showed that saponification value of Brassica seed oil is 173 that support the present result. The saponification values of Dolfin, Local 2 and Local 4 exceed the range. Thus, it can be said that high saponification value containing samples may be adulterated with other oil.

The mean peroxide value for Radhuni, Dolfin, Teer, Fresh, Local 1, Local 2, Local 3 and Local 4 are 0.0, 0.0, 0.0, 0.0, 0.0, 4.5, 0.0 and 0.0 respectively. The standard range of peroxide value in mustard oil is 10.0 milliequivalents peroxide oxygen/kg as recognized by BSTI. In this study, all of the peroxide value except Local 2 does not show any result. Only Local 2 gives a result that is within the standard range of peroxide value. Geier (2004) has illustrated in his classical experiment that high peroxide value is mostly due to the age of the seeds. Another research report of Arya *et al* (2007) showed that peroxide value is correlated with the organoleptic evaluation of rancidity. So, none of the samples used for this study does not show rancidity.

Average iron content of Radhuni, Dolfin, Teer, Fresh, Local 1, Local 2, Local 3 and Local 4 are 5.03, 6.63, 5.19, 5.07, 11.09, 9.37, 3.72 and 4.96, respectively. The standard range of iron content in mustard oil is maximum 5.00 mg/kg according to BSTI. The result of Dolfin, Local 1 and Local 2 go over the standard range. Biswas *et al* (2001) observed that iron content of brassica seed oil is 8.44 mg that support the result of Dolfin. Iron level is quite detrimental to the color and flavors stability of the oils. It leads to pro-oxidative effects resulting in poor color and poor flavor stability in mustard oil. So, relatively high concentrations of iron often occur because of the inevitable contact of oils with iron in the course of extraction, storage and transport.

The detailed analysis of commercial mustard oils obtained from market is done for adulterant analysis of mustard oils. There are three types of adulterant analysis is done in this study. In our study, the test of synthetic allyl isothiocyanate that is used to prepare artificially made commercial mustard oil is most valuable. All of the samples except Local 3 and Local 4 show the absence of synthetic allyl isothiocyanate. So, our finding from this study is that all of the samples used for this test are not artificially prepared. The finding of argemone oil contamination with mustard oil shows that four samples like Dolfin, Local 1, Local 2 and Local 4 are adulterated with this oil. Shukla *et al* (2005) in their review have stated that mustard oil containing argemone oil causes epidemic dropsy in human. The research work done by Babu *et al* (2006) report on health hazards that occurs epidemic dropsy in human. The result of adulteration with mineral oil gives us that all of the samples included for the test are not contaminated with mineral oil. Generally, mineral oil found in the mustard oil due to the poor transportation procedure.

In conclusion, it can be said that the overall quality of Radhuni, Teer, Fresh and Local 3 is good. Among the samples taking for analysis, comparatively the quality of Dolfin, Local 1, Local 2 and Local 4 is poor. Thus, the quality studies can ensure whether oil producers and marketers are correctly supply their products in the market or not.

References

Ackman R G, 1977 "Rapeseed oil: Chemical and physical characteristics." *Proceeding Symposium on Rapeseed Oil, Meal and By- Product Utilization Rapeseed Association of Canada*, Vol. 45: 12.

Addis P B, 1986 "The chemistry of polymerized oils." *Food Chemistry Toxicology*, Vol. 24: 1021-1030.

Addison K, 2007 "Oil yields and characteristics." In: *Vegetable Oil Yields, Characteristics Journey to Forever*, Web site: http://journeytoforever.org/.

Agriculture Marketing Information Network, 2004 "Post harvest profile of mustard-rapeseed." Agriculture Marketing Information Network, Government of India, Web site: http://www.agmarknet.nic.in/mustard-rapeseed-profile.pdf. Accessed in, 2007.

Ahmed U K, Absar N, Haque R; Islam R M, 2000 "Fatty acid composition of different varieties of mustard and rapeseed." *Bangladesh Journal Agricultural Research*, Vol. 25 (3): 415 - 422.

Anonymous H, 1987 "Pearson's chemical analysis of foods." 8th (Ed.), Longman Scientific and Technical Group Limited, Harlow.

Arya S S, Ramanujam S; Vijayaraghavan P K, 2007 "Refractive index as an objective method for evaluation of rancidity in edible oils and fats." *Journal of American Oil Chemist's Society*, Vol. 46 (1).

