COMPARATIVE STUDY ON THE CHEMICAL COMPOSITION OF DIFFERENT VARIETIES AND ADVANCED LINE OF RAPESEED AND MUSTARD *(Brassica spp.)*

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CERTIFICATE

This is to certify that the thesis entitled ***"COMPARATIVE STUDY ON THE CHEMICAL COMPOSITION OF DIFFERENT VARIETIES AND ADVANCED LINE OF RAPESEED AND MUSTARD (Brassica spp.)"*** *submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207, in partial fulfillment 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* ***MD. SAHEDI RAHMAN****, Registration No.* ***10-04055,*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma in any other institutes.*

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

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DEDICATED TO

MY BELOVED PARENTS AND

ALL OF MY TEACHERS

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ABSTRACT

An experiment was conducted to study the chemical composition of two released varieties and four advanced line of rapeseed and mustard (*Brassica spp.*). Among these released varieties and advanced line, the highest grain weight was obtained from (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (4.500 g) and lowest grain weight obtained from BARI Sarisha-15 (4.46 g). In case of proximate analysis, the highest protein content and the highest carbohydrate were recorded from (SAU Sarisha-1 x SAU Sarisha-2)-F7 (29.37%) and BARI Sarisha-9 x BARI Sarisha-6 (19.95%) respectively. The oil content of different released varieties and advanced line of mustard and rapeseed varied from 35.81% to 40.58%. (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (537.56 kcal/g) contained the highest amount of Gross Energy. The highest amount of calcium content BARI Sarisha-15 (1.09%) and the highest amount of magnesium content (0.61%) were collected from BARI Sarisha-15. The highest amount of saponification value (169.6) and the highest amount of iodine value (108.8) were recorded from BARI Sarisha-14; however, the highest amount of acid value was recorded from (SAU Sarisha-1 x SAU Sarisha-2)-F7 (1.78). Erucic acid was in the range of 37.37% to 47.75%, oleic acid was the highest in (SAU Sarisha-1 x SAU Sarisha-2)-F7 contained 17.15%, while BARI Sarisha-9 x BARI Sarisha-6 containd the highest amount of the unsaturated linoleic acid (19.37%) and containd the highest amount of linolenic acids (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (7.83%). Moreover palmitic acid, stearic acid , arachidic acid and behenic acid were also present in small amount. It may be concluded that, released and advanced line of (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2, BARI Sarisha-15, (SAU Sarisha-1 x SAU Sarisha-2)-F7**,** BARI Sarisha-14 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.

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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

CHAPTER I

INTRODUCTION

There are many oil seed crops like mustard, sesame, groundnut, linseed, niger, safflower, sunflower and soybean which are being cultivated in Bangladesh. Among these, mustard is considered as the major oil crop. Mustard is an oil seed crop that belongs to the genus *Brassica* of the family *Brassicaceae*. It is one of the main cultivable edible oil seed crops of Bangladesh. It covers the land area of 216800 hectares in Bangladesh producing about 183500 metric tons of oilseeds (BBS, 2007). Bangladesh occupies the 5th place in respect of total oilseed production in the world and occupies the first position in respect of area and production among the oil crops (BBS, 2010). It is the most important popular oil crop which is grown in rabi season in Bangladesh. Only a few decades ago, in Bangladesh mustard oil was the exclusive cooking oil, medicinal ingredient and supplied fat in our daily diet. However, the yield of this crop in Bangladesh is much lower as compared to other countries. The average yield of rapeseed-mustard in Bangladesh is very low (0.76 t ha-1) that is less than 50% of the world average (FAO, 2004).

Bangladesh is deficit in edible oil, which cost valuable foreign currency for importing seeds and oil. Annually country is producing about 2.80 lac M tons of edible oil as against the requirement of 9.80 lac M tons. Thus importing edible oil is a regular phenomenon of this country (BBS, 2010). Every year Bangladesh imports 2085864 metric tons of edible oil to meet up the annual requirement of the country, which costs Tk. 64430 million (BBS, 2007). Both the acreage and production of the crop have been decreasing since 1990 mainly due to ingression of cereal crops like- rice, maize, wheat etc. Delayed harvest of transplanted aman rice and wetness of soil are other reasons which hinder mustard cultivation in rabi season (BARI, 2008). Recently BARI has released some mustard varieties, which have high yield potential under the circumstance the farmers are getting lower yield of mustard with their local varieties with poor crop management practices. 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. 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).

It produces 9 kcal 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. In traditional medicines, it is used to relieve the pain associated with arthritis, muscle sprains and strains. Seed paste applied on wounds whereas paste of leaf said to heal cattle wounds (Sood *et al.,* 2010). Rapeseed mustard oil quality is determined by the constituent fatty acids including palmitic, stearic, oleic, linoleic, linolenic, eicosenoic and erucic acids; and is highly affected by 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).

Rapeseed and mustard are common names used for different species of the family Cruciferae (*Bracicaceae*). Rapeseed includes *Brassica compestris* and *B. napus* and 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). 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). The yield of mustard can be augmented by adopting modern and recommended technologies along with the use of high yielding varieties. Fertilizer is the depending source of nutrient that can be used to boost up growth and yield of rapeseed (Sinha *et al.,* 2003).

In addition there are some cultivars viz. BARI Sarisha-14, BARI Sarisha-1, SAU-1 and SAU-2, are now cultivating in different regions of our country. The nutritional quality of all these released and advanced line 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 burning question arises about the physico-chemical quality of BARI and SAU 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 advanced line to ensure their nutritional status.

Objectives

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

1. To evaluate the physical and chemical composition of different varieties and advanced line of rapeseed and mustard varieties.
2. To compare the physico-chemical parameters and nutritional quality of different varieties and advanced line of rapeseed and mustard varieties .
3. To compare the gross energy obtain from different varieties and advanced line of rapeseed and mustard varieties.
4. To identify nutritionally potential rapeseed and mustar dvariety for the welfare of human being.

CHAPTER II

REVIEW OF LITERATURE

Grain Weight

Thousand seed weight is a very important character of rapeseed and mustard, where highest consideration is one of the seed yield. 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. They found that thousand grain weights were determined at 13% moisture level. The highest thousand grain weight was found in BARI Sarisha- 13 (4.38 g), this was significantly higher than all others released variety and the lowest thousand grain weight was found in BARI Sarisha- 9 (3.06 g). Statistically similar results were shown by BARI Sarisha- 11 (3.56 g), BARI Sarisha- 14 (3.75 g) and Sarisha-16 (3.75 g).

