

**COMPARATIVE STUDY ON THE PHYSICO-
CHEMICAL CHARACTERISTICS OF SOYBEAN (*Glycine
max*) GENOTYPE**

BY

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**COMPARATIVE STUDY ON THE PHYSICO-CHEMICAL
CHARACTERISTICS OF SOYBEAN (*Glycine max*)
GENOTYPE**

BY

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CERTIFICATE

This is to certify that the thesis entitled, “**COMPERATIVE STUDY ON THE PHYSICO-CHEMICAL CHARACTERISTICS OF SOYBEAN (*Glycine max*) GENOTYPES**” submitted to the Department of **Agricultural Biochemistry**, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN BIOCHEMISTRY**, research work carried out by **TANJINA AFRIN** bearing **Registration No. 18-09059** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

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COMPARATIVE STUDY ON THE PHYSICO-CHEMICAL CHARACTERISTICS OF SOYBEAN (*Glycine max*) GENOTYPES

ABSTRACT

A comparative study was conducted to find out the physico-chemical characteristics of five different soybean varieties collected from various sources. In this study, seed weight, moisture%, ash%, oil%, protein%, carbohydrate, chemical constant (saponification, acid, and iodine value), fatty acid composition and mineral composition of five soybean genotypes and their cakes were estimated. Among the five varieties the highest thousand seed weight was 159.00 ± 1.0 g found in BINA Soybean-2. Moisture content varied from $9.29 \pm 0.12\%$ (Shohag) to $10.49 \pm 0.28\%$ (BINA Soybean-2). Maximum ash content was observed in Shohag ($5.18 \pm 0.12\%$). Oil content varied from $12.67 \pm 1.15\%$ (BARI Soybean-5) to $16.73 \pm 0.64\%$ (BINA Soybean-1). Maximum oil cake was found in BARI Soybean-5 ($87.33 \pm 1.15\%$). Protein percentage ranged from $37.99 \pm 0.36\%$ (BARI Soybean-6) to $41.50 \pm 0.18\%$ (BINA Soybean-1). Carbohydrate content was ranged from $27.19 \pm 0.45\%$ (BINA Soybean-1) to $34.72 \pm 0.58\%$ (BARI Soybean-6). Highest iodine value was observed in BINA Soybean-1 (95.69 ± 0.10). Highest saponification value was found in BINA Soybean-1 (191.79 ± 0.21). Lowest acid value was found in BINA Soybean-1 (0.91 ± 0.01). BINA Soybean-1 content highest amount of oleic acid ($24.72 \pm 0.03\%$), linoleic acid ($35.63 \pm 0.03\%$) and linolenic acid ($9.24 \pm 0.03\%$). In terms of mineral composition, the variety Shohag showed highest amount of phosphorus ($0.82 \pm 0.82\%$), potassium ($0.80 \pm 0.04\%$), manganese (32.03 ± 0.06 ppm), copper (29.00 ppm) and zinc (54.70 ppm). This study suggests that Shohag contained notable mineral compositions but in the matter of qualitative aspect BINA Soybean-1 performed better over other varieties.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	ACKNOWLEDGEMENTS	i
	ABSTRACT	ii
	CONTENTS	iii-v
	LIST OF TABLES	v-vi
	LIST OF FIGURES	vi
	LIST OF ABBREVIATION	vii
I	INTRODUCTION	1-4
II	REVIEW OF LITERATURE	5-19
III	MATERIALS AND METHODS	19-39
	3.1 Materials	20
	3.1.1 Brief description of varieties	20-23
	3.2 Experiment location & experimental design	24
	3.3 Physical Analysis	24
	3.3.1 Determination of 1000 grain weight	24
	3.3.2 %Moisture content	24
	3.3.3 Determination of Ash	24
	3.4 Chemical Analysis	25
	3.4.1 Estimation of Oils/Fats	25
	3.4.2 Estimation of total protein content by Micro Kjeldahl method	27
	3.4.3 Estimation of Carbohydrate	29
	3.4.4 Chemical constant	29
	3.4.4.1 Saponification value (SPV)	29
	3.3.4.2 Iodine value	31
	3.3.4.3 Acid value	33

CHAPTER	TITLE	PAGE
	3.4.5 Estimation of fatty acid composition	35
	3.4.6 Estimation of minerals	36
	3.4.6.1 Preparation of reagents	36
	3.4.6.2 Digestion of soybean sample for determination of P, K, Ca, Mg, S, Fe, Zn, Cu, Mn, N	38
	3.4.7 Statistical Analysis	39
IV	RESULTS AND DISCUSSION	32-45
	4.1 Physical characteristics of different varieties of Soybean (<i>Glycine max</i>)	40
	4.1.1 1000 Grain weight	40
	4.1.2 Moisture	40
	4.1.3 Ash	41
	4.2 Chemical characteristics of different varieties of Soybean (<i>Glycine max</i>)	42
	4.2.1 Oil content	42
	4.2.2 Oil cake	43
	4.2.3 Protein	43
	4.3 Carbohydrate	44
	4.4 Chemical constant	45
	4.4.1 Saponification Value	45
	4.4.2 Iodine value	46
	4.4.3 Acid Value	46
	4.5 Fatty acid composition	47

CHAPTER	TITLE	PAGE
	4.5.1 Saturated fatty acid composition	48
	4.5.2 Unsaturated fatty acid composition	49
	4.6 Mineral composition	50-55
V	SUMMARY AND CONCLUSION	56-57
	RECOMMENDATION	58
VI	REFERENCES	59-64
VII	APPENDIX	65

LIST OF TABLES

SL.NO.	TITLE OF THE TABLE	PAGE
1	Weight of 1000 seed, Moisture percentage and Ash percentage of different varieties of soybean (<i>Glycine max</i>).	42
2	Oil content (%), oil cake (%), and protein (%) of different varieties soybean (<i>Glycine max</i>).	44
3	Carbohydrate content of different varieties of soybean (<i>Glycine max</i>).	45
4	Saponification value, Iodine value, and Acid value of different varieties of soybean (<i>Glycine max</i>).	47
5	P (%), K (%) content of different varieties of soybean (<i>Glycine max</i>).	52