Babu C K, Khanna K S; Das M, 2006 "Safety evaluation studies on argemone oil through dietary exposure for 90 days in rats." *Food and Chemical Toxicology*, Vol. 44 (7): 1151-1157.

Bangladesh Bureau of Statistics, Oil world, 2007 *Global Oils and Fats Business Magazine*, Vol.34 (2), Published on, 2008.

Barr S L, Ramakrishnan R; Johnson C, Halleran S, Dell R B ;Ginsberg H N, 1992 "Reducing total dietary fat without reducing saturated fatty acids does not significantly lower total plasma cholesterol concentrations in normal males." *American Journal Clinical Nutrition*, Vol. 55: 575-581.

Biswas T K, Sana N K, Badal R K; Haque E M, 2001 "Biochemical study of some oil seeds (Brassica, Sesame and Linseed)." *Pakistan Journal of Biological Science*, Vol. 4 (8): 1002 – 1005.

Bonanone A, Pagnan A, Biffani S, Opportuno A, Sogato H; *et al*, 1992 "Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma LDL to oxidative modification." *Arteriosclerosis and Thrombosis*, Vol.12: 529 –533.

Brabban A D; Edwards C, 1995 "The effects of glucosinolates and their hydrolysis products on microbial growth." *Journal of Applied Microbiology*, Vol. 79 (2): 171 – 177.

Brand G; Jacquot L, 2002 "Sensitization and desensitization to allyl isothiocyanate (mustard oil) in the nasal cavity." *Chemistry Senses*, Vol. 27: 593-598.

Budavari S, (Ed.) 1996 "The Merck index." 12th (Ed.), Whitehouse Station, NJ, Merck & Co., Inc., P. 54.

Caggiula AW; Mustad V A, 1997 "Effects of dietary fat and fatty acids on coronary artery disease risk and total lipoprotein cholesterol concentrations: epidemiologic studies." *American Journal Clinical Nutrition*, Vol. 65 (5):1597S-1610S.

Chemical Information Services, 1995 "Directory of world chemical producers." 1995/96 Standard Edition, Dallas, TX, P. 442.

Cheong L S; Seng O C, 1998 "Medicinal plants: the mustard seeds." Khuddaka Nikaya, China, Chapter VIII, P. 114.

Downey R K, 1964 "Genetic control of fatty acid biosynthesis in rapeseed." *Journal of the American Oil Chemist's Society*, Vol. 41: 475-478.

Downey R K; Stefansson B R, 1988 "Canola." In: Evans D; Brown N, The Canadian Encyclopedia, 2nd (Ed.), *Hurtig Publishers Limited.*

Enas A E, 1996 "Cooking oils, cholesterol and CAD: facts and myths." *Indian Heart Journal*, Vol.48: 423-27.

Enas A E, Senthilkumar A, Chennikkara H ; Bjurlin A M, 2003 "Prudent diet and preventive nutrition from pediatrics to geriatrics: current knowledge and practical recommendations." *Indian Heart Journal*, Vol.55: 310 -338.

Enig M G, 2000 "Benefits of saturated fats." *Wise Traditions in Food, Farming and the Healing Arts Magazine*, Vol. 1 (2): 49.

Erickson K L, 1985 "Dietary fat influences on murine myeloma growth and lymphocyte mediated cytotoxicity." *Journal Nutrition Clinical International*, Vol. 72: 115-120.

Eskin, N A M, McDonald B E, Przybylski R, Malcolmson L J, Scarth R, Mag T, Ward K; Adolph D, 1996 "Canola oil." In: Hui Y H, (Ed.), Edible oil and fat products: oil and oil seeds. John Wiley and Sons Inc., New York, P. 1–96.

Failor R A, Childs M T; Bierman E L, 1988 "The effects of omega 3 and omega 6 fatty acid- enriched diets on plasma lipoproteins and apoproteins in familial combined hyperlipidemia." *Metabolism*, Vol. 37: 1021–1028.

FAO, 2005 "Rapeseed." In: Wikipedia, The Free Encyclopedia, 2008, Web site: http://faostat.fao.org/faostat/form/.

FDA, 1990 "In: canola oil and rapeseed." Oil Color (Ed.), Mikkelson B, 2005, Web site: http://www.snopes.com/medical/toxins/canola.asp.

Firestone D, 1996 "Official methods and recommended practices of the American Oil Chemists Society." 4th (Ed.), *American Oil Chemist's Society*, Champaign, Method Ca 5a–40, Free Fatty Acids.