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

Banga *et al*. (2009) observed that seed yield in different genotypes was mainly governed by seed yield per plant. The 1000-seed weight was the maximum in RL-1359 (5.15 g) whereas it was at par in other genotypes.

Chowdhury *et al.* (2008) carried out an experiment on evaluation of rapeseed and mustard varieties. The research work indicated that the highest thousand grain weight was found in BARI Sarisha-9 (4.9 g) and BARI Sarisha-15 (2.5 g) which showed lowest thousand grain weight.

Siddiqui *et al*. (2004) evaluated some important characteristics of *Brassica carinata* cultivars (IGC-01 and PusaGaurav), *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.

Ozer *et al.* (2003) studied two cultivars (Tower and Lirawell) of rapeseed to investigate the effect of sowing dates with four levels of nitrogen (0, 80, 160 and 240 kg N ha-1). Result revealed that adequate N fertilization was important in increasing 1000- seed weight in summer oilseed rape and suggested that the rate of 160 kg N ha-1 will be

adequate for the crop to meet its N requirements. 1000-seed weight differs with nitrogen levels that enhanced yield.

Singh *et al.* (2002) conducted an experiment with Varuna variety of mustard with 5 levels of nitrogen (0, 30, 60, 90 and 120 kg ha-1) and P (0, 15, 30, 45 and 60 kg ha-1). Application of N and P increased 1000-seed weight. However, the significant increase in 1000 seed weight was recorded in 120 kg N ha-1.

Shukla *et al.* (2002) conducted an experiment to study the integrated nutrient management for Indian mustard *(B. juncea).* They obtained maximum 1000-seed weight with the application of 120 kg N ha-1.

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 variety and advanced 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 (3.42 g) 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* reported 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 s*eeds ranges from 2.6 g - 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) found that moisture content of different released and line cultivars of mustard and rapeseed was ranged from 4.00% to 5.20%. They found the highest

moisture content (5.20%) was observed from BARI Sarisha-15; while the lowest moisture content (4%) was found in BARI Sarisha-16.

According to Sarker *et al.* (2015) conducted an experiment that moisture content of black and yellow mustard cakes were 9.20 ± 0.5% and 9.73 ± 0.6%. They found that the results of the moisture content were slightly higher than the moisture content 8.3 ± 0.2% reported in literature.(Mahmud et al. 2012).

Barrozo *et al.* (2008) carried out an experiment on 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 ºC and 45 ºC, respectively .

Huda (2001) reported an experiment the moisture content of rapeseed mustard varieties and advanced lines. They suggested 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 seeds 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% .They found that BARI Sarisha-14 (9.6%) contained highest amount of oil.

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. These data are comparable (7.12 ± 0.12%) to the results reported in literature (Datta *et al.*, 2013).

Abul-Fadl *et al*. (2011) reported 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

Fats and oil are important to maintain body temperature. Its provide 9 kcal energy from each gram of oil.

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

Arif *et al*. (2012) carried out an experiment to find out the potential nutrients rich of oil seeds varieties and they found highest amount of crude oil in *B. juncea*and *B. napus* .i.e. 45.67% and 43.87%, respectively.

Gadei *et al.,* (2012) examined the composition and physical properties of different oil seeds. They observed that oil 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.

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 gave the highest oil content (40%) among these.

Sengupta *et al.* (2003) reported that oil content of mustard seeds ranges from 40% - 41.5% and kernel 45% -47.1%.

Bhowmik *et al.* (2003) conducted an experiment on some released rapeseed and mustard varieties and advanced 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.

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%; more or less similar result were found by Kumar and Tsunoda, (1980) .

BARI (2001) reported that variable amount of oil are present in different rapeseed and 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.

Niraz *et al.* (2001) conducted an experiment to compare the 21 genotypes of Indian mustard. Results showed 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%).

Rathore *et al.* (2000) reported that oil content of sarson, toria and rai ranges from 43-45%, 30-35% and 31-35%, respectively.

Ullah *et al.* (1997) examined that twelve released varieties and line cultivars of rapeseed 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 whereas lowest was found in *B. juncea.*

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

Mazzoncini *et al.* (1993) carried out an experiment on seed oil content of *Brassica carinata* ranged from 32.5% to 40.6%. They reported that seed oil content was higher in rapeseed which ranged from 40.5% to 47.3%.

BARI (1992-93) determined the oil content of eight brown seeds of *B. campestris* lines. Oil contented 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) reported the oil content of seven yellow seeds of *B. campestris* lines including two check varieties and advanced lines. They observed that there was no significant difference among the lines with respect to its oil content (41.99% to 42.52%).

Vijay *et al*. (1992) evaluated the oil contents in 65 released varieties and advanced line of rapeseed and mustard. The result showed that many released varieties and advanced line cultivars had high oil content (more than 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 fourteen *Brassica campestries* genotypes. They found that highest oil contented 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.

Oil cake

Oil cake is the nutritious feed items for cattle and fish. It is also used as a good organic matter.

Hossain *et al.* (2015) reported an experiment to evaluate the percentage of oil cake of different varieties of mustard and rapeseed and they found that, the BARI Sarisha-13 contained significantly highest amount of oil cake (61.25%). The lowest value was found in BARI Sarisha-15 (57.75%). Similar results were found by Chowdhury *et al.* (2008) and Appelqvist *et al*. (1992).

Chowdhury *et al*. (2008) conducted an experiment to evaluate the percentage of oil cake of different released varieties 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 by 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%). Appelqvist *et al*. (1992) carried out a typically rape seed oil (Kind of mustard seed) contain 58% cake.

Chemical properties of Oil

The chemical characteristics of oil determine the quality and stability of oil.

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

Chowdhury *et al. (*2008), determined the chemical constant of mustard and rapeseed oil. They observed 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.

Richet *et al*. (1987) used fifteen different solvents for the extraction of oil from rapeseeds under identical condition. They also recorded 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) carried out an experiment on extracted oil from rapeseed to examine the physico-chemical parameters of the oil. They found that iodine value, saponification value, acid value and unsaponifiable matter were 112.0, 170.0, 2.6 and 0.66, respectively.

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), they 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.

Bachheti *et al.* (2012), conducted the Gas liquid chromatography (GLC) analysis of oil seeds which revealed that it contained oleic acid (73.58%), linoleic acid (19.26%), palmitic acid (3.31%), myristic acid (1.18%) and stearic acid (2.68%). Mustard oil possess 60% monounsaturated fatty acids of which 42% Erucic acid and 12% Oleic acid, it had also 21% polyunsaturated of which 6% was the omega-3 alpha-Linolenic acid and 15% omega-6 linoleic acid along with 12% saturated fats were conductucded (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.