SL. NO.	TITLE OF THE TABLE	PAGE
6	S (%), Mg (%), and Ca (%) content of different varieties of soybean (<i>Glycine max</i>).	53
7	Fe (ppm), Mn (ppm), Cu (ppm), and Zn (ppm) content of different varieties of soybean (<i>Glycine max</i>).	55

LIST OF FIGURES

SL. NO.	TITLE OF THE FIGURES	PAGE
1	BINA Soybean-2	22
2	BINA Soybean-1	22
3	BARI Soybean-5	23
4	BARI Soybean-6	23
5	Shohag	23
6	Oil extraction by using soxhlet method	26
7	Determination of acid value of soybean oil	34
8	Saturated fatty acid comparison of five different soybean varieties.	49
9	Unsaturated fatty acid comparison of five different soybean varieties.	50

Some commonly used Abbreviations in this study

Full Word Abbreviation

And others	<i>et al.</i>
Coefficient of variation	CV
Poly Unsaturated Fatty Acid	PUFA
Daily Value	DV
World Health Organization	WHO
Non-Protein Nitrogen	NPN
Liter	L
Titer Value	TV
Saponification Value	SPV
Normality	N
Analysis of variance	ANOVA
Association of Official Analytical Chemist	AOAC
Bangladesh Agriculture Research Institute	BARI
Bangladesh Institute of Nuclear Agriculture	BINA
Degree Celsius (Centigrade)	°C
Least significant difference	LSD
Milliliter	ml
Parts per Million	ppm
Food and Agriculture Organization	FAO
Microgram	µg
Microliter	µl

CHAPTER I

INTRODUCTION

Soybean (*Glycine max*) is native to East Asia, widely grown for its edible bean, which has numerous uses. Soybean is one of the most important legume crops in the world is gaining increased cultivation attention in the humid tropics (Ayanwole, 2007) because of its high-quality protein, 85% cholesterol free oil content (Malik *et al.*, 2007) and nutritional value for humans and livestock as well as its ability to maintain soil fertility (Ngalamu *et al.*, 2013). The soybean is one of the richest and cheapest sources of protein and is a staple in the diets of people and animals in numerous parts of the world. Because soybeans contain less starch, they are a good source of protein for diabetics. In East Asia the bean is extensively consumed in the forms of soy milk, a whitish liquid suspension, and tofu, a curd somewhat resembling cottage cheese. Soybeans are also sprouted for use as a salad ingredient or as a vegetable and may be eaten roasted as a snack food. Young soybeans, known as edamame, are commonly steamed or boiled and eaten directly from the pod. Soy sauce, a salty brown liquid, is produced from crushed soybeans and wheat that undergo yeast fermentation in salt water for six months to a year or more; it is a ubiquitous ingredient in Asian cooking. Other fermented soy foods include tempeh, miso, and fermented bean paste.

Bangladesh is a country of about 170 million people and annually consumes about 3.0 million tons of oils and fats, which include both edible and inedible. As revealed from the statistics of Oil World - 2019, globally recognized source of information on oils and fats, consumption of oils and fats in Bangladesh shows an increasing trend, which is highest among the developing countries. In 2019, total consumption of oils and fats was 3.04 million tons which is about 2.97 percent higher compared to

2018. The average per capita consumption of oils and fats is seen approaching 18.7 kgs. Consumption of oils and fats, in general, is on a growing trend keeping in pace with population growth, economic development and increase of purchasing power of the general consumers. It is worth mentioning that due to insufficient indigenous production, Bangladesh is mostly dependent on import to meet up the requirements of oils and fats. About 90 to 92 per cent of the annual requirements of oils and fats are met through import. Presently there are three major edible oils consumed in the country, namely, palm oil, soybean oil and rapeseed/canola oil and import shares of which were at a ratio of 58:37:5 respectively, as per 2019 statistics. Among the three kinds of oils, palm oil and soybean oils are imported in crude/refined form and marketed in refined form through processing locally (Alam, 2020).

Soybean plays a significant role in boosting the immune system and general health of the human body (Murray-Kolb *et al.*, 2003) as well as serves as dietary supplement for diabetics (Azadbakht *et al.*, 2003). Soybean possess a very high nutritional value. It contains about 20% oil and 40% high quality protein (as against 7.0% in rice, 12% in wheat, 10% in maize and 20-25% in other pulses). Soybean protein is rich in valuable amino acid lysine (5%) in which most of the cereals are deficient (Singh *et al.*, 2010)

According to the US Food and Drug Administration, soy is a good source of protein for vegetarians and vegans or for people who want to reduce the amount of meat they eat (US Food and Drug Administration, 1999). Soy protein products can be good substitutes for animal products because, unlike some other beans, soy offers a 'complete' protein profile. Soy protein products can replace animal-based foods which also have complete proteins but tend to contain more fat, especially saturated fat without requiring major adjustments elsewhere in the diet. Soybean oil is

comprised of primarily polyunsaturated fatty acids (PUFAs), particularly linoleic acid (C_{18:2}), an omega-6 (ω6) fatty acid that makes up ~55% of soybean oil. Unfortunately, eating too much omega-6 can raise blood pressure, lead to blood clots that can cause heart attack and stroke, and cause our body to retain water. Soybean oil contains some omega-3 fats also, which are heart-healthy fats often found in salmon and sardines, but are less common in plant-based sources of food (Deol *et al.*, 2017).

Soybeans are an exceptional source of essential nutrients, providing in a 100 gram serving (raw, for reference) high contents of the Daily Value (DV) especially for- protein (36% DV), dietary fiber (37%), iron (121%), manganese (120%), phosphorus (101%) and several B vitamins, including folate (94%). High contents also exist for vitamin K, magnesium, zinc and potassium. Raw soybeans are 20% fat, including saturated fat (3%), monounsaturated fat (4%) and polyunsaturated fat, mainly as linoleic acid. For human consumption, soybeans must be cooked with "wet" heat to destroy the trypsin inhibitors (serine protease inhibitors). Raw soybeans, including the immature green form, are toxic to all monogastric animals (Circle and Smith, 1972).

The importance of soybean in our diet is significant. However, we have found infrequent studies related to the comparison of mineral content and fatty acid composition of soybean oil in Bangladesh (Prodhan *et al.*, 2015). Present research work was conducted for the assessments of effects of genotypes on physico-chemical characteristics of soybean with the following objectives-

1. To assess the physical and chemical characteristics, minerals content, oil percentage and fatty acid profiles of collected varieties of soybean.