Freese R; Mutanen M, 1997 "Alpha – linolenic acid and murine chain n-3 fatty acid differs only slightly in their diet homeostatic factors in healthy subjects." *American Journal Clinical Nutrition*, Vol. 66: 591-598.

Ganesh M S, 2006 "New developments in rapeseed mustard." *Current Science*, Vol. 90 (9): 1174 -1175.

Gardner C D; Kramer H C, 1995 "Monounsaturated verses polyunsaturated dietary fat and serum lipids." *A Meta Analysis- Arterioscler Thrombosis Vascular Biology*, Vol. 15: 1917 –1927.

Gaul L E, 1964 "Contact dermatitis from synthetic oil of mustard." *Archeology Dermatology*, Vol. 90: 158–159.

Geier H, 2004 "Canola quality in Alaska (2001 Harvest)." Agriculture and Forestry Experiment Station (AFES), *Research Progress Report 42*, University of Alaska Fairbanks, Web site: www.uaf.edu/snras.

Grifen R C, 1927 "Technological methods of analysis." Director of Analytical Department, 2nd (Ed). USA, P.170 -180.

Grundy S M, 1986 "Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol." *Nutrition England Journal Medical*, Vol. 314: 745–748.

Grundy S M; Denke M A, 1990 "Dietary influences on serum lipids and lipoproteins." *Journal Lipid Research*, Vol. 31 (7): 1149-72.

Gur M I; Harwood J L, 1991 "Lipid Biochemistry: an introduction." London: Chapman and Hall, P.406.

Gurr M, 1992 "Dietary lipid and Coronary Heart Disease." *Prop. Lipid Research*, Vol. 31: 195-243.

Gutkin S S, 1950 "Properties and uses of high iodine number." Falkidin drying oils, *Journal of American Oil Chemist's Society*, Vol. 27: 542.

Government of India, Annual report, 2001-2002 Ministry of Health and Family Welfare, Nirman Bhawan, New Dhilli , "In: The Prevention of Food Adulteration Act, 1954 (Amended in 1964, 1976, 1986)."

Hariharan K S; Purushothama P L, 1996 "Studies on red palm oil: Effect of partial supplementation of saturated fats upon lipids and lipoproteins." *Nutrition Research*, Vol.18 (8): 1381 – 1392.

Hildich T P, 1949 "The industrial chemistry of fats and waxes." 3rd (Ed.), Baillier Tindal and Cox, London, P. 80 - 83, 159.

Holley R A, 2008 "Antimicrobial activity." In: *Food Technology Intelligence Inc.*, Winnipeg, MB, Canada.

ISO 660, 1983 (Ed.) "Animal and vegetable fats and oils – determination of acid value and acidity." ISO, Geneva.

Jacobs M B, 1959 "The chemical analysis of foods and food products." 3rd (Ed.) D. Van Nostrand Company Limited, London, P.393.

Jha P, Flather M, Lonn E , Farkouh M; Yusuf S, 1995 "The antioxidant vitamins and cardiovascular disease, a critical review of epidemiologic and clinical trial data." *Annual International Medical*, Vol.123: 860–872.

Jimmy C, Jiang Y Z, Li R; Chan S M, 2003 "Chemical composition of the essential oils of Brassica juncea (L.) Coss. grown in different regions, Hebei, Shaanxi and Shandong, of China." *Journal of Food and Drug Analysis*, Vol. 11(1): 22-26.

Kalua C M, Bedwood D R, Bishop A G; Pienzler P D, 2008 "Changes in virgin oil quality during low temperature fruit storage." *Journal Agricultural Food Chemistry*, Vol.10: 1021.

Khosla M I, 2000 "The nutritional benefits of organic mustard oil." In: Wikipedia, The Free Encyclopedia, Whole Foods, New Delhi, Published on: 21st March 2007.

Khosla P; Sundram K, 1996 "Effects of dietary fatty acid composition on plasma cholesterol." *Progress Lipid Research*, Vol. 35 (2): 93-132.

Kramer J K, *et al*, 1998 "Hematological and lipid changes in new born piglets fed milk-replacer diets containing erucic acid." *Journal Lipid Research*, Vol. 33 (1): 1-10.

Kris-Etherton P M, Harris W S; Appel L J, 2003 "Omega-3 fatty acids and cardiovascular disease: new recommendations from the American Heart Association." *Arteriosclerosis Thrombosis Vascular Biology*, Vol.23: 151–152.