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 yellow and brown mustard seed oils, respectively. Moreover, linoleic acid was 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 contained 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) found that the fatty acid composition 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%).

Nasr *et al*. (2006), carried out an experiment on five important fatty acids, i.e. oleic acid, linoleic acid, linolenic acid, stearic acid and palmitic acid were commonly found in ten rapeseed released varieties and advanced line which oleic acid and stearic acid had the highest and lowest percentages, respectively. Oleic acid levels in different rapeseed released varieties and advanced line 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. They reported the differences 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) conducted on nutritional analysis a different rapeseed varieties such as *B. carinata* cultivars (IGC-01 and PusaGaurav), *B. juncea* cultivars (Jagannath, Kranti, Rohini and TERI (OE) M 21-Swarna and *B. napus* CV. (Hyola PAC-401). They found 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) carried out 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 showed that amount of linoleic acid (18:2) was ranges from 11% - 22%.

Cardone *et al.* 2002, reported that an important source of vegetable oil for human nutrition, oilseed rape was a key source of healthy high-calorie mono-unsaturated fatty acids (MUFA) like oleic acid (18:1). These fats constituted about 61% of total fats and helped to increase high-density lipoprotein (HDL ; so called “good

cholesterol") and reduce low-density lipoprotein (LDL; "bad cholesterol") in the blood.

Niraz *et al.* (2000) evaluated the twenty one genotypes of Indian mustard. They found that considerablevariation 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) found 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 ranged 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 *et al.* (2003) described 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 *et al.* (1980) evaluated an experiment to determine the fatty acid composition. He reported that fatty acid composition of mustard released varieties and advanced 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 found out 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.

Lyons *et al*. (1964*)* carried out an experiment on the nomenclature that was traditionally utilized to represent the different fatty acids was as follows: Within the parenthesis, the letter C was followed by a number that represents the chain length, i.e., how many carbon molecules were contained within the chain. After the colon there was a second number representing the degree of saturation of the fatty acid chain. For example, linolenic acid was represented as (C18:3). This means that this fatty acid was composed of a carbon chain containing 18 carbon molecules with three double bonds somewhere in the chain. If there were no double bonds, and the second number was 0, the chain was fully saturated. As a rule of thumb, at room temperature, saturated fatty acids were solid because straight saturated chains were joined by the attraction of the carbon and the hydrogen atoms in close proximity.

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.

BILJANA *et al.* (2015), carried out an experiment on there was an internationally regulated definition of canola that differentiates it from rapeseed, based upon its less than two percent of erucic acid (C22:1) and less than 30 μmol/g glucosinolates. Oilseed products that do not meet this standard cannot use the trade marked term Canola. Like

soybean, canola contained high oil contented as well as a high protein content. It contained about 40 % oil and 23 % protein compared to 20 % and 40 %, respectively, for soybean consumption.

Sarker *et al.* (2015), conducted on 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 *et al.* reported comparatively equal amount of crude protein in mustard cakes (Kumar *et al.,* 2002; Chowdhury *et al.* 2010).

Al-Jasass *et al.* (2012*)* reported that, cress, mustard, black cumin and black pepper contained higher protein content ranged from 26.61% to 25.45%, as compared to fenugreek (12.91%) and clove (6.9%).Mustard seeds contained 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 were two main types of storage proteins present in mustard seeds: legumin type globulins (11S, cruciferins) and napin-type proteins (2S, napins), which were water soluble and had an isoelectric point around a pH value of 7.

Abul-Fadl *et al*. (2011) carried out an experiment to evaluate 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%).

Latif *et al*., 2008 examined on 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.

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

Bell *et al*., 1999 conducted on 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.

Mirza *et al*. (1998) carried out an experiment to evaluate the protein content of rapeseed oil. They stated that significant differences were found among protein contents which are negatively correlated with oil content.

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

Carbohydrate

Rapeseed and mustard contain relatively lower amount carbohydrate.

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

Bachheti *et al. (*2012) carried out an experiment to evaluate thephysico-chemical properties of some conventional food oils and found that mustard seeds contained 23.8% carbohydrate.

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

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%.

Ara *et al* (2014), carried out an experiment that the highest seed weight plant $^{-1}$, 1000 seed weight and oil content percent was observed at 120 kg N/ha but higher dose of N 180 kg/ha failed to produce better results as 120 kg N/ha whereas the highest dose of B 2 kg/ha gave the highest value of seed weight plant^{-1}, 1000 seed weight and oil content percent.

Arif *et al*. (2012), carried out an experiment where they reported 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 (Gulshan98), 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 evaluate the physico-chemical properties of some conventional food oils and they 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) found an experiment to determine the minerals content of rapeseed and mustard varieties and advanced lines. They reported 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 *et al.* (1988) conducted an experiment on the mineral content of defatted rapeseed meals. They 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 examine the minerals content of different oilseed varieties and advanced lines cultivars. They showed that different released

varieties and advanced 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.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

Two released varieties and four advanced lines of mustard *(Brassica Campestries*) namely BARI Sarisha-14, BARI Sarisha-15, BARI Sarisha-9 x BARI Sarisha-6, (SAU Sarisha-1 x SAU Sarisha-2)-F7, BARI Sarisha-9 x BARI Sarisha-6-F12, (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 were selected for the study. The seeds were collected from the oilseeds Research centre of BARI, Gazipur. Seed were cleaned sun dried and stored into plastic container in a cool place until used for the chemical analysis.

3.1.1 Brief description of varieties

BARI Sarisha-14: This is a composite variety evolved by BARI in recent. Its grain colour is yellow and round in shape. The grain is large in size.

BARI Sarisha-15: This is a composite variety evolved by BARI in recent. Its grain colour is yellow and oval in shape. The grain is small in size.

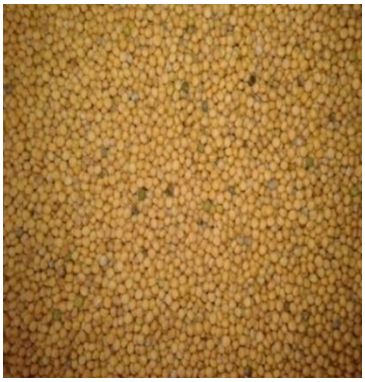
BARI Sarisha-9 x BARI Sarisha-6: This advanced line evolved by BARI. Its grain color is blackish and oval in shape. The grain is large in size.

(SAU Sarisha-1 x SAU Sarisha-2)-F7: This advanced line evolved by SAU. Its grain is yellow in color and round in shape. The grain size is medium.

