

**CHARACTERIZATION, PROXIMATE COMPOSITION AND  
MINERAL PROFILE ANALYSIS OF DIFFERENT SUNFLOWER  
(*Helianthus annuus* L.) GENOTYPES**

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**CERTIFICATE**

*This is to certify that the thesis entitled “**CHARACTERIZATION, PROXIMATE COMPOSITION AND MINERAL PROFILE ANALYSIS OF DIFFERENT SUNFLOWER (*Helianthus annuus* L.) GENOTYPES**” submitted to the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in BIOCHEMISTRY**, embodies the result of a piece of bona fide research work carried out by **MUNSHI MOHAMMAD SUMON**, Registration No. **13-05332** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

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***DEDICATED TO-***

***My Beloved Parents and  
Respected Research Supervisor***

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**June, 2020**

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**ABSTRACT**

A comparative study was done to find out physical-chemical properties of hybrid, high yielding (HY) and indigenous varieties of sunflower seeds collected from three local markets. In this study seed weight, moisture%, ash%, protein%, and fat% of whole seed, chemical constant, and fatty acid profile of oil, and minerals composition of oilcake were investigated. Among the varieties, BARI Shurjomukhi-2 (HY) showed the highest thousand grain weight ( $69.79 \pm 2.06$  g), moisture content ( $6.29 \pm 0.09\%$ ), oil content ( $36.46 \pm 0.40\%$ ), and crude protein ( $25.03 \pm 0.89\%$ ). In case of chemical constant, for sunflower oil, BARI Shurjomukhi-2 contained the highest saponification value ( $237.01 \pm 2.17$ ), iodine value ( $131.64 \pm 0.46$ ), and lower acid value ( $0.60 \pm 0.04$ ) than in other oil. For fatty acid composition, BARI Shurjomukhi-2 contained the highest stearic acid ( $9.60 \pm 0.39\%$ ) and oleic acid ( $46.13 \pm 0.89\%$ ) while Canadian (hybrid) showed the highest content of palmitic acid ( $9.15 \pm 0.14\%$ ) and BARI Shurjomukhi-3 linoleic acid ( $53.80 \pm 1.05\%$ ). Maximum ash content was recorded in Taiwan (hybrid) ( $3.20 \pm 0.03\%$ ). In terms of mineral composition, the highest amount of magnesium ( $0.57 \pm 0.01\%$ ), sulfur ( $0.090 \pm 0.001\%$ ), potassium ( $0.90 \pm 0.03\%$ ), phosphorous ( $1.42 \pm 0.01\%$ ), copper ( $47.75 \pm 0.05$ ppm), zinc ( $73.36 \pm 0.03$ ppm), and iron ( $119.75 \pm 0.05$ ppm) observed in the oilcake of hybrid (Taiwan) variety. The highest content of calcium ( $0.2300 \pm 0.01\%$ ) and manganese ( $45.50 \pm 0.6$ ppm) was found highest in Local variety. Therefore, Hybrid (Taiwan) showed best result for mineral composition but in terms of total physico-chemical aspect, HY (BARI-Shurjomukhi-2) performed better over hybrid (Taiwan) and Local varieties.

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## LIST OF ABBREVIATIONS AND ACRONYMS

### Full Word Abbreviation

And others	<i>et al.</i>
Coefficient of variation	CV
Poly Unsaturated Fatty Acid	PUFA
Days after flowering	DAF
World Health Organization	WHO
Non-Protein Nitrogen	NPN
Hours	h
Cultivars	cv.
Fatty acid	FA
Liter	L
Titer Value	TV
Saponification Value	SV
Iodine value	IV
Acid value	AV
Normality	N
Analysis of variance	ANOVA
United States Department of Agriculture	USDA
Association of Official Analytical Chemist	AOAC
Bangladesh Agriculture Research Institute	BARI
Degree Celsius (Centigrade)	°C
Least significant difference	LSD
Gram	g
Miligram	mg
Milliliter	ml
Parts per Million	ppm
Food and Agriculture Organization	FAO
Microgram	µg
Microliter	µl

## CHAPTER I

### INTRODUCTION

Oil crops supply many nutrients and minerals in both human and animal nourishment (Kollathova *et al.*, 2019; Das *et al.*, 2017). The sunflower is one of the major cultivated oilseeds crops with global production of about 21.2 million metric tons in 2019/20 (STAT, 2020). In Bangladesh, annual sunflower production is about 1.8 thousand metric tons (BBS, 2019).

The cultivated sunflower (*Helianthus annuus* L.) belongs to the family of Asteraceae (Compositae). *Helianthus* comes from the Greek word Helios, meaning sun, and anthos, meaning flower, and from Latin *annuus*, meaning annual. The property of this flower ‘turning with the sun’ is clearly expressed by the French and Spanish languages when naming this plant. The flower is appreciated through the world (Sanchez-Muniz *et al.*, 2016).

Sunflower has high demand as it contains low cholesterol that’s why it is good for health (Khatun *et al.*, 2016). High-calorie nutrients in sunflower oil have become a vital constituent of the human diet. The presence of polyunsaturated fatty acids  $\omega$ -6 and  $\omega$ -9 in sunflower oil protects us from atherosclerosis and improves the activity of the liver, the kidney, and the gall bladder and also has the ability to control blood cholesterol level and prevent heart disease (Ahmadian *et al.*, 2019; Gunstone, 2011). In contrast to other edible oils, sunflower oil has high levels of Linoleic acid that is resistant to oxidation during storage (Martinez-Force *et al.*, 2015). Unsaturated fatty acids such as oleic and linoleic may reduce the risk of cardiovascular diseases (Farvid *et al.*, 2014). Oleic acid also has oxidative stability during the extraction and

refining processes and storage and for use in fried foods (Carvalho *et al.*, 2019; Perdomo *et al.*, 2015). In particular, a diet rich in monounsaturated fatty acids decreases the cholesterol level connected with low-density lipoproteins (“harmful cholesterol”) and has zero effect on the level of the triglycerides or on the cholesterol connected with high density lipoprotein, if compared to a diet rich in saturated fatty acids (Grundy, 1986). Recent studies have clarified the antioxidant properties of oleic acid (Berry, 1997). When oleic acid content increase in the tissues, in conditions of high oxygen stress (oxygen toxicity) that helps to form better cellular protection than a similar increase in polyunsaturated fatty acids (Kinter *et al.*, 1996). Some scientist have demonstrated that there is an inverse relationship between the consumption of monounsaturated fatty acids and occurrence of colon cancer (Baldini *et al.*, 2002; Chyou *et al.*, 1996).

Not only the oil but also the whole seed can be used as medicinal ointments, bread, pigments and a snack food, as an element in baked foodstuffs, salads, and candies and for feeding birds and small animal pets. Press cake and oil meal are protein-rich animal feed and a rich reservoir of essential amino acids such as lysine, methionine, cystine, tryptophan, as well as of calcium, phosphorus, and B group vitamins (Sanchez-Muniz *et al.*, 2016). Sunflower oil meal is a good source of vitamin E (Demirel *et al.*, 2004). Average of 39.6% of protein, 1.6% of fat, 12.3% of crude fiber, 23.5% of nitrogen-free extractives, and 6.0% of ash can be easily obtained from sunflower press cake (Sanchez-Muniz *et al.*, 2016). Sunflower oilcakes are also good source of minerals but many factor such as species, soil, climatic conditions, agro-technics and breeding influence the mineral content in oilcakes (Galik *et al.*, 2016). Although many researchers studied the composition of sunflower oil,

comparative analysis of the chemical composition of seed and mineral profile of cake among different genotypes in Bangladesh. But we found very few studies related to the comparison of proximate composition and mineral profiling of available High yielding varieties, Hybrid and Local sunflower varieties found in different markets in Bangladesh. The purpose of our qualitative comparison study was to obtain protein rich feed from by-products and quality oil from sunflower seed.

### **Objectives**

1. To compare the physico-chemical parameters of different of sunflower varieties.
2. To determine fatty acid composition of different genotype of sunflower oil.
3. To determine the mineral composition of different varieties of sunflower oilcake.

## CHAPTER II

### REVIEW OF LITERATURE

Vegetable oils and fats constitute an important component of human diet, ranking third after cereals and animal products. Sunflower is important oil crop in human and animal nutrition because of its high level of fats/oil, fatty acid, protein and other nutritionally valuable substances. Physical properties, chemical analysis, and estimation of minerals of different types of sunflower observed by other researcher are described in this section.

#### **Grain Weight**

Thousand seed weight is a very prominent character of sunflower, where highest consideration is one of the seed yields. A good number of literature are available on the variability of this trait.

Baydar and Erbas (2005) conducted an experiment to evaluate dry weight of sunflower seeds obtained from different maturity stages and positions on the head. The heads were harvested sequentially 10, 15, 20, 25, 30, 35, 40, and 45 days after flowering (DAF) in 2002 and 2003. Dry weight per seed at 2002 was observed 36.10-56.40 mg where at 2003 was 22.80-49.60 mg. In 2002, seed weight increased remarkably at 25 DAF and maximized at 30 DAF, at 56.40 mg. In 2003, seed weight increased gradually up to the last harvest date at 52.00 mg, respectively.

Seiler (1983) examined the effects of flowering date on seed weight for 38 wild *H. annuus* populations grown at a single location. Average dry weight per seed (mg) remained relatively constant from the first flowering date through the eighth date and ranges from (4.30-8.70 mg). Thereafter, there was a decline to a low at the last flowering date. Seed weight for cultivated sunflower followed a similar pattern and ranges

from (27.20-47 mg).The continued decrease in weight per seed may be due to the strong influence that number of seeds per head has on seed weight. Wild *H. annuus* is multiple-headed and flowers over several weeks with larger heads with more seeds being produced early, and smaller heads with less seeds being produced later.

Earle *et al.* (1968) showed the comparative data on the composition of achene from six sunflower varieties. They found that 1000 seeds weight of Armavirec, Peredovik, Vniimk 8931, Smena, Krasnodarets, Arrowhead and Mingren were 59 g, 86 g, 62 g, 65 g, 86 g, 91 g, and 77 g, respectively.

### **Moisture**

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

Nadeem *et al.* (2010) was conducted a research work to characterize the sunflower hybrids and assess their suitability for the preparation of bread. They summarized that moisture content in sunflower oilseeds varied from 3.06 to 6.32%.

Anjum *et al.* (2006) was observed the microwave roasting effects on the physicochemical composition of sunflower seed oil. The moisture contents of the unroasted sunflower oilseeds of variety KL-39 and FH-330 were 7.00 and 6.30%, respectively.

Earle *et al.* (1968) investigated that moisture content of different variety of sunflower was ranged from 5.00% to 8.20%. They found the highest moisture content (8.20%) was observed from Mingren; while the lowest moisture content (5.00%) was found in Krasnodarets.



## **Ash**

Ash define as 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.

Kollathova *et al.* (2019) worked to investigate mineral profile of oilseeds and their by-products as feeding sources for animal nutrition. They found that ash content in different oilseeds such as sunflower, soybean, flaxseed and rapeseed were 3.6%, 5.3%, 3.9%, and 3.6%, respectively.

Nadeem *et al.* (2010) was conducted a research word to characterize the sunflower hybrids and assess their suitability for the preparation of bread. They summarized that ash content in different sunflower hybrid ranged from 2.68-3.13%.

Anjum *et al.* (2006) was observed the microwave roasting effects on the physicochemical composition of sunflower seed oil. Content of ash of the unroasted sunflower oilseeds of variety KL-39 and FH-330 were 5.00% and 5.50%, respectively.