Kris-Etherton P M; Yu S, 1997 "Individual fatty acid effects on plasma lipids and lipoproteins: human studies." *American Journal Clinical Nutrition*, Vol. 65 (5 Suppl): 1628S-1644S.

Lawson H, 1997 "Food oils and fats – technology." *Utilization and Nutrition*, 1st (Ed.), P. 263.

Leonard C, 1994 "Sources and commercial applications of high erucic vegetable oils." *Lipid Technology*, Vol. 4: 79–83.

Li D, Sinclair A, Wilson A, Nakkote S, Kelly F, Abedin L; *et al*, 1999 "Effect of dietary alpha-linolenic acid on thrombotic risk factors in vegetarian men." *American Journal Clinical Nutrition*, Vol.69: 872–882.

Lichtenstein A H, Ausman L M, Carrasco W, Jenner J L, Gualtieri L J, Goldin B R; *et al*, 1993 "Effects of canola, corn, and olive oils on fasting and postprandial plasma lipoproteins in humans as part of a National Cholesterol Education Program Step 2 diet." *Arterioscler Thrombosis*, Vol.13: 1533–1542.

Manchanda S C, 2006 "Mustard oil can reduce the risk of heart diseases." All India Institute of Medical Sciences (AIIMS), New Delhi, *Current Science*, Vol. 90 (9).

Mattil K F, Norris F A, Stirton A J; Swern D, 1964 "Bailey's industrial oil and fat products." *International Science Publication*, Agricultural Division, John Wiley and Sons, New York, P. 772-773.

Mattson F H; Grundy S M, 1985 "Comparison of effects of dietary saturated, monounsaturated and polyunsaturated fatty acids on plasma lipids and lipoprotein in man." *Journal Lipid Research*, Vol. 26: 194-202.

Mayton H S, Oliver C, Vaughn S F; Loria R 1996 "Correlation of fungicidal activity of *Brassica* species with allyl isothiocyanate production in macerated leaf tissue." *Phytopathology*, Vol. 86: 267-271.

Mortuza M G, 2006 "Tocopherol and sterol content of some rapeseed / mustard cultivars developed in Bangladesh." *Pakistan Journal of Biological Sciences*, Vol. 9 (9): 1812 – 1816.

National Toxicology Program, 1991 "NTP Chemical repository data sheet: Allyl isothiocyanate." *Research Triangle Park*, NC.

Niewiadomski H, 1990 "Rapeseed chemistry and technology." *Elsevier, PWN, Polish Scientific Publisher*, New York.

Nowsheen I, 1994 "Quality analysis of selected cooking oils available in the market." M. Sc. Thesis, Department Food and Nutrition, Home Economics College, Lahore, Pakistan.

Nugon-Baudon L; Rabot S, 1994 "Glucosinolates and glucosinolates derivatives: Implications for protection against chemical carcinogenesis." *Nutrition Research Review*, Vol.7: 205-231.

Ohlson R, 1972 "Non- industrial uses of rapeseed oil and rapeseed fatty acid." In: "Rapeseed: cultivation, composition, processing and utilization." Appleqvist L A and Ohlson R, (Ed.) Elsevier, Amsterdam, P. 274.

Olivier C, Vaughn S F, Mizubuti E S G; Loria R, 1999 "Variation in allyl isothiocyanate production within Brassica species and correlation with fungicidal activity." *Journal Chemistry Ecology*, Vol. 25: 2687-2701.

Peter M C, 1993 "Rapeseed: nutritional aspects of a novel oil." *CSIRO Human Nutrition, Publication P.O. Box.10041*, BC Adelaide, South Australia.

Prabhu H R, 2000 "Lipid peroxidation in culinary oils subjected to thermal stress." *Indian Journal of Clinical Biochemistry*, Vol. 15 (1): 1-5.

Prakash S, Kumar P R, Sethi M, Yadav R K, Singh C; Tandon R K, 2000 "Mustard oil – The gem among cooking oils, a review." *Mustard Research and Promotion Consortium*, Department of Gastroenterology and Human Nutrition, All India Institute of Medical Sciences, Ansari Nagar, New Delhi.

Parathasarathy S; Rankin S M, 1992 "A high-monounsaturated-fat/low-carbohydrate diet improves peripheral insulin sensitivity in non-insulin dependent diabetic patients." *Progress Lipid Research*, Vol. 31: 127-143.

PS&D Database, FAS/USDA, 2003 US Department of Agriculture, Economics and Statistics Service, China: Fibers and Oilseed Statistics, April 2003.