BARI Sarisha-9 x BARI Sarisha-6-F12: This advanced line evolved by BARI. Its grain is in blackish colour. The grain size is small to medium.

(SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2: This advanced line evolved by SAU. Its grain is in blackish to ash colour. The grain size is medium.

BARI Sarisha-14

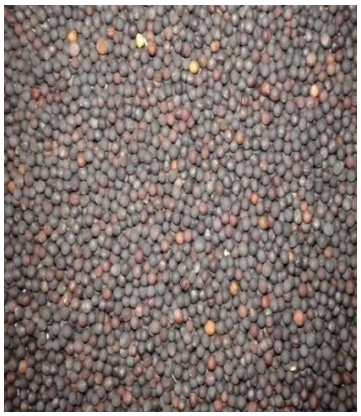


BARI Sarisha-15

BARI Sarisha-9 x BARI Sarisha-6

(SAU Sarisha-1 x SAU Sarisha-2) F7

BARI Sarisha-9 xBARI Sarisha-6-F12



(SAU Sarisha-1 x SAU Sarisha-2) X F1 X SAU Sarisha-2

Plate 1. Photograph showing variation in seed coat color, seed size and shape of some selected released varieties and advanced lines of (*Brassica campestris)*

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 seed 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^{o} 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^{o} C. The dishes were transferred to desiccators to cool. Aluminum dish was weighed after cooling (w_3).

Calculation

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

3.2.2 Determination of ash

The sample is ignited at 600^{o} 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^{o}C and crucible was heated for 1h and transferred into a desiccator; cooled them to room temperature and weighted (W_1). About 2 g sample was put into the crucible weighted (W_2). The sample was burned in a muffle furnace at 600^{o} C for about 2 h. The crucibles were transferred into the desiccator 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 = $W_3 - W_1$

$$\% \text{ Ash} = \frac{\text{Weight of the ash}}{\text{Weight of the sample taken}} \times 100$$

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

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 1984) 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 alkaline 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 ^{o}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 sulphate (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

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

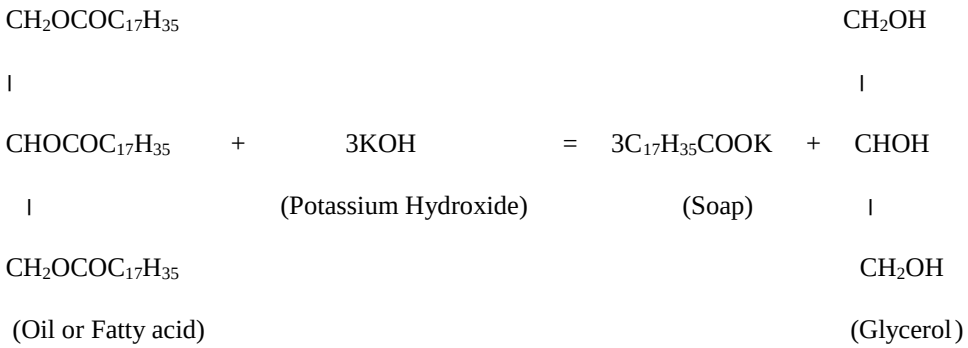
Where 14.007 is the equivalent weight of nitrogen.

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. Weight 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

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

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) x .0127 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

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

3.3.4 Estimation of fatty acid composition

Seed sample of rapeseed and mustard were received from ORC, BARI, Joydebpur, Gazipur. Fatty acid composition was determined by Gas-liquid chromatographic method (Uppstrom *et al*.1978).

Reagent

1. Ethylate reagent (Petroleum ether / 0.02M sodium hydroxide in ethanol (2/3)
2. A Salt solution (80 g NaCl and 3g Sodium hydrogen Sulphate in 1 litre water)

Procedure

1. About 12 mg of oil or equivalent amount of oil seeds was taken (seed was crushed in an oil paper and then transferred into a test tube).
2. The sample was extracted and transesterified at the same time with 5 ml ethylated reagent and shaked.
3. The samples were kept for overnight at room temperature.
4. 10 ml salt solution was added and shaked. As soon as the two layers were separated, the benzene phase was transferred to small test tubes.
5. A Philips PU 4500 chromatograph instrument was used with flame ionization detector (FID).
6. A glass column (1.5m x 4mm) was packed with BDS. With this column the injection post, column and detector temperature was set at 220^{o} C, 185^{o} C and 240^{o} C, respectively.
7. Nitrogen flow (used as career gas) rate was 22 ml/min, the injection volume was 2µl.
8. Peak areas were measured with an electronic digital integrator (Shinadzu C-R6A chromatopac).

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 + Fat + 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% 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 $BaCl_2$. 2 H_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

Nitric-perchloric solution

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

3.3.7.1 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^{o} 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.

3.4 Statistical Analysis

The recorded data 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

Two released and four advanced line 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 advanced line were presented in different tables. The data had also been estimated on moisture free basis in order to allow for better comparison of the different fraction. The data mentioned were the average of three replication and had been presented and discussed.

4.1 Physical characteristics of rapeseed and mustard released and advanced line

4.1.1 Seed weight

Weight of thousand grains of different released and advanced line of mustard and rapeseed had been compared in table 1. It was found that seed weight varied with their size and shape. Weights of thousand grains were determined at 13% moisture level. The highest weight of thousands of seed weight was found in (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (4.50g). This was higher than all others released varieties & advanced lines and statistically similar results were shown by (SAU Sarisha-1 x SAU Sarisha-2)-F7 (4.30g), BARI Sarisha-9 x BARI Sarisha-6-F12 (4.721g) and BARI Sarisha-14 (3.790g). The lowest weight of thousand seeds was found in BARI Sarisha-15(4.46). The present values were consisted 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*. (2008) reported range of weight of thousand seed 2.5 g to 4.9 g, 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 5.15 g and 3.95 g

respectively. The present values were higher than the reported value of Mondal and Wahhab (2001).

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 advanced line of mustard and rapeseed had been presented in table 1. The moisture content of different released and advanced line of mustard and rapeseed was ranged from 4.40% to 5.02%. The highest moisture content (5.02%) was observed from BARI Sarisha-9 x BARI Sarisha-6 , which was followed (4.55%,4.55%, 4.45%, and 4.44%) by BARI Sarisha-9 x BARI Sarisha-6-F12**,** BARI Sarisha-14 , BARI Sarisha-15, (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2; while the lowest moisture content (4.40%) was found in (SAU Sarisha-1 x SAU Sarisha-2)-F7. 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 advanced line of mustard and rapeseed that have been presented in table 1. Significantly highest amount of dry matter contained was recorded in (SAU Sarisha-1 x SAU Sarisha-2)-F7 (95.60%), followed by (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (95.56%), BARI Sarisha-15 (95.55%), (SAU Sarisha-1 x SAU Sarisha-2)-F7 (95.45%) and BARI Sarisha-15 (95.45%). The lowest amount of dry matter contained was found in BARI Sarisha-9 x BARI Sarisha-6 (94.9%) which was significantly lowest among all the variety & advanced lines. These variations might be due to environmental factor.

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

Name of the released and line cultivars (Treatments)	Weight of 1000 seeds (gm)	Moisture (%)	Dry matter (%)
BARI Sarisha-14	3.79 d	4.55 b	95.45 a
BARI Sarisha-15	3.45 e	4.45 b	95.55 a
BARI Sarisha-9 x BARI Sarisha-6	3.46 e	5.02 a	94.98 b
(SAU Sarisha-1 x SAU Sarisha-2)-F7	4.30 b	4.40 b	95.60 a
BARI Sarisha-9 x BARI Sarisha-6-F12	3.98 c	4.55 b	95.45 a
(SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2	4.50 a	4.44 b	95.56 a
LSD $_{(0.05)}$	0.10	0.42	0.42
CV (%)	1.45	5.02	0.24

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 rapeseed 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 advanced line of mustard and rapeseed were extracted by petroleum ether (40-60^{0}C) varied from 35.81% to 40.58% (table 2). The variety (SAU Sarisha-1 x SAU Sarisha-2)-F7 had the lowest amount of oil contained (35.81%) , while the variety (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 contained significantly highest amount of oil (40.58%), followed by BARI Sarisha-15 (40.13%) and BARI Sarisha-14 (39.67%). The results clearly indicated that variety (SAU

Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (40.58%), BARI Sarisha-15 (40.13%) and BARI Sarisha-14 (39.67%) can be considered as better source of oil. Present values were 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% and 45-46% 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-1 x SAU Sarisha-2)-F7 contained highest amount of oil cake (64.19%), followed by BARI Sarisha-9 x BARI Sarisha-6 (63.76%) and BARI Sarisha-9 x BARI Sarisha-6-F12 (61.36%). The lowest value was found in (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (59.42%). 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 had been presented in table 2. The highest value was obtained from (SAU Sarisha-1 x SAU Sarisha-2)-F7 (59.79%), followed by BARI Sarisha-9 x BARI Sarisha-6 (58.74%) and BARI Sarisha-9 x BARI Sarisha-6-F12 (56.81%). The lowest value obtained from (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (54.99%) 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 different released and advanced line of rapeseed and mustard (*Brassica* spp.)

Name of the released and line cultivars (Treatments)	Oil content (%)	Oil cake (%)	Dry wt. of cake (%)
BARI Sarisha-14	39.67 c	60.33 d	55.78 d
BARI Sarisha-15	40.13 b	59.87 e	55.42 de
BARI Sarisha-9 x BARI Sarisha-6	36.24 e	63.76 b	58.74 b
(SAU Sarisha-1 x SAU Sarisha-2)-F7	35.81 f	64.19 a	59.79 a
BARI Sarisha-9 x BARI Sarisha-6-F12	38.64 d	61.36 c	56.81 c
(SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2	40.58 a	59.42 f	54.99 e
LSD $_{(0.05)}$	0.33	0.33	0.48
CV (%)	0.47	0.30	0.47

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 advanced line

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

advanced line were ranges from 162.6 to 169.6 and had been presented in table 3. The statistically highest Saponification value was found in BARI Sarisha-14 (169.6). There were significant variations between the varieties and advanced lines, (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (167.5), (SAU Sarisha-1 x SAU Sarisha-2)-F7 (166.4), but the values recorded for these released and line cultivars were significantly higher than BARI Sarisha-9 x BARI Sarisha-6 (162.3). The present values were 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 were 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 table 3. The highest amount of iodine value were observed in BARI Sarisha-14 (108.8), followed by (SAU Sarisha-1 x SAU Sarisha-2)-F7 (105.7) and BARI Sarisha-15 (103.6). The lowest amount of iodine value recorded in BARI Sarisha-9 x BARI Sarisha-6 (97.37), followed by (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (99.57). The observed values were supported by the reported values of Chowdhury *et al. (*2008), Khan *et al*. (2013) and Richet *et al*. (1987).

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 advanced line of mustard and rapeseed had been presented in table 3. The highest acid value was found from (SAU Sarisha-1 x SAU Sarisha-2)-F7 (1.78), followed by BARI Sarisha-14 (1.58); whereas the lowest acid value was found from BARI Sarisha-9 x BARI Sarisha-6-F12 (1.28) followed by BARI Sarisha-9 x BARI Sarisha-6 (1.32). 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).

Table 3. Chemical constant of oil of the different released and advanced line of rapeseed and mustard (*Brassica* spp.)

Name of the released and line cultivars (Treatments)	Saponification value	Iodine value	Acid value
BARI Sarisha-14	169.6 a	108.8 a	1.58 ab
BARI Sarisha-15	165.8 d	103.6 c	1.44 bc
BARI Sarisha-9 x BARI Sarisha-6	162.3 f	97.37 f	1.32 bc
(SAU Sarisha-1 x SAU Sarisha-2)-F7	166.4 c	105.7 b	1.78 a
BARI Sarisha-9 x BARI Sarisha-6-F12	163.6 e	101.4 d	1.28 c
(SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2	167.5 b	99.57 e	1.54 abc
LSD $_{(0.05)}$	0.37	0.20	0.30
CV (%)	0.12	0.11	10.97

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

4.2.2 Fatty acid composition

Comparison of fatty acid composition determined through gas chromatography are demonstrated in table 4. According to results, there was a significant difference between the studied rapeseed and mustard released and advanced line in terms of their fatty acid compounds. Significantly the highest amount of palmitic acid was observed in BARI Sarisha-9 x BARI Sarisha-6 (4.43%); followed by BARI Sarisha-9 x BARI Sarisha-6-F12 (3.88%) and (SAU Sarisha-1 x SAU Sarisha-2)-F7 (3.28%); Lowest amount of palmitic acid content was observed in BARI Sarisha-15 (2.78%). The concentration of stearic acid varied from 1.18% to 1.66%; whereas arachidic acid contents ranged from 4.56% to 5.63%. (SAU Sarisha-1 x SAU Sarisha-2)-F7 contained the highest amount (1.49%) of behenic acid. (SAU Sarisha-1 x SAU Sarisha-2)-F7 contained the highest

amount (17.15%) of oleic acid; followed by BARI Sarisha-9 x BARI Sarisha-6 (16.97%) and the lowest amount was found in BARI Sarisha-15 (14.36%) which was significantly lowest among all the varieties and advanced lines. Lenoleic acid content of the released and advanced line ranged from 14.67% to 19.37%. The highest amount of lenoleic acid contents was found in BARI Sarisha-9 x BARI Sarisha-6 (19.37%) which was significantly highest among all the released and advanced line and lowest amount (14.67%) was found in BARI Sarisha-9 x BARI Sarisha-6-F12. 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 6.45% to 7.83%; whereas erucic acid contents ranged from 37.37% to 47.75%. Differences were found among the released and advanced line of rapeseed and mustard in respect of erucic acid content. BARI Sarisha-15 contained the highest amount of erucic acid (47.75%), followed by BARI Sarisha-14 (47.43%) and lowest amount was found in BARI Sarisha-9 x BARI Sarisha-6 (37.37%); which was significantly lowest among all the varieties and advanced lines. GLC analytical data indicated that the major fatty acid composition of the two released and four advanced line of mustard and rapeseed oils included unsaturated fatty acid ranging from 80.16% to 86.54%. The highest amount of total unsaturated fatty acid contained BARI Sarisha-14 (86.54%), the lowest amount of total unsaturated fatty acid contained BARI Sarisha-9 x BARI Sarisha-6 (80.16%).while only a minor fraction by saturated fatty acids (9.94% to 11.92%). 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 were in conformity with the results by Wani Mubashir (2012), Abul-fadl *et al.* (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 had 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 oil.

Table 4. Fatty acid composition of different released and advanced line of rapeseed and mustard (*Brassica* spp.)

Name of the released and advanced line (Treatments)	Percentage of fatty acids									
	Palmitic Acid ($C_{16:0}$)	Stearic Acid ($C_{18:0}$)	Arachidic Acid ($C_{20:0}$)	Behenic acid ($C_{20:1}$)	TSFA	Oleic Acid ($C_{18:1}$)	Linoleic Acid ($C_{18:2}$)	Linolenic Acid ($C_{18:3}$)	Erucic Acid ($C_{22:1}$)	TUSFA
BARI Sarisha-14	2.87 d	1.46 d	5.63 a	1.06 c	11.02 b	16.53 c	15.09 c	7.49 b	47.43 a	86.54 a
BARI Sarisha-15	2.78 e	1.57 c	4.58 cd	1.28 b	10.21 c	14.36 f	14.77 de	7.53 b	47.75 a	84.41 b
BARI Sarisha-9 x BARI Sarisha-6	4.43 a	1.61 b	4.84 bc	0.84 d	11.72 a	16.97 b	19.37 a	6.49 d	37.37 e	80.16 d
(SAU Sarisha-1 x SAU Sarisha-2)-F7	3.28 c	1.66 a	5.49 a	1.49 a	11.92 a	17.15 a	16.21 b	7.79 a	45.05 b	86.20 a
BARI Sarisha-9 x BARI Sarisha-6-F12	3.88 b	1.29 e	4.93 b	0.93 d	11.03 b	15.43 d	14.67 e	6.69 c	43.40 d	80.19 d
(SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2	2.84 de	1.18 f	4.56 d	1.45 a	9.94 d	14.67 e	14.93 cd	7.83 a	44.39 c	81.82 c
LSD $_{(0.05)}$	0.08	0.05	0.27	0.12	0.22	0.16	0.21	0.20	0.36	0.36
CV (%)	1.16	0.01	2.96	5.42	1.10	0.56	0.72	1.52	0.45	0.24

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

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 5.

4.3.1 Ash

Ash content of different released and advanced line of mustard and rapeseed were variable and ranged from 11.24% to 13.46% (table 5). Significantly highest amount of ash contained was recorded in (SAU Sarisha-1 x SAU Sarisha-2)-F7 (13.46%), and followed BARI Sarisha-9 x BARI Sarisha-6-F12 (12.38%), BARI Sarisha-15 (11.92%), But these values were significantly higher than the BARI Sarisha-9 x BARI Sarisha-6 (11.24%). 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 advanced line 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 5. The statistically highest amount of protein was obtained from (SAU Sarisha-1 x SAU Sarisha-2)-F7 (29.37%) and followed by BARI Sarisha-9 x BARI Sarisha-6-F12 (28.38%), (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (28.19%). On the other hand BARI Sarisha-9 x BARI Sarisha-6 (25.56%), which was statistically lowest amount of protein. The present values were 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.

Table 5. Proximate analysis of protein, ash and carbohydrate content of different released and advanced line of rapeseed and mustard (*Brassica spp.*)

Name of the released and line cultivars (Treatments)	Ash (%)	Protein (%)	Carbohydrate (%)
BARI Sarisha-14	11.65 d	28.00 c	16.13 d
BARI Sarisha-15	11.92 c	26.63 d	16.88 c
BARI Sarisha-9 x BARI Sarisha-6	11.24 e	25.56 e	19.95 a
(SAU Sarisha-1 x SAU Sarisha-2)-F7	13.46 a	29.37 a	18.86 b
BARI Sarisha-9 x BARI Sarisha-6-F12	12.38 b	28.38 b	16.08 d
(SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2	11.86 cd	28.19 bc	14.89 e
LSD $_{(0.05)}$	0.22	0.38	0.12
CV (%)	1.00	0.75	0.36

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

4.3.3 Carbohydrate

Carbohydrate content of different released and advanced line of rapeseed and mustard were determined moisture free basis. The data had been presented in table 5. The higher amount of carbohydrate found in BARI Sarisha-9 x BARI Sarisha-6 (19.95%) was highest than other released and line cultivars of rapeseed and mustard but statistically similar with (SAU Sarisha-1 x SAU Sarisha-2)-F7 (18.86%). The lowest amount of

carbohydrate was obtained from (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (14.89%). Agronomics practices, environmental factors as well as variation among the released and line cultivars might be influenced the carbohydrate content. The present values were 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.

4.3.4 Minerals

Different major and minor minerals were analyzed in this work. The amounts of major minerals content of rapeseed had been illustrated in table 6. It was well known that rape seed contained small amount of minerals.

Major minerals

Calcium (Ca)

In case of calcium content of different released and advanced line of rapeseed and mustard was ranged from 0.98% to 1.09% (table 6). Significantly highest amount of calcium (Ca) content was observed in BARI Sarisha-15 (1.09%), followed by BARI Sarisha-14 (1.07%), BARI Sarisha-9 x BARI Sarisha-6 (1.05%), BARI Sarisha-9 x BARI Sarisha-6-F12 (1.04%) and (SAU Sarisha-1 x SAU Sarisha-2)-F7 (1.03%). Lowest amount of calcium content was obtained from (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (0.98%). 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 contained 492.1mg and 490 mg 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 advanced line of rapeseed and mustard had been presented in table 6. Magnesium content of different released and advanced line was ranged from 0.55% to 0.61%. The highest amount of Magnesium content was found in BARI Sarisha-15 (0.61%); followed by BARI Sarisha-14 (0.60%) and the lowest amount in (SAU Sarisha-

1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (0.55%); followed by (SAU Sarisha-1 x SAU Sarisha-2)-F7 (0.58%). BARI Sarisha-15 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 6. Proximate analysis of major minerals content of different released and advanced line of rapeseed and mustard (*Brassica* spp.)

Name of the released and line cultivars (Treatments)	Ca (%)	Mg (%)	P(%)
BARI Sarisha-14	1.07 b	0.60 b	0.77 f
BARI Sarisha-15	1.09 a	0.61 a	0.88 d
BARI Sarisha-9 x BARI Sarisha-6	1.05 c	0.59 c	0.81 e
(SAU Sarisha-1 x SAU Sarisha-2)-F7	1.03 e	0.58 d	0.90 b
BARI Sarisha-9 x BARI Sarisha-6-F12	1.04 d	0.59 c	0.89c
(SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2	0.98 f	0.55 e	0.99a
LSD $_{(0.05)}$	0.05	0.05	0.05
CV (%)	0.39	0.01	0.01

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

Phosphorus (P)

In case of Phosphorus content of different released and advanced line of rapeseed and mustard was ranged from 0.77% to 0.99% (table 6). Significantly highest amount of Phosphorus (P) content was observed in (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (0.99%), followed by (SAU Sarisha-1 x SAU Sarisha-2)-F7 (0.90%), BARI Sarisha-9 x BARI Sarisha-6-F12 (0.89%). Lowest amount of phosphorus content was obtained from BARI Sarisha-14 (0.77%). 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).

Minor minerals

Copper (Cu)

Copper contained of different released and advanced line of rapeseed and mustard was ranged from 5.06 to 5.68 ppm (table 7). Significantly highest amount of Cu contained observed in BARI Sarisha-15 (5.68 ppm) which was followed by BARI Sarisha-14 (5.57 ppm), BARI Sarisha-9 x BARI Sarisha-6 (5.45 ppm) and BARI Sarisha-9 x BARI Sarisha-6-F12 (5.43 ppm). Lowest amount of Cu contained observed in (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (5.06 ppm) which was followed by (SAU Sarisha-1 x SAU Sarisha-2)-F7 (5.36 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 advanced line of rapeseed and mustard were ranged from 121.7 ppm to 149.1 ppm (Table 7). Significantly highest amount of Fe contained was observed in (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (149.1 ppm) which was followed by BARI Sarisha-9 x BARI Sarisha-6-F12 (144.9 ppm) and (SAU Sarisha-1 x SAU Sarisha-2)-F7 (137.1 ppm). The variety BARI Sarisha-14 showed lowest amount of Fe (121.7 ppm) which was followed by BARI Sarish- 15 (129.4 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 18 ppm respectively.

Table 7. Proximate analysis of minor minerals content of different released and advanced line of rapeseed and mustard (*Brassica* spp.)

Name of the released and line cultivars (Treatments)	Cu (ppm)	Fe (ppm)	Mn (ppm)
BARI Sarisha-14	5.57 b	121.7 f	78.48 f
BARI Sarisha-15	5.68 a	129.4 e	83.45 e
BARI Sarisha-9 x BARI Sarisha-6	5.45 c	131.8 d	85.13 d
(SAU Sarisha-1 x SAU Sarisha-2)-F7	5.36 e	137.1 c	88.42 c
BARI Sarisha-9 x BARI Sarisha-6-F12	5.43 d	144.9 b	93.45 b
(SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2	5.06 f	149.1 a	96.19 a
LSD $_{(0.05)}$	0.02	0.31	0.16
CV (%)	0.15	0.12	0.10

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

Manganese (Mn)

The Manganese content of different released and advanced line of rapeseed and mustard were ranges from 78.48 ppm to 96.19 ppm in (Table 7). Significantly highest amount of Mn contained was found in (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (96.19 ppm) which was followed by BARI Sarisha-9 x BARI Sarisha-6-F12 (93.45ppm). The lowest amount was found in BARI Sarisha-14 (78.48 ppm) which was followed by BARI Sarisha-15 (83.45 ppm) and BARI Sarisha-9 x BARI Sarisha-6 (85.13 ppm). These treatments are statistically similar. The present values were supported by the reported value of Bachheti *et al.* (2012) and Josefson (1988).

4.4 Gross energy

Energy from carbohydrate of mustard and rapeseed varied significantly due to different released and advanced line (table 8). Significantly the highest amount of energy from carbohydrate found in two cultivars BARI Sarisha-9 x BARI Sarisha-6 (79.80 kca/g) and (SAU Sarisha-1 x SAU Sarisha-2)-F7 (75.44kca/g); while significantly lowest amount found in (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (59.56 kca/g). The significantly highest energy from protein observed from (SAU Sarisha-1 x SAU Sarisha-2)-F7 (117.5 kca/g), followed by BARI Sarisha-9 x BARI Sarisha-6-F12 (113.5 kca/g); whereas significantly the lowest amount of energy (102.2 kca/g) from protein observed from BARI Sarisha-9 x BARI Sarisha-6. The highest amount of energy from fat was observed in (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (365.2 kcal/g), which was significantly higher than all other released and advanced line cultivars, followed by BARI Sarisha-15 (361.2 kcal/g); whereas the lowest amount counted from (SAU Sarisha-1 x SAU Sarisha-2)-F7 (322.3 kca/g). The study found that gross energy of different released and advanced line cultivars of rapeseed and mustard ranged from 518.9 to 540.3 kcal/g. The statistically highest amount of gross energy found from (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (537.56 kcal/g), followed by BARI Sarisha-15(535.21 kca/g); while lowest amount of gross energy recorded from BARI Sarisha-9 x BARI Sarisha-6 (508.20 kcal/g) followed by (SAU Sarisha-1 x SAU Sarisha-2)-F7 (515.24 kcal/g).

Table 8. Proximate analysis of Gross energy from carbohydrates, Proteins and oils of different released and advanced line of rapeseed and mustard (*Brassica* spp.)