Earle *et al.* (1968) observed that ash content of different variety of sunflower was ranged from 1.80% to 3.70%. They reported the ash content of different variety were 3.30%, 3.20%, 3.60%, 3.70%, 2.80%, 2.40%, and 1.80% for Armavirec, Peredovik, Vniimk 8931, Smena, Krasnodarets, Arrowhead, and Mingren, respectively.

## **Oil**

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

Akkaya *et al.* (2018) determine the oil ratios of sunflowers cultivated in the warm climate of the Cukurova region at different sowing dates. The mean crude oil ratios ranged from 38.82% to 44.42%, the highest crude

oil ratio (44.42%) was found in the standard type sunflower variety, Tunca. When we evaluated the crude oil ratios according to sunflower types, the highest crude oil ratio was found in standard type sunflowers with 42.57%. When we evaluated the data according to sowing time, the highest crude oil ratio was determined to be 47.90% in SD1, for the standard type sunflower variety, Tunca.

Mandal *et al.* (2006) evaluated a total of 167 samples of sunflower (*Helianthus annuus* L.) genotypes, introduced from France were grown during the winter season (rabi) 2002-03 and (rabi) 2003-04 at the farm of NBPGR, New Delhi, and evaluated for seed oil content. Promising lines, with more than 40% oil content in seed were identified.

Baydar and Erbas (2005) conducted an experiment to evaluate variations in the contents of oil of sunflower seeds obtained from different maturity phases and positions on the head. The heads were harvested successively 10, 15, 20, 25, 30, 35, 40, and 45 days after flowering (DAF) in 2002 and 2003. In changes in oil contents in 2002, a sudden rise was commenced at 15 DAF and persistent until 35 DAF. Significant increases in oil content from 30.50% to 45.80% were detected in these phases of seed development. The oil content did not increase significantly from 30 to 35 DAF, after which it started to decline gradually from 45.80% to 42.60%. The accumulation pattern of the oil was similar in 2003, and the highest oil content was determined at 30 DAF with a value of 47.90%. Although the oil contents were higher from 10 to 30 DAF in 2003, 2002 was a better year than 2003 in terms of the oil content of the final seeds.

Seiler (1983) described that environmental factors, especially temperature, during the period of seed development and maturation, have been shown to affect both content and composition of oil in maturing cultivated sunflower seed. The effects of temperature on oil content,

however, have been variable. The average seed oil content for all populations was 25.7% and ranged from 17.20 to 34.20%. Oil content remained relatively stable from the 6 July flowering date through the 21 September date. Thereafter, there were slight increases in seed oil content to a maximum of 27.6% for the 5 October sampling. The oil content of wild *H. annuus* populations remained more stable and did not vary by flowering date as much as the oil content did in the commercial hybrid. Canvin (1965) reported that sunflower grown at a constant 21<sup>0</sup> C had a higher oil content than those grown at either a lower or higher temperature. Harris *et al.* (1978) showed that oil content decreased as temperature increased.

Thompson *et al.* (1979) reported that cultivated sunflower seeds were significantly related seed oil for wild *H. annuus* ranges from 19.00% to 35.90% with total solar radiation and day length with a mean of 25.40%. They found that oil content decreased as temperatures decreased. They postulated that these differences in responses may have been due to temperature effects at specific developmental stages or to factors other than temperature. Other factors indirectly related to temperature, such as total solar energy or day length, may influence the oil content and its quality.

Earle *et al.* (1968) found that oil content of different variety of sunflower was ranged from 21.00% to 51.40%. They found the highest oil content (51.40%) was observed from Peredovik; while the lowest oil content (21.00%) was found in Mingren.

Canvin *et al.* (1965) showed that the effect of temperature on the oil content of the oils from several oil seed crops. Plants of rape, safflower, sunflower, flax, and castor bean were cultivated at temperatures of 10, 16, 21, and 26.50<sup>0</sup>C for the period of seed development. When mature, the

seed was harvested from individual plants and analyzed for oil content. The oil content of sunflower was  $36.80 \pm 0.70\%$ ,  $37.30 \pm 1.50\%$ ,  $40.40 \pm 1.00\%$ ,  $36.40 \pm 1.30\%$  that wasn't stricken by temperature.

### **Chemical properties of Oil**

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

Rehab (2012) found that the effects of deep frying on physicochemical parameters such as acid value, iodine value were evaluated over 30 hours of the frying process. The pure oils were heated at  $180\text{ }^{\circ}\text{C}$ , then frozen French fried potatoes were fried every 30 minutes. Oil samples were taken every 5 hours and the entire continuous frying period was 30 hours. The initial acid values of the fresh oils under investigation were found according to frying period 0, 5, 10, 15, 20, 25, and 30 hour that was  $0.13 \pm 0.04$ ,  $0.25 \pm 0.03$ ,  $0.30 \pm 0.03$ ,  $0.35 \pm 0.05$ ,  $0.42 \pm 0.07$ ,  $0.53 \pm 0.03$ ,  $0.69 \pm 0.03$  respectively and iodine value were  $123.00 \pm 0.29$ ,  $121.50 \pm 1.05$ ,  $119.55 \pm 0.21$ ,  $118.10 \pm 0.31$ ,  $117 \pm 0.35$ ,  $114 \pm 0.90$ ,  $111.10 \pm 0.38$  respectively.

Galucio *et al.* (2011) said that sunflower oil (*Helianthus annuus*) is rich in unsaturated fatty acids considered essential to human health. The aim of this work is to characterize physical-chemically the sunflower oil. Iodine value ( $\text{g I}_2/100\text{g}$ ), Saponification value ( $\text{mg KOH/g}$ ), and Free Fatty Acid (%) were 122.8, 191.7, and 0.262, respectively.

Ali *et al.* (2009) determined the chemical constant of the seed oil of two sunflower lines when subjected to water stress at different growth stages where found iodine value  $112.08 \pm 3.38$ - $119.87 \pm 5.57$  in case of Gulshan 98 genotype and  $113.99 \pm 4.39$ - $128.34 \pm 3.78$  in case of Suncross. The iodine value of the oil decreased significantly in cv. Suncross with the

imposition of water stress at the reproductive stage, but this decreasing effect was not observed in cv. Gulshan-98. Saponification value were  $184.42 \pm 4.56$ - $188.52 \pm 4.89$  in case of Gulshan 98 where  $178.45 \pm 3.56$ - $186.67 \pm 4.56$  for Suncross genotype. The oil saponification value remained unaffected due to water stress. But water stress at reproductive stage there were significant difference between two genotype in case of saponification value.

Anjum *et al.* (2006) was observed the microwave roasting effects on the physicochemical composition of sunflower seed oil. Saponification values of the control sunflower oil samples of varieties KL-39 and FH-330 were 189.0 and 185.0 mg KOH/g, respectively. Iodine values of control oils of KL-39 and FH-330 were 138.0 and 140.0 g of I<sub>2</sub>/100 g of oil, respectively.

Earle *et al.* (1968) found that iodine value of different variety of sunflower was ranged from 114.6 to 136.5. They found the highest iodine value was observed from Vniimk 8931; while the lowest iodine value was found in Krasnodarets.

### **Fatty acid composition**

Sunflower oil has a special fatty acid composition. It contains different types of saturated and unsaturated fatty acid. Oleic acid, linoleic acid, palmitic acid and stearic acid are the most important fatty acids in sunflower oil.

Akkaya *et al.* (2018) determine the fatty acid composition of sunflowers cultivated in the warm climate of the Cukurova region at different sowing dates. The average percentages of oleic acid obtained in the study were 60.08%, 74.35% and 88.01% in standard, mid-oleic and high-oleic sunflower varieties. When the variation in oleic acid ratios were

examined according to the sowing date, it was seen that the sowing date in both of the high-oleic sunflowers, and the Saray variety of the mid-oleic sunflowers, did not affect the oleic acid ratio ( $p > 0.05$ ).

Reis Akkaya and Akkaya (2018) conducted an experiment to determine the fatty acid composition of oils extracted from sunflowers grown in East Mediterranean region. Samples were taken from 6 different sunflower varieties planted in three different locations during the harvest season. Oleic acid contents of oils extracted from sunflowers were 41.04-46.15% for standard-oleic acid types and 81.99% for high-oleic acid containing type in average. Average linoleic acid contents were 40.72-46.73% for standard-oleic type and 8.16% for high-oleic acid type. The results of this study showed that oils extracted from sunflower grown in East Mediterranean region had higher oleic acid content. Pre-sowing sunflower seeds classified in standard type turned out to be mid-oleic acid type when grown in this region.

Rehab (2012) found that the amount of C16:0, C18:0, C18:1, C18:2, C18:3, SAFE, MUFA, PUFA were  $7.60 \pm 0.41$ ,  $4.92 \pm 0.09$ ,  $19.50 \pm 0.31$ ,  $67.48 \pm 1.22$ ,  $0.50 \pm 0.02$ ,  $12.52 \pm 0.50$ ,  $19.50 \pm 0.31$ ,  $67.98 \pm 1.24$  percent respectively. Sunflower oil had significantly ( $P < 0.05$ ) the highest level of linoleic acid.

Gonzalez-perez and Vereijken (2007) showed in a review paper title “Sunflower proteins: overview of their physicochemical, structural and functional properties” that fatty acid composition in sunflower oil were Palmitic, Stearic, Oleic, Linoleic 5–7%, 2–6%, 15-37%, 51-73%, respectively.

Anjum *et al.* (2006) was observed the microwave roasting effects on the physicochemical composition of sunflower seed oil. The principal fatty acid components of the control sunflower oil varieties KL-39 and FH-330

were palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic acid (C18:2) in concentrations of 8.06, 7.42; 4.04, 3.35; 30.82, 39.68; and 53.93, 49.53%, respectively.

Baydar and Erbas (2005) showed that the composition of fatty acids changed significantly during seed development. Palmitic acid, stearic acid, oleic acid and linoleic acid comprised over 99% of total lipids on the average, and of these oleic and linoleic acids comprised over 85% of total fatty acids. In the first year, palmitic and stearic acid contents decreased significantly with seed development, reaching minimum values of 6.60% at 35 DAF and 4.80% at 30 DAF, respectively. In the second year, while palmitic acid fluctuated up to late maturity stages, stearic acid showed a regular decrease from 8.80% to 3.50%.

Seiler (1983) examined the effects of flowering date on fatty acid composition for 38 wild *H. annuus* populations and cultivated sunflower Hybrid 896 grown at a single location. In wild *H. annuus*, palmitic acid appeared variable (4.80-5.60%). It was high at the first flowering date and then decreased and became cyclic. A similar trend was observed in the commercial hybrid. Stearic acid in wild *H. annuus* seeds remained relatively stable for the first eight flowering dates and then increased and leveled off during the last three samplings. It ranges from (2.40-3.70%) and averaging 2.90%. Stearic acid in the commercial hybrid was more erratic in the early samplings but ended up at about the same level as in the wilds. It ranges from (1.80-3.50%) and averaging 2.40%. Oleic acid in wild *H. annuus*, varied from 12.80 to 31.10%, averaging 22.20%, and linoleic acid varied from 63.70 to 77.90%, averaging 69.10%. In case of commercial hybrid oleic acid varied from 15.80 to 49.70%, averaging 32.80%, and linoleic acid varied from 41.60 to 75.00%, averaging 58.50%. The general trend for linoleic acid was opposite that for oleic

acid. Linoleic acid increased from the first date of flowering to a maximum at the last date. Linoleic acid levels of wild *H. annuus* seeds began at a higher level than occurred in the commercial hybrid seeds. However, it increased about the same level in both wild and cultivated seeds by the last date.