Reddy K S, 2004 "Mustard oil made healthier." In: Wikipedia, The Free Encyclopedia, DDI News.

Rodriguez-Estrada M T, Penazzi G, Goboni M F, Bertacco G; Lercker G, 1997 "Effect of different cooking methods on some lipids and protein components of hamburgers." *Meat Science*, Vol. 45: 365-375.

Salunkhe D K; Desai B B, 1986 "Post harvest Biotechnology of oil seeds." *CRC Press*, Boca Raton, Florida, United States, P. 71 - 89.

Schmidt E B, 1994 "The potential of omega-3 fatty acids to reduce CAD." *Proceedings of the Conference on Omega-3 Fatty Acid in Nutrition and Vascular Biology*, Houston, Texas, P. 208–211.

Shukla A K, Singh R P; Johar G S, 2002 "A simple non-instrumental technique to differentiate a natural mustard oil sample from a synthetically made artificial mustard oil." *Journal of Oil Technologists' Association of India*, Vol. 34: 147-148.

Shukla K A, Dixit A K; Singh R P, 2005 "Detection of adulteration in edible oils." *Journal of Oleo Science*, Vol. 54 (6): 317-324.

Singh G, Shashi K, Palanisamy M, Valery I; Vera V, 2008 "Antioxidant and antimicrobial activities of essential oil and various oleoresins of Elettaria

cardamomum (seeds and pods)." *Journal of the Science of Food and Agriculture*, Vol. 88 (2): 280-289.

Smouse T H, 1994 "Factors affecting oil quality and stability." In: Methods to assess quality and of oils and fat – containing foods, Warner K, and Eskin N A M, (Ed.), AOCS Press, Champaign, IL, USA, P.17 - 36.

Sonntag N O V, 1991 "Erucic, behenic: Feedstocks of the 21st century." *Information*, Vol. 5: 449–463.

Sonntag N O V, 1995 "Industrial utilization of long-chain fatty acids and their derivatives." P. 339–352. In: Kimber D S; McGregor D I, (Ed.) "Brassica oilseeds." CAB International, Oxon, UK.

Sultana R, Bhatti N, Hashmi A S; Javed M T, 1998 "Study on the effect of rapeseed oils (Sarsoon and Canola) and hydrogenated fat on serum lipid fractions of rats." *Pakistan Journal of Biological Sciences*, Vol. 1 (4): 376- 379.

Taylor D C , Mac Kenzie S L, McCurdy A R, McVetty P B E, Giblin E M, Pass E W, Stone S J, Scarth R, Rimmer S R; Pickard M D, 1994 "Stereospecific analyses of seed triacylglycerols from high-erucic acid Brassicaceae: detection of erucic acid at the sn-2 position in *Brassica oleracea* L. genotypes." *Journal of American Oil Chemist's Society*, Vol. 71:163–167.

Timms C, Schladt L, Robertson L, Rauch P, Schramm H; Oesch F, 1987 "The regulation of rat liver epoxide hydrolases in relation to that of other drug-metabolizing enzymes." In: Drug Metabolism; From Molecules to Man, P.5548, [Benford D, Bridges J W; Gibson G G, (Ed.)], London: Taylor and Francis.

USDA Food Data, 2000 "Rapeseed." In: Wikipedia, The Free Encyclopedia, Web Site: http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl.

Valsta L M; *et al*, 1992 "Effects of a monounsaturated rapeseed oil and a polyunsaturated sunflower oil diet on lipoprotein levels in humans." *Arterioscler Thrombosis*, Vol.12 (1):50-57.

Vaughan J G; Hemingway J S, 1959 "The utilization of mustards." *Economic Botany*, Vol. 13: 196–204.

Vogel A I, 1961 "A text book of quantitative inorganic analysis." 3rd (Ed.), Longman, London, P. 787, 806-810.

Wagner K H, Isnardy B; Elmadfa I, 2004 "Gamma and delta tocopherols are more effective than alpha tocopherol on the oxidation of a 10% rapeseed oil triacylglycerol in water emulsion with and without radical initiator." *European Journal Lipid Science Technology*, Vol. 106: 44-51.

Wattenberg L W, Hanky A B, Barany G, Spamins V L, Lam L K T; Fenwick G R, 1986 "Inhibition of carcinogenesis by some minor dietary components." In: *Diet, Nutrition and Cancer*, [Hayashi Y, *et al*, (Ed.)], *Journal Tokyo*: *VNU Science*, P.13-21.