-
2. To compare the physico-chemical parameters and nutritional quality of collected varieties of soybean.

CHAPTER II

REVIEW OF LITERATURE

In the common terminology soybean is vocabularies as the oilseeds while the beans are grouped as pulses. Soy come about of different size and coating colors. The percentage of oil along with protein both report nearly 60% of dry soybeans weight. From the total composition, the main biomolecules are protein generally about 40% and oil for 20%. The residual composition includes around 35% carbohydrates and nearly around 5% amount of ash.

Grain Weight

Sharma *et al.* (2014) carried out a research on Soybean, a major source of high-quality protein and oil. Soybean seed quality determine by seed nutritional and antinutritional parameters. The objective of this study was to investigate the physical characteristics and nutritional composition of some new soybean genotypes. Hundred seed weight of soybean genotypes ranged from 8.7 to 11.1 gm.

Rani *et al.* (2008) conducted a study of nine varieties of soybean to analyze moisture, 1000 grain weight, grain hardness, cooking time, seed density, hydration capacity, hydration index, swelling capacity, swelling index, protein, fat, crude fibre, ash and minerals. These soybean varieties contained 74.0-112.4 g 1000 grain weight.

Kulkarni (2019) observed a total of three soybean genotypes including two black -Kalitur and DSM with one yellow –DSb 21 procured during kharif, 2017, University of Agricultural Sciences, Dharwad for physicochemical composition, cooking quality and acceptability. A

significant variation was found in physical and functional characteristics of black and yellow soybean genotypes. The DSM genotype had highest 100 seed weight (16.85 g).

Krishna *et al.* (2003) performed a research with seven new varieties of soybean exhibited 1000 grain weight 118.3-145.6 g.

Sultan *et al.* (2015) investigated some agro-morphological characters, besides seed oil content and fatty acid composition of two local soybean genotypes labeled as S/D-18 and S/D-22 grown under rainfed conditions of Kashmir Himalayas in India. The genotype S/D-22 matured earlier than that of S/D-18. 100-seed weight of S/D-18 and S/D-22 are 14.3 g and 14.7 g respectively.

According to Deshpande *et al.* (1993) the physical properties of soybean on thousand grain mass ranges from 0.110 to 0.127 kg.

Moisture

A study was carried out by Anwar *et al.* (2016) to evaluate the variation of quality attributes among oils from different soybean varieties (Bovender special, Foster and F-8827). Results indicated that the contents of moisture of soybean genotypes are ranged from 8.4–10.2%.

Rani *et al.* (2008) conducted a study of nine varieties of soybean to analyze moisture, 1000 grain weight, grain hardness, cooking time, seed density, hydration capacity, hydration index, swelling capacity, swelling index, protein, fat, crude fibre, ash and minerals. These soybean varieties contained moisture ranged from 8.60-10.10%.

Kulkarni (2019) observed a total of three soybean genotypes including two black-Kalitur and DSM with one yellow–DSb 21 for physicochemi-

cal composition, cooking quality and acceptability. A significant variation was found in physical and functional characteristics of black and yellow soybean genotypes. The highest moisture was recorded in Kalitur (12 %).

According to Deshpande *et al.* (1993) the moisture content of soybean varieties ranges from 8.7 to 25.0%.

According to Sharma & Miglani (2016) moisture content varied from 6.117.9% and three black, one brown and one green soybean genotype exhibited moisture content higher than 15% among twenty different soybean genotypes.

Ashaolu & Noibi (2013) examines the effect of moisture content on some mechanical properties of soybean. Two soybean varieties – TGx1987-10F and TGx1987-62F were conditioned at two moisture content level of 6.51% and 10.35%.

Zhu *et al.* (2018) reported that moisture content for the 90 selected samples of soybeans were ranged from 8.47–10.67%. For the 216 samples in the calibration set, moisture content was 7.42–13.71%. For the 54 samples in the external validation set, moisture, content was 6.92–11.24%.

A research was conducted by Etiosa *et al.* (2018) aiming to analyse the minerals and proximate content of soybean in order to explore its nutritional values in human and animal diets. The result revealed that soya bean contained 8.07% of moisture.

Ash

Anwar *et al.* (2016) evaluated the variation of quality attributes among oils from different soybean varieties (Bovender special, Foster and F-8827). Oils were extracted using *n*-hexane as solvent. Results indicated that contents of ash ranged from 5.5–6.9%.

Rani *et al.* (2008) conducted a study of nine varieties of soybean to analyze moisture, 1000 grain weight, grain hardness, cooking time, seed density, hydration capacity, hydration index, swelling capacity, swelling index, protein, fat, crude fibre, ash and minerals. The ash content of different soybean varieties ranged from 4.45-5.60% on dry matter basis.

Kulkarni (2019) observed a total of three soybean genotypes including two black -Kalitur and DSM with one yellow –DSb 21 were procured during kharif, 2017, University of Agricultural Sciences, Dharwad to analyze the physicochemical composition, cooking quality and acceptability. A significant variation was found in physical and functional characteristics of black and yellow soybean genotypes. Black soybean DSM genotype had significantly high content of ash (5.82 g).

Krishna *et al.* (2003) performed a research with the seven new varieties of soybean. The varieties contained ash ranged from 4.2-5.2%.

According to Sharma *et al.* (2016) the ash content in twenty genotypes of soybean varied from 2.06-8.6%.

According to Jahan *et al.* (2013) the proximate composition of ash of soybean meal used in different diets (% on dry matter basis) is 5.71.

A research was carried out by Kuzniar *et al.* (2016) to determine the influence of the chemical composition and moisture content in seeds from

selected soybean cultivars on their mechanical properties. According to the research the ash content in ten different soybean varieties ranged from 4.86 to 5.49%.

A research was conducted by Etiosa *et al.* (2018) aiming to analyse the minerals and proximate content of soybean in order to explore its nutritional values in human and animal diets. The result revealed that soya bean contained 4.29% of ash.

A research was conducted in the topic of Nutrient Analysis of Raw and Processed Soybean and Development of Value-Added Soybean Noodles by Gupta *et al.* (2013). According to the research soybean content 5.6% of ash.