Harris *et al.* (1978) reported that palmitic acid decreased from 19.60% to 6.20% and that stearic acid increased from 1.80% to 5.40% during seed development in sunflower. A rapid decrease in the synthesis of oleic acid and a rapid increase in the synthesis of linoleic acid were observed during the development of sunflower seeds, with no abrupt fluctuation. When the seeds developed from 10 to 45 DAF, oleic acid content decreased from 38.30% to 21.40% in 2002 and from 40.90% to 20.20% in 2003, linoleic acid increased from 46.00% to 65.60% in 2002 and 33.70% to 64.90% in 2003. Thereafter, oleic acid content significantly declined, while linoleic acid content significantly increased during seed development.

Dorrell and Whelan (1978) reported that oleic acid varied from 12.30 to 19.20%, averaging 14.80%, and linoleic acid varied from 74.40 to 82.60%, averaging 79.70%. They also reported that palmitic acid varies from 2.60 to 6.10%, averaging 3.50%, and stearic acid varies from 1.30 to 2.60%, averaging 2.00%.

Earle *et al.* (1968) showed the comparative data on the composition of achenes from sunflower varieties Armavirec, Peredovik, Vniimk 8931, Smena, Krasnodarets, Arrowhead and Mingren. Hand-separated achene components were analyzed. Kernel oil from seed raised in northern United States or southern Canada typically contains about 70% of linoleic acid. The result showed that the sunflower oil contain different amount of fatty acid such as myristic acid (0.10-0.20%), palmitic acid (5.00-6.00%),



palmitoleic acid (0.10%), stearic acid (4.40-6.50%), oleic acid (15.90-41.40%), linoleic acid (46.00-71.50%).

## **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.

Gonzalez-perez and Vereijken (2007) showed in a review paper title “Sunflower proteins: overview of their physicochemical, structural and functional properties” that dehulled seed of sunflower contain 20.40-40.00% crude protein where whole seed contain 10.00-27.10%, the value being strongly affected by the sunflower variety. About 87–99% of this is true protein. The other 1–13% originates from peptides, amino acids or other nitrogenous substances.

Anjum *et al.* (2006) was observed the microwave roasting effects on the physicochemical composition of sunflower seed oil. Contents of protein of the unroasted sunflower oilseeds of variety KL-39 and FH-330 were 24.94 and 21.00, respectively.

Earle *et al.* (1968) carried out an experiment to determine the protein content of different sunflower variety. They reported the protein content (%N×6.25) of different variety were 16.90%, 19.80%, 16.00%, 23.80%, 22.20%, 18.10%, and 15.90% for Armavirec, Peredovik, Vniimk 8931 , Smena, Krasnodarets, Arrowhead, Mingren, respectively. The crude protein contents are calculated by use of the conventional factor of 6.25 times the nitrogen percentage.

Canvin *et al.* (1965) showed that the effect of temperature on the fatty acid composition of the oils from several oil seed crops. Plants of rape, safflower, sunflower, flax, and castor bean were cultivated at temperatures of 10, 16, 21, and 26.5 °C for the period of seed development. The protein content of the seeds of all the species increased with increasing temperature. Protein content (% N × 6.25) of sunflower was 13.90 ± 0.40, 15.70 ± 0.70, 15.90 ± 0.30, 20.30 ± 0.50 at temperatures of 10, 16, 21, and 26.5°C.

### **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 sulfur and trace minerals are iron, manganese, zinc, and boron.

Kollathova *et al.* (2019) worked to investigate mineral profile of oilseeds and their by-products as feeding sources for animal nutrition. In case of macro minerals, whole seed of sunflower contained calcium, phosphorous, magnesium and potassium were 0.23%, 0.69%, 0.34%, and 0.87%, respectively where cake contained same macro miner were 0.33%, 1.44%, 0.42%, and 1.38%, respectively. Micro minerals such as copper, iron, manganese, and zinc for whole seed were 2.12 mg%, 3.38 mg%, 1.50 mg%, and 4.70 mg%, respectively and same minerals for cake were 3.19 mg%, 5.87 mg%, 2.105 mg%, and 5.89 mg%, respectively.

Nadeem *et al.* (2010) was conducted a research word to characterize the sunflower hybrids and assess their suitability for the preparation of bread.

The iron, zinc, copper, manganese, calcium, sodium, potassium and magnesium contents ranged from 5.57 to 8.85 mg% and 6.40 to 10.19 mg%, 9.05 to 14.19 mg% and 9.53 to 17.86 mg%, 6.83 to 8.73 mg% and 6.92 to 12.17 mg%, 2.11 to 3.72 mg% and 2.50 to 6.99 mg%, 3.19 to 3.90 mg% and 3.45 to 4.10 mg%, 44.02 to 45.86 mg% and from 44.43 to 46.65 mg%, 4.68 to 6.81 mg% and from 5.14 to 7.0 mg% and 7.34 to 16.36 mg% and 8.21 to 18.06 mg% between the whole sunflower seeds and dehulled seeds, respectively among different sunflower hybrids. On account of mineral content, FH-331 was found to be the best.

USDA (2008) gave the following composition for mineral contents in sunflower seeds. Per 100 g seeds contains calcium 78 mg, iron 5.25 mg, magnesium 325 mg, phosphorus 660 mg, potassium 645 mg, sodium 9 mg, zinc 5 mg, copper 1.80 mg, manganese 1.95 mg.

Gonzalez-perez and Vereijken (2007) showed in a review paper title “Sunflower proteins: overview of their physicochemical, structural and functional properties” that dehulled sunflower seed contain amount of macro minerals such as Potassium, Phosphorus, Sulfur, Magnesium, Calcium, and Sodium were 0.67–0.75%, 0.60–0.94%, 0.26–0.32%, 0.35–0.41%, 0.08–0.10%, and 0.02%, respectively.

## CHAPTER III

### MATERIALS AND METHODS

#### 3.1 Sample collection and preparation

The investigation was conducted at the Biochemistry laboratory of the Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh and the laboratory of Soil Resource Development Institute (SRDI), Khamarbari, Dhaka, Bangladesh between October, 2019 to February, 2020. Two high yielding varieties (HYV) released from Bangladesh Agricultural Research Institute (BARI) namely BARI Surjomukhi-2, BARI Surjomukhi-3, two hybrid (Candian OP and Taiwan KB2) and one Local variety of sunflower (*Helianthus annuus* L.) were selected for the study. Seeds were collected from different local market of Bangladesh. Sample were as T<sub>1</sub> = BARI Surjomukhi-3, T<sub>2</sub> = Local variety, T<sub>3</sub> = Candian (Hybrid), T<sub>4</sub> = Taiwan (Hybrid) and T<sub>5</sub>=BARI Surjomukhi-2.

#### 3.2 Brief description of varieties

(a) **BARI Surjamukhi-2:** Developed by Bangladesh Agriculture Research Institute (BARI), Gazipur, Bangladesh following open pollination and selection method. The seeds are 9-10 mm long and 4 mm broad. cylindrical or drop-shaped. Its grain is black in color. The grain size is medium or large

(b) **BARI Surjamukhi-3:** This is a composite variety evolved by BARI in recent. Its grain colour is bright black and near oval in shape. The grain is medium in size.

(c) **Canadian (Hybrid):** This is hybrid variety. The seeds are 10-11 mm long and 4 mm broad, cylindrical or drop-shaped. Its grain color is striped grey/black. The grain is large in size.



**(a) BARI Surjamukhi-3**



**(b) Canadian (Hybrid)**



**(c) BARI Sujramukhi-2**



**(d) Taiwan (Hybrid)**



**(e) Local**

Plate 1. Photograph of sunflower seeds.

**(d) Taiwan (Hybrid):** This is also hybrid variety. The seeds are 9-11 mm long and 4 mm broad, cylindrical or drop-shaped. Its grain is black in color. The grain size is medium or large.

**(e) Local:** This is local variety found in different district of Bangladesh mainly southern region. The seeds are 9-11 mm long and 4 mm broad, cylindrical or drop-shaped. Its grain is in striped grey colour. The grainsize is medium to large.

### **3.3 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.

### **3.4 Determination of thousand grain weight**

The mass was determined by randomly selecting 100 seed samples and weighing in an electronic balance of 0.0001 g sensitivity. The weight was then converted into 1000 seed mass.

### **3.5 Determination of moisture**

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

#### **Materials**

1. Aluminum moisture dish
2. Electrical weighing balance
3. Oven
4. Desiccator

#### **Procedure**

Empty aluminum moisture dish was weighted (W1) and 2.5 g sample (dried at normal temperature after harvest) was taken in a moisture dish and weighted (W2). The sample was spreaded evenly and placed without lid in oven and dried samples overnight at 100°C. The dishes were transferred to desiccators to cool. Aluminum dish was weighed after cooling (W3) (Aziz *et al.*, 2018).

Calculation:

$$\% \text{ Moisture} = \frac{W2 - W3}{W2 - W1} \times 100$$

### **3.6 Determination of ash**

In this method, destruction of organic matter is accomplished by high temperature oxidation. The critical factors are (i) nature of the ashing vessel, (ii) placement in the muffle furnace and (iii) ashing temperature and time. The vessels used for ashing ranges from silica to platinum crucible or dishes. The sample size can vary from 0.5 to 2.0 g, depending on expected concentration of elements to be determined. The temperature

used for ashing vary from 475 to 600<sup>0</sup>C or even more and time required for digestion can be from 4 to 12 hours (depending on the sample weight and type of sample) (Paul *et al.*, 2017).

Materials:

1. Silica crucible
2. Balance
3. Muffle furnace
4. Desiccators.

### **Procedure**

First a silica crucible was weighted and then 2.0 g of dried ground sample was taken in the silica crucible. The crucibles are placed in a cool muffle furnace (Protech, Model:PT-1700M). Care should be taken to see that the crucibles are away by at least 2 cm from the walls of the furnace. This is to avoid localized over-heating. Ashing is usually done by heating the furnace at 600<sup>0</sup>C for about 5 hours (temperature and duration of heating need to be standardized as these two factors vary from sample to sample). The crucibles were transferred into the desiccator and cooled them to room temperature and weighted. It was done immediately to prevent moisture absorption. The incineration repeated until constant weight was obtained.

Calculation:

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



## 3.7 Chemical Analyses

### 3.7.1 Oil extraction and cake preparation

Materials:

1. Electrical weighing balance
2. Soxhlet apparatus
3. Menthol heater
4. Petroleum ether (40-60<sup>0</sup>C)

#### Procedure

The sunflower oil was extracted using Soxhlet method (Aziz *et al.*, 2018) with some modifications. First, sunflower seed (50 g) was taken in aluminum dish and dried for 1 hour at 100°C temperature. Sunflower flour was prepared by using blender. Then sample was taken into 25x100mm cellulose soxhlet extraction thimble (Whatman 2800-250). The thimble was put into the soxhlet and 250 ml petroleum ether (AR grade) was poured into the soxhlet flask. Then it was connected to round bottom flask followed with condenser and total apparatus was placed on menthol heater (40-60°C temperature) for 16-20 hours. When extraction of oil was completed menthol heater turned off and permitted to cool. Allow the round bottom flask open for 1 days to remove the solvent from the extracted oil. Thimble was removed from the soxhlet and dried the sample in normal air. Finally, sunflower oil cake (a byproduct after getting oil) was collected. The yield of sunflower oil was calculated by following formula.

$$\text{Oil percentage of seeds} = \frac{\text{Weight of extracted oil}}{\text{Weight of seeds}} \times 100$$

### **3.7.2 Estimation of total protein content by Microkjeldhal method**

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

#### **Reagents**

Digestion mixture: 100 g of potassium sulphate ( $K_2SO_4$ ) was thoroughly mixed with 20 g of copper sulfate ( $CuSO_4 \cdot 5H_2O$ ) and 2.5 g selenium dioxide ( $SeO_2$ ) was added with it.