Wattenberg L W; Loub W D, 1978 "Inhibition of polycyclic aromatic hydrocarbon induced neoplasia by naturally occurring indoles." *Cancer Research*, Vol.38: 1410-1413.

Web Site: "http://en.wikipedia.org/wiki/Allyl_isothiocyanate." In: Wikipedia, The Free Encyclopedia, Published on, September, 2007.

Web site: "http://www.mayoclinic.com/health/, Canola oil: Is it harmful to your health?" Published on, 2006.

Web Site: "http://www.sciencelab.com/, Mustard oil, synthetic, MSDS (Material Safety Data Sheet)." Published on, 6th June, 2008.

Web Site: "http://www.Seatons.com/, Natural oils for industry, Technical, Analytical test methods." Published on, 24th February, 2009.

Web Site: "http://www.tis-gdv.de/tis_e/sitemap/sitemap.htm, Mustard oil." In: Transport Information Service, Gesamtverband der Deutschen Versicherungswirtschaft (GDV), Berlin, 2002-2008.

Weiss E A, 1983 "Oilseed crops." Tropical Agriculture Series, Longman, London, P. 161.

Weiss J T, 1983 "Food oils and their uses." 2nd (Ed.), *AVI Publishing Company Inc.*, Westport, Connecticut.

Welter J, 2007 "Oil yields and characteristics." In: Vegetable Oil Yields, Characteristics Journey to Forever, Web Site: http://journeytoforever.org/.

White T J, 1991 "Methods of measuring change in deep fat frying oils." *Food Technology*, Vol. 45(2): 75-80.

Wijesundera C, Ceccato C, Fagan P, Shen Z, Burton W; Salisbury P, 2008 "Canola quality Indian mustard oil (Brassica juncea) is more stable to oxidation than conventional canola oil (Brassica napus)." *Journal American Oil Chemist's Society*, Vol. 85: 693–699.

Williams K A, 1966 "Oils, fats and fatty foods – their practical examination." 4th (Ed.), *American Elsevier Publishing Company Inc.*, 52 Vanderbilt Avenue, New York, P. 176.

Yano T, Yajima S, Virgona N, Yano Y, Otani S, Kumagai H, Sakurai H, Kishimoto M; Ichikawa T, 2000 "The effect of 6-methylthiohexyl isothiocyanate isolated from Wasabia japonica (wasabi) on 4-(methylnitrosamino)- 1- (3-pyridyl)-1- buatnone-induced lung tumorigenesis in mice." *Cancer Letter*, Vol. 155: 115-120.

Youngs C G, Mallard T M, Craig B M; Sallans H R, 1951 "Component of fatty acid composition of rapeseed oil." *Canadian Journal of Chemistry*, Vol.29: 124.

Appendices

Appendix 1: Analysis of moisture content for various samples

ANOVA

Parameter	Sum of Squares	Degree of freedom	Mean Square	F value	Significance level
Between Groups	.139	7	.020	21.923	*
Within Groups	.014	16	.001		
Total	.153	23			

* Significance at 1% level

Appendix 2: Analysis of specific gravity for various samples

ANOVA

Parameter	Sum of Squares	Degree of freedom	Mean Square	F value	Significance level
Between Groups	.002	7	.000	23.675	*
Within Groups	.000	16	.000		
Total	.002	23			

* Significance at 1% level

Appendix 3: Analysis of refractive index for various samples

ANOVA

Parameter	Sum of Squares	Degree of freedom	Mean Square	F value	Significance level
Between Groups	.000	7	.000	10.043	*
Within Groups	.000	16	.000		
Total	.000	23			

* Significance at 1% level

Appendix 4: Analysis of color value for various samples

ANOVA

Parameter	Sum of Squares	Degree of freedom	Mean Square	F value	Significance level
Between Groups	857.073	7	122.439	13.495	*
Within Groups	145.167	16	9.073		
Total	1002.240	23			

* Significance at 1% level

Appendix 5: Analysis of acid value for various samples

ANOVA

Parameter	Sum of Squares	Degree of freedom	Mean Square	F value	Significance level
Between Groups	13.401	7	1.914	33.888	*
Within Groups	.904	16	.056		
Total	14.305	23			

* Significance at 1% level

Appendix 6: Analysis of iodine value for various samples

ANOVA

Parameter	Sum of Squares	Degree of freedom	Mean Square	F value	Significance level
Between Groups	4216.347	7	602.335	201.188	*
Within Groups	47.902	16	2.994		
Total	4264.249	23			