Name of the released and line cultivars (Treatments)	Energy from Oil (kcal/g)	Energy from Protein (kcal/g)	Energy from carbohydrae (kcal/g)
BARI Sarisha-14	357.1 c	112.0 c	64.52 d
BARI Sarisha-15	361.2 b	106.5 d	67.51 c
BARI Sarisha-9x BARI Sarisha-6	326.2 e	102.2 e	79.80 a
(SAU Sarisha-1 x SAU Sarisha-2)-F7	322.3 f	117.5 a	75.44 b
BARI Sarisha-9 x BARI Sarisha-6-F12	347.7 d	113.5 b	64.31 d
(SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2	365.2 a	112.8 bc	59.56 e
$LSD_{(0.05)}$	1.52	1.52	0.45
CV (%)	0.47	0.75	0.36

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

CHAPTER V

SUMMARY AND CONCLUSION

From my research work we observed that, the highest 1000 grains weight was found in (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (4.50 g) and the lowest thousand grains weight was found in BARI Sarisha-15 (4.46 g). The highest moisture content (5.02%) was observed from BARI Sarisha-9 x BARI Sarisha-6; whereas the lowest moisture content (4.40%) was found in (SAU Sarisha-1 x SAU Sarisha-2)-F7. Significantly highest amount of dry matter contained was recorded in (SAU Sarisha-1 x SAU Sarisha-2)-F7 (95.60%); while the lowest amount of dry matter contained was found in BARI Sarisha-9 x BARI Sarisha-6 (94.98%).

The oil content of different released and advanced line of mustard and rapeseed varied from 35.81% to 40.58% .The variety (SAU Sarisha-1 x SAU Sarisha-2)-F7 (35.81%) had the lowest amount of oil contained, while the variety (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (40.58%) contained significantly highest amount of oil. The (SAU Sarisha-1 x SAU Sarisha-2)-F7 contained statistically highest amount of oil cake (64.19%), and the lowest value was found in (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (59.42%). The highest dry weights of cake were obtained from SAU Sarisha-2 (56.56%). The highest value was obtained from (SAU Sarisha-1 x SAU Sarisha-2)-F7 (59.79%) and the lowest value obtained from (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (54.99%).

The highest saponification value was found in BARI Sarisha-14 (169.6). Values recorded for all released and advanced line were significantly higher than BARI Sarisha-9 x BARI Sarisha-6 (162.3). The highest amount of iodine value were observed in BARI Sarisha-14 (108.8) and the lowest amount of iodine value recorded in BARI Sarisha-9 x BARI Sarisha-6 (97.37). The highest acid value was found from (SAU Sarisha-1 x SAU Sarisha-2)-F7 (1.78); whereas the lowest acid value was found from BARI Sarisha-9 x BARI Sarisha-6-F12 (1.28).

Significantly the highest amount of palmitic acid was observed in BARI Sarisha-9 x BARI Sarisha-6 (4.43%) and the lowest amount of palmitic acid content was observed in BARI Sarisha-15 (2.78%). The concentration of stearic acid varied from 1.18% to 1.66%; whereas arachidic acid contents ranged from 4.56% to 5.63%. (SAU Sarisha-1 x

SAU Sarisha-2)-F7 contained the highest amount (1.49%) 0f behenic acid. (SAU Sarisha-1 x SAU Sarisha-2)-F7 contained the highest amount (17.15%) of oleic acid and the lowest amount was found in BARI Sarisha-15 (14.36%) which was significantly lowest among all the varieties and advanced lines. The highest amount of lenoleic acid contained was found in BARI Sarisha-9 x BARI Sarisha-6 (19.37%), and lowest amount (14.67%) was found in BARI Sarisha-9 x BARI Sarisha-6-F12. The concentration of linolenic acid varied from 6.45 to 7.83%. BARI Sarisha-15 contained the highest amount of erucic acid (47.75%) and the lowest amount was found in BARI Sarisha-9 x BARI Sarisha-6 (37.37%) which was significantly lowest among all the varieties and advanced lines. Mustard and rapeseed oils included unsaturated fatty acid ranging from 80.16% to 86.54%; while only a minor fraction by saturated fatty acids (10.03% to 11.92%).

Ash content of different released and advanced line of mustard and rapeseed were variable and ranged from 11.24% to 13.46%. Highest amount of protein was obtained from (SAU Sarisha-1 x SAU Sarisha-2)-F7 (29.37%), the lowest amount of protein content (25.56%) showed by BARI Sarisha-9 x BARI Sarisha-6. The amount of carbohydrate contained found in BARI Sarisha-9 x BARI Sarisha-6 (19.95%) was highest than other released and advanced line of rapeseed and mustard. The lowest amount of carbohydrate was obtained from (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (14.89%).

Significantly highest amount of calcium (Ca) content was observed in BARI Sarisha-15 (1.09%), while the lowest amount of calcium content was obtained from (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (0.98%).The highest amount of magnesium content was found in BARI Sarisha-15 (0.61%) and the lowest amount in (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (0.547%). Significantly highest amount of phosphorus (P) content was observed in (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (0.99%) and the lowest amount of phosphorus content was obtained from BARI Sarisha-14 (0.77%) .Significantly highest amount of Cu contained observed in BARI Sarisha-15 (5.68 ppm), and the lowest amount observed in (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (5.06 ppm). Significantly highest amount of Fe contained was observed in (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (149.1 ppm). The variety BARI Sarisha-14 showed lowest amount of Fe (121.7 ppm).Significantly highest amount of Mn contained was found in (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (96.19 ppm), while the lowest amount was found in

BARI Sarisha-14 (78.48 ppm). The highest amount of gross energy found from (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 (537.56 kcal/g); while lowest amount of gross energy recorded from BARI Sarisha-9 x BARI Sarisha-6 (508.20 kcal/g).

From my experiment, it was observed that none of the released and advanced line of rapeseed and mustard performed the best by all nutrient parameters. But the advanced line (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 performed the best considering the oil contents & gross energy. In case of fatty acid composition, BARI Sarisha-14 performed good results and content good erucic acid fraction. (SAU Sarisha-1 x SAU Sarisha-2)-F7, BARI Sarisha-15 showed the good performance for the most mineral contents. BARI Sarisha-14 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 advanced line of (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2, BARI Sarisha-15, (SAU Sarisha-1 x SAU Sarisha-2)-F7**,** BARI Sarisha-14 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 the experiment we can recommend that (SAU Sarisha-1 x SAU Sarisha-2) x F1 x SAU Sarisha-2 and BARI Sarisha-15 are the best.
- 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.

CHAPTER VI

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CHAPTER VII

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)