STUDY ON THE PHYSICO-CHEMICAL CHARACTERISTICS OF THE OILS AND OIL CAKES OF THE DIFFERENT RELEASED AND LINE CULTIVARS OF MUSTARD AND RAPESEED *(Brassica spp.)*

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JUNE 2015

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Registration Number: 14-06356

A Thesis
Submitted to the Department of Biochemistry
Sher-e-Bangla Agricultural University, Dhaka
In partial fulfilment of the requirements
for the degree
of

MASTER OF SCIENCE (MS)
IN
BIOCHEMISTRY

SEMESTER: JANUARY-JUNE, 2015

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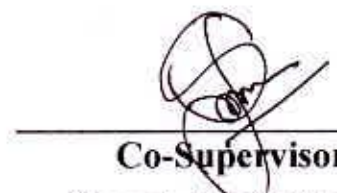

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CERTIFICATE

This is to certify that the thesis entitled **"Study on the Physico-Chemical Characteristics of the Oils and Oil Cakes of the Different Released And Line Cultivars of Mustard And Rapeseed *(Brassica spp.)*"** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN BIOCHEMISTRY**, embodies the result of a piece of bonafide research work carried out by **S.M. SHAFIQUL ISLAM**, Registration No. **14-06356** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

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Dedicated To
My Beloved Parents

Some commonly used Abbreviations

Full Word Abbreviation	
And others	*et al*
Association of Official Analytical Chemist	AOAC
Atomic Absorption	AA
Bangladesh Agriculture Research Institute	BARI
Bangladesh Bureau of Statistics	BBS
Food and Agriculture Organization	FAO
Bangladesh Council of Scientific and Industrial Research	BCSIR
Oilseeds Research Laboratory	ORL
Calcium	Ca
Copper	Cu
Crude protein	CP
Cubic Centimeter (Solid materials)	CC
Coefficient of variation	CV
Degree Celsius (Centigrade)	°C
Fatty acid	FA
Gas chromatography	GC
Gram	gm
Hour	hr
Institute of food and Nutrition	IFN
Iron	Fe
Kilogram	kg
Least significant difference	LSD
Liter	L
Magnesium	Mg
Manganese	Mn
Milliliter	ml
Molar	M
Molecular weight	Mw
Normality	N
Parts per Million	ppm
Phosphorus	P
Potassium	K
Sodium	Na
Sulphur	S
Tri chloro acetic acid	TCA
Zinc	Zn

ACKNOWLEDGEMENTS

All praises to Almightly and Kindful trust on to "Almighty Allah" for His never-ending blessing, the author deems it a great pleasure to express his profound thankfulness to his respected parents, who entiled much hardship inspiring for prosecuting his studies, receiving proper education. The author likes to express his deepest sense of gratitude to his respected supervisor, ***Dr. Kamal Uddin Ahmed****,Professor and Chairman, Department of Biochemistry, Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh for his scholastic guidance, support, encouragement and valuable suggestions and constructive criticism throughout the study period and gratuitous labor in conducting and successfully completing the research work and in the preparation of the manuscript writing. The author also expresses his gratefulness to his respected Co-Supervisor,* ***Nasrat Jahan Shelly****, Assistant Professor, Department of Biochemistry, SAU, Dhaka for her scholastic guidance, helpful comments and constant inspiration, valuable suggestions throughout the research work.The author also express heartful thanks to* ***Mst. Farhana Nazneen****, Assistant Professor, Department of Biochemistry, SAU, Dhaka for her valuable suggestions, instructions, cordial help and encouragement during the period of the study.The author would like to gratefully acknowledged respected sir* ***Md. Arif Hussain,*** *for supply seed to study this research. The author would like to gratefully acknowledge respected sir* ***Md. Sajedur Rahman,*** *for helping to analysis data. Oil seed Research Centre and Soil Science Division, BARI, Joydebpur, Gazipur is gratefully acknowledged for their kind cooperation regarding the provide Laboratory facilities for Biochemical analysis.The author expresses his sincere appreciation to his another teachers, brothers, sisters, relatives, well wishers and friends for their inspiration, help and encouragement throughout the study period.*

The Author

STUDY ON THE PHYSICO-CHEMICAL CHARACTERISTICS OF THE OILS AND OIL CAKES OF THE DIFFERENT RELEASED AND LINE CULTIVARS OF MUSTARD AND RAPESEED*(Brassica spp.)*

ABSTRACT

To study the physico-chemical characteristics of four released and two line cultivarsof rapeseed (*Brassica campestris* and *Brassica napus*) and mustard (*Brassica juncea*), an experiment was conducted.Among these released and line cultivars, the highest grain weight was obtained from BARI Sarisha-9 X BARI Sarisha-6 (4.892g) and lowest grain weight obtained from SAU Sarisha-2 (4.459g). In case of proximate analysis, the highest protein content and the highest carbohydrate were recorded from SAU Sarisha-2 (28.05%)and Tori-7(19.08%) respectively. The oil content of different released and line cultivarsof mustard and rapeseed varied from 38.74% to 40.55%.BARI Sarisha-9 (540.3kcal/g) contained the highest amount of Gross Energy. The highest amount of calcium content (1.006%) and the highest amount of Magnesium content (0.838%) were attained from Tori-7. The highest amount of saponification value(168.8) and the highest amount of iodine value (110.2) were recorded from Tori-7; however, the highest amount of acid value was recorded from Tori-7 X BARI Sarisha-6(1.610). Erucic acid was in the range of 41.11 – 50.67%, oleic acid was the highest in Tori-7 contained 18.56%, while BARI Sarisha-9 containd the highest amount of the unsaturated linoleic (17.75%) and linolenic (15.83%) acids. Moreover, palmitic acid, stearic acid and arachidic acid were also present in small amount. Substantial genetic variability exists for chemical composition and nutritional traits which could be utilized to suggest the future strategy for the nutritionist, health advisors and breeders.

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CHAPTER I

INTRODUCTION

Mustard (*Brassica spp.*) is one of the most important oilseed crops throughout the world after soyabean and groundnut (FAO, 2004). It has a remarkable demand for edible oil in Bangladesh. It occupies first position of the list in respect of area and production among the oilseed crops grown in this country (BBS, 2004). In the year of 2003-04 it covered 1.79 lakhs hectare (ha) land and the production was 2.11 lakhs metric ton (Mt), where as the total oilseed production was 4.07 lakhs Mt and total area covered by oilseed crops was 3.89 lakhs ha. In the year of 2004-05 it covered 3.95 lakhs ha land and the production was 3.79 lakhs Mt. (BBS, 2005). Rape seed and mustard are common names used for different species of the family Cruciferae (Bracicaceae). Rape seed includes *Brassica compestris* and *B. napus*. Mustard specifically refers to *Brassica juncea* and *Eruca sativa*. There are considerable differences in agronomic characteristics, yield, and fatty acid (FA) composition of seed oil between species and between varieties (Bauer, 2015).

The *Brassica* oil-seed crops belong to the family Bracicaceae (synonymous with Cruciferea, common name crucifers) has traditionally attracted the attention of plant breeders and oil chemists, because of its high oil content. These crops, including rape (*B. campestris* L., and *B. napus* L.) and mustard (*B.juncea*) are the third major source of edible vegetable oils in the world, after soybean and oily palm (FAO, 2011).The *Brassica* oil-seed crops have been grown in Bangladesh since long. The tender leaves of these cultivars serve as vegetable, while the seeds as a source of lubricating and cooking oil. The residue left after oil extraction (i.e., oil cake or meal) being rich in protein (Durrani and Khalil, 1990) can be used as livestock feed.

It produces 9kcal energy from 1gm of oil per unit in comparison with other diets (carbohydrate and Protein). In a balanced diet for human health 20-25% of

calories should come from fats and oils. The mustard oil is not used only for cooking purpose but also is used for hair dressing, body massing and for different types of pickles preparation. It is also used as medicinal values. The protein quality and quantity obtained from oil cake of *B. campestries* is high. (Chowdhury *et al.*, 2010)

Mustard is a high yielding oilseed with a reasonably high content of oil (Riley, 2004). Mustard seeds have high energy content, having 28-32% oil with relatively high protein content (28-36%). The amino acid composition of mustard protein is well balanced; it is rich in essential amino acids. Until now mustard seeds have been used mainly for condiment production. This advantageous chemical composition and its relatively low price offer wide possibilities for usage of this valuable seed, for example in human foods as additive (Gadei *et al.*, 2012) and to feed animals.

Oil is one of the necessary nutrients for the human body, which is supplied by animal and plant sources (Nabipour *et al.*, 2007). Herbal oils are the main sources of fats and fat-soluble vitamins, which have a substantial role in the human diet (Stuchlik, 2002). After cereals, oily seeds are the second food sources throughout the world, whose oil is of rich fatty acid types (Siavash, 2005). Rapeseed oil has a high nutritional quality due to its lowest levels of saturated fatty acid, balanced amount of unsaturated fatty acids and being free from cholesterol (Starner *et al.*, 2002). With the considerable amounts of unsaturated fatty acids in the seed oil of the studied rapeseed released and line cultivars and also their less than 1% erucic acid content taken into account, the obtained oil can be a good replacement for animal fat or even other herbal oils in the human diet (Sanavi *et al.*, 2004).

Rapeseed-mustard oil quality is determined by the constituent fatty acids including palmitic, stearic, oleic, linoleic, linolenic, eicosenoic and erucic acids; and is highly affectedby the variety type (Nasr *et al.*, 2006; Javidfar *et al.*, 2007). One of the main breeding objectives regarding rapeseed besides the oil quantity is

to increase its oil quality (Azizi *et al.*, 1999). From the nutritional perspective, linoleic acid is the most important unsaturated fatty acid. Linoleic and linolenic acids are essential fatty acids not synthesized by our body, it has to be supplied through meals. In addition, oleic acid is among unsaturated fatty acids whose antioxidant effects have been proved (Berry *et al.*, 1997). Erucic acid, although, anti-nutritional and should be <2% in the edible oil, higher erucic acid is of considerable industrial importance. Information regarding concentration of saturated fat, trans fat, linoleic (ω-6) to linolenic (ω-3) acids ratio, saturated fatty acids (SFA)/monounsaturated fatty acids (MUFA)/polyunsaturated fatty acids (PUFA) ratio, concentration of anti-nutritional factors (erucic acid, glucosinolates, phytic acid, sinapic acid and tannins etc.), presence of various phenolic compounds acting as an antioxidants and taste determinants, reducing seed meal fibre content, association of glucosinolates with different biotic and abiotic stress factors and their role in plant defense systems and other biological activities including anti-cancerous activities (Kumar *et al.*, 2011).

In addition there are some cultivars viz. BARI Sarisha-6, BARI Sarisha-9, Tori-7, SAU-2 are now cultivating in different parts of the country. The nutritional quality of all these released and line cultivars is not yet analyzed. If the nutritional quality of rapeseed and mustard is known, its consumption as well as its multipurpose uses will be increased which play a vital role in improving the nutritional status of the people of our country. Now a crucial question arises about the physico-chemical quality of BARI released and line cultivars rapeseed and mustard. Therefore the present study was undertaken with a view to determine physico-chemical properties of some popular selected released and line cultivars to ensure their nutritional status.

Objectives

In order to fulfill the above mentioned aim, experiments have been undertaken with the following objectives:

1. To investigate the physical and chemical properties, mineral content, oil percentage and fatty acid profiles of selected released and line cultivars of rapeseed and mustard.
2. To compare the physico-chemical parameters and nutritional quality of selected released and line cultivars of rapeseed and mustard.
3. To compare the Gross Energy obtain from selected released and line cultivars of rapeseed and mustard.

CHAPTER II

REVIEW OF LITERATURE

Vegetable oils and fats constitute an important component of human diet, ranking third after cereals and animal products. *Brassica* is considered to be the most important source of vegetable and protein rich meal worldwide (sovero, 1993; Cardoza and Stewart, 2003). Mustard and rapeseed is important oil crop in human and animal nutrition because of its high level of fats/oil, fatty acid, protein and other nutritionally valuable substances. As a high energy component of food, edible oil is important for meeting the caloric requirement. Physical properties, chemical analysis, estimation of minerals and total energy of different types of rapeseed and mustard observed by other researcher are described in this section.

Grain Weight

Thousand seed weight is a very important character of rapeseed and mustard, where highest consideration is one of the seed yield. This character has been found to vary widely from genotypes to genotypes and from environment to environment. A good number of literatures are available on the variability of this trait.

Hossain *et al.* (2015) found that seed weight varied with their size and shape. Thousand grain weights were determined at 13% moisture level. The highest thousand grain weight was found in BARI Sarisha13 (4.38g), this was significantly higher than all others released variety and the lowest thousand grain weight was found in BARI Sarisha-9(3.06g). Statistically similar results were shown by BARI Sarisha-11 (3.567g), BARI Sarisha-14 (3.751g) and Sarisha-16 (3.756g)

Chowdhury *et al.* (2014) found that seed weight varied with their size and shape. Thousand grain weights were determined at 13% moisture level. The highest

thousand grain weight was found in BARI Sarisha-9 (4.9 g). This was significantly higher than all others released variety and advanced lines of *Brassica campestris*, followed by BARI Sarisha-14 (4.2 g). Statistically similar results were shown by BARI Sarisha-12 (3.3 g), Din-2 (3.3 g) and BC-2193 (3.5 g) but these values were significantly higher than the BARI Sarisha-15 (2.5 g) which showed lowest thousand grain weight.

Damian (2014) said that, 1000 seed mass of the mustard seeds increased from 4.3 to 5.1g, as the moisture content increased from 7.0 to 15.99% (d.b.).