Oil content

Sharma *et al.* (2014) carried out a research on Soybean, a major source of high-quality protein and oil where soybean seed quality determine by seed nutritional and antinutritional parameters. The objective of this study was to investigate the physical characteristics and nutritional composition of some new soybean genotypes. The genotypes contained % oil (14.0–18.7).

A study was carried out by Anwar *et al.* (2016) to evaluate the variation of quality attributes among oils from different soybean varieties (Bovender special, Foster and F-8827). Oils were extracted using *n*-hexane as solvent. Results indicated that contents of seed oil among the tested varieties varied from 15.85% to 19.49%.

Sharma *et al.* (2013) revealed that the effects of soaking and cooking methods on physicochemical characteristics, nutrients and antinutrients in

twenty soybean genotypes were varied from one another. Raw soybean genotypes exhibited 20.7-22.2% oil.

Sultan *et al.* (2015) investigated some agro-morphological characters, besides seed oil content and fatty acid composition of two local soybean genotypes labeled as S/D-18 and S/D-22 grown under rainfed conditions of Kashmir Himalayas in India. Seeds of genotype S/D-18 yielded an oil content of 19.54% while in genotype S/D-22 oil content of 19.74% was recorded.

Amos-Tautua and Onigbinde (2013) extracted crude oils from three vegetable seeds namely: soybeans (*Glycine max*), groundnut (*Arachis hypogaea*) and maize (*Zea mays*) purchased from a local market in Sagamu Ilisan, Ogun State. The results of physical analysis showed that soybean oil had the highest percentage of oil yield (14. 51%).

Oil cake

Sharma *et al.* (2014) carried out a research on Soybean, a major source of high-quality protein and oil where soybean seed quality determine by seed nutritional and antinutritional parameters. The objective of this study was to investigate the physical characteristics and nutritional composition of some new soybean genotypes. The genotypes contained % oil cake (81.3-86).

A study was carried out by Anwar *et al.* (2016) to evaluate the variation of quality attributes among oils from different soybean varieties (Bovender special, Foster and F-8827). Oils were extracted using *n*-hexane as solvent. Results indicated that contents of seed oil cake among the tested varieties varied from 80.51% to 84.15%.

Sharma *et al.* (2013) experimented the effects of soaking and cooking methods on physicochemical characteristics, nutrients and antinutrients in twenty soybean genotypes. Raw soybean genotypes exhibited 77.80% to 79.3% oil cake.

Protein

A study was carried out by Anwar *et al.* (2016) to evaluate the variation of quality attributes among oils from different soybean varieties (Bovender special, Foster and F-8827). Results indicated that contents of protein ranged from 41.67 – 45.64%.

Rani *et al.* (2008) conducted a study of nine varieties of soybean to analyze moisture, 1000 grain weight, grain hardness, cooking time, seed density, hydration capacity, hydration index, swelling capacity, swelling index, protein, fat, crude fibre, ash and minerals. Crude protein varied from 35.42 - 41.78%.

Sharma *et al.* (2014) conducted a study to investigate the physical characteristics and nutritional composition of some new soybean genotypes. The genotypes contained % crude protein (39.4–44.4).

Kulkarni (2019) observed a total of three soybean genotypes including two black -Kalitur and DSM with one yellow –DSb 21 were procured during kharif, 2017, University of Agricultural Sciences, Dharwad to find out the physico-chemical composition, cooking quality and acceptability. A significant variation was found in physical and functional characteristics of black and yellow soybean genotypes. Significantly high amount of protein was observed in the DSb 21 (43.63 g).

Research was conducted by Rao *et al.* (1998) with the objective of determining yield, seed protein, and fatty acid composition of soybean genotypes selected for tofu production. Twelve soybean genotypes were planted in plots arranged in a randomized complete block design with four replications at the Agricultural Research Station, Fort Valley State University, Georgia, USA during 1994 and 1995. The protein content varied between 314.2 and 480.7 g/kg seed.

Sharma *et al.* (2013) revealed the effects of soaking and cooking methods on physicochemical characteristics, nutrients and antinutrients in twenty soybean genotypes. Batches of seeds were soaked for 18 h in distilled water, 1% citric acid and 2% sodium bicarbonate solutions at room temperature and then boiled in water. Raw soybean genotypes exhibited 36.5-43.2% protein.

Krishna *et al.* (2003) performed a research with the seven new varieties of soybean. The varieties contained (%) protein 37.19 - 41.56.

According to Sharma & Miglani (2016) total soluble protein content of black, brown, green and yellow groups from twenty different soybean genotype varied from 29.5-40.56, 29.37-30.43, 28.03-43.1 and 30.53-43.23 g/100 g, respectively. Highest mean total soluble protein (37.55%) and oil (21.9%) was recorded in yellow coloured soybean genotypes and values were significantly higher as compared to black, brown and green groups.

Zhu *et al.* (2018) reported that moisture, crude fat, and protein content for the 90 selected samples of soybeans- protein content ranged from 37.37–43.20%. For the 216 samples in the calibration set, protein content was ranged from 37.37–43.21%. For the 54 samples in the external validation set, protein content was ranged from 37.04–43.56%.

Jahan *et al.* (2013) conducted an experiment to examine the mineral content of different oilseed varieties and advanced lines cultivars and found that proximate composition of crude protein of soybean meal used in different diets (% on dry matter basis) is 43.30.

A research was carried out by Kuzniar *et al.* (2016) to determine the influence of the chemical composition and moisture content in seeds from selected soybean cultivars on their mechanical properties. According to the research the protein content in ten different soybean varieties ranged from 31.35 to 36.97%.

A research was carried out by Kim (1988) on Physico-chemical, nutritional, and flavor properties of soybean extracts processed by rapid-hydration hydrothermal cooking. According to this research the protein content of soybean is about 41.7%.

A research was conducted by Etiosa *et al.* (2018) aiming to analyse the minerals and proximate content of soybean in order to explore its nutritional values in human and animal diets. The result revealed that soya bean contained 37.69% of protein.

A research was conducted in the topic of Nutrient Analysis of Raw and Processed Soybean and Development of Value-Added Soybean Noodles by Gupta *et al.* (2013). According to the research soybean content about 42.9% of dry protein.