Sodium hydroxide solution (60%): 600 g sodium hydroxide and 50 g sodium thiosulphate were dissolved in distilled water, cooled and made the volume up to 1 liter.

Boric acid: 40 g of boric acid was dissolved in water and made up to 1 liter.

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.

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 sunflower cake sample (100 mg) was 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).

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

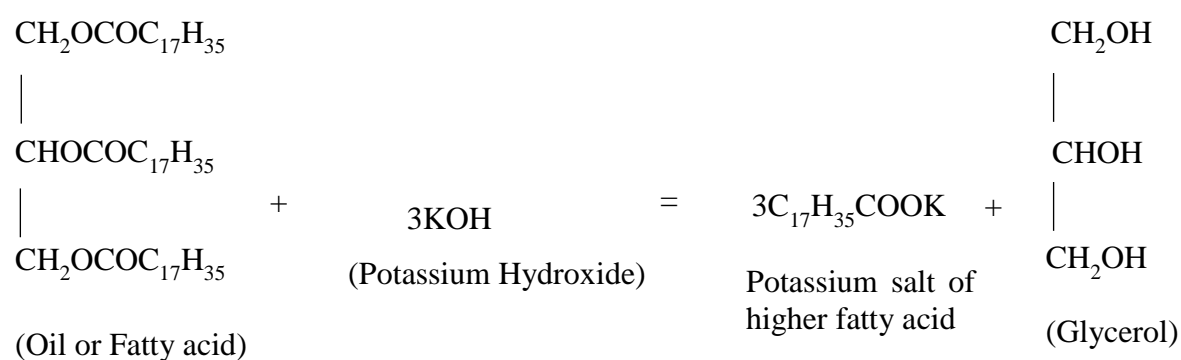
Where 14.007 is the equivalent weight of nitrogen.

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

### 3.7.3 Chemical constant

#### 3.7.3.1 Determination of Saponification value and saponification

**equivalent:** 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.



The Saponification equivalent (groups of saponified per equivalent of base) is proportional to the average molecular weight of the fatty acids present in the lipid.

#### Reagents

Hydrochloric acid 0.5 N: 20.9 ml 37% HCl mixed with water for 500ml solution.

Alcoholic solution of Potassium hydroxide. Take 14.02 g potassium hydroxide and dissolve it in very little water. Make up to 500 ml by adding rectified spirit (C<sub>2</sub>H<sub>5</sub>OH) of specific gravity 0.81.

Phenolphthelin solution, 1% in alcohol: 0.2g phenolphthelin mixed with ethanol to make up to 20 ml.

## Procedure

Oil was taken (1-2 g) in bottom flask. Added 25 ml of 0.5 N alcoholic potash solutions and fit the flask with a cork and a long air condenser. Reflux the contents of the flask for about 30 minutes by heating on boiling water bath. 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 experiment (B) without fat (25 ml of the same alcoholic KOH heated in a similar way is titrated, against 0.05 N acid) (Sharif *et al.*, 2017).

Calculation:

$$\text{Saponification value} = \frac{56.1 \times (B - A) \times \text{strenght of acid}}{\text{Weight of substance in g}}$$

$$\text{Saponification Equivalent} = \frac{56100}{\text{Saponification value}}$$

### 3.7.3.2 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 (Sharif *et al.*, 2017).

### Reagents required

Sodium thiosulphate (0.1 N): 12.41 g sodium thiosulphate pentahydrate mixed with water to make up to 500 ml.

Starch solution (indicator): Starch (1g) was dissolved in 50 ml hot water and diluted to 100 ml with cold water.

Hanus solution: Iodine (13 g) was dissolved in glacial acetic acid. Bromine (3 ml) was added to it and the solution was diluted with glacial acetic acid to 1 liter.

15% Potassium iodide solution: Dissolve 75 g KI crystals in water and make up the volume to 500 ml.

### **Procedure**

In a 500 ml dry glass stoppered bottle, added 5ml of given oil sample (the oil sample was dissolved in 10 ml chloroform (the concentration of the oil sample is 5%).

To the content of the bottle 25 ml of Hanus solution was added and the mixture was allowed to stand in the dark for exactly 30 minutes with occasional shaking.

Potassium iodine (10 ml) solution was mixed to it and the mixture was shaken well.

Freshly boiling cooled water (100 ml) was added to the mixture and the content of the bottle was titrated with 0.1 N sodium thiosulphate solution, using starch solution as indicator.

A blank experiment (without the oil) was performed exactly in the same manner as described above.

Calculations:

$$\text{Iodine value} = \frac{S \times (X - Y) \times 0.127 \times 100}{W}$$

Where, S=Strength of the sodium thiosulphate solution.

X= ml of sodium thiosulphate solution required in the blank test.

Y= ml of sodium thiosulphate solution required in true test.

W=Weight of the oil taken in grams.

### **3.7.3.3 Acid value and Free fatty acid (as oleic acid)**

The acid number or value of a fat or oil tells the amount of free fatty acid present in it. The acid number is expressed as the Number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. The acid number of a fat can give the extent of rancidity in a stored sample. A fat which has been both processed and stored properly has a very low acid number. During storage fats may become rancid as a result of peroxide formation at the double bonds by atmospheric oxygen and hydrolysis by micro-organisms with the liberation of free fatty acid. The amount of free fatty acid present therefore gives an indication of the age and quality of the fat.

#### **Reagents**

Phenolphthalein: 1 percent solution in alcohol neutralized with 0.1 N NaOH.

Denatured alcohol (Neutral): Mix 10 volumes of ethyl alcohol with 1 volume of methyl alcohol and neutralize with N/4 NaOH using phenolphthalein as indicator.

0.25N Sodium hydroxide and 0.1N Sodium hydroxide.

0.05 N potassium hydroxide solution

#### **Procedure**

Oil sample (5-7 g) in 250 ml conical flask was taken and added 50 ml denatured alcohol (neutral) and shaken well. Then 2 ml of phenolphthalein as indicator was added and titrated against 0.05 N potassium hydroxide with vigorous shaking after each addition till a permanent light pink color was produced which persists for at least 1 minute (Sharif *et al.*, 2017).

Calculation:

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

Where, N= Strength of potassium hydroxide

V= Number of ml of aqueous potassium hydroxide required for titration

W= Weight of the oil taken in the gram

### **Free fatty acid**

Percentage of free fatty acid (oleic acid) was calculated by using the formula:

$$\% \text{ Free fatty acid (as oleic acid)} = \frac{\text{Acid value} \times M}{561}$$

Where,

M= molecular weight of the oleic acid.

### **3.7.4 Estimation of fatty acid composition**

Fatty acid composition was determined by Gas-liquid chromatographic method (Uppstrom *et al.*, 1978).

### **Reagent**

Ethylate reagent (Petroleum ether / 0.02M sodium hydroxide in ethanol (2/3)

A Salt solution (80 g NaCl and 3g Sodium hydrogen Sulphate in 1 litre water).



## **Procedure**

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).

The sample was extracted and transesterified at the same time with 5 ml ethylated reagent and shaken.

The samples were kept for overnight at room temperature.

10 ml salt solution was added and shaken. As soon as the two layers were separated, the benzene phase was transferred to small test tubes.

A Philips PU 4500 chromatograph instrument was used with flame ionization detector (FID).

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° C, 185° C and 240° C, respectively.

Nitrogen flow (used as carrier gas) rate was 22 ml/min, the injection volume was 2µl. Peak areas were measured with an electronic digital integrator (Shinadzu C-R6A chromatopac).

### **3.8 Estimation of minerals**

#### **3.8.1 Preparation of reagents**

##### **3.8.1.1 Reagents for P determination**

###### **Reagent A**

Added antimony trioxide (45 g) in 400ml water then added 150ml conc. H<sub>2</sub>SO<sub>4</sub> and allowed to cool.

Ammonium molybdate (7.5 g) was dissolved in 300ml water. Cool antimony solution and molybdate solution was mixed by adding 1 liter of water.

## **Reagent B**

Gelatin (1 g) was dissolved in 100 ml hot water.

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

### **3.8.1.2 Reagent for Ca and Mg determination**

#### **1% Lanthanum solution**

Fifty nine gram of lanthanum oxide ( $\text{La}_2\text{O}_3$ ) were added in 50 ml of water then added 250 ml conc.  $\text{H}_2\text{SO}_4$  gradually and carefully. It was made up to 5 liters by adding water.

### **3.8.1.3 Reagents for S determination**

#### **Mixed acid seed solution**

Firstly 65 ml of conc.  $\text{HNO}_3$  and 250 ml glacial acetic acid were added to about 500 ml of water. Then 3 ml of 1000 ppm S standard solution was added and made volume to one liter with water.

#### **Turbidimetric reagent**

Ten g of polyvinyl pyrrolidone (PVP K30) was dissolved in about 100 ml of hot water along with 150 g of  $\text{BaCl}_2 \cdot 2 \text{H}_2\text{O}$  was dissolved in about 500 ml of water. The PVP and barium chloride solutions were mixed and were made to liter with water

### **3.8.2 Preparation of standards**

For standard calibration Cu, Fe, Mn and Zn were prepared together in water following concentration as  $2\mu\text{g}$  Cu/ml,  $10\mu\text{g}$  Fe/ml,  $4\mu\text{g}$  Mn/ml,  $2\mu\text{g}$  Zn/ml.

The P, K and S were also prepared together in water as  $20\mu\text{g}$  P/ml,  $100\mu\text{g}$  K/ml,  $20\mu\text{g}$  S/ml.

Finally, Ca and Mg as 100 µg Ca/ml, 40 µg Mg/ml were prepared in the same solution.

### **3.8.3 Digestion solution**

Nitric-perchloric solution

Conc. Perchloric acid (100 ml) was added to 500 ml concentrated HNO<sub>3</sub> to prepare nitric-perchloric solution.

#### **3.8.3.1 Digestion of sample for determination of P, K, Ca, Mg, S, Cu, Fe, Zn and Mn**

##### **Digestion procedure**

Oil cake (500 mg) was taken into a 50 ml boiling flask. 5 ml of nitric-perchloric acid (5:1) solution was added and placed in digestion chamber. Started digestion for 1 hour and 30 minutes at 370°C temperature. After completely digestion removed from digestion chamber and was cooled and 15 ml water was added. For collection of aliquot the flask was slowly agitated and heated to suspend the ash and filter.

##### **3.8.4 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 4 ml of turbid metric 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 and K 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 by using spectrophotometer at 680 nm for P and by using a flame photometer (Model AnA-135, OSK, Japan) at

766.5 nm for K. 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 Atomic Absorption Spectrometer contraAA 700 (Analytik Jena). For Fe, Mn and Zn determination, the original filtrate was used to analyze these elements by Atomic Absorption Spectrometer (Sharif *et al.*, 2017).

### **3.9 Statistical Analysis**

The recorded data for each character from the experiments was analyzed statistically with a one way ANOVA to find out the variation resulting from experimental treatments using statistix 10 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 (LSD) Least Significance difference test (Gomez *et al.*, 1984).

## CHAPTER IV

### RESULTS AND DISCUSSION

Five released cultivars of sunflower (*Helianthus annuus* L.) were taken for the determination of physical and chemical characteristics. The seeds were stored in the refrigerator under a suitable storage condition. The proximate composition and some other nutrients compositions of sunflower seeds are also testified.

#### **Analytical studies of the whole seeds**

The proximate composition of whole sunflower seeds of different released cultivars is presented in different tables. The data have also been estimated on a moisture-free basis in order to allow for a better comparison of the different fractions. The data taken are the average of three replication and have been presented and discussed.

#### **4.1 Physical characteristics of sunflower released cultivars**

##### **4.1.1 Thousand grain weight**

The significant variations were observed among the different varieties in terms of the effect on the thousand-grain weight (Table 1). The highest weight of thousands grains was found in BARI Shurjomukhi-2 ( $69.79 \pm 2.06$  g), which was statistically identical with Taiwan (hybrid) ( $69.13 \pm 1.47$  g) and Canadian (hybrid) ( $69.67 \pm 0.82$  g). The lowest weight of thousand grains was found in BARI Sujomukhi-3 ( $54.94 \pm 0.73$  g), which also statistically identical with Local ( $56.16 \pm 1.41$  g) variety. The present values were supported by the reported values (Earle *et al.*, 1968; Baydar and Erbas, 2005). Seiler (1983) reported lower value (27.2-47 g) than the present investigation and demonstrated that grain weight vary due to flowering date. Maturity stages and positions of seeds on the head can

change the grain weight (Baydar and Erbas, 2005). Seed weight varied with their size and shape (Chowdhury *et al.*, 2014). Grain weight increased from 4.3 to 5.1 g as the moisture content increased from 7.00 to 15.99% (Damian, 2014).

#### **4.1.2 Moisture**

The moisture percentage of different sunflower genotypes was ranged from  $4.78 \pm 0.15\%$  to  $6.29 \pm 0.09\%$  (Table 1). The highest value of moisture percentage was found in BARI Shurjomukhi-2 ( $6.29 \pm 0.09\%$ ), which was statistically similar to BARI Shurjomukhi-3 ( $5.85 \pm 0.34\%$ ) and also followed by Local ( $5.59 \pm 0.37\%$ ) variety. The lowest value of moisture content was found in Canadian (hybrid) ( $4.78 \pm 0.15\%$ ), which was statistically identical with Taiwan (hybrid) ( $5.02 \pm 0.38\%$ ). Present results was similar with (Earle *et al.*, 1968 and Nadeem *et al.*, 2010). The reason of changing the moisture content may be due to too much drying the grain after harvest, storage period (Bhardwaj *et al.*, 1999). Apart from this maturation process, can change the moisture content of the grains by a series of morphological, physiological and functional changes that started from fertilization, continuing until the grains reach sufficient moisture for harvest (Ciabotti *et al.*, 2016).

#### **4.1.3 Dry matter**

A statistically significant variation was observed for dry matter content of different sunflower genotypes that have been presented in (Table 1). The highest dry matter content ( $95.22 \pm 0.15\%$ ) were observed from Canadian (hybrid), which was statistically identical ( $94.97 \pm 0.38\%$ ) to Taiwan (hybrid) and followed ( $94.14 \pm 0.34\%$  and  $94.40 \pm 0.37\%$ ) by BARI Shurjomukhi-3 and Local, respectively. The lowest dry matter content ( $93.71 \pm 0.10\%$ ) was found in BARI Shurjomukhi-2. Kollathova *et al.* (2019) reported the similar result.

**Table 1. Weight of thousand seed, moisture and dry matter of different released cultivars of sunflower.**

Name of the released cultivars (Treatments)	Weight of 1000 seeds (g)	Moisture %	Dry matter %
BARI Shurjomukhi-3	54.94 <sup>b</sup> $\pm$ 0.73	5.85 <sup>ab</sup> $\pm$ 0.34	94.14 <sup>bc</sup> $\pm$ 0.34
Local	56.16 <sup>b</sup> $\pm$ 1.41	5.59 <sup>b</sup> $\pm$ 0.37	94.40 <sup>b</sup> $\pm$ 0.37
Canadian (hybrid)	69.67 <sup>a</sup> $\pm$ 0.82	4.78 <sup>c</sup> $\pm$ 0.15	95.22 <sup>a</sup> $\pm$ 0.15
Taiwan (hybrid)	69.13 <sup>a</sup> $\pm$ 1.47	5.02 <sup>c</sup> $\pm$ 0.38	94.97 <sup>a</sup> $\pm$ 0.38
BARI Shurjomukhi-2	69.79 <sup>a</sup> $\pm$ 2.06	6.29 <sup>a</sup> $\pm$ 0.09	93.71 <sup>c</sup> $\pm$ 0.10
LSD (0.05)	2.54	0.54	0.54
CV (%)	2.17	5.34	0.31

In a column, means with a similar letter (s) are not significantly different and means with different letter (s) are significantly different by LSD at 5% level of significance. SD = standard deviation; CV = Coefficient of Variation; LSD = Least significant difference.

#### 4.1.4 Oil content

Different sunflower genotypes varied significantly in terms of oil content. The oil content of different sunflower genotypes was varied from 23.29  $\pm$  0.77% to 36.46  $\pm$  0.40% (Table 2). The highest amount of oil content was found (36.46  $\pm$  0.40%) to BARI Shurjomukhi-2, which was statistically similar (35.16  $\pm$  1.30%) to Taiwan (hybrid) and followed (34.36  $\pm$  1.20%) by Canadian (hybrid). The lowest amount of oil content was observed (23.29  $\pm$  0.77) by BARI Shurjomukhi-3, which was followed (29.86  $\pm$  1.40) by Local variety. The present values were accepted by the reported values of (Akkaya *et al.*, 2018). Earle *et al.* (1968) found that the oil% sunflower genotypes was ranged from 21.00% to 51.40%. The reason of such variation among genotypes for fluctuation of temperatures which have inverse relationship with oil content during seed

development but other environmental factors such water stress; that affect the oil production by changing growth and seed development, can interfere with temperature effect (Harris *et al.*, 1978). Besides of these maturity phases and positions of seed on the head changed oil content (Baydar and Erbas, 2005).

#### **4.1.5 Oil Cake**

A statistically significant variation was observed for oil cake of different sunflower genotypes that have been presented in (Table 2). Oil cake/meals are used for numerous purposes. Oil cake is a nutritive food item for cattle and fish. It is also used as good organic fertilizer and constituent of composts. The BARI Shurjomukhi-3 contained the highest amount of oil cake ( $76.707 \pm 0.77\%$ ) and followed ( $70.140 \pm 1.40\%$ ) by Local variety. The lowest amount of oil cake was observed ( $63.540 \pm 0.40\%$ ) by BARI Shurjomukhi-2, which was statistically similar ( $64.840 \pm 1.30\%$ ) to Taiwan (hybrid) and followed ( $65.640 \pm 1.20\%$ ) by Canadian (hybrid). The present values were supported by the reported values of (Akkaya *et al.*, 2018; Seiler, 1983 and Earle *et al.*, 1968).

#### **4.1.6 The dry weight of the cake**

Dry cakes are used to calculate the content of different nutrients which are crucial for our poultry feed, organic fertilizer, and other numerous purposes. The dry weights of the cake had been presented in (Table 2). The highest value was obtained from BARI Shurjomukhi-3 ( $70.85 \pm 0.59\%$ ), followed by Local variety ( $64.54 \pm 1.03\%$ ). The lowest value obtained from BARI Shurjomukhi-2 ( $57.25 \pm 0.50\%$ ), which was significantly lower than all the varieties and followed by Taiwan (hybrid) ( $59.81 \pm 1.16\%$ ) and Canadian (hybrid) ( $60.86 \pm 1.18\%$ ). The last two are statistically identical.



**Table 2. Oil content of sunflower seeds, oilcake content and dry weight of oilcake of different sunflower genotypes.**

Name of the released cultivars (Treatments)	Oil content (%)	Oil Cake (%)	Dry oil cake (%)
	Mean $\pm$ SD (n=3)		
BARI Shurjomukhi-3	23.29 <sup>d</sup> $\pm$ 0.77	76.707 <sup>a</sup> $\pm$ 0.77	70.85 <sup>a</sup> $\pm$ 0.59
Local	29.86 <sup>c</sup> $\pm$ 1.40	70.140 <sup>b</sup> $\pm$ 1.40	64.54 <sup>b</sup> $\pm$ 1.03
Canadian (hybrid)	34.36 <sup>b</sup> $\pm$ 1.20	65.640 <sup>c</sup> $\pm$ 1.20	60.86 <sup>c</sup> $\pm$ 1.18
Taiwan (hybrid)	35.16 <sup>ab</sup> $\pm$ 1.30	64.840 <sup>cd</sup> $\pm$ 1.30	59.81 <sup>c</sup> $\pm$ 1.16
BARI Shurjomukhi-2	36.46 <sup>a</sup> $\pm$ 0.40	63.540 <sup>d</sup> $\pm$ 0.40	57.25 <sup>d</sup> $\pm$ 0.50
LSD (0.05)	1.97	1.97	1.70
CV (%)	3.39	1.58	1.49

In a column, means with a similar letter (s) are not significantly different and means with different letter (s) are significantly different by LSD at 5% level of significance. SD = standard deviation; CV = Coefficient of Variation; LSD = Least significant difference.

#### 4.1.7 Protein

The highest amount of protein was obtained from BARI Shurjomukhi-2 (25.03  $\pm$  0.89%) which was statistically identical with Local (24.71  $\pm$  1.36%), Taiwan (hybrid) (24.42  $\pm$  0.69) and BARI Shurjomukhi-3 (24.20  $\pm$  1.54). On the other hand Canadian (hybrid) (22.38  $\pm$  1.31%) was contained statistically lowest amount of protein (Table 3). The present values were more or less similar to the reported values of (Earle *et al.*, 1968). Gonzalez-perez and Vereijken (2007) reported that the seed of sunflower contains 10.00-27.10% crude protein, the value being strongly affected by the sunflower variety. Protein content in oilseeds is genetically defined, environmental factors can vary in the nitrogen availability during grain formation (Hayati *et al.*, 1995). Some studies revealed that rainfall during the grain-filling stage may changes protein content rather than temperature effect, so this parameter can be linked to water stress that finally may affect productivity (Pipolo, 2002)

#### 4.1.8 Ash

Ash content of different sunflower genotype was variable and ranged from  $2.96 \pm 0.02\%$  to  $3.20 \pm 0.03\%$  (Table 3). Significantly highest amount of ash contained was recorded in Taiwan (hybrid) ( $3.20 \pm 0.03\%$ ), and followed Canadian (hybrid) ( $3.14 \pm 0.02\%$ ) and BARI Shurjomukhi-2 ( $3.11 \pm 0.03\%$ ). On the other hand, BARI Shurjomukhi-3 ( $2.96 \pm 0.02\%$ ), which was statistically the lowest amount of ash and followed by Local ( $3.06 \pm 0.02\%$ ). The present values was supported by (Earle *et al.*, 1968; Nadeem *et al.*, 2010 and Kollathova *et al.*, 2019). Anjum *et al.* (2006) showed that ash conten for unroasted sunflower oilseeds were 5.00-5.50%. In terms of quality, safety and availability of nutrients in nutrition, however, a lower content of crude ash in nutrient sources is desired (Galik *et al.*, 2016). Differences in crude ash content compared to the literature sources may be due to topographic and climatic factors Kollathova *et al.* (2019).