Banga *et al.* (2009) observed the seed yield in different genotypes was mainly governed by seed yield per plant. The 1000-seed weight was the maximum in RL-1359 (5.15g) whereas it was at par in other genotypes. The experiment consisted of four genotypes of Indian mustard (RL-1359, TAC-437, Kranti and NRCDR-601).

Siddiqui *et al.* (2004) introduces some important characteristics of *Brassica carinata* cultivars (IGC-01 and Pusa Gaurav), *B. juncea* cultivars (Jagannath, Kranti, Rohini, and TERI (OE) M21-Swarna) and *B. napus* cultivars (Hyola PAC-401) in case of grain weight. They reported that highest grain weight observed TERI (OE) M21-Swarna (3.95g) among these cultivars.

Mondal *et al.* (2001) found that weight of 1000 seeds varied from variety to variety and species to species. They found that thousand seed weight of 2.50-2.65 g in case of improved Tori-7 (*B. campestris*) and 1.50-2.80 g in Rai-5 (*B. napus).*

Karim *et al.* (2000) reported that released and line cultivars showed significant difference in weight of thousand seeds. They found higher weight of 1000 seed in J-3023 (3.43 g), J-3018 (3.42g) and J-4008 (3.50 g).

According to Kumar and Singh (1994) in *B. juncea* , Kudla (1993) in sewede rape, Andarhennadia *et al.* (1991) in brown mustard, Biswas (1989) in *B. campestris*, Labowitz (1989) in *B. rapa*, Yin (1989) in *B. rapa* and Chowdhury *et al.* (1987) in *B. rapa* found different degrees significant variations of thousand seed weight due to variable genotypes.

Kaul *et al.* (1986) carried out an experiment to evaluate thousand grain weights of *Brassica campestris* seeds. They found that grain weight of *Brassica* seeds ranges from 2.6-3.1 g for brown-seeded form and 2.5- 4.5 g for yellow-seeded form, respectively.

Moisture

In storage condition, the permeable moisture level of different oil seeds are 10-12%. Moisture content is important factor than other nutrients as they vary with it. It is also important for insect infestation and disease.

Hossain *et al.* (2015) moisture content of different released and line cultivars of mustard and rapeseed was ranged from 4 to 5.2%. The highest moisture content (5.2%) was observed from BARI Sarisha-15; while the lowest moisture content (4%) was found in BARI Sarisha-16.

According to Sarker *et al.* (2015) moisture content of black and yellow mustard cakes were 9.20 ± 0.5% and 9.73 ± 0.6%. The results of the moisture content were slightly higher than the moisture content 8.3 ± 0.2% reported in literature.(Mahmud et al. 2012; Marnoch and Diosady, 2006).

Mustard intended for long-term storage should be kept at less than 9% moisture. If the mustard needs to be dried for safe storage, the drying air temperature and seeds 'temperature should not exceed 65 and 450C, respectively (Barrozo *et al.* 2008).

Huda (2001) conducted an experiment the moisture content of rapeseed mustard varieties and advanced lines. They suggest that seeds having moisture content from 8-10% stored well and safe moisture content for sealed storage is 4-8%.

BARI (1987-88) carried out an experiment to determine the moisture content of rapeseed-mustard varieties and advanced lines. They reported the moisture content of different released and line cultivars i.e., 7.06%, 8.36%, 7.66%, 7.98%, 7.41%, 8.38%, 7.81%, and 7.82% for SS-75, Krisna, PT-303, Varuna, TS-72, Kranti, S-5 and Tori-7, respectively.

Ash

Ash is the residue that remains after the complete combustion of the organic compound of a food product. The estimation of ash content in oil crops enables the classification of oil.

Hossain *et al.* (2015) found that, ash content of different released varieties of mustard and rapeseed were variable and ranged from 9.6% to 12.5% . There were no significant variations among the released and line cultivars BARI Sarisha-13 (12.5%), BARI Sarisha-9 (12.4%), BARI Sarisha-16 (12.37%), BARI Sarisha- 11 (12%), BARI Sarisha-15 (11.8%). But these values were significantly higher than the BARI Sarisha-14 (9.6%)

Sarker *et al.* (2015) observed that, ash content of black and yellow mustard cakes were 7.10 ± 0.3% and 5.90 ± 0.3% respectively. (AMB Express 5:22) .These data are comparable (7.12 ± 0.12%) to the results reported in literature (Datta *et al.*, 2013)

Abul-Fadl *et al.* (2011) observed that the tested mustard seeds of both yellow and brown varieties containing an adequate percentage of ash, dietary fiber and total carbohydrates which were found to be as 4.08, 5.87 and 16.60% in yellow variety and 3.88, 6.34 and 16.49% in brown variety; respectively.

The ash content was variable among the five oil seed samples. *B. napus* (Durr-e-NIFA) contained the maximum amount of ash (6.93%) followed by *B. juncea* (NIFA-Raya) (6.73%) and sunflower (Gulshan 98), (6.00 %). While cotton seed (Niab, 98) contained (4.56%) and the least amount was found in corn (Jalal), i.e., (1.50%). The results were in agreement to those reported by Besbes *et al.* (2003).

Sosulski *et al.* (1991) conducted an experiment to evaluate ash content of different rapeseed-mustard varieties and advanced lines. They reported that ash content of *B. campestris and B. rapa* ranges from 7.0-8.4% and 7.3-8.0%, respectively.

Nehrins *et al.* (1990) reported that ash content of Canadian wild mustard and rapeseed were found to be 4.38% and 7.3%, respectively.

Kaul *et al.* (1986) carried out an experiment to evaluate partial analysis of India cruciferous oilseeds. They found that ash content of *Brassica campestris* variety dichotoma BS-2 and Toria T-36 were 3.74% and 3.22%, respectively.

Oil

Hossain *et al.* (2015) conducted an experiment to evaluate composition and physical properties of different oil seeds. The oil content of different varieties of mustard and rapeseed varied from 38.75% to 42.25%.

Gadei *et al.*, (2012) conducted an experiment to evaluate composition and physical properties of different oil seeds. They observed that Mustard seeds have high energy content, having 28–32% oil with relatively high protein content (28–36%). B.R. Moser *et al.* (2009) also found the similar result.

Arif *et al.* (2012) carried out an experiment to evaluate potential nutrients of oil seeds and they observed highest amount of crude oil in *B. juncea* (Durr-e-NIFA) and *B. napus* (NIFA Raya) i.e., 45.67% and 43.87% respectively.

Mandal *et al.* (2002) said that one of the most remarkable characteristics of *Brassica* species is the high oil content in the seeds, ranging in wild types from about 21.5-46.7%;but according to Kumar and Tsunoda, (1980) it is range from about 17 >40%.

Siddiqui *et al.* (2004) introduced some features of *B. carinata* (IGC-01 and Pusa Gaurav) cultivars, *B. juncea* cultivars (Jagannath, Kranti, Rohini and TERI (OE) M21-swarna) and *B. napus* cv. (Hyola PAC-401). They reported that cultivars Pusa Gaurav recorded the highest oil content (40%) among these.

Sengupta *et al.* (2003) evaluated that oil content of rapeseed-mustard varieties and advanced lines. The results indicated that oil content of mustard seed ranges from 40-41.5% and Kernel 45-47.1%.

Bhowmik (2003) experiment conducted on some released rapeseed and mustard released and line cultivars and their oil content. Results revealed that oil content of brown sarson, yellow sarson and toria ranges from 44-45%, 42-46% and 42.44%, respectively.

Rathore (2000) reported that oil content of different released rapeseed-mustard released and line cultivars varieties and advanced lines. Results stated that oil content of sarson, toria and rai ranges from 43-45%, 30-35% and 31-35%, respectively.

BARI (2001) reported that variable amount of oil are present in different rape seed mustard varieties and advanced lines. Results showed that oil content of different released and line cultivars i.e., 40-42%, 41-42%, 44-45%, 40-41% and 43-44% for Tori-7, Kalyania (TS-72), Sonali (SS-75), BARI sarisha-10 and BARI Sarisha-11, respectively.

Tyagi *et al.* (2001) was analyzed proximate composition of oil and crude protein. The highest amount of oil (38.96%) and crude protein (46.23%) were recorded for Toria Kranti and Toria shgamgarh, respectively.

Ullah *et al.* (1997) said that twelve released and line cultivars of rape seed mustard including three species, namely *B. campestris; B. juncea and B. napus*; significant differences were found among the released and line cultivars in respect of oil yield. They reported that *B. campestris* produced highest amount of free and bound oil where as lowest was found in *B. juncea.*

Niraz *et al.* (2001) evaluated 21genotypes of Indian mustard. Results indicated that considerable variation in oil content was found and ranging from 36.49% to 40.19% at per with vardan (39.14%) and varuna (38.13%).

Mazzoncini *et al.* (1993) described seed oil content of *Brassica carinata* ranged from 32.5% to 40.6%. They reported that seed oil content was higher in rape ranging from 40.5% to 47.3%.

Vijay *et al.* (1992) evaluated the oil contents in 65 released and line cultivars of rapeseed-mustard. The result indicated that many released and line cultivars have high oil content (more then 40%) and particularly RH, RK and DIRA had low oil content, mostly in the range of 35-38%.

BARI. (1986-87) carried out an experiment on oil content of 14 *Brassica campestries* genotypes. Results showed that highest oil content i.e., 42.87% and 42.69% for the lines OTBC-0893 and OTBC-1493 and others advanced lines were not significantly different from the first lines.

BARI (1992-93) determines the oil content of eight brown seeded *B. campestris* lines. Oil content of different advanced lines is 41.77%, 42.40%, 42.43%, 41.37%, 41.75% and 41.88% for BC-2192, BC-2493, BC-2592, BC-2693, BC-2892 and

BC-2093. Results revealed that there was no significant difference among the advanced lines with respect to its oil content.

BARI (1992-93) determined the oil content of seven yellow seeded *B. campestris* lines including two check varieties and advanced lines. Results revealed that there was no significant difference among the lines with respect to its oil content (41.99 to 42.52%).

Novoselov *et al.* (1994) analyzed the oil content of different rapeseed oil. Results revealed that oil content of rapeseed ranges from 45-46%.

Oil cake

Hossain *et al.*(2015) conducted an experiment to evaluate the percentage of oil cake of different varieties of mustard and rapeseeds and they found that, the BARI Sarisha13 contained significantly highest amount of oil cake (61.25%). The lowest value was found in BARI Sarisha-15 (57.75%).The present values were supported by the reported values of Chowdhury et al. (2014) and Appelqvist et al. (1992).

Chowdhury *et al.*(2014) conducted an experiment to evaluate the percentage of oil cake of different released and line cultivars of mustard and rapeseeds and they found that,the BARI Sarisha-12 contained significantly highest amount of oil cake (59.95%), followed BARI Sarisha-9 (59.47%) and BARI Sarisha-15 (59.25%). The lowest value was found in BARI Sarisha-14 (57.75%), followed by advanced lines Din-2 (58.14%).

Oil cake is the nutritious feed items for cattle and fish. It is also used as a good organic matter. Appelqvist *et al.* (1992) reported that typically rape seed oil (Kind of mustard seed) contain 58% cake.

Carbohydrate

Rapeseed and mustard contain relatively lower amount carbohydrate. It consists mainly glucose, sucrose and fructose crude fibre is also considerate as a constituent of carbohydrates.

Hossain *et al.* (2015) conducted an experiment to evaluate the percentage of oil cake of different varieties of mustard and rapeseeds and they found that, seeds contained 12.75-17.02% carbohydrate.

Bachheti *et al.* (2012) conducted an experiment to study physico-chemical properties of some conventional food oils and found that mustard seeds contain 23.8% carbohydrate.

Gopalan *et al.* (1995) in Nutritive value of Indian foods stated that dry mustard seeds contained 20-23% carbohydrate.

Fatty acid composition

Mustard and rapeseed oil has a special fatty acid composition. It contains different types of saturated and unsaturated fatty acid. oleic acid, linoleic acid, linolenic acid, palmitic acid and stearic acid are the most important and essential fatty acids in rapeseed oil.

Amir *et al.* (2012) ,in their study, identified fatty acids in the seed oil of studied spring and winter rapeseed released and line cultivars were oleic acid (63.62-67.38%), linoleic acid (15.87-19.06%), linolenic acid (7.55-9.76%), palmitic acid (3.55-4.51%) and stearic acid (1.54-2.3%), respectively. Moreover, arachidic acid, erucic acid, gadoleic acid and palmitoleic acid were among fatty acids with the lowest percentages (Less than 1%) of rapeseed varieties and advanced lines.

Mustard oil possess 60% monounsaturated fatty acids of which 42% Erucic acid and 12% Oleic acid, it has also 21% polyunsaturated of which 6% is the omega-3

alpha-Linolenic acid and 15% omega-6 linoleic acid along with 12% saturated fats.(Wani Mubashir, 2012)

The concentration of oleic acid (18:1), a beneficial monounsaturated fatty acid, ranges from 3.6-32.2% in rapeseed-mustard oil (Chauhan and Kumar, 2011).

Abul-fadl *et al.* (2011) reported that, erucic acid was predominant fatty acid in yellow and brown mustard seeds oils, which was represented about 37.89 and 23.90%,respectively.Both yellow and brown mustard seeds oils contained a little amounts (ranged between 8.45 to 8.94%) of saturated fatty acids as compared to the other edible oils. Oleic acid was the prevalent unsaturated fatty acids, which was ranged between 19.08 to 20.24% of total fatty acid profiles in both yelloe and brown mustard seed oils, respectively. Moreover, linoleic acid is the second dominant unsaturated fatty acid recorded about from 12.37 to 21.36 in both yellow and brown mustard seed oils, respectively.