Carbohydrate

Krishna *et al.* (2003) performed a research with the seven new varieties of soybean. The varieties contained (%) carbohydrates (by difference) 17.58-22.47.

Middelbos & Fahey Jr. (2008) reported that Soybean contained approximately 30–35% carbohydrates and in Soybean meal, carbohydrate content may be as high as 40%. Carbohydrates are usually divided into two main groups based on their physicochemical properties in plant material. The first group, the nonstructural carbohydrates, includes low molecular weight sugars, oligosaccharides, and storage polysaccharides. The second group comprises of the structural polysaccharides and includes dietary fiber components.

Rani *et al.* (2008) conducted a study of nine varieties of soybean to analyze moisture, 1000 grain weight, grain hardness, cooking time, seed density, hydration capacity, hydration index, swelling capacity, swelling index, protein, fat, crude fibre, ash and minerals. The carbohydrate content of different soybean varieties ranged from 21.40-29.83 percent on dry matter basis.

A research was conducted by Etiosa *et al.* (2018) aiming to analyse the minerals and proximate content of soya bean in order to explore its nutritional values in human and animal diets. The result revealed that soya bean contained 16.31% of carbohydrate.

A research was conducted in the topic of Nutrient Analysis of Raw and Processed Soybean and Development of Value-Added Soybean Noodles by Gupta *et al.* (2013). According to the research soybean content about 19.8 g of carbohydrate.

Chemical properties of Oil

A study was carried out by Anwar *et al.* (2016) to evaluate the variation of quality attributes among oils from different soybean varieties (Bovender special, Foster and F-8827). The physical and chemical characteristics among the tested oils varied as: iodine value (119–128 g of I/100 g of oil, saponification value (181–187 mg KOH/g).

Amos-Tautua and Onigbinde (2013) extracted crude oils from three vegetable seeds namely: soybeans (*Glycine max*), groundnut (*Arachis hypogaea*) and maize (*Zea mays*) purchased from a local market in Sagamu Ilisan, Ogun State were investigated. Extraction of the oils from the seeds was carried out by soxhlet method using petroleum ether as the extractant. The physio-chemical properties of the oils were determined using Gas Chromatography (GC) and other standard methods. The chemical analysis showed that the saponification values were 228.19, 227.49 and 211.37 mg KOH/g, acid values were 19.21, 4.63 and 65.50 mg KOH/g and iodine values were 73.02, 38.65 and 47.25 for soybean, groundnut and maize oils respectively.

Prodhan *et al.* (2015) investigated the chemical characteristics of six brand soybean oil such as Mustafa, Muskan, Pusti, Teer, Fresh and Rupchanda collected from retail market in Bangladesh. The studied parameters were iodine value, saponification value, acid value and Reichert-Meissl number and unsaponifiable matter. The acid value, saponification value, Reichert-Meissl number, unsaponifiable matters were determined by the standard methods. Hanus method was followed to determine the iodine value of the oil. Saponification values of Mustafa, Muskan, Pusti, Teer, Fresh and Rupchanda were (202, 208, 224, 213, 210 and 237 respectively). Acid values of Mustafa, Muskan, Pusti, Teer,

Fresh and Rupchanda soyabean oils were (0.374, 0.748, 0.561, 0.374, 0.374 and 0.423 respectively). Iodine values of Mustafa, Muskan, Pusti, Teer, Fresh and Rupchanda soyabean oils were (85.45, 105.70, 89.64, 90, 105.26 and 110 respectively).

Belsare and Badne (2017) stated that fats and oils are nutritionally important because they form one of the three major classes of food. Oils are used in a variety of ways. For all oils, saponification value 175 to 251 mg KOH/g, Acid value 0.56 to 1.12 mg KOH/g.

According to Abitogun *et al.* (2008) the saponification value 199.63 ± 1.81 mg KOH/g oil, acid value 2.72 ± 0.17 mg KOH/g oil, iodine value 119.21 ± 0.40 WIJ (WIJ's solution is analytical reagent used to determine the iodine value of a substance).

Fatty acid composition

Research was conducted by Rao *et al.* (1998) with the objective of determining yield, seed protein, and fatty acid composition of soybean genotypes selected for tofu production. Twelve soybean genotypes were planted in plots arranged in a randomized complete block design with four replications at the Agricultural Research Station, Fort Valley State University, Georgia, USA during 1994 and 1995. Both BARC-8 and BARC-9 had significantly higher protein content than other genotypes. V71-370 had the highest oleic acid concentration and a high ratio (0.92) of monounsaturated to polyunsaturated fatty acids. The concentration of linoleic and linolenic acids ranged from 406.5 to 531.0 and 37.1 to 63.0 g/kg oil, respectively.

Özcan & Al Juhaimi (2014) observed that free fatty acid contents of sprouted soybean oil were found between 1.26% (Adasoy) and 4.20%

(Nazlıcan and Türksoy). Palmitic, oleic and linoleic acids were found as major fatty acids of soybean genotypes. Oleic acid contents of samples were found between 19.07% (roasted Adasoy) and 35.31% (roasted A3935), linoleic contents of oils ranged between 42.17% (roasted Nazlıcan) and 54.76% (sprouted A3127).

Sultan *et al.* (2015) investigated some agro-morphological characters, besides seed oil content and fatty acid composition of two local soybean genotypes labeled as S/D-18 and S/D-22. Fatty acids of myristic, palmitic, stearic, oleic, linoleic and linolenic were detected and quantified in the oil extracted from the seeds of these genotypes. Saturated fatty acids constituted 11.88% (S/D18) and 11.42% (S/D-22) while unsaturated fatty acids constituted 88.12% (S/D-18) and 88.58% (S/D-22) of the oil.

Amos-Tautua and Onigbinde (2013) extracted crude oils from three vegetable seeds namely: soybeans (*Glycine max*), groundnut (*Arachis hypogaea*) and maize (*Zea mays*). Gas Chromatography analysis gave the following fatty acid profiles: oleic (26.2%), linoleic (8.5%), stearic (5.6%) for soybean.

According to Abitogun *et al.* (2008) fatty acid detected includes myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic and traces of arachidic and eicosienic acids and the values were 0.016%, 7.269%, 0.014%, 2.635%, 21.166%, 39.900%, 6.767%, 0.010%, 0.0000988% respectively for crud soybean oil.