**Table 3. Protein and ash content in different sunflower genotypes.**

Name of the released cultivars (Treatments)	Protein%	Ash%
	Mean $\pm$ SD (n=3)	
BARI Shurjomukhi-3	24.20 <sup>ab</sup> $\pm$ 1.54	2.96 <sup>d</sup> $\pm$ 0.02
Local	24.71 <sup>a</sup> $\pm$ 1.36	3.06 <sup>c</sup> $\pm$ 0.02
Canadian (hybrid)	22.38 <sup>b</sup> $\pm$ 1.31	3.14 <sup>b</sup> $\pm$ 0.02
Taiwan (hybrid)	24.42 <sup>ab</sup> $\pm$ 0.69	3.20 <sup>a</sup> $\pm$ 0.03
BARI Shurjomukhi-2	25.03 <sup>a</sup> $\pm$ 0.89	3.11 <sup>b</sup> $\pm$ 0.03
LSD (0.05)	2.19	0.04
CV (%)	4.98	0.68

In a column, means with a similar letter (s) are not significantly different and means with different letter (s) are significantly different by LSD at 5% level of significance. SD = standard deviation; CV = Coefficient of Variation; LSD = Least significant difference.

## 4.2 Chemical characteristics of sunflower oil

### 4.2.1 Saponification value and Saponification equivalent

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 sunflower genotypes were ranges from  $150.04 \pm 2.17$  to  $237.01 \pm 2.17$  and had been presented in (Figure 1). The statistically highest Saponification value was found in BARI Shurjomukhi-2 ( $237.01 \pm 2.17$ ), which was followed by Taiwan (hybrid) ( $198.86 \pm 2.09$ ) and BARI Shurjomukhi-3 ( $195.70 \pm 2.17$ ) but both were statistically identical. The lowest value obtained from Local ( $150.04 \pm 2.17$ ) genotype and followed by Canadian (hybrid) ( $152.19 \pm 1.49$ ), but both were also statistically identical. Galucio *et al.* (2011); Ali *et al.* (2009); Anjum *et al.* (2006) observed similar result for sunflower oil.

The saponification equivalents of the five varieties sunflower seed oils are also given in (Figure 1), which is calculated from the saponification value as described in the materials and methods. It is directly proportional to the average chain length of the fatty acid present. Fats or oils consisting largely of C<sub>18</sub> fatty acids along with some palmitic acid, a little unsaponifiable matter, and a low acidity generally have saponification equivalent around 290.80; a higher value indicates the presence of an appreciable quantity of higher acids. Findings of the results clearly indicated that all five varieties of sunflower seeds contained mainly fatty acids of higher molecular weight (C<sub>18</sub> and above).

#### 4.2.2 Iodine value

Iodine value is defined as grams of iodine absorbed by 100 g fats/oil. It helps to estimate the degree of unsaturation. The iodine values of different sunflower genotypes have been presented in (Figure 1). The significant variations were observed among the different varieties in terms of the effect on iodine value. The statistically highest amount of iodine value was observed in BARI Shurjomukhi-2 ( $131.64 \pm 0.46$ ) followed by Taiwan (hybrid) ( $116.04 \pm 0.21$ ) and BARI Shurjomukhi-3 ( $110.54 \pm 0.49$ ). The lowest amount of iodine value recorded in Local ( $101.80 \pm 0.23$ ) genotype, followed by Canadian (hybrid) ( $103.25 \pm 0.23$ ). Earle *et al.* (1968) found that iodine value of different variety of sunflower was ranged from 114.6 to 136.5. Rehab (2012); Galucio *et al.* (2011); Ali *et al.* (2009) and Anjum *et al.* (2006) was observed the similar result.

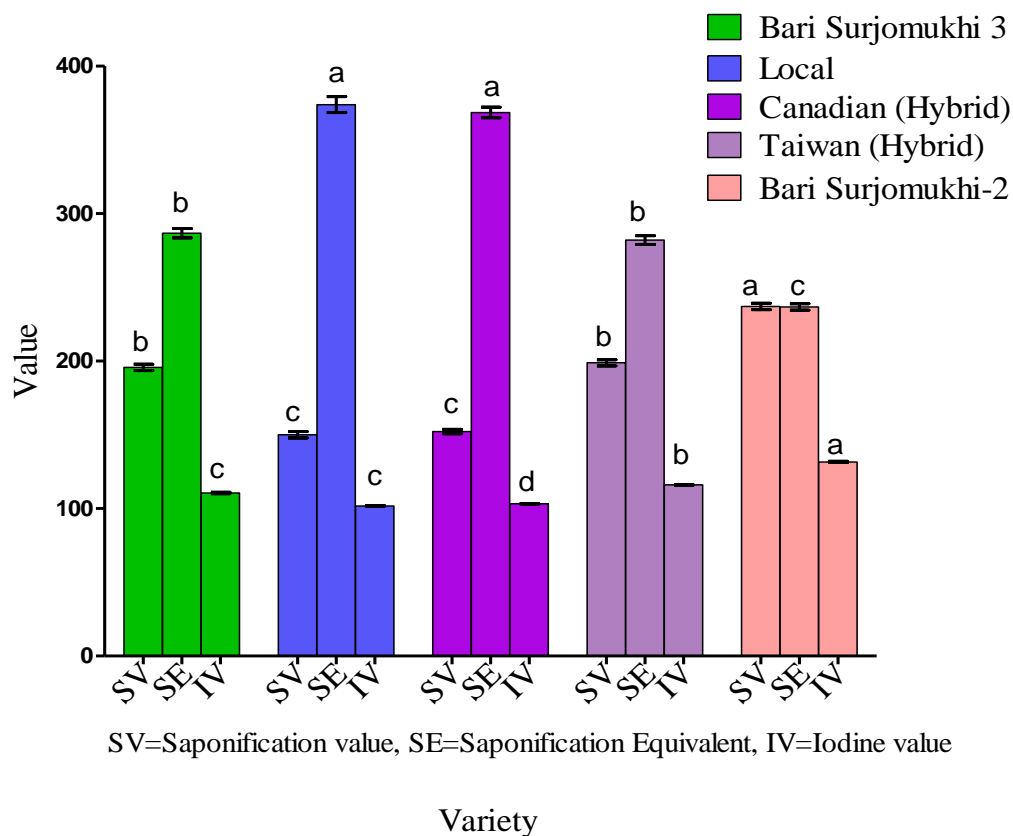


Figure 1. The chemical constants of sunflower oils.

### 4.2.3 Acid value and percentage of free fatty acid (as oleic)

The acid value is defined as the milligrams of KOH required to neutralize the free fatty acids present in 1 g of fats/oil. This value is used in determining the rancidity due to free fatty acids. Acid values of different sunflower genotypes had been presented in (Figure 2). The significant variations were observed among the different varieties in terms of the effect on Acid value. The highest acid value was found from Local variety ( $1.31 \pm 0.03$ ), followed by Taiwan (hybrid) ( $1.15 \pm 0.05$ ) and Canadian (hybrid) ( $0.80 \pm 0.02$ ); whereas the lowest acid value was found from BARI Shurjomukhi-2 ( $0.60 \pm 0.04$ ) followed by BARI Shurjomukhi-3 ( $0.70 \pm 0.02$ ). As shown in Figure 2, the percentage of free fatty acid as oleic is calculated from acid value and it was found to be varied from  $0.30 \pm 0.02\%$  to  $0.66 \pm 0.02\%$ . A high percentage of free fatty acid (above 1.5%) is a determinant or indication of the unsuitability of the oil for edible purposes. So the oils of the five varieties of sunflower seeds might be suitable for edible purposes. Our findings supported by (Galucio *et al.*, 2011; Rehab, 2012).

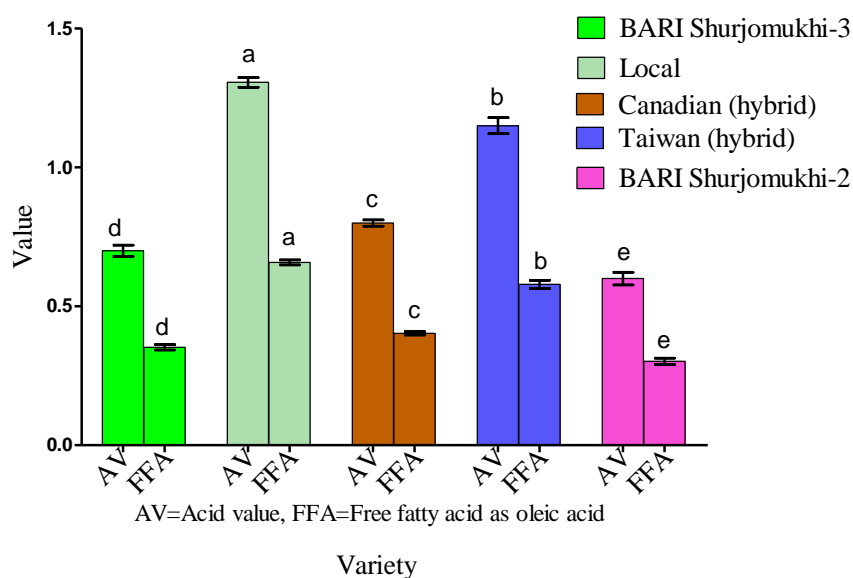


Figure 2. Acid value and free fatty acids content of sunflower oils.

### 4.3 Fatty acid composition

Comparison of fatty acid composition estimated through gas chromatography are showing in (Table 4). There was a significant difference between the studied genotypes in terms of their fatty acid compositions. Significantly the highest amount of palmitic acid was seen in Canadian (hybrid) ( $9.15 \pm 0.14\%$ ); followed by Taiwan (hybrid) ( $7.60 \pm 0.05\%$ ) and BARI Shurjomukhi-3 ( $5.45 \pm 0.47\%$ ); lowest amount of palmitic acid content was found in BARI Shurjomukhi-2 ( $3.40 \pm 0.03\%$ ) which was also closely followed by Local ( $4.84 \pm 0.13\%$ ) genotype. The amount of palmitoleic acid ranged from  $0.31 \pm 0.01\%$  to  $6.6 \pm 0.10\%$  where high amount found in Taiwan (hybrid) and lowest amount in BARI Shurjomukhi-2. The concentration of stearic acid varied from  $3.40 \pm 0.30\%$  to  $9.60 \pm 0.39\%$  whereas the highest concentration was observed in BARI Shurjomukhi-2 ( $9.60 \pm 0.39\%$ ). It was followed by Taiwan (hybrid) ( $7.63 \pm 0.19\%$ ) and Local ( $6.57 \pm 0.07\%$ ) variety. The lowest concentration of stearic acid was found in BARI Shurjomukhi-3 ( $3.40 \pm 0.30\%$ ) which was also followed by Canadian (hybrid) ( $5.82 \pm 0.02\%$ ). BARI Shurjomukhi-2 contained the highest amount ( $46.13 \pm 0.89\%$ ) of oleic acid; followed by Canadian (hybrid) ( $18.34 \pm 0.02\%$ ) and Taiwan (hybrid) ( $38.59 \pm 1.03\%$ ). The lowest amount was found in Local ( $33.76 \pm 0.89\%$ ) variety which was significantly lowest among all the varieties and also followed by BARI Shurjomukhi-3 ( $35.96 \pm 0.25\%$ ). The highest amount of linoleic acid contents was found in BARI Shurjomukhi-3 ( $53.80 \pm 1.05\%$ ) which was significantly highest among all the released cultivars and lowest amount ( $38.60 \pm 0.08\%$ ) was found in Taiwan (hybrid) which was statistically identical with Canadian (hybrid) ( $39.21 \pm 0.03\%$ ) and BARI Shurjomukhi-2 ( $39.51 \pm 0.19\%$ ). Akkaya *et al.* (2018); Reis Akkaya and Akkaya. (2018); Anjum *et al.* (2006);

**Table 4. Fatty acid composition of sunflower genotypes.**

Name of the released cultivars (Treatments)	Palmitic Acid (%) (C <sub>16:0</sub> )	Stearic Acid (%) (C <sub>18:0</sub> )	Palmitoleic Acid (%) (C <sub>16:1</sub> )	Oleic Acid (%) (C <sub>18:1</sub> )	Linoleic Acid (%) (C <sub>18:2</sub> )
	Mean ± SD (n=3)				
BARI Shurjomukhi-3	5.45 <sup>c</sup> ±0.47	3.40 <sup>e</sup> ±0.30	0.92 <sup>d</sup> ±0.03	35.96 <sup>d</sup> ±0.25	53.80 <sup>a</sup> ±1.05
Local	4.84 <sup>d</sup> ±0.13	6.57 <sup>c</sup> ±0.07	1.32 <sup>c</sup> ±0.01	33.76 <sup>e</sup> ±0.89	49.57 <sup>b</sup> ±0.70
Canadian (hybrid)	9.15 <sup>a</sup> ±0.14	5.82 <sup>d</sup> ±0.02	3.82 <sup>b</sup> ±0.02	41.41 <sup>b</sup> ±0.57	39.21 <sup>c</sup> ±0.03
Taiwan (hybrid)	7.60 <sup>b</sup> ±0.05	7.63 <sup>b</sup> ±0.19	6.61 <sup>a</sup> ±0.10	38.59 <sup>c</sup> ±1.03	38.60 <sup>c</sup> ±0.08
BARI Shurjomukhi-2	3.40 <sup>e</sup> ±0.03	9.60 <sup>a</sup> ±0.39	0.31 <sup>e</sup> ±0.01	46.13 <sup>a</sup> ±0.89	39.51 <sup>c</sup> ±0.19
LSD (0.05)	0.42	0.43	0.09	1.42	1.05
CV (%)	3.76	3.59	1.86	1.99	1.31

In a column, means with a similar letter (s) are not significantly different and means with different letter (s) are significantly different by LSD at 5% level of significance. SD = standard deviation; CV = Coefficient of Variation; LSD = Least significant difference.

Baydar and Erbas. (2005); Seiler (1983); Dorrell and Whelan (1978); Harris *et al.* (1978) and Earle *et al.* (1968) observed similar trend of results.

#### **4.4 Minerals**

Different major and minor minerals were analyzed in this work. The amounts of major minerals content of sunflower had been illustrated in Table 5 and Figure 3. It was well known that sunflower seed contained a small amount of micro minerals.

##### **4.4.1 Major minerals**

###### **4.4.1.1 Calcium (Ca) content**

Statistically, significant variation was recorded in terms of calcium content in the different sunflower genotypes (Table 5). The highest calcium content ( $0.23 \pm 0.01\%$ ) was observed from Local variety which was statistically identical ( $0.22 \pm 0.01\%$ ) with BARI Shurjomukhi-3 and followed ( $0.20 \pm 0.01\%$ ) by Canadian (hybrid). The lowest amount of calcium content ( $0.14 \pm 0.01\%$ ) was obtained from Taiwan (hybrid) which was followed ( $0.18 \pm 0.01\%$ ) by BARI Shurjomukhi-2 (Table 5). Some study showed that sunflower cake contained 0.33% by (Kolláthova *et al.*, 2019), 0.03-0.04% by (Nadeem *et al.*, 2010), 0.08–0.10% by (Gonzalez-perez and Vereijken, 2007) of calcium. According to USDA (2008), Sunflower seeds contains 0.078% of calcium. Differing climatic, soil conditions, and analytical techniques can vary the result between different authors (Nadeem *et al.*, 2010).

###### **4.4.1.2 Magnesium (Mg) content**

Different sunflower genotypes showed statistically significant variation for magnesium content (Table 5). The highest magnesium content ( $0.57 \pm 0.01\%$ ) was recorded from Taiwan (hybrid) which was statistically



identical ( $0.55 \pm 0.02\%$ ) BARI Shurjomukhi-2. Lowest magnesium content was observed ( $0.42 \pm 0.01\%$ ) by Local genotype which was also statistically identical ( $0.43 \pm 0.01\%$  and  $0.44 \pm 0.01\%$ ) to BARI Shurjomukhi-3 and Canadian (hybrid), respectively. The magnesium content in sunflower cake 0.42% find out by (Kollathova *et al.*, 2019) and 0.35-0.41% by (Gonzalez-perez and Vereijken, 2007). According to USDA (2008), Per 100 g seeds contains 325 mg of magnesium. Many researchers observed that differing climatic, soil conditions, and analytical techniques can vary the result (Nadeem *et al.*, 2010).

**Table 5. Major minerals (calcium, magnesium and sulfur) content in sunflower oilcake.**

Name of the released cultivars (Treatments)	Ca (%)	Mg (%)	S (%)
	Mean $\pm$ SD (n=3)		
BARI Shurjomukhi-3	0.2200 <sup>a</sup> $\pm$ 0.01	0.43 <sup>b</sup> $\pm$ 0.01	0.048 <sup>d</sup> $\pm$ 0.002
Local	0.2300 <sup>a</sup> $\pm$ 0.01	0.42 <sup>b</sup> $\pm$ 0.01	0.063 <sup>c</sup> $\pm$ 0.002
Canadian (hybrid)	0.2000 <sup>b</sup> $\pm$ 0.01	0.44 <sup>b</sup> $\pm$ 0.01	0.074 <sup>b</sup> $\pm$ 0.001
Taiwan (hybrid)	0.1400 <sup>d</sup> $\pm$ 0.01	0.57 <sup>a</sup> $\pm$ 0.01	0.090 <sup>a</sup> $\pm$ 0.001
BARI Shurjomukhi-2	0.1800 <sup>c</sup> $\pm$ 0.01	0.55 <sup>a</sup> $\pm$ 0.02	0.050 <sup>d</sup> $\pm$ 0.001
LSD (0.05)	0.02	0.02	0.003
CV (%)	5.15	2.62	2.28

In a column, means with a similar letter (s) are not significantly different and means with different letter (s) are significantly different by LSD at 5% level of significance. SD = standard deviation; CV = Coefficient of Variation; LSD = Least significant difference.

#### 4.4.1.3 Sulfur (S) content

Statistically, significant variation was recorded in terms of sulfur content due to different sunflower genotypes (Table 5). The statistically significant highest Sulfur content ( $0.090 \pm 0.001\%$ ) was recorded from Taiwan (hybrid) which was followed ( $0.074 \pm 0.001\%$ ) by Canadian (hybrid). The lowest Sulfur content was observed ( $0.048 \pm 0.002\%$ )

BARI Shurjomukhi-3 genotype which was also statistically identical ( $0.050 \pm 0.001\%$ ) to BARI Shurjomukhi-2 and followed ( $0.063 \pm 0.002\%$ ) by Local genotype. Gonzalez-perez and Vereijken (2007) reported a higher amount (0.26–0.32%) of sulfur in the sunflower seed.

#### **4.4.1.4 Phosphorus (P) content**

Statistically, significant variation was recorded in terms of phosphorus content due to different sunflower genotypes (Figure 3). The statistically significant highest phosphorus content ( $1.42 \pm 0.01\%$ ) were recorded from Taiwan (hybrid) which was followed ( $1.23 \pm 0.03\%$ ) by BARI Shurjomukhi-2, while the lowest phosphorus content was recorded ( $0.83 \pm 0.01\%$ ) from Canadian (hybrid), which was followed ( $0.87 \pm 0.02\%$  and  $0.90 \pm 0.02\%$ ) by Local and BARI Shurjomukhi-3 but both are statistically identical. This result was supported by Gonzalez-perez and Vereijken (2007) and Kollathova *et al.* (2019). According to USDA (2008) Per 100 g seeds contains 660 mg of phosphorus.

#### **4.4.1.5 Potassium (K) content**

Statistically, significant variation was recorded in terms of Potassium content due to different sunflower genotypes (Figure 3). The statistically significant highest Potassium content ( $0.90 \pm 0.03\%$ ) were recorded from Taiwan (hybrid) which was statistically identical ( $0.89 \pm 0.01\%$ ) to BARI Shurjomukhi-2 and followed by BARI Shurjomukhi-3. The lowest Potassium content was observed ( $0.75 \pm 0.01\%$ ) Canadian (hybrid), which was also statistically identical ( $0.78 \pm 0.02\%$ ) to Local genotype. This result showed similar result with Gonzalez-perez and Vereijken (2007); Kollathova *et al.* (2019); Nadeem *et al.* (2010) and USDA (2008).

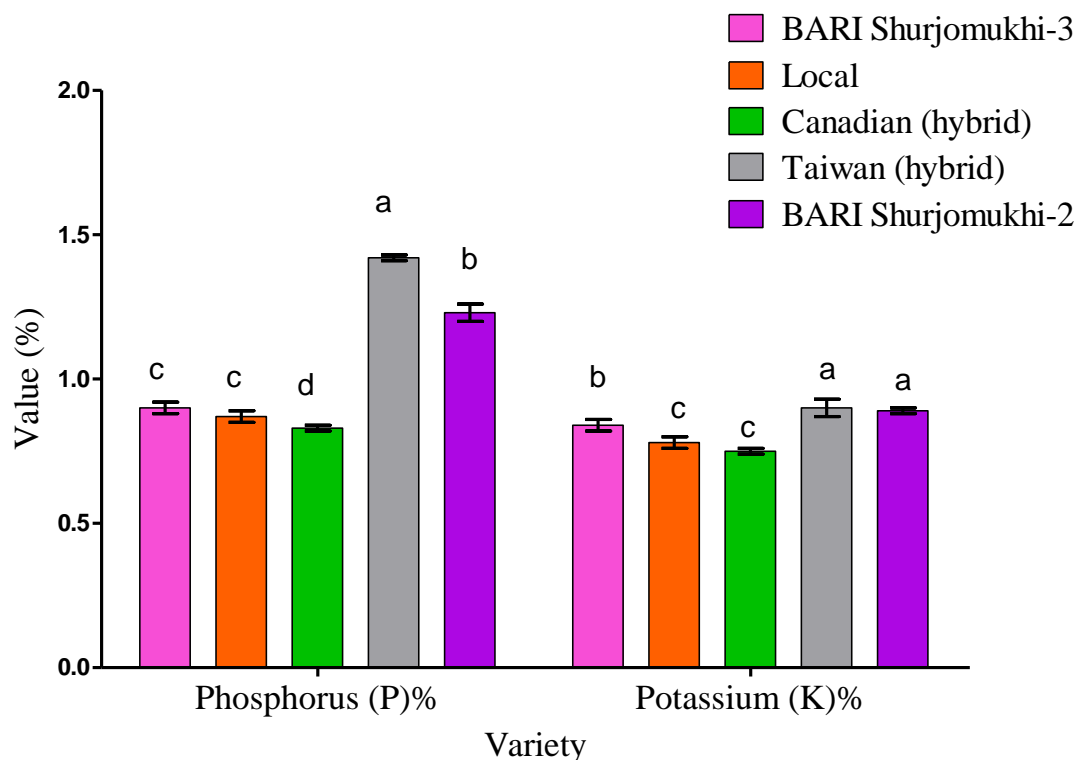


Figure 3. Major minerals (Potassium and phosphorous) content of different sunflower oilcake.

#### 4.4.2 Micro minerals

##### 4.4.2.1 Manganese (Mn) content

The manganese content of different sunflower genotype were ranges from  $19.00 \pm 0.50$  ppm to  $45.50 \pm 0.60$  ppm in (Table 6). Significantly highest amount of Mn contained was found in Local genotype ( $45.50 \pm 0.60$  ppm) which was followed by Canadian (hybrid) ( $29.25 \pm 0.01$  ppm) and BARI Shurjomukhi-3 ( $27.00 \pm 0.20$  ppm). The lowest amount was found in Taiwan (hybrid) ( $19.00 \pm 0.50$  ppm) which was followed by BARI Shurjomukhi-2 ( $24.00 \pm 0.32$  ppm). The present values were supported by the reported value of (Kollathova *et al.*, 2019 and Nadeem *et al.*, 2010). According to USDA (2008), sunflower seeds contains manganese 19.5 ppm.

#### 4.4.2.2. Copper (Cu) content

Copper contained of different sunflower oil cake was ranged from  $40.00 \pm 0.11$  ppm to  $47.75 \pm 0.05$  ppm (Table 6). Significantly highest amount of Cu was observed in Taiwan (hybrid) ( $47.75 \pm 0.05$  ppm) which was followed by BARI Shurjomukhi-2 ( $45.00 \pm 0.12$  ppm). The lowest amount of Cu contained observed in BARI Shurjomukhi-3 ( $40.00 \pm 0.11$  ppm). Our result was higher than Kollathova *et al.* (2019) findings. They reported that amount of copper in sunflower oil cake 31.98 ppm. According to USDA (2008) sunflower seeds contains copper 18.0 ppm.

**Table 6. Manganese and copper contents in sunflower oilcake.**

Name of the released cultivars (Treatments)	Mn (ppm)	Cu (ppm)
	Mean $\pm$ SD (n=3)	
BARI Shurjomukhi-3	$27.00^c \pm 0.20$	$40.00^d \pm 0.11$
Local	$45.50^a \pm 0.60$	$41.25^c \pm 0.05$
Canadian (hybrid)	$29.25^b \pm 0.01$	$41.25^c \pm 0.02$
Taiwan (hybrid)	$19.00^e \pm 0.50$	$47.75^a \pm 0.05$
BARI Shurjomukhi-2	$24.00^d \pm 0.32$	$45.00^b \pm 0.12$
LSD (0.05)	0.7058	0.1453
CV (%)	1.34	0.19

In a column, means with a similar letter (s) are not significantly different and means with different letter (s) are significantly different by LSD at 5% level of significance. SD = standard deviation; CV = Coefficient of Variation; LSD = Least significant difference.

#### 4.4.2.3 Zinc (Zn)

The zinc content of different sunflower oil cake was ranged from  $73.36 \pm 0.03$  ppm to  $53.37 \pm 0.03$  ppm (Table 7). Significantly highest amount of zinc was found in Taiwan (hybrid) ( $73.36 \pm 0.03$  ppm). The lowest amount of zinc was found ( $53.37 \pm 0.03$  ppm) in Local genotype. The

present investigations were supported by studied of (Kollathova *et al.*, 2019) and USDA (2008). But Nadeem *et al.* (2010) detected higher zinc content in sunflower seeds that was 117.81ppm.

#### 4.4.2.4 Iron (Fe)

The iron content of different sunflower oil cake was ranged from  $71.75 \pm 0.05$  ppm to  $119.75 \pm 0.05$  ppm (Table 7). Significantly highest amount of iron was found in Taiwan (hybrid) ( $119.75 \pm 0.05$ ppm). The lowest amount of iron was found ( $71.75 \pm 0.05$ ppm) in BARI Shurjomukhi-3 (HYV). The present investigations were higher than A value of (Kollathova *et al.*, 2019; Nadeem *et al.*, 2010 and USDA, 2008). Our results are different from many others studies we assume the reasons may be due to the differing climatic and soil conditions as well as analytical techniques used here.

**Table 7. Zinc and iron content in sunflower oilcake.**

Name of the released cultivars (Treatments)	Zn (ppm)	Fe (ppm)
	Mean $\pm$ SD (n=3)	
BARI Shurjomukhi-3	$71.21^b \pm 0.04$	$71.75^e \pm 0.05$
Local	$53.37^e \pm 0.03$	$108.50^d \pm 0.10$
Canadian (hybrid)	$65.50^c \pm 0.02$	$119.00^b \pm 0.05$
Taiwan (hybrid)	$73.36^a \pm 0.03$	$119.75^a \pm 0.05$
BARI Shurjomukhi-2	$57.64^d \pm 0.02$	$118.75^c \pm 0.05$
LSD (0.05)	0.05	0.12
CV (%)	0.05	0.06

In a column, means with a similar letter (s) are not significantly different and means with different letter (s) are significantly different by LSD at 5% level of significance. SD = standard deviation; CV = Coefficient of Variation; LSD = Least significant difference.

## CHAPTER V

### SUMMARY AND CONCLUSION

The research was conducted at the Biochemistry laboratory of the Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh and the laboratory of Soil Resource Development Institute (SRDI), Khamarbari, Dhaka, Bangladesh between October, 2019 to February, 2020. Two high yielding varieties (HYV) released from Bangladesh Agricultural Research Institute (BARI) namely BARI Surjomukhi-2, BARI Surjomukhi-3, two hybrid (Canadian OP and Taiwan KB2) and one Local variety of sunflower (*Helianthus annuus* L.) were selected for the study. The highest weight of thousands grains was found in BARI Shurjomukhi-2 ( $69.79 \pm 2.06\text{g}$ ) and the lowest weight of thousand grains was found in BARI Sujomukhi-3 ( $54.94 \pm 0.73\text{g}$ ). The highest value of moisture percentage was found in BARI Shurjomukhi-2 ( $6.29 \pm 0.09\%$ ); whereas the lowest value of moisture content was found in Canadian (hybrid) ( $4.78 \pm 0.15\%$ ). Significantly highest amount of dry matter content ( $95.22 \pm 0.15\%$ ) were observed from Canadian (hybrid); while the lowest dry matter content ( $93.71 \pm 0.10\%$ ) was found from BARI Shurjomukhi-2. The highest amount of oil content was found ( $36.46 \pm 0.40\%$ ) to BARI Shurjomukhi-2. The lowest amount of oil content was observed ( $23.29 \pm 0.77\%$ ) by BARI Shurjomukhi-3. The BARI Shurjomukhi-3 contained the highest amount of oilcake ( $76.707 \pm 0.77\%$ ) and the lowest amount of oilcake was observed ( $63.540 \pm 0.40\%$ ) by BARI Shurjomukhi-2. The highest value of dry weight of the cake was obtained from BARI Shurjomukhi-3 ( $70.85 \pm 0.59\%$ ). The lowest value obtained from BARI Shurjomukhi-2 ( $57.25 \pm 0.50\%$ ). The highest amount of protein was obtained from BARI Shurjomukhi-2 ( $25.03 \pm 0.89\%$ ) and on the other hand Canadian (hybrid) ( $22.38 \pm 1.31\%$ ) was

contained statistically lowest amount of protein. The highest amount of ash contained was recorded in Taiwan (hybrid) ( $3.20 \pm 0.03\%$ ) and on the other hand, BARI Shurjomukhi-3 ( $2.96 \pm 0.02\%$ ) had the lowest amount of ash. The statistically highest saponification value was found in BARI Shurjomukhi -2 ( $237.01 \pm 2.17$ ) and the lowest value obtained from Local ( $150.04 \pm 2.17$ ) variety. The highest amount of iodine value was observed in BARI Shurjomukhi-2 ( $131.64 \pm 0.46$ ) and the lowest amount of iodine value recorded in Local ( $101.80 \pm 0.23$ ). The highest acid value was found from Local variety ( $1.31 \pm 0.03$ ); whereas the lowest acid value was found from BARI Shurjomukhi-2 ( $0.60 \pm 0.04$ ).

The highest amount of palmitic acid was seen in Canadian (hybrid) ( $9.15 \pm 0.14\%$ ) and the lowest amount of palmitic acid content was found in BARI Shurjomukhi-2 ( $3.40 \pm 0.03\%$ ). The amount of palmitoleic acid ranged from  $0.31 \pm 0.01\%$  to  $6.6 \pm 0.10\%$  where high amount found in Taiwan (hybrid). The content of stearic acid varied from  $3.40 \pm 0.30\%$  to  $9.60 \pm 0.39$  whereas the highest content was observed in BARI Shurjomukhi-2 ( $9.60 \pm 0.39$ ) and the lowest concentration of stearic acid was found in BARI Shurjomukhi-3 ( $3.40 \pm 0.30$ ). BARI Shurjomukhi-2 contained the highest amount ( $46.13 \pm 0.89\%$ ) of oleic acid and the lowest amount of oleic acid was found in local ( $33.76 \pm 0.89\%$ ) variety. The highest amount of linoleic acid contents was found in BARI Shurjomukhi-3 ( $53.80 \pm 1.05\%$ ) which was significantly highest among all the released cultivars and lowest amount ( $38.60 \pm 0.08\%$ ) was found in Taiwan (hybrid). The highest calcium content ( $0.23 \pm 0.01\%$ ) was observed from Local variety, while the lowest amount of calcium content ( $0.14 \pm 0.01\%$ ) was obtained from Taiwan (hybrid). The highest magnesium content ( $0.57 \pm 0.01\%$ ) was recorded from Taiwan (hybrid) and lowest magnesium content was observed ( $0.42 \pm 0.01\%$ ) Local

genotype. The statistically significant highest Sulfur content ( $0.090 \pm 0.001\%$ ) were recorded from Taiwan (hybrid) and the lowest Sulfur content was observed ( $0.048 \pm 0.002\%$ ) BARI Shurjomukhi-3. The highest phosphorus content ( $1.42 \pm 0.01\%$ ) were recorded from Taiwan (hybrid), while the lowest phosphorus content was recorded ( $0.83 \pm 0.01\%$ ) from Canadian (hybrid). The highest Potassium content ( $0.90 \pm 0.03\%$ ) were recorded from Taiwan (hybrid) and the lowest Potassium content was observed ( $0.75 \pm 0.01\%$ ) Canadian (hybrid). Significantly highest amount of Mn contained was found in Local genotype ( $45.50 \pm 0.60$  ppm) and the lowest amount was found in Taiwan (hybrid) ( $19.00 \pm 0.50$  ppm). The highest amount of Cu was observed in Taiwan (hybrid) ( $47.75 \pm 0.05$  ppm) and the lowest amount of Cu contained observed in BARI Shurjomukhi-3 ( $40.00 \pm 0.11$  ppm). The zinc content of different sunflower oil cake was ranged from  $73.36 \pm 0.03$  ppm to  $53.37 \pm 0.03$  ppm. Significantly highest amount of zinc was found in Taiwan (hybrid) ( $73.36 \pm 0.03$  ppm) and the lowest amount of zinc was found ( $53.37 \pm 0.03$  ppm) in Local genotype. Significantly highest amount of iron was found in Taiwan (hybrid) ( $119.75 \pm 0.05$  ppm). The lowest amount of iron was found ( $71.75 \pm 0.05$  ppm) in BARI Shurjomukhi-3 (HY)

From my experiment, it was observed that none of the released cultivars performed the best by all nutrient parameters. BARI Shurjomukhi-2 (HYV) observed the highest thousand grain weight, moisture content, oil content and crude protein. In case of chemical constant, for sunflower oil, BARI Shurjomukhi-2 contained the highest saponification value, iodine value, and lower acid value than in other oil. For fatty acid composition, BARI Shurjomukhi-2 contained the highest stearic acid and oleic acid while Canadian (hybrid) showed the highest content of palmitic acid and



BARI Shurjomukhi-3 linoleic acid. Maximum ash content was recorded in Taiwan (hybrid) in contrast with it BARI Shurjomukhi-3 (HY) was lowest. In terms of mineral composition highest amount of Mg, S, K, P, Cu, Zn, Fe observed in hybrid (Taiwan) variety with an exception of Ca and Mn which found highest in Local variety. However, this study suggested that although Hybrid (Taiwan) contained notable mineral composition but in terms of qualitative aspect, HY (BARI-Shurjomukhi-2) performed better over hybrid (Taiwan) and Local varieties.

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