Moser *et al.* (2009) observed that, mustard oil has a special fatty acid composition, it contains about 20–28% oleic acid, 10–12% linoleic, 9.0–9.5% linolenic acid, and 30–40% erucic acid, which is indigestible for human and animal organisms.

Pospišil *et al.* (2007) concluded that the fatty acid compounds in rapeseed hybrids and its double low released and line cultivars were affected by released and line cultivars to a great extent. In the new rapeseed varieties and advanced lines, instead of erucic acid, the level of other fatty acids such as oleic acid (more than 60%) and linoleic acid (10-20%) increased, while the level of linolenic acid had decreased (less than10%).

In the study by Nasr *et al.* (2006), five important fatty acids, i.e. oleic acid, linoleic acid, linolenic acid, stearic acid and palmitic acid were commonly found in 10 rapeseed released and line cultivars which oleic acid and stearic acid had the

highest and lowest percentages, respectively. Oleic acid levels in different rapeseed released and line cultivars were 51% to 62%, while there was 18-32% linoleic acid, 2-16% linolenic acid, 0.15-2.2% stearic acid and 4-8% palmitic acid. In addition, Nasr *et al.* (2006), reported a difference in terms of seed oil percentages among rapeseed released and line cultivars and mentioned the mean variation of seed oil percentages to be 37-42 in them.

Siddiqui *et al.* (2004) carried out some nutritional analysis on different rapeseed varieties such as *B. carinata* cultivars (IGC-01 and Pusa Gaurav), *B. juncea* cultivars (Jagannath, Kranti, Rohini and TERI (OE) M 21-Swarna and *B. napus* CV. (Hyola PAC-401). They reported that Jagannath, Kranti and Rohini content highest amount of linolenic acid (22.76%), erucic acid (43.30%) and palmitic acid (5.63%).

Sengupta *et al.* (2003) conducted an experiment on fatty acid composition in seven edible oils. They reported that mustard seed content saturated, mono-unsaturated and poly-unsaturated fatty acid was 6.73 and 21 respectively. They noted that amount of linoleic acid (18:2) was ranges from 11-22%.

Niraz *et al.* (2000) evaluated 21 genotypes of Indian mustard. They reported that considerable variation was found in fatty acid profile and low amount of erucic acid was observed in many genotypes ranging from 40.12 to 49.7%.

Ullah *et al.* (1997) conducted an experiment on twelve released and line cultivars of rapeseed mustard including three species namely *B. campestris, B. juncea and B. napus*. Significant differences were observed among these released and line cultivars in respect of fatty acid composition. They reported that newly introduced variety Nap-8509 (*B. napus*) contained lowest (36.4%) erucic acid and tradition Tori-7 contained lowest amount of linoleic acid.

Mazzoncini *et al.* (1993) conducted an experiment in different rapeseed released and line cultivars and reported that seed oil contained a high percentage of erucic acid (34-35%), linoleic acid (21-22%) and linolenic acid (18-19%).

BARI (1992-93) carried out an experiment on fatty acid composition of eight brown seeded *B. campestris* lines (BC-2192, BC-2492, BC-2592, BC-2692, BC-2892 and BC-2092) and seven yellow seeded *B. campestris* lines including two check varieties and advanced lines. Results revealed that there was no remarkable variation in fatty acid composition.

Sanches *et al.* (1990) reported that lipid content of different rapeseed released and line cultivars ranges from 36.8 to 41.35% with significant differences between cultivars. Palmitic acid comprised 50-70% of total saturated fatty acid; oleic acid comprised 64.7 to 72.1% of mono-unsaturated fatty acid and linolenic acid 13.0 to 17.5% of total poly unsaturated fatty acid. Erucic acid ranged from non-detectable to 1.08%.

Wahhab *et al.* (1980) introduced some features with low content of erucic acid in rape seed-mustard varieties and advanced lines. Results revealed that one seed was identified containing as low as 29.61% of erucic acid. Either oleic or linoleic acid has replaced erucic acid content as a major constituent.

Bhowmik (2003) reported that Indian rapeseed and mustard oils are inferior in quality as they contain high amount of erucic acid (28.0-53.0%) and linolenic acids (8.5-22.7%) although they also reported seed oil contain linoleic (12.0-21.0%) and oleic acid (10.0-24.0%) which are nutritionally good.

Appelqvist (1980) conducted an experiment to determine the fatty acid composition. He found that fatty acid composition of mustard released and line cultivars i.e., 3.0%, 0.8%, 9.9%, 13.5%, 9.8%, 6.3% and 52.3% for palmitic acid,

stearic acid, oleic acid, linoleic acid, linolenic acid, eicosenoic acid and erucic acid, respectively.

Rollet *et al.* (1995) determined the composition of French rape oil (*Brassica sp.*). They reported that fatty acid content in rape seed oil was 51%, 29%, 16%, 1%, 1.5% and 1.5% for erucic acid, oleic acid, linoleic acid, lenolenic acid, palmitic acid and lignoceric acid, respectively.

Protein

Around 28-32% of the mustard seed total weight is composed of proteins. Proteins are polymers of amino acids. Proteins form the structural elements of cells and tissue in the human body and are considered as the basis of life, but they are also essential components in different food systems.

In study by Sarker *et al.* (2015), the protein contents in black and yellow mustard cakes were 38.17% and 28.80% and their pepsin digestibility was 80.33% and 77.43%, respectively. The proteins were extracted at different pH and maximum proteins (89.13% of 38.17% and 87.76% of 28.80% respectively) isolated from black and yellow mustard cakes at pH 12.

The Crude protein content of mustard cakes obtained were 38.17% and 28.80% which were lower (45.0% and 34.0%) than those reported by many other authors (Marnoch and Diosady, 2006; Prapakornwiriya and Diosady, 2004). However, Chowdhury et al. and Kumar ent al. reported comparatively equal amount of crude protein in mustard cakes (Kumar *et al.*, 2002; Chowdhury *et al.* 2010). The variation observed could be because of the variety of mustard raised and the differences in sampling adopted, influence of season of harvest etc. Mean ether extract content of black and yellow mustard cakes were also low compared to many other authors (Latif *et al.*, 2008; Ramachandran *et al.*, 2007).

Al-Jasass *et al.* (2012) reported that,Cress, mustard, black cumin and black pepper contain higher protein contents ranging from 26.61 to 25.45%, as compared to fenugreek (12.91%) and clove (6.9%).

Mustard seeds contain 28-32% protein by weight and 30-35% of oil, although these values can vary slightly between varieties, growing regions and crop years (Canadian Grain Commission, 2012).

There are two main types of storage proteins present in mustard seeds: legumin type globulins (11S, cruciferins), and napin-type proteins (2S, napins), which are water soluble and have an isoelectric point around a pH value of 7 (Wanasundara, 2011).

Abul-Fadl *et al.* (2011) carried out an experiment to compare of protein content of mustard seeds between yellow and brown varities . They noticed that the yellow mustard seed had a higher content of protein (36.73%) than this found in brown mustard seed (32.48%).

Sengupta *et al.* (2003) reported that protein content of rapeseeds variety was variable laboratory analysis revealed that protein content of rapeseed were ranges from 44.2-44.7%.

Of the proteins in mustard seed, around 70% is composed of storage proteins, cruciferin and napin, which are found inside the protein bodies and have no catalytic functions. Up to 10% is considered to be oleosin, a main structural component of the membrane surrounding the oil bodies (Bell *et al.*, 1999)

Sosulski *et al.* (1991) reported that proximate composition of different released and line cultivars of rapeseed and turnip rape. The results showed that protein content of different released and line cultivars were variable i.e., *B. campestris* content 40.8% and *B. napus* content 45.5% protein in seed oil. Mirza *et al.* (1998)

carried out an experiment to evaluate the protein content of rapeseed oil. They noted significant differences were found among protein contents which are negatively correlated with oil content.

Mineral

Unlike other nutrient elements, mineral cannot be synthesized by living organism. Mineral are inorganic elements required by the animal body for maintenance of vital processes essential for life. The general functions of minerals are structural component of body organ and tissues, constituents of body fluids and tissues, electrolytes, and catalysts in enzyme and hormone system. The major minerals are calcium, magnesium, phosphorus, potassium and sulpher and trace minerals are iron, manganese, zinc and boron. Sarker *et al.* (2015) reported that, total minerals content of black mustard cake and yellow mustard cake were found to be 7.10% and 5.90%.

In study by Arif *et al.* (2012), a higher (0.01%) of potassium (K) was found in corn (Jalal) variety while *B. juncia* and *B. napus* contained the next higher same amount of (0.005%) of K. Cotton (Niab 98) contained (0.004%) K, while least amount was determined in sunflower (Gulshan 98), i.e., (0.003%). *B. napus* (Durr-e-NIFA) and sunflower (Gulshan, 98) contained the same high amount of P (0.86%) as compared to other oil seeds. Corn (Jalal) was poorest with respect to its P content (0.53%). Highest amount of Na was estimated in corn (Jalal), while least amount of (0.01%) was found in Sunflower (Gulshan, 98).

Bachheti *et al.* (2012) conducted an experiment to study physico-chemical properties of some conventional food oils and found that mineral content of mustard seed oil(*Brassica Compestris*) i.e , 694.3(g/100g), 4.86(g/100 g), 492.1(g/100g), 0.034(g/100g), 0.019 (g/100g), 0.007(g/100 g),8.11(g/100g) and 0.84(g/100g) for P,Zn,Ca ,Mg ,K,Na, Fe and Cu, respectively.

Sengupta *et al.* (2003) carried out an experiment to determine the minerals content of rapeseed and mustard varieties and advanced lines. They found that minerals content of rapeseed in 100g edible portion was 4.2 gm (calcium 490 mg, phosphorus 700 mg and Iron 17.9 mg and other minerals were present in negligible quantity).

Josefson (1988) evaluated the mineral content of defatted rapeseed meals. He found that mineral content of rape seed released and line cultivars i.e., 1.72%, 1.10%, 0.70%, 1.61%, 68 ppm and 18 ppm for sulpher, phosphorus, calcium, potassium, zinc and Iron, respectively.

Kaul *et al.* (1986) conducted an experiment to evaluate minerals content of different oilseed varieties and advanced lines. They found that different released and line cultivars content variable quantity of minerals i.e., 5.76%, 0.49%, 0.93%, 0.82% and 0.25% for N, P, K, Ca and Mg, respectively.

Chemical properties of Oil

The chemical characteristics of oil determine the quality and stability of oil. Chowdhury *et al.* (2014), determined the chemical constant of mustard and rapeseed oil. They found that, Saponification values of different released variety and advanced lines were ranges from 154-168.3; Iodine values of different released variety and advanced lines were ranges from 93.45-110.2 and acid values of different released variety and advanced lines were ranges from 1.31-1.61.

In study by Khan *et al.* (2013), chemical analysis of the mustard oil showed that the saponification value, iodine number, acid value are >170, >100 and <0.5 respectively.

Richet *et al.* (1987) used 15 different solvents for the extraction of oil from rapeseeds under identical condition. They also determined the acid value,

Saponification value and iodine value of the extracted oils. The values were 2.7 and 7.6 in benzene and methyl ester for acid value, 170.4 and 182.4 in propyl alcohol and toluene for saponification value and 82.1 and 98.5 in butyl oxide and acetone in CCl_4 for iodine value, respectively.

Martin *et al.* (1995) extracted oil from rapeseed to evaluate the physico-chemical parameters of the oil. They reported that iodine value, saponification value, acid value and unsaponifiable matter were 112.0, 170.0, 2.6 and 0.66, respectively.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

Four released and two line cultivars of mustard *(Brassica spp.)* namely BARI Sarisha-6, BARI Sarisha-9, BARI Sarisha-9 x BARI Sarisha-6, Tori-7, Tori-7 x BARI Sarisha-6, SAU-2 were selected for the study. The seeds were collected from the Department of Biochemistry of SAU. Seed were cleaned and sun-dried and stored into plastic container in a cool place until used for the chemical analysis.

3.1.1 Brief description of varieties and advanced lines

BARI Sarisha-6: This is a composite variety evolved by BARI. Its grain color is yellow and round shape. The grain size ranges from small to medium.

BARI Sarisha-9: This is a composite variety evolved by the BARI. Its grain color is brownish and oval shape. The grain is medium in size.

BARI Sarisha-9 x BARI Sarisha-6: This advanced lines evolved by BARI. Its grain color is blackish and oval in shape. The grain is large in size.

Tori-7: This is a composite variety evolved by BARI. Its grain color is blackish and round in shape. The grain is large in size.

Tori-7 x BARI Sarisha-6: This advanced lines evolved by BARI. Its grain color is brownish and oval in shape. The grain is medium in size.

SAU Sarisha-2: This is a composite variety evolved by SAU. Its grain is yellow in color and round in shape. The grain size is medium.

BARI Sarisha-6

BARI Sarisha-9

BARI Sarisha-9 x BARI Sarisha-6

Tori-7

Tori-7 x BARI Sarisha-6

SAU Sarisha-2

Plate 1. Photograph showing variation in seed coat colour, seed size and shape of some selected released and line cultivars of rapeseed and mustard *(Brassica spp.)*

3.2 Physicochemical Properties

The nutritive value of a food-grain is indicated by its composition shown by chemical analysis. The degree to which the chemical analysis indicates the nutritive value is dependent on the constituents determined. Some constituents are determined fairly, easily and rapidly: while others require much more time and analytical ability. Consequently most laboratories are faced with a choice between making a few rather simple determinations of a large number of samples or complete analysis of a limited number of samples. Before undertaking an analysis, the results of which will represent the composition of a crop or a consignment of a food-grain, it is necessary to ensure that the sample taken is randomly selected so as to be truly representative of the original bulk, and is sufficient in amount.

3.2.1 Determination of 1000 grain weight

The mass was determined by randomly selecting 100 samples and weighing in an electronic balance of 0.001 g sensitivity. The weight was then converted into 1000 seed mass.

3.2.2 Determination of moisture

Moisture content if mustard sample was determined by conventional method i.e., drying in an oven at 100° C for overnight.

Procedure

Empty aluminum moisture dish was weighted (w_1) and 2.5 g sample was taken in a moisture dish and weighted (w_2). The sample was spread evenly and placed without lid in oven and dried samples overnight at 100° C. The dishes were transferred to desiccators to cool. Aluminum dish was weighed after cooling (w_3).

Calculation

$$\% \text{ Moisture} = \frac{(W_2 - W_3)}{(W_2 - W_1)} \times 100$$

3.2.3 Determination of ash

The sample is ignited at 600° C to burn off all organic material. The inorganic material which does not volatilize at that temperature is called ash. The procedure was described by Ranganna (1986).

Equipments

1. Balance
2. Muffle furnace
3. Desiccators.

Procedure

The temperature of the muffle furnace was fixed to 600°C and crucible was heated for 1h and transferred into a desiccators; cooled them to room temperature and weighted (W_1). About 2 g sample was put into the crucible weighed (W_2). The sample was burned in a muffle furnace at 600° C for about 2 h. The crucibles were transferred into the desiccators and cooled them to room temperature and weighted (W_3). It was done immediately to prevent moisture absorption. The incineration repeated until constant weight was obtained.

Calculation

Weight of the sample taken = $W_2 - W_1$

Weight of the ash obtained = $W_3 - W_1$

$$\% \text{ Ash} = \frac{(W_3 - W_1)}{(W_2 - W_1)} \times 100$$

3.3 Chemical Analyses

3.3.1 Estimation of oils/fats

Reagents & Equipments

1. Anhydrous ethyl ether
2. Soxhlet, flask and condenser
3. Hot plate

Procedure

Dried mustard flour sample was weighed out into an extraction thimble. Weight of thimble and sample were recorded in laboratory book. The thimble was placed into the soxhlet. 50-100 ml ethyl ether was added to the soxhtet flask, then it was connected to holder and condenser. Soxhlet flask was placed on hot plate and distilled at low temperature for 16-20 hours. After extraction it was turned off and allowed to cool. When distillation was ceased, the extraction thimble was removed and allowed to air dry for 30-40 minutes the thimble was weighed out. The loss of weight was cured fat.

% Crude fats/Oil (on a dry weight basis) =

$$\frac{\text{Wt. of thimble \& sample before extraction - Wt. of thimble \& sample after extraction}}{\text{Weight of sample before extraction}} \times 100$$

The fat determined by the above procedure (Hughes, 1965) contains usual lipids including waxes pigments, certain gums and resins. A better name for these constituents would be "ether soluble extract."

3.3.2 Estimation of total protein content by Microkjeldhal method

The protein content of food stuff is obtained by estimating the nitrogen content of the material and multiplying the nitrogen value by 6.25 (according to the fact that nitrogen constitutes on average 16% of a protein molecule). This is referred to as crude protein content, since the non protein nitrogen (NPN) present in the material is not taken in consideration. The estimation of nitrogen is done by Kjeldhal method (AOAC 2010) which depends upon the fact that organic nitrogen when digested with sulphuric acid in the presence of catalyst selenium oxide, mercury or copper sulfate is converted into ammonium sulphate. Ammonia liberated by making the solution alkalines is distilled into a known volume of a standard acid which is then back titrated.

The nitrogen present in the sample is converted to ammonium sulphate by digestion at (380°C) with sulphate acid in presence of a catalyst, potassium sulphate and mercuric oxide. Ammonia liberated by distilling the digest with sodium hydroxide solution is absorbed by boric acid and is titrated for quantitative estimation.

Equipments

1. Balance
2. Microkjeldhal (Mkj) digestion set
3. Mkj distillation set.

Reagents

1. Digestion mixture: 100 g of potassium suphate (K_2SO_4) was thoroughly mixed with 20 g of copper sulfate ($CuSO_4.5H_2O$) and 2.5 g selenium dioxide (SeO_2) was added with it.
2. 60% Sodium hydroxide solution: 600 g sodium hydroxide and 50 g sodium thiosulphate were dissolved in distilled water, cooled and made the volume up to 1 liter.
3. Boric acid: 40 g of boric acid was dissolved in water and made up to 1 liter.

4. Double indicator: 200 mg each methyl red and bromocresol green was dissolved separately in 100 ml of 70% ethanol. One part of methyl red and five parts of bromocresol green were mixed before use.

5. Hydrochloric acid (0.02 N HCl): 8.5 ml concentrated hydrochloric acid was added to 5 liter of distilled water. Standardized to 0.02 N acids by titrating it against standard sodium carbonate (0.02 N) solution.

Procedure

A known quantity of the finely mustard ground sample (100 mg) weighted out in an Mkj digestion flask. About 2 g digestion mixture was added with it 2 ml of concentrated sulphuric acid was dispensed into the flask. Then it was digested for about 2 hrs in Mkj digestion set and was cooled the clear digest. The digest was dissolved in minimum amount of distilled water and carefully transferred to an Mkj distillation set. 10 ml of sodium hydroxide solution was added and distilled it. The distillate was collected for 5 min into 5 ml boric acid containing 2 drops of mixed indicator in a 50 ml conical flask, till the color of solution was changed. The distillate was titrated against a standard hydrochloric acid and noted the titer value (TV).

Calculation

$$N\ \% = \frac{(14.007) \times (\text{normality of the acid, } 0.02) \times (TV) \times 100}{\text{Weight of sample (mg)}}$$

Where 14.007 is the equivalent weight of nitrogen.

Nitrogen % is converted into protein by multiplying with a factor 6.25 for cereals and pulses.

3.3.3 Chemical constant

3.3.3.1 Saponification value

Saponification value is the number of milligrams of KOH required to completely saponify 1 g of oil. The method is based upon the principle that fat, on treatment with excess of alcoholic KOH is used up. The excess of KOH left unused may then be found by titrating it against a standard acid.

Reagents

1. Hydrochloric acid (0.5N).
2. Alcoholic solution of Potassium hydroxide. Take 28 g potassium hydroxide and dissolve it in very little water. Make up to one liter by adding rectified spirit (C_2H_5OH) of specific gravity 0.81.

Procedure

1. Weigh accurately 2 g of fat in 250 ml conical flask. Add 25 ml of 0.5 N alcoholic potash solutions and fit the flask with a cork and a long air condenser.
2. Refux the contents of the flask for about 30 minutes by heating on boiling water bath so that the contents just simmer. Cool the flask and add 1 ml of 1% solution of phenolphthalein and titrate the excess of the alkali against standard N/2 acid (a).

At the same time and under similar conditions carry out a blank expt (b) without fat (25 ml of the same alcoholic KOH heated in a similar way is titrated, against .05 N acid). 1 ml of 0.5 N HCL was equivalent to 0.02805g of KOH.

Calculation

$$\text{Saponification value} = \frac{(b\text{-}a) \times 0.02805 \times 1000}{\text{Wt. of substance in g}}$$

3.3.3.2 Iodine value

Iodine value or Iodine absorption number is the percentage of iodine monochloride (ICI) in terms of iodine absorbed by the oil. Some oils and fats contain many unsaturated fatty acid constituents such as oleic and linoleic acids which take up halogen to form saturated compounds. The extent of this combination however, depends on the degree of unsaturation.

Regents required

1. 0.1N sodium thiosulphate.
2. Starch solution (indicator).
3. Wij's solution: The Wij's solution required for this process is prepared as dissolve 8.5 of iodine and 7.8 g of iodine trichloride in separate portions of about 450 ml of glacial acetic acid. Mix the solutions and make up to one liter.
4. Potassium iodide solution: Dissolve 104 g KI crystals in water and make up the volume to 1litre.

Procedure

1. Accurately weight the oil or fat into a glass stoppered bottle of 200 ml capacity.
2. Add 5 ml of carbon tetrachloride to dissolve the oil and then add 25 ml of Wij's solution.
3. Allow it to stand for at least 1 hour in a dark place. Also put a blank expt containing 5 lm of carbon tetrachloride and 25 ml of Wij's solution but no oil.
4. Now add 5 ml of 10% potassium iodide solution and 50 ml water to each bottle and titrate against 0.1N $Na_2S_2O_3$ using starch solution as the indicator, near the end when the colour of titre becomes pale yellow. Blue colour disappears which indicates the end point.

Calculations

Vol. of 0.1N $Na_2S_2O_3$ used in blank expt = V_1 ml

Vol. of 0.1N $Na_2S_2O_3$ used in the case of oil = V_2 ml

Difference = (V_1-V_2) ml of 0.1 N sodium thiosulphate

= (V_1-V_2) ml of 0.1N I_2 absorbed

$$= (V_1-V_2) \times \frac{127}{10} \times \frac{1}{1000} \text{ g of iodine}$$

$$= (V_1-V_2) \times .0127 \text{ g of iodine.}$$

$$\text{Iodine value} = \frac{100}{\text{Wt. of oil}} \times 0.0127 \times (V_1-V_2)$$

3.3.3.3 Acid value

Acid value of oil is determined by titration of a known weight of it against N/.4 sodium hydroxide using phenolphthalein as the indicator.

$$C_{17}H_{33}COOH + NaOH = C_{17}H_{33}COONa + H_2O$$

Reagents:

1. Phenolphthalein: 1 percent solution in alcohol neutralized with 0.1 N NaOH.
2. Denatured alcohol (Neutral): Mix 10 volumes of ethyl alcohol with 1 volume of methyl alcohol and neutralize with N/40 NaOH using phenolphthalein as indicator.
3. N/4 Sodium hydroxide and N/10 Sodium hydroxide.

Procedure

Weigh 5-7 g of oil in 250 ml conical flask and add 50 ml denatured alcohol (neutral) and shake well. Now add 2 ml of phenolphthalein as indicator and titrate against N/4 NaOH with vigorous shaking after each addition till a permanent light pink color is produced which persists for at least 1 minute (a).

Calculation

$$\text{Acid value} = \frac{\text{a X 0.00561}}{\text{Wt. of oil}} \times 1000$$

3.3.4 Estimation of fatty acid composition

Seed sample of rapeseed and mustard were received from Department of Biochemistry of SAU. Fatty acid composition was determined by Gas chromatographic method (Cocks and Rede, 1966).

Preparation of Reagents

40mL of methanol were taken in 50mL conical flask. It was placed on ice water and then 10mL of sulphuric acid was added in it and this solution was saved for further use.

Methyl Esters Preparation

Methylation of fatty acids in the oils under study was carried out according to the procedure with some modifications as described by Were et al. (2006) with some modifications. The procedure adopted was as under: 200mg (0.2mL) oil was taken in a 50mL screw capped Pyrex glass tubes having 50cm length and 1cm internal diameter. Then 2mL of methanolic sulphuric acid added in each tube and glass vials were put in a pre-heated oven at 80°C for 1 hour and shake after 15min. The glass vials taken out, cooled and 2mL of dist. water were added in each tube to stop the reaction. Then esterifies fatty acids were extracted with 1mL of petroleum ether (40-60°C) thrice. After that the ether content was evaporated and remaining oily surface was injected into gas chromatography for fatty acid profile.

Gas chromatography

The upper layer (1µL) was injected into a gas chromatograph (Massachusetts, Model GC- Clarus 500 Perkin Elmer Incorporate, USA) equipped with a polar capillary a flame ionization detector and column ELITE-5(30m x 0.25mm ID x 0.25µm, Perkin Elmer, USA) to obtain FA methyl ester peaks. The column

temperature was 150°C and detector temperature was 250°C and held for 0.5min and increased at the rate of 10°C/min to 250°C and held for 5min and a run time of 20-50min. Comparing the retention times with those of standards individual peaks of FA methyl esters were identified. By individual FA composition was calculated using the peak areas of the FA species that appear in the chromatogram as a relative percentage of the total peak areas of all the FA in the oil sample (Cocks and Rede, 1966).

3.3.5 Estimation of Carbohydrate

Total carbohydrate Estimation: The method was described by Raghuramulu *et al.* (2003). The content of the available carbohydrate was determined by the following equation:

Carbohydrate = 100- [(Moisture + Protein + Ash + Oil/Fats) g/100g]

3.3.6 Estimation of minerals

Preparation of reagents

a. Reagents for P determination

Reagent A

1. Forty five gm antimony trioxide and 400 ml water were mixed in 1 liter volumetric flask and 150 ml conc. H_2SO_4 was added then it was allowed to cool.
2. Ammonium molybdate (7.5 gm) was dissolved in 300 ml water.
3. Cool antimony solution and molybdate solution was mixed by adding 1 liter of water.

Reagent B

1. One gm gelatin was dissolved in 100 ml hot water.

2. Reagent A (150 ml) dissolved to about 500 ml water and dissolved gelatins were mixed and finally, 1 gm of ascorbic acid was dissolved with it to make volume 1 liter.

b. Reagent for Ca and Mg determination

1. 1% Lanthanum solution:

Fifty nine gm of lanthanum oxide (La_2O_3) were added with about 50 ml of water. Slowly and cautiously, 250 ml conc. HCL was added to dissolve the La_2O_3. It was made to 5 liters with water.

c. Reagents for S determination

Mixed acid seed solution

Sixty five ml of conc. HNO_3 and 250 ml glacial acetic acid ware added to about 500 ml of water. 3 ml of 1000 ppm S standard solution was added and made volume to liter with water.

d. Turbidimetric reagent

Ten gm of polyvinyl pyrolidone (PVP K30) was dissolved in about 100 ml of hot water. 150 gm of $BaCl_2$. $2H_2O$ was dissolved in about 500 ml of water. The PVP and barium chloride solutions were mixed and were made to liter with water.

e. Preparation of standards

1. For convenience the Cu, Fe, Mn and Zn were prepared together in water. The high concentration for these elements was as follows: 2 µg Cu/ml, 10µg Fe/ml, 4µg Mn/ml, 2 µg Zn/ml.
2. The P, K and Na were prepared together in water with high concentrations as follows: 20µg P/ml, 100 µg K/ml, 40 µg Na/ml.
3. S was prepared in the same solution with high concentrations as follows. 20 µg S/ml.