Anwar *et al.* (2016) reported the free fatty acid content among oils from different soybean varieties (Bovender special, Foster and F-8827) ranged from 0.39–0.67% as oleic acid.

Gunstone (1996) found that the Soybean contained near about 81% unsaturated fatty acid and 14% saturated fatty acid. Typical variety of Soybean contained: oleic (43%), linoleic (35%), linolenic acid (3%), palmitic (10%) and stearic acid (4%).

According to Martin *et al.* (2008) soybean oil content palmitic acid (11.29-11.77%), stearic acid (3.23-3.83%), arachidic acid (0.26-0.38%), and behenic acid (0.40-0.50%).

Mineral

Krishna *et al.* (2003) performed a research with the seven new varieties of soybean. Components determined include (mg/100 g seeds) calcium 246.60-280.00, phosphorus 502.00-540.86 and iron 10.00-13.36.

Rani *et al.* (2008) conducted a study of nine varieties of soybean to analyze moisture, 1000 grain weight, grain hardness, cooking time, seed density, hydration capacity, hydration index, swelling capacity, swelling index, protein, fat, crude fibre, ash and minerals. The total calcium, phosphorus, iron and zinc content ranged between 230.2-255.2, 496.1-514.2, 8.40-11.20 and 7.16-7.89 mg/100g, respectively. Results indicate that variety, PK-1024 had better physicochemical properties among the nine varieties studied.

Özcan & Al Juhaimi (2014) observed that macro and micro element contents of sprouted, oven roasted and raw (untreated) soybean seeds were determined by Inductively Coupled Plasma Atomic Emission Spectrometry. The potassium contents of soybean seeds ranged between 16,375 mg/kg (raw Adasoy) and 20,357 mg/kg (sprouted A3127), while phosphorus contents of seeds varied from 5427 mg/kg (oven roasted

Türksoy) to 7759 mg/kg (sprouted Nova). The micro element contents of samples were found to be different depending on the processing procedures and soybean genotypes.

A research was conducted by Etiosa *et al.* (2018) aiming to analyse the minerals and proximate content of soya bean in order to explore its nutritional values in human and animal diets. The result revealed that soya bean contained 300.36 mg/100 g of Calcium, 258.24 mg/100 g of Magnesium, 16.4 mg/100 g of Iron, 3.0 mg/100 g of Sodium, 2.7 mg/100 g of Zinc, 695.20 mg/100 g of Phosphorus.

A research was conducted by Garcia *et al.* (1998) where commercial soybean product soybean protein isolate, soybean flour, textured soybean, whole soybeans, and soybean dairy-like products (liquid and powdered milks, shake, yogurt, and infant formulas)-have been analysed for their content in solids, ash, pH, acidity, protein, fat, phosphorus, and some metal ions (calcium, copper, iron, potassium and zinc).The result stated that copper contents are minimal for all soybean products (≤ 2.00 mg/ 100 g) and potassium (≥ 234 mg/100g).

A research was conducted in the topic of Nutrient Analysis of Raw and Processed Soybean and Development of Value-Added Soybean Noodles by Gupta *et al.* (2013). According to the research soybean content about 9.60mg of Iron and 245 mg of Calcium.

Uwem *et al.* (2017) conducted a research on the issue of proximate composition, phytoconstituents and mineral contents of soybean (*Glycine Max*) flour grown and processed in Northern Nigeria. The result indicates the minerals analyzed were: Ca (231.6), Fe (5.790), Mg (249.8), Zn (2.414) and Mn (0.651) which were within the FAO/WHO standards for metals in foods.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

Five varieties of Soybean (*Glycine max*) namely BINA Soybean-1, BINA Soybean-2, BARI Soybean-5, BARI Soybean-6, Shohag were selected for the study. The seeds were collected from BARI, Joydebpur, Gazipur and BINA, Mymensingh. Seeds were cleaned and sun-dried and stored in plastic container in a cool place until used for the chemical analysis.

3.1.1 Brief description of varieties

BINA Soybean-1

BINA Soybean-1 is moderately resistant to yellow mosaic virus (YMV) and tolerant to stem rot diseases released in 2011. The plant is shorter in height, deep green leaflet and light-yellow seed coat. Hundred seed weight is 15.17 gm. This variety is can be grown both Kharif (mid July) and Rabi (mid January) seasons. Maturity period ranges from 105-110 days. It can be grown in wide ranges of land and soil types from sandy to loam soils. It can produce seed yield of 3.0-3.3 t/ha. The seed contains 44.5% protein, 27.0% starch and 19.0% oil. This variety can be cultivated all over the country but more suitable for high and Charland of South and South-western regions of Bangladesh.

BINA Soybean-2

BINA Soybean-2 is moderately resistant to yellow mosaic virus (YMV) which was released in 2011. The plant is shorter in height,

deep green leaves, hilum very clear, black color and brighter yellow seed coat. Hundred seed weight is 15.9 gm. This variety can be grown both Kharif (Mid July) and Rabi (Mid January) seasons. Maturity period ranges from 95-100 days. It can be grown in wide ranges of land and soil types from sandy to loam soils. It can produce seed yield of 2.4-2.8 t/ha. The seed contains 43.0% protein, 27.0% starch and 18.0% oils. This variety can be cultivated all over the country but more suitable for high and Charland of South and Southwestern regions of Bangladesh.

BARI Soybean-5

BARI Soybean-5 was released in 2002. The plant height ranges from 40-60 cm. Hundred seed weight is 12.2 gm. The seed is creamy in color. The seed contains 40-45% protein and 21-22% oils. This variety can be grown both Kharif (Mid July) and Rabi (mid January) seasons. Maturity period ranges from 90-100 days. It can produce seed yield of 1.6-2.0 t/ha.

BARI Soybean-6

BARI Soybean-6 is resistant to yellow mosaic virus (YMV) which was released in 2009. The plant height ranges from 50-55 cm. Hundred seed weight is 11.93 gm. The seed color is creamy color. The seed contains 42-44% protein and 20-21% oils. This variety can be grown both Kharif (Mid July) and Rabi (mid January) seasons. Maturity period ranges from 100-110 days. It can produce seed yield of 1.8-2.10 t/ha.