4. Ca and Mg were prepared in the same solution with high concentrations as follows; 100 µg Ca/ml, 40 µg Mg/ml.

f. Digestion solution

1. Nitric-perchloric solution.
   Conc. Perchloric acid (100 ml) was added to 500 ml concentrated HNO_3 to prepare nitric-perchloric solution.

3.3.6.1 Digestion of mustard seed sample for determination of P, K, Ca, Mg, S, Fe, Zn and B

a. Digestion procedure

Weighted 500 gm dry seed sample and put into a 50 ml boiling flask. 5 ml of nitric-perchloric solution was allowed on cool hot plate and turned temperature to 375° C. It was allowed to digest for 1 hour and 30 minutes. The flask was removed from digestion chamber and was cooled and 15 ml water was added. The flask was agitated and heated to dissolve the ash and filter.

b. Analytical procedure

By using a combination diluter-dispenser, 1 ml aliquot was taken from filtrate and 19 ml water (dilution 1) was added. The other dilutions were made in the following order. For S determination, 7 ml of aliquot from dilution 1, 9 ml of acid seed solution and 4ml of turbidimetric solution were mixed together thoroughly. It was allowed to stand 20 minutes and not longer than one hour. The reading was taken in turbid meter or in colorimeter at 535 nm using a cuvette with 2 cm light path. For P, K and Na determination, 1 ml aliquot from dilution 1, 9 ml of water and 10 ml of color reagent were mixed together. It was allowed to stand about 20 minutes and reading was taken of spectrophotometer at 680 nm.

For Ca and Mg determination, 1 ml aliquot from dilution 1, 9 ml of water and 10 ml of 1% lanthanum solution were mixed together. It was analyzed by AA procedure.

For Fe, Mn and Zn determination, the original filtrate was used to analyze these elements by AA procedure.

3.3.7 Estimation of Energy:

The chemical energy content of food ingredients is usually expressed in terms of heat units (since all forms of energy are convertible into heat energy).The gross food energy was estimated by multiplying the crude protein, crude fat and total carbohydrate by at water factors 4, 9 and 4 respectively (Okwu, 2006; Osborne and Vooget, 1978).

3.4 Statistical Analysis

The recorded date for each character from the experiments was analyzed statistically to find out the variation resulting from experimental treatments using MSTAT package program. The mean for all the treatments were calculated and analysis of variance of characters under the study was performed by F variance test. The mean differences were evaluated by least significance difference test.

CHAPTER IV

RESULTS AND DISCUSSION

Four released and two line cultivars of rapeseeds and mustard *(Brassica spp.)* were taken for the determination of physical and chemical characteristics. The seeds were stored in the store house under a suitable storage condition. The proximate composition and some other nutrients compositions of mustard seeds are also reported.

Analytical studies of the whole seeds

The proximate composition of whole rapeseed and mustard seeds of different released and line cultivars are presented in different tables. The data have also been estimated on moisture free basis in order to allow for better comparison of the different fraction. The data mentioned are the average of three replication and have been presented and discussed.

4.1 Physical characteristics of rapeseed and mustard released and line cultivars

4.1.1 Grain weight

Weight of thousand of grains of different released and line cultivars of mustard and rapeseed has been compared in Table 1. It was found that seed weight varied with their size and shape. Weight of thousand of grains were determined at 13% moisture level. The highest weight of thousand of grains was found in BARI Sarisha-9 x BARI Sarisha-6 (4.892g). This was higher than all others released variety & advanced lines and statistically similar results were shown by Tori-7 (4.721g), BARI Sarisha-6 (4.721g) and Tori-7 x BARI Sarisha-6 (4.740g). The lowest weight of thousand of grains was found in SAU-2 (4.459). The present values are consistent with the results reported by Banga *et al.* (2013), Siddiqui *et*

al. (2004), Kumar and Singh (1994), Andarhennadi *et al.* (1991), Biswas (1989), Chowdhury *et al.* (1987) and Kaul *et al.* (1986). Chowdhury *et al.* (2014) reported range of weight 0f thousand seed 2.5to4.9g, among different Bangladeshi mustard varieties and advanced lines. Banga *et al.*(2009) and Siddiqui *et al.* (2004) found that the highest amount of 1000 seed weight were 5.15g and 3.95g respectively.The present values are higher than the reported value of Mondal and Wahhab (2001); who found that thousand seed weight varies from 2.50-2.65 g .

4.1.2 Moisture

Seed deterioration increased as moisture content is increased. When seeds contain moisture above 18% then attacked by molds and insects. Seed stores responsible for 6-8 months in temperate climate in open storage at a moisture content from 10-13%. The moisture content of different released and line cultivars of mustard and rapeseed has been presented in Table 1. The moisture content of different released and line cultivars of mustard and rapeseed was ranged from 4.3 to 5.1%. The highest moisture content (5.1%) was observed from BARI Sarisha-6, which was followed (4.9%, 4.7%, 4.6% and 4.5%) by Tori-7 x BARI Sarisha-6, SAU Sarisha-2, BARI Sarisha-9 and Tori-7; while the lowest moisture content (4.3%) was found in BARI Sarisha-9 x. BARI Sarisha-6. The results of the moisture content were significantly lower than that of Sarker *et al.* (2015), Al Mahmud et al. (2012); Marnoch and Diosady (2006), BARI annual report (1987-88). According to Sarker *et al.* (2015) moisture content of mustard cakes were 9.20 ± 0.5% and 9.73 ± 0.6%; and the moisture content 8.3 ± 0.2% reported in literature by Al Mahmud *et al.* (2012); Marnoch and Diosady, (2006). BARI (1987-88) reported that moisture content ranges from 7.41 to 8.38%. These may be influenced by different level of sun drying after harvesting.

4.1.3 Dry matter

A statistically significant variation was observed for dry matter content of different released and line cultivars of mustard and rapeseed that has been presented in Table 1. Significantly highest amount of dry matter contained was recorded in BARI Sarisha-9 x BARI Sarisha-6 (95.7%), followed by Tori-7 (95.5%), BARI Sarisha-9 (95.4%), SAU Sarisha-2 (95.3%), Tori-7 x BARI Sarisha-6 (95.1%) and BARI Sarisha-11 (95).The lowest amount of dry matter contained was found in BARI Sarisha-6 (94.9%) which was significantly lowest among all the variety & advanced lines. These variations might be due to environmental factor, soil and crop management practices.

Table 1. Weight of 1000 seed, Moisture and Dry matter of the different released and line cultivars of rapeseed and mustard (*Brassica* spp.)

Name of the released and line cultivars (Treatments)	Weight of 1000 seeds (at 13% moisture level) (gm)	Moisture (%)	Dry matter (%)
BARI Sarisha-6	4.721 ab	5.100 a	94.90 e
BARI Sarisha-9	4.542 b	4.600 cd	95.40 bc
BARI Sarisha-9 x BARI Sarisha-6	4.892 a	4.300 e	95.70 a
Tori-7	4.643 ab	4.500 d	95.50 b
Tori-7 x BARI Sarisha-6	4.740 ab	4.900 b	95.10 d
SAU-2	4.459 b	4.700 c	95.30 c
$LSD_{(0.05)}$	0.3355	0.1908	0.1908
CV (%)	3.96	2.21	0.11

Figure in a column followed by a common letter do not differ significantly at 5% level by DMRT

4.1.4 Oil content

The oil content of the mustard and rapeseeds depends on many factors like genetic factor; agro-ecological conditions including cultivation sites and crop management system etc. The oil content of different released and line cultivars of mustard and rapeseed were extracted by petroleum ether (40-60^{0}C) varied from 38.74% to 40.55% (Table 2). The variety SAU Sarisha-2 had the lowest amount of oil contained (38.74%) , while the variety BARI Sarisha-9 contained significantly highest amount of oil (40.55%), followed by Tori-7 (39.62%) and BARI Sarisha-9 x BARI Sarisha-6 (39.44%). The results clearly indicated that variety BARI Sarisha-9 (40.55%), Tori-7 (39.62%) and BARI Sarisha-9 x BARI Sarisha-6 (39.44%) can be considered as better source of oil. Present values are higher than the reported value of Gadei *et al.*, (2012) and B.R. Moser *et al.* (2009), who found that oil content of mustard seed ranges from 28–32%; whereas Arif *et al.* (2012), Bhowmik (2003), Novoselov *et al.* (1997) reported that oil content of rapeseed ranges from 45.67- 43.87%, 42-46%and45-46% respectively, which are slightly higher than present results. On the other hand, The present investigations were more or less similar the reported values of Vijay *et al.* (1992), Rathore (1999-2000). Niraz *et al.* (2001), BARI report (2001), Sengupta *et al.* (2003), Mandal *et al.* (2002). These variations might be due to biological factor, environmental factor, soil and crop management practices.

4.1.5 Oil cake

Oil cake/meals are used for various purposes. Oil cake is a nutritious food items for cattle and fish. It is also used as a good organic fertilizer and ingredient of composts. The SAU Sarisha-2 contained highest amount of oil cake (61.26%), followed by Tori-7 x BARI Sarisha-6 (61.20%) and BARI Sarisha-6 (60.78%). The lowest value was found in BARI Sarisha-9 (59.45%). The present values were supported by the reported values of Chowdhury *et al.* (2014) and Appelqvist *et al.* (1992). Chowdhury *et al.* (2014) found that percentage of oil cake range from 58.14 to 59.95% and Appelqvist *et al.* (1992), reported that typically rape seed oil (Kind of mustard seed) contain 58% cake.

4.1.6 Dry weight of cake

Dry cakes are used to evaluate the content of different nutrient which are essential for our poultry feed, organic fertilizer and other various purposes. The dry weights of cake have been presented in Table 2. The highest value was obtained from SAU Sarisha-2 (56.56%), followed by Tori-7 x BARI Sarisha-6 (56.30%) and BARI Sarisha-9 x BARI Sarisha-6 (56.26%). The lowest value obtained from BARI sarisha-9 (54.68%) which was significantly lower than all the varieties and advanced lines.

Table 2. Proximate analysis of oil content, oil cake and Dry wt. of cake of the different released and line cultivars of rapeseed and mustard (*Brassica* spp.)

Name of the released and line cultivars (Treatments)	Oil content (%)	Oil cake (%)	Dry wt. of cake (%)
BARI Sarisha-6	39.22 bc	60.78 ab	55.68 b
BARI Sarisha-9	40.55 a	59.45 c	54.68 c
BARI Sarisha-9x BARI Sarisha-6	39.44 bc	60.56 ab	56.26 ab
Tori-7	39.62 b	60.38 b	55.88 ab
Tori-7 x BARI Sarisha-6	38.80 c	61.20 a	56.30 ab
SAU-2	38.74 c	61.26 a	56.56 a
$LSD_{(0.05)}$	0.7254	0.7256	0.6880
CV (%)	1.01	0.66	0.68

Figure in a column followed by a common letter do not differ significantly at 5% level by DMRT

4.2 Chemical characteristics of rapeseed and mustard released and line cultivars

4.2.1 Chemical constant of oil

Saponification value

Saponification value of oil/fats refers to the number of mg of KOH required to saponify one gram of fats /oil. It is inversely proportionate to the molecular weight or chain length of the fatty acids present in the fats/oil. Saponification values of different released and line cultivars were ranges from 158.6 to 168.8 and have been presented in Figure 1. The statistically highest Saponification value was found in Tori-7 (168.8). There were significant variations between the varieties and advanced lines, BARI Sarisha-9 (166.8), BARI Sarisha-9 x BARI Sarisha-6 (166.6), but the values recorded for these released and line cultivars were significantly higher than SAU Sarisha-2 (161.7). The present values are lower than the reported values of Khan *et al.* (2013) and Richet *et al.* (1987). They determined that, the Saponification value of the extracted mustard oils were >170,170 and 182.4 respectively. However these values are supported by Hossain *et al.* (1998) and Chowdhury *et al.* (2014).

Iodine value

Iodine value is defined as grams of iodine absorbed by 100 gm fats/oil. It helps to estimate the degree of unsaturation. The iodine values of different released and line cultivars of rapeseed and mustard have been presented in Figure 1. The highest amount of iodine value were observed in Tori-7 (110.2), followed by BARI Sarisha-9 (106.4) and BARI Sarisha-9 x BARI Sarisha-6 (105.6). The lowest amount of iodine value recorded in Tori-7 x BARI Sarisha-6 (96.33), followed by SAU Sarisha-2 (100.3). The observed values were supported by the reported values of Chowdhury *et al.* (2014), Khan *et al.* (2013) and Richet *et al.* (1987)

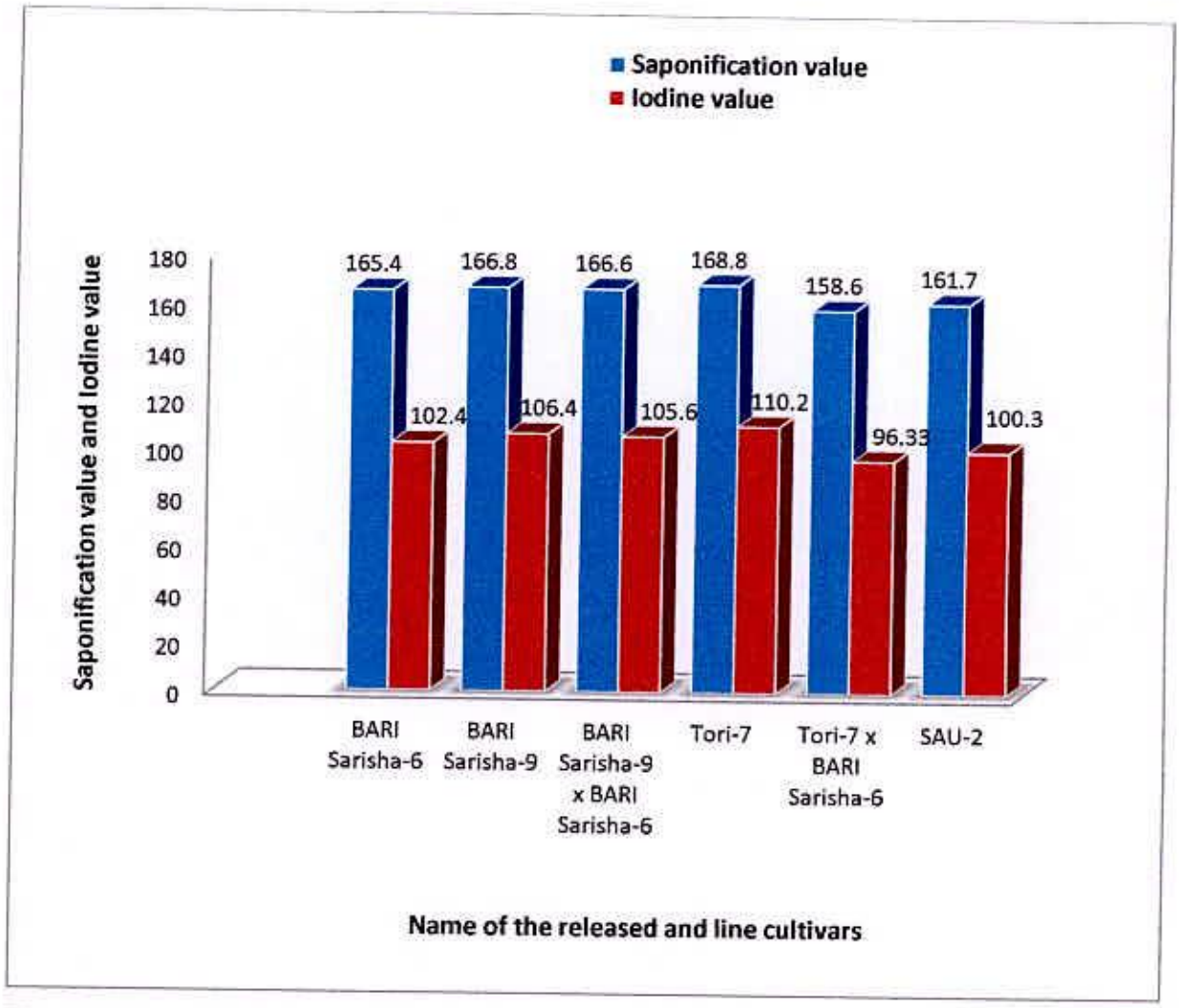

Figure 1. Chemical constant of oil of the different released and line cultivars of rapeseed and mustard (*Brassica* spp.)

Acid value

It is defined as the milligrams of KOH required to neutralize the free fatty acids present in 1 gm of fats/oil. This value is used in determining the rancidity due to free fatty acids. Acid values of different released and line cultivars of mustard and rapeseed have been presented in Figure 2. The highest acid value was found from Tori-7 x BARI Sarisha-6 (1.610), followed by SAU Sarisha-2 (1.580); whereas the lowest acid value was found from BARI Sarisha-9 (1.410) followed by BARI Sarisha-6 (1.450). Chowdhury *et al.* (2014) and Khan *et al.* (2013) found the more or less similar result. Although the present values were lower than the reported values of Richet *et al.* (1987).

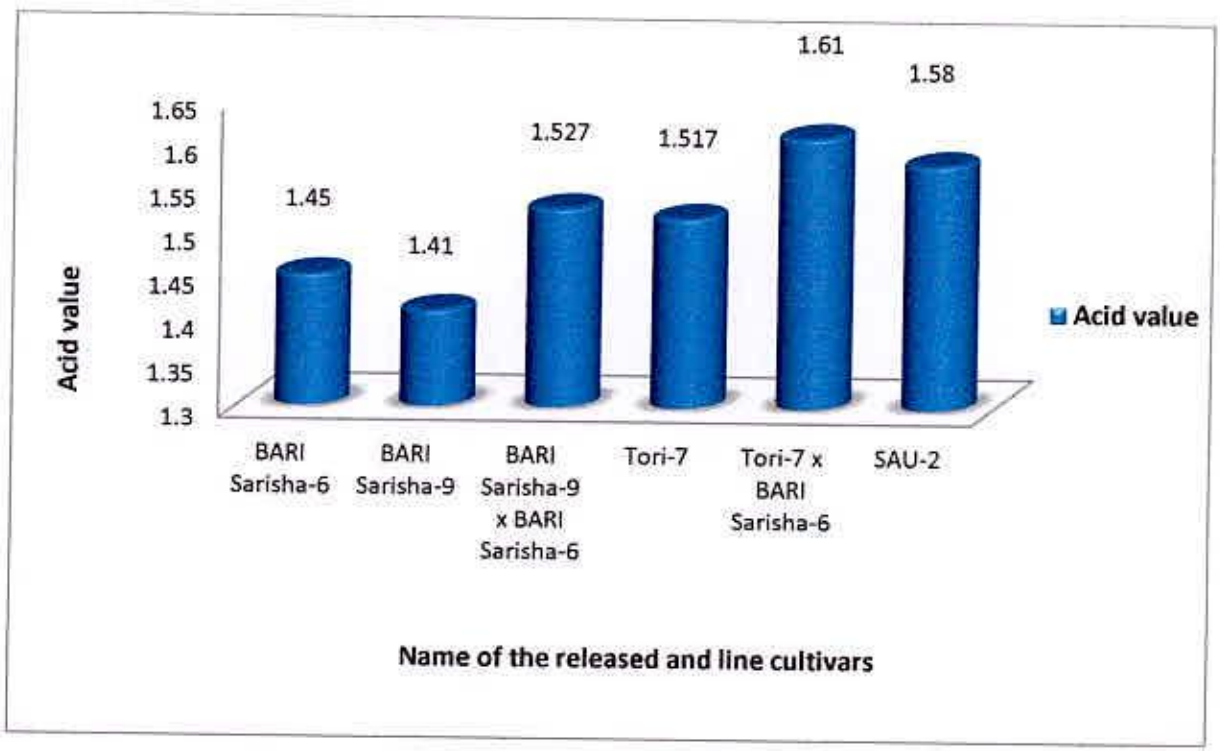

Figure 2. Acid value of oil of the different released and line cultivars of rapeseed and mustard (*Brassica* spp.)

4.2.2 Fatty acid composition

Comparison of Gas chromatography results are demonstrated in Table 3. According to results, there was a significant difference between the studied rapeseed and mustard released and line cultivars in terms of their fatty acid compounds. Significantly the highest amount of palmitic acid was observed in Tori-7 x BARI Sarisha-6 (3.440%); followed by BARI Sarisha-9 (2.880%) and SAU Sarisha-2; Lowest amount of palmitic acid content was observed in BARI Sarisha-6 (1.769%). The concentration of stearic acid varied from 0.00 to 1.770%; whereas arachidic acid contents ranged from 0.740 to 4.737%. Tori-7 contained the highest amount (18.56%) of oleic acid ; followed by SAU Sarisha-2 (17.65%) and the lowest amount was found in BARI Sarisha-9 (9.032%) which was significantly lowest among all the varieties and advanced lines. Lenoleic acid content of the released and line cultivars ranged from 12.70 to 17.75%. The Highest amount of lenoleic acid contents was found in BARI Sarisha-9 (17.75%) which was significantly highest among all the released and line cultivars and lowest amount (12.70%) was found in BARI Sarisha-6. The linoleic acid content is important from the stand point of utilization of oil for food products. Linolenic acid, erucic acids were also present in these varieties and advanced lines. The concentration of linolenic acid varied from 6.270 to 15.83%; whereas erucic acid contents ranged from 41.11 to 50.67%. Differences were found among the released and line cultivars of rapeseed-mustard in respect of erucic acid content. BARI Sarisha-6 contained the highest amount of erucic acid (50.67%), followed by Tori-7 (50.22%) and lowest amount was found in BARI Sarisha-9 (41.11%); which was significantly lowest among all the varieties and advanced lines. GLC analytical data indicated that the major fatty acid composition of the four released and line cultivars and two advanced lines of mustard and rapeseed oils included unsaturated fatty acid ranging from 83.72 to 87.48%; while only a minor fraction by saturated fatty acids (4.397 to 8.444%). From the present data, it might be suggested that all the *Brassica* oil seeds are suitable for edible purpose as they contained higher amount of unsaturated fatty acid. These findings are in conformity with the results by Wani Mubashir (2012), Abul-fadl *et al.*

(2011),Chauhan and Kumar (2011), Moser *et al.* (2009), Niraz *et al.* (2003) and Appelqvist (1980). Wani Mubashir, (2012) stated that mustard oil possess 42% Erucic acid and 12% Oleic acid, it has also 6% omega-3 alpha-Linolenic acid and 15% omega-6 linoleic acid along with 12% saturated fats. Chauhan and Kumar (2011) observed that the concentration of oleic acid (18:1), a beneficial monounsaturated fatty acid, ranges from 3.6-32.2% in rapeseed-mustard oil . Abul-fadl *et al.* (2011) reported that, erucic acid was in yellow and brown mustard seeds oils was represented about 37.89 and 23.90%, respectively. Oleic acid ranged between 19.08 to 20.24% of total fatty acid profiles in both yellow and brown mustard seed oils respectively. Moreover linoleic acid was recorded from 12.37 to 21.36 in both yellow and brown mustard seed oils respectively. Moser *et al.* (2009) observed that mustard oil has a special fatty acid composition. It contains about 20–28% oleic acid, 10–12% linoleic, 9.0–9.5% linolenic acid, and 30–40% erucic acid. Moreover Appelqvist (1980) found that fatty acid composition of mustard released and line cultivars i.e., 3.0%, 0.8%, 9.9%, 13.5%, 9.8%, 6.3% and 52.3% for palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, eicosanoic acid and erucic acid respectively.

Table 3. Fatty acid composition of the different released and line cultivars of rapeseed and mustard (*Brassica* spp.)

Name of the released and line cultivars (Treatments)	Percentage of fatty acids						
	Palmitic Acid ($C_{16:0}$)	Stearic Acid ($C_{18:0}$)	Arachidic Acid ($C_{20:0}$)	Oleic Acid ($C_{18:1}$)	Linoleic Acid ($C_{18:2}$)	Linolenic Acid ($C_{18:3}$)	Erucic Acid ($C_{22:1}$)
BARI Sarisha-6	1.769 f	0.000 d	4.737 a	13.68 e	12.70 f	7.2810 d	50.67 a
BARI Sarisha-9	2.880 b	1.082 c	4.482 b	9.032 f	17.75 a	15.83 a	41.11 f
BARI Sarisha-9 x BARI Sarisha-6	2.320 d	1.197 b	0.8800 e	17.16 c	14.19 b	7.660 c	48.47 d
Tori-7	2.220 e	1.220 b	4.000 c	18.56 a	13.08 e	7.940 b	50.22 b
Tori-7 x BARI Sarisha-6	3.440 a	1.260 b	0.7400 f	15.51 d	14.03 c	6.270 f	47.92 e
SAU-2	2.610 c	1.770 a	1.400 d	17.65 b	13.74 d	6.610 e	49.23 c
LSD $_{(0.05)}$	0.01819	0.08136	0.08136	0.01819	0.01819	0.01819	0.01819
CV (%)	0.34	4.28	1.53	0.04	0.05	0.04	0.02

Figure in a column followed by a common letter do not differ significantly at 5% level by DMRT

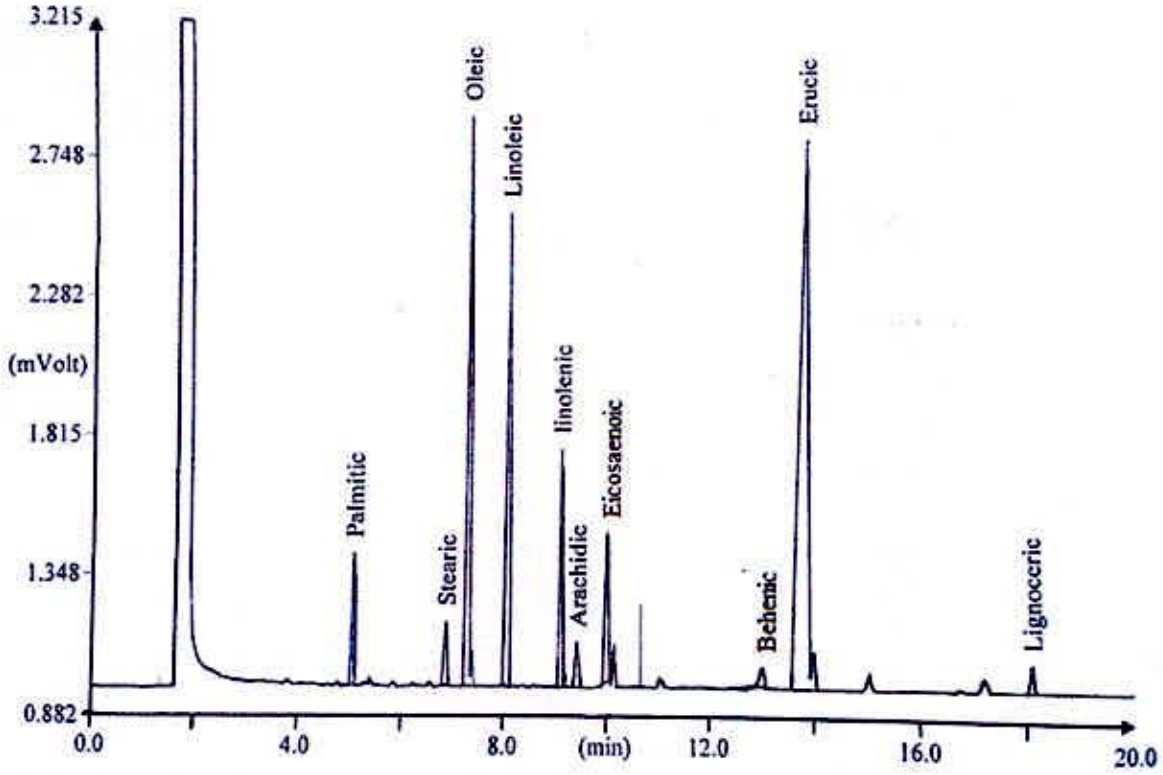

Figure 3. Fatty acid composition SAU Sarisha-2 *(Brassica spp.)*

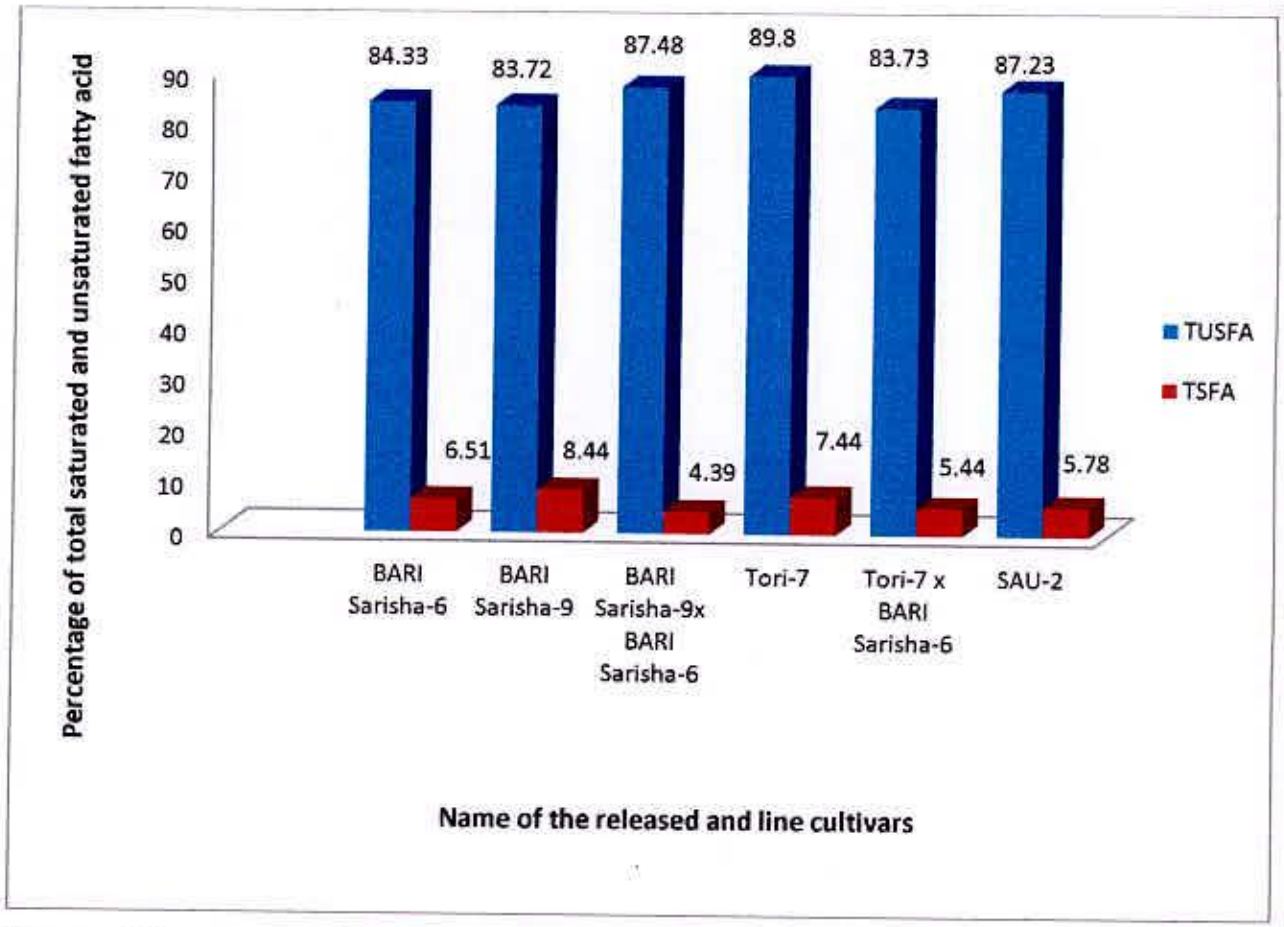

Figure 4. Percentage of total saturated and unsaturated fatty acid of oil of the different released and line cultivars of rapeseed and mustard (*Brassica* spp.)

4.3 Analysis of oil cake

After extraction of oil the seeds gave the defatted cakes of varying amounts. The proximate composition of the oil cakes was determined by standard procedure. The results of cake analysis were presented in Table 4.

4.3.1 Ash

Ash content of different released and line cultivars of mustard and rapeseed were variable and ranged from 11.00% to 14.00% (Table 4). Significantly highest amount of ash contained was recorded in SAU Sarisha-2 (14.00%), and followed Tori-7 x BARI Sarisha-6 (13.50%), BARI Sarisha-9 x BARI Sarisha-6 (12.20%), But these values were significantly higher than the BARI Sarisha-9 (11.00%). The present values were significantly higher than the reported value of Sarker *et al.* (2015), Abul-Fadl *et al.* (2011), Nehrins *et al.* (1990), Sosulski *et al.* (1991) and Kaul *et al.* (1986).

4.3.2 Protein

Protein is the major nutrient of different released and line cultivars of rapeseed and mustard. Protein content is genetically controlled. It is also influenced by nitrogen fertilizer application and agronomics practices. The protein content was determined on moisture free basis. Protein content of different released and line cultivars of rapeseed and mustard have been presented in Table 4. The statistically highest amount of protein was obtained from SAU Sarisha-2 (28.05%) and followed by Tori-7 x BARI Sarisha-6 (27.15%), BARI Sarisha-6 (26.60%). On the other hand BARI Sarisha-9 (25.05%), which was statistically lowest amount of protein. The present values are more or less similar with the reported values of Sarker *et al.* (2015), Chowdhury *et al.* (2010), Nehrins *et al.* (1990), Sosulki *et al.* (1991), and Mirza *et al.* (1998). However these result are lower than those reported by many other authors: Prapakornwiriya and Diosady (2004) determined the protein 45.0%,34.0% respectively and Sengupta *et al.* (2003) revealed that protein content of rapeseed were ranges from 44.2-44.7%. This might be due to the nitrogen fertilizer application, ecology and agronomics practices.

4.3.3 Carbohydrate

Carbohydrate content of different released and line cultivars of rapeseed and mustard were determined moisture free basis. The data have been presented in Table 4. The higher amount of carbohydrate found in Tori-7(19.08%) was highest than other released and line cultivars of rapeseed and mustard but statistically similar with BARI Sarisha-9 (18.80%). The lowest amount of carbohydrate was obtained from SAU Sarisha-2 (14.51%). Agronomics practices, environmental factors as well as variation among the released and line cultivars might be influenced the carbohydrate content. The present values are slightly lower than the reported values of Bachheti *et al.* (2012) and Gopalan *et al.* (1981). Bachheti *et al.* (2012) found that mustard seeds contain 23.8% carbohydrate and Gopalan *et al.* (1995) stated that dry mustard seeds contained 20-23% carbohydrate.

Table 4. Proximate analysis of protein, ash and carbohydrate content of the different released and line cultivars of rapeseed and mustard (*Brassica* spp.)

Name of the released and line cultivars (Treatments)	Ash (%)	Protein (%)	Carbohydrate (%)
BARI Sarisha-6	12.10 d	26.60 c	16.98 c
BARI Sarisha-9	11.00 f	25.05 f	18.80 a
BARI Sarisha-9 x BARI Sarisha-6	12.20 c	26.15 d	17.91 b
Tori-7	11.25 e	25.55 e	19.08 a
Tori-7 x BARI Sarisha-6	13.50 b	27.15 b	15.65 d
SAU-2	14.00 a	28.05 a	14.51 e
$LSD_{(0.05)}$	0.01819	0.01819	0.6928
CV (%)	0.08	0.001	2.22

Figure in a column followed by a common letter do not differ significantly at 5% level by DMR

4.3.4 Minerals

Different major and minor minerals were analyzed in this work. The amounts of major minerals content of rapeseed have been illustrated in Table 5. It is well known that rape seed contained small amount of minerals.

Major minerals

Calcium (Ca)

In case of calcium content of different released and line cultivars of rapeseed and mustard was ranged from .722% to 1.006% (Table 5). Significantly highest amount of calcium (Ca) content was observed in Tori-7 (2.7%), followed by BARI Sarisha-6 (0.840%), BARI Sarisha-9 (0.820%). Tori-7 x BARI Sarisha-6 (0.797%) and BARI Sarisha-9 x BARI Sarisha-6 (0.738%). lowest amount of calcium content was obtained from SAU Sarisha-2 (0.722%). The present investigations were supported by reported value of Sarker *et al.* (2015), M.Arif *et al.*(2012), Bachheti *et al.* (2012), Josefson (1988), Sengupta *et al.* (2003) and Pathak *et al.* (1973). Bachheti *et al.* (2012), Sengupta *et al.* (2003) reported that mustard contain 492.1mg and 490mg respectively, while Josefson (1988) stated that mustard contain 0.7% Ca.

Magnesium (Mg)

Magnesium is the major minerals for human nutrition. Magnesium content of different released and line cultivars of rapeseed and mustard have been presented in Table 5. Magnesium content of different released and line cultivars was ranged from 0.370% to 0.838%. The highest amount of Magnesium content was found in Tori-7 (0.838%); followed by BARI Sarisha-6 (0.730%) and the lowest amount in BARI Sarisha-9 (0.370%); followed by SAU Sarisha-2 (0.602%). Tori-7 was significantly highest than all other varieties and advanced lines. The present investigations were supported by reported value of Sarker *et al.* (2015), M.Arif *et al.* (2012), Bachheti *et al.* (2012), Josefson (1988), Sengupta *et al.* (2003) and Pathak *et al.* (1973).

Table 5. Proximate analysis of major minerals content of the different released and line cultivars of rapeseed and mustard (*Brassica* spp.)

Name of the released and line cultivars (Treatments)	Ca (%)	Mg (%)
BARI Sarisha-6	0.8400 b	0.7300 b
BARI Sarisha-9	0.8200 c	0.3700 e
BARI Sarisha-9 x BARI Sarisha-6	0.7380 e	0.6170 d
Tori-7	1.006 a	0.8380 a
Tori-7 x BARI Sarisha-6	0.7970 d	0.6640 c
SAU-2	0.7220 e	0.6020 d
$LSD_{(0.05)}$	0.01819	0.01819
CV (%)	0.64	0.67

Figure in a column followed by a common letter do not differ significantly at 5% level by DMRT

Minor minerals

Copper (Cu)

Copper contained of different released and line cultivars of rapeseed and mustard was ranged from 1.205-3.955 ppm (Table 6). Significantly highest amount of Cu contained observed in Tori-7 x BARI Sarisha-6 (3.955 ppm) which was followed by BARI Sarisha- 9 (3.25 ppm), BARI Sarisha-6 (3.20 ppm) and Tori-7 (3.001 ppm). Lowest amount of Cu contained observed in SAU Sarisha-2 (1.205 ppm) which was followed by BARI Sarisha-9 x BARI Sarisha-6 (2.105 ppm). The present investigations were supported by reported value of Sarker *et al.* (2015), M.Arif *et al.* (2012), Bachheti *et al.* (2012), Josefson (1988), Sengupta *et al.* (2003) and Pathak *et al.* (1973).

Iron (Fe)

Iron contained of different released and line cultivars of rapeseed and mustard were ranged from 30.0 to 101.3 ppm (Table 6). Significantly highest amount of Fe contained was observed in Tori-7 (101.3 ppm) which was followed by BARI Sarish-9 x BARI Sarish-6 (78.54 ppm) and Tori-7 x BARI Sarish-6 (75.30 ppm). The variety BARI Sarisha-9 showed lowest amount of Fe (30.00 ppm) which was followed by BARI Sarish- 6 (32.00 ppm). These might be influenced the different levels of Fe in soil, Fertilizer and variation among the varieties and advanced lines. The present values were higher than the reported values of Bachheti *et al.* (2012) and Josefson (1988); who found that

Fe content of mustard seed oil were 8.11(g/100g) and 18ppm respectively.

Zinc (Zn)

The zinc content of different released and line cultivars of rapeseed and mustard were ranges from 47.94 to 66.9 ppm in (Table 6). Significantly highest amount of Zn contained was found in BARI Sarisha-16 (66.9 ppm) which was followed by BARI Sarish- 13(57ppm). The lowest amount was found in BARI Sarisha-14 (47.94 ppm) which was followed by BARI Sarisha -11 (48.93 ppm) and BARI

Sarisha-15 (51.48 ppm). These treatments are statistically similar. The present values were supported by the reported value of Bachheti *et al.* (2012) and Josefson (1988).

Table 6. Proximate analysis of minor minerals content of the different released and line cultivars of rapeseed and mustard (*Brassica* spp.)

Name of the released and line cultivars (Treatments)	Cu (ppm)	Fe (ppm)	Zn (ppm)
BARI Sarisha-6	3.200 c	32.00 e	42.10 c
BARI Sarisha-9	3.250 b	30.00 f	44.30 b
BARI Sarisha-9 x BARI Sarisha-6	2.105 e	78.54 b	40.02 d
Tori-7	3.001 d	101.3 a	33.42 e
Tori-7 x BARI Sarisha-6	3.955 a	75.30 c	51.12 a
SAU-2	1.205 f	37.86 d	24.30 f
$LSD_{(0.05)}$	0.01819	0.09965	0.1286
CV (%)	0.26	0.09	0.19

Figure in a column followed by a common letter do not differ significantly at 5% level by DMRT

4.4 Gross energy

Energy from Carbohydrate of mustard and rapeseed varied significantly due to different released and line cultivars (Table 7). Significantly the highest amount of energy from carbohydrate found in two cultivars Tori-7 (76.33kca/g) and BARI Sarisha-9(75.20kca/g); while significantly lowest amount found in SAU Sarisha-2 (58.05kca/g).The significantly highest energy from protein observed from SAU Sarisha-2 (112.2kca/g), followed by Toro-7 x BARI Sarisha-6 (108.6kca/g); whereas significantly the lowest amount of energy (100.2kca/g) from protein observed from BARI Sarisha-9. The highest amount of energy from fat was observed in BARI Sarisha-9 (365.0kcal/g), which was significantly higher than all other released and line cultivars, followed by Tori-7 (356.6kcal/g); whereas the lowest amount counted from SAU Sarisha-2 (348.6kca/g). The study found that Gross energy of different released and line cultivars of rapeseed and mustard ranged from 518.9 to 540.3kcal/g. The statistically highest amount of gross energy found from BARI Sarisha-9 (540.3kcal/g), followed by Tori-7 (535.1kca/g); while lowest amount of gross energy recorded from SAU Sarisha-2 (518.9kcal/g) followed by Tori-7 x BARI Sarisha-6 (520.4kcal/g), which was significantly similar.

Table 7. Proximate analysis of Gross energy from carbohydrates, Proteins and oils of the different released and line cultivars of rapeseed and mustard (*Brassica* spp.)

Name of the released and line cultivars (Treatments)	Energy from Carbohydrate (kcal/g)	Energy from Protein (kcal/g)	Energy from Oil (kcal/g)
BARI Sarisha-6	67.92 c	106.4 c	353.0 bc
BARI Sarisha-9	75.20 a	100.2 f	365.0 a
BARI Sarisha-9x BARI Sarisha-6	71.63 b	104.6 d	355.0 bc
Tori-7	76.33 a	102.2 e	356.6 b
Tori-7 x BARI Sarisha-6	62.59 d	108.6 b	349.2 c
SAU-2	58.05 e	112.2 a	348.6 c
$LSD_{(0.05)}$	2.767	0.0257	6.522
CV (%)	2.22	0.001	1.01

Figure in a column followed by a common letter do not differ significantly at 5% level by DMRT

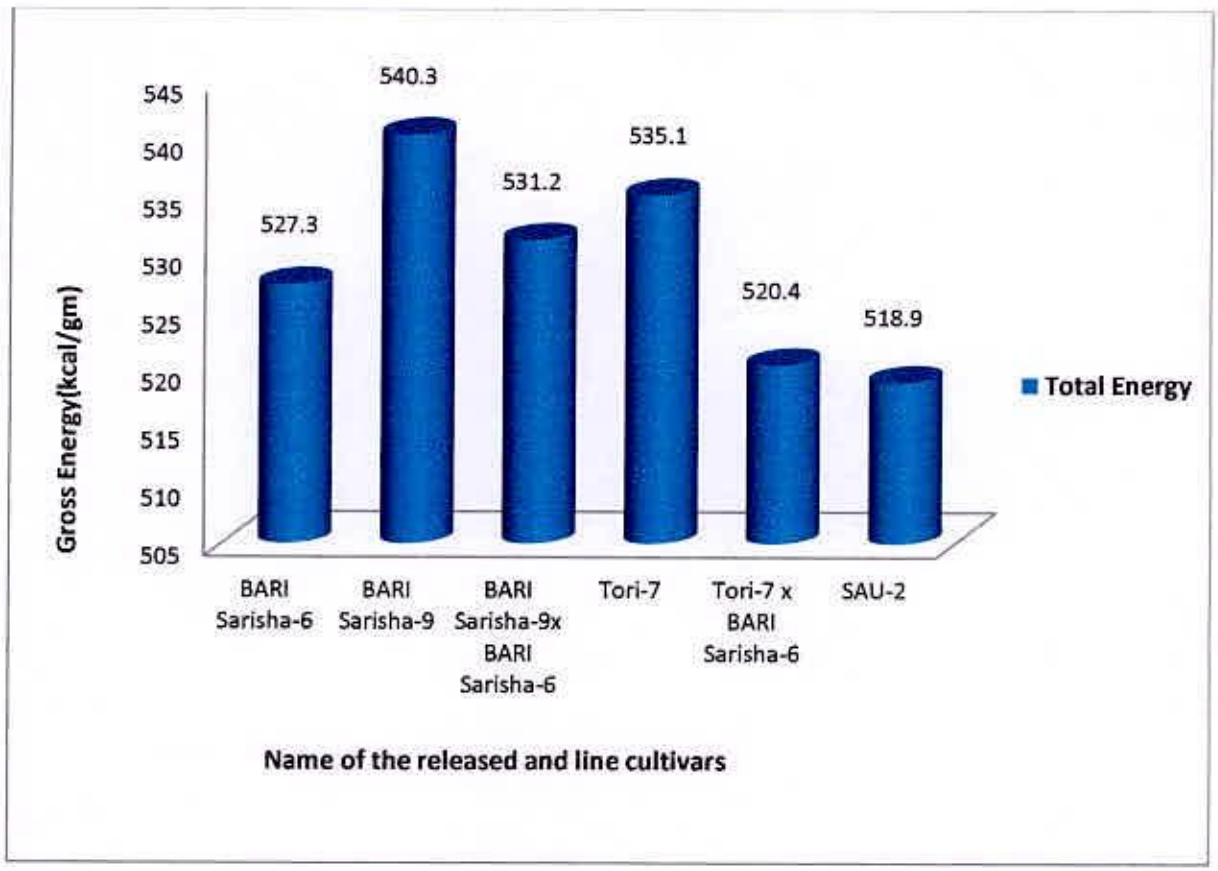

Figure 5. Gross Energy of the different released and line cultivars of rapeseed and mustard (*Brassica* spp.)

CHAPTER V
SUMMARY AND CONCLUSION

The study was conducted at the Biochemistry laboratory and research room at the Department of Biochemistry, Sher-e-Bangla Agricultural University and also in the Oil seed Research Centre and Soil Science Division, BARI, Joydebpur, Gazipur during the period from July to December 2014 to evaluate the physicochemical parameters of the rapeseed and mustard. Four released and two line cultivars were selected growing in large scale in Bangladesh, which released and line cultivars were evolved by BARI. Among these released and line cultivars, the highest thousand grain weight was found in BARI Sarisha-9 x BARI Sarisha-6 (4.892g) and the lowest thousand grain weight was found in SAU Sarisha-2 (4.459g). The highest moisture content (5.1%) was observed from BARI Sarisha-6; whereas the lowest moisture content (4.3%) was found in BARI Sarisha-9 x BARI Sarisha-6. Significantly highest amount of dry matter contained was recorded in BARI Sarisha-9 x BARI Sarisha-6 (95.70%); while the lowest amount of dry matter contained was found in BARI Sarisha-6 (94.90%).

The oil content of different released and line cultivars of mustard and rapeseed varied from 38.74% to 40.55% .The variety SAU Sarisha-2 (38.74%) had the lowest amount of oil contained, while the variety BARI Sarisha-9 (40.55%) contained significantly highest amount of oil. The SAU Sarisha-2 contained statistically highest amount of oil cake (61.26%), and the lowest value was found in BARI Sarisha-9 (59.45%). The highest dry weights of cake were obtained from SAU Sarisha-2 (56.56%).

The highest Saponification value was found in Tori-7 (168.8). Values recorded for all released and line cultivars were significantly higher than Tori-7 x BARI Sarisha-6(158.6). The highest amount of iodine value were observed in Tori-7 (110.2) and the lowest amount of iodine value recorded in Tori-7 x BARI Sarisha-6(96.33). The

highest acid value was found from Tori-7 x BARI Sarisha-6(1.610); whereas the lowest acid value was found from BARI Sarisha-9 (1.410).

Significantly the highest amount of palmitic acid was observed in BARI Tori-7 x BARI Sarisha-6(3.44%) and the lowest amount of palmitic acid content was observed in BARI Sarisha-6 (1.760%).The concentration of stearic acid varied from 0.00 to 1.770%; whereas arachidic acid contents ranged from 0.740 to 4.737%. Tori-7 contained the highest amount (18.56%) of oleic acid and the lowest amount was found in BARI Sarisha-9 (9.032%) which was significantly lowest among all the varieties and advanced lines. The Highest amount of lenoleic acid contained was found in BARI Sarisha-9 (17.75%), and lowest amount (12.70%) was found in BARI Sarisha-6. The concentration of linolenic acid varied from 6.270 to 15.83%. BARI Sarisha-6 contained the highest amount of erucic acid (50.67%) and the lowest amount was found in BARI Sarisha-9 (41.11%) ;which was significantly lowest among all the varieties and advanced lines. Mustard and rapeseed oils included unsaturated fatty acid ranging from 83.72 to 89.80%; while only a minor fraction by saturated fatty acids (4.397 to 8.444%).

Ash content of different released and line cultivars of mustard and rapeseed were variable and ranged from 11.0% to 14.0% .Highest amount of protein was obtained from SAU Sarisha-2(28.05%), the lowest amount of protein content (25.05%) showed by BARI Sarisha-9. The amount of carbohydrate contained found in Tori-7 (19.08%) was highest than other released and line cultivars of rapeseed and mustard. The lowest amount of carbohydrate was obtained from SAU Sarisha-2 (14.51%).

Significantly highest amount of calcium (Ca) content was observed in Tori-7 (1.006%), while the lowest amount of calcium content was obtained from SAU Sarisha-2 (0.722%).The highest amount of Magnesium content was found in Tori-7 (0.838%) and the lowest amount in BARI Sarisha-9 (0.370%).Significantly highest

amount of Cu contained observed in Tori-7 x BARI Sarisha-6 (3.955 ppm), and the lowest amount observed in SAU Sarisha-2 (1.205 ppm). Significantly highest amount of Fe contained was observed in Tori-7 (101.3 ppm). The variety BARI Sarisha-9 showed lowest amount of Fe (30.0 ppm).Significantly highest amount of Zn contained was found in Tori-7 x BARI Sarisha-6 (51.12 ppm), while the lowest amount was found in SAU Sarisha-2 (24.3 ppm). The highest amount of gross energy found from BARI Sarisha-9(540.3 kcal/g); while lowest amount of gross energy recorded from SAU Sarisha-2 (518.9 kcal/g).

From the above discussion, it was observed that none of the released and line cultivars of rapeseed and mustard performed the best by all nutrient parameters. But BARI Sarisha-9 performed the best considering the oil contents & gross energy. In case of fatty acid composition, BARI Sarisha-9 performed good results and content low erucic acid fraction. Tori-7, Tori-7 x BARI Sarisha-6, BARI Sarisha-6 showed the good performance for the most mineral contents. Tori-7 contained highest amount of saponification value, Iodine value and total unsaturated fatty acid. Based on the information mentioned above, it may be concluded that, released and line cultivars of BARI Sarisha-9, Tori-7, Tori-7 x BARI Sarisha-6, with appropriate qualitative and quantitative properties in their seed oil contents, can be grown in large scale as they contained the highest amount of different nutrient contents.

RECOMMENDATION

- From this experiment we can recommend that BARI Sarisha-9 and Tori-7 are the best cultivars among the released and line cultivars.
- Further analysis of different mustard variety should be done to know their nutrient content.
- Nutritional analysis is also important for breeders to evolve more nutrients rich mustard variety.
- Chemical composition and nutritional traits suggests the future strategy for the nutritionist, health advisors and dieticians as to how to make best use of the rapeseed and mustard.

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APPENDICE

Appendice 1. Chemical composition of some *Brassica* oilseeds

Species	Seed (%)				Free fatty acid (%)	Mineral content (%)				
	Moisture	Oil	Protein	Ash		N	P	K	Ca	Mg
B. campestris	6.0	46.38	17.38	3.74	0.28	5.18	0.71	1.45	0.35	0.27
B. napus	7.3	39.37	22.99	5.31	1.18	6.07	0.59	1.77	0.40	0.27
B. juncea	6.0	44.30	23.60	3.84	4.45	6.78	0.65	1.13	0.48	0.31
B. carinata	6.4	39.89	21.67	4.65	0.56	5.76	0.49	0.98	0.82	0.25
B. nigra	6.7	28.96	28.77	3.76	0.71	6.48	0.79	1.29	0.60	0.22

Source: Pathak, et al. (1973)