Shohag

Shohag is resistant to yellow mosaic virus (YMV) which was released in 1991. The plant height ranges from 50-60 cm. Hundred seed weight is 11.73 gm. The seed color is bright yellow. The seed contains 40-45% protein and 21-22% oils. This variety can be grown both Kharif (Mid July) and Rabi (mid January) seasons. Maturity period ranges from 90-100 days. It can produce seed yield of 1.5-2.0 t/ha.



Fig:1 BINA Soybean-1



Fig:2 BINA Soybean-2



Fig:3 BARI Soybean-5



Fig:4 BARI Soybean-6



Fig:5 Shohag

3.2 Experiment location & experimental design

The experiment was carried out at the Biochemistry laboratory of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka, and also in the laboratory of Soil resource development institute (SRDI), Khamar Bari Rd, Dhaka 1215, Bangladesh during October 2019 to February 2020.

The experiment was conducted in a completely randomized design (CRD) with three replications.

3.3 Physical Analysis

3.3.1 Determination of 1000 grain weight

The mass was determined by randomly selecting 1000 seed samples and weighing in an electronic balance of 0.00 g sensitivity.

3.3.2 %Moisture content

Empty aluminum moisture dish was weighted (W1) and 2 g sample was taken in a moisture dish and weighted (W2). The sample was spread evenly and placed without lid in oven and dried samples overnight at 100°C. The dishes were transferred to desiccators to cool. Aluminum dish was weighed after cooling (W3). The moisture was determined from the following formula

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

3.3.3 Determination of Ash

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

Equipment

1. Balance
2. Muffle furnace
3. Desiccators
4. Crucible

Procedure

The temperature of the muffle furnace was fixed to 600°C and crucible was heated for 1 h and transferred into a desiccator; cooled them to room temperature and weighted (W1). About 2 g sample was put into the crucible weighted (W2). The sample was burned in a muffle furnace at 600°C for about 2 hrs. The crucibles were transferred into the desiccator and cooled them to room temperature and weighted (W3). It was done immediately to prevent moisture absorption. The incineration repeated until constant weight was obtained.

Calculation Weight of the sample taken = W2–W1

Weight of the ash obtained = W3–W1

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

3.4 Chemical Analysis

3.4.1 Estimation of Oils/Fats

Reagents and Equipment

- I. Petroleum ether
- II. Soxhlet apparatus: Soxhlet, flask and condenser
- III. Hot plate

Soybean oil was extracted by using Soxhlet method described by Aziz *et al.* (2018). Dried and grinded soybean sample were weighted out into an extraction thimble. Weight of thimble and sample were recorded in

laboratory work book. The thimble was placed into the Soxhlet. 250 ml petroleum ether was added to the Soxhlet flask, then it was connected to holder and condenser. Soxhlet flask was placed on the hot plate and distilled at low temperature (40-60°C) for 3-4 hours for each sample. 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 weighted out. The loss of weight was crude fat.



Fig 6: Oil extraction by using soxhlet method.

3.4.2 Estimation of total protein content by Micro Kjeldahl 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 Kjeldahl 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.

Equipment

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 gm of copper sulfate ($CuSO_4 \cdot 5H_2O$) 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 liters 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 grinded soybean sample (100 mg) weighted out in an Mkj digestion flask. About 2 gm 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

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

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.

3.4.3 Estimation of Carbohydrate

Total carbohydrate Estimation

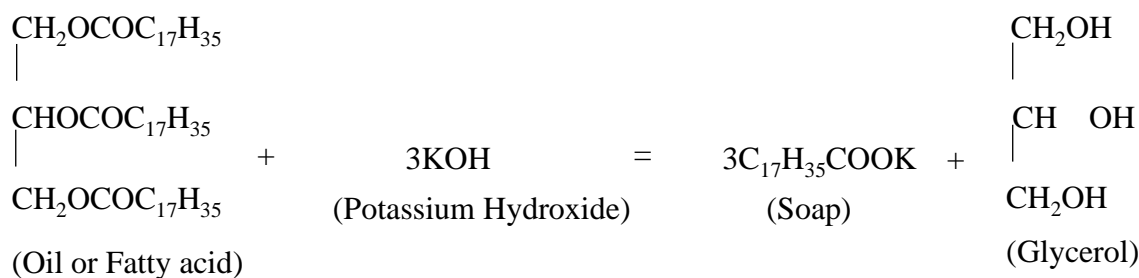
The method was described by Raghuramulu *et al.* (2003). The content of the available carbohydrate was determined by the following equation:

$$\text{Carbohydrate} = 100 - [(\text{Moisture} + \text{Protein} + \text{Ash} + \text{Oil/Fats}) \text{ g}/100\text{g}]$$

3.4.4 Chemical constant

3.4.4.1 Saponification value (SPV)

It is defined as the number of milligrams of KOH required to saponify 1 gm of fat or oil completely. It provides information on the average chain length and hence of molecular weight of fatty acids in the fat. The higher is the saponification number the shorter the average chain length of fatty acids, and the lower the average molecular weight of the fatty acids and vice-versa.



Reagents

1. Hydrochloric acid 0.5 N
2. Alcoholic solution of Potassium hydroxide (0.5 N KOH).
3. Few drops of phenolphthalein solution.

Procedure

1 gm oil was taken in 100 ml bottom flask 25 ml 0.5 N alcoholic KOH solution was added. Air condenser was connected tightly with the bottom flask. It was then heated for 30 minutes on the heater and shaken occasionally. After heating, air condenser was removed and the bottom flask was cooled at room temperature, the content of the bottom flask was titrated with 0.5 N HCl.

Here, few drops of phenolphthalein solution were added as indicator. Blank determination was conducted (without oil).

$$\text{Saponification value} = \frac{(B - T) \times 0.5\text{N} \times 56.1}{\text{Weight of oil}}$$

Here,

B = ml of HCl required for each blank

T = ml of HCl required for each oil sample

3.4.4.2 Iodine value

Iodine value is defined as the grams of iodine absorbed by 100 gm of fat or oil. The iodine value is a measure of the degree of unsaturation in an oil. Iodine value is a useful parameter in studying oxidative rancidity of oils since higher the unsaturation the greater the possibility of the oils to go rancid. The most important application of the iodine value is to determine the amount of unsaturation contained in fatty acids. This unsaturation is in the form of double bonds which react with iodine compounds. The higher the iodine value, the more unsaturated fatty acid bonds are present in a fat.

Reagents

1. Chloroform (CHCl_3) (few drops)
2. 15% KI solution (10 ml)
3. Starch (1 gm) dissolved in 100 ml hot water and diluted to 100 ml cold water
4. 0.1 N Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) solution (titration)
5. 0.1 N Potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) solution
6. Concentrated Hydrochloric acid (HCl)
7. Sodium bicarbonate (NaHCO_3)
8. Hanus solution

Preparation of Hanus I (iodine) solution

About 13 gm I (iodine) added into 3 ml of Bromine. Then added rest amount of glacial acetic acid (CH_3COOH) to make 1 L of solution.

Procedure

Less than 1 gm of oil was weighed into 500 ml glass stoppered I (iodine) flask and dissolved in few drops of chloroform and mixed properly. 25 ml of the Hanus I (iodine) solution was added and the mixture allowed to stand for 30 minutes in dark, shaking occasionally. 10 ml of 15% KI solution was added and mixed thoroughly, 100 ml freshly boiled and cooled water was added and washing down any free iodine on stopper. The iodine was titrated with 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$, adding it gradually, with constant shaking, until yellow solution turned almost colorless. Few drops of starch indicator were added and titration was continued until blue color entirely disappeared. Bottle was shaken violently, so that any iodine remaining in solution in the CHCl_3 might be taken up by the KI solution.

Two bland were conducted along with the sample. Percent weight of I (iodine) absorbed by the oil was calculated by the following formula:

$$\text{Iodine Number} = \frac{(\text{B}-\text{S}) \times \text{N} \times 0.127 \times 100}{\text{W}}$$

Where,

B = ml 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$ required by blank

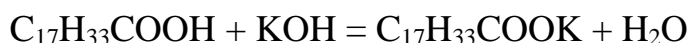
S = ml 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$ required by oil sample

N = Normality of $\text{Na}_2\text{S}_2\text{O}_3$

W = Weight of sample in gram.

3.4.4.3 Acid value

Acid value is defined as the mg of KOH required to completely neutralize free fatty acids present in 1 gm of fat or oil. Oils decompose on long standing due to bacterial or fungal contamination or chemical decomposition. Acid number is higher for oils stored for longer duration. Refined oils should be free from any free fatty acids and have negligible acid number. Acid value of oil is determined by titration of a known weight of it against 0.05 N potassium hydroxide using phenolphthalein as the indicator.



Reagents

1. 95% neutralized alcohol (ethanol, $\text{C}_2\text{H}_5\text{OH}$).
2. Potassium hydroxide (KOH) solution (0.05 N KOH)
3. Phenolphthalein ($\text{C}_{20}\text{H}_{14}\text{O}_4$) solution, 1% in 95% alcohol (ethanol, $\text{C}_2\text{H}_5\text{OH}$)

Procedure

Weigh 3-5 gm of oil in 250 ml round bottom flask and add 50 ml denatured alcohol (ethanol) and shake well. Now add 1 ml of phenolphthalein as indicator and titrate against 0.05 N KOH with vigorous shaking after each addition till a permanent light pink color is produced which persists for at least 1 minute.



Fig 7: Determination of acid value of soybean oil

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

Here,

N = Normality of KOH

W = wt. of sample in gram.

V = Variation (Final reading – initial reading)

3.4.5 Estimation of fatty acid composition

Fatty acid composition was determined by Gas-liquid chromatographic method (Uppstrom & Aa, 1978).

Reagent

1. Ethylate reagent (Petroleum ether / 0.02 M sodium hydroxide in ethanol (2/3))
2. A Salt solution (80 gm NaCl and 3 gm Sodium hydrogen Sulphate in 1 lit 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 shook.
3. The samples were kept for overnight at room temperature.
4. 10 ml salt solution was added and shook. 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 (1.5 m x 4 mm) was packed with BDS (Base Deactivated Silica). With this column the injection post, column and detector temperature was set at 220°C, 185°C and 240°C, respectively.
7. Nitrogen flow (used as carrier gas) rate was 22 ml/min, the injection volume was 2µl.

8. Peak areas were measured with an electronic digital integrator (ShinadzuC-R6A chromatopac).

3.4.6 Estimation of minerals

3.4.6.1 Preparation of reagents

a. Reagents for P determination

Reagent A

1. 45 gm antimony trioxide and 400 ml water were mixed in 1L volumetric flask and 150 ml concentrated 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. 1 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 concentrated HCL was added to dissolve the La_2O_3 . It was made to 5 liters with water.

c. Reagents for S determination

Reagent A

Mixed acid seed solution 65 ml of concentrated HNO₃ and 250 ml glacial acetic acid were added to about 500 ml of water. 3 ml of 1000 ppm S standard solution was added and made volume to 1 liter with water.

Reagent B (Turbidimetric reagent)

10 gm of polyvinyl pyrolidone (PVP K30) was dissolved in about 100 ml of hot water. 150 gm of BaCl₂.2H₂O was dissolved in about 500 ml of water. The PVP and barium chloride solutions were mixed and were made to 1 liter with water (Islam, 2015).

d. 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, 10 µg Fe/ml, 4 µg Mn/ml, 2 µg Zn/ml.
2. P and K were prepared together in water with high concentrations as follows: 20 µg P/ml, 100 µg K/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.

e. Digestion solution

Nitric-perchloric solution Concentrated Perchloric acid (100 ml) was added to 500 ml concentrated HNO₃ to prepare nitric-perchloric solution.

3.4.6.2 Digestion of soybean sample for determination of Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sulfur (S), Iron (Fe), Zinc (Zn), Copper (Cu), Manganese (Mn), Nitrogen (N).

a. Digestion procedure

500 gm oil cake was taken and put into a 50 ml boiling flask. 5 ml of nitric-perchloric (5:1) solution was added and placed in digestion chamber at 370°C temperature. 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 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 min and reading was taken of spectrophotometer at 680 nm for P and by using a flame photometer (Model AnA-135, OSK, Japan) at 766.5 nm for K. 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 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 700 (ANALYTIK JENA).

For Fe, Mn and Zn determination, the original filtrate was used to analyze these elements by AA (Atomic Absorption spectrophotometer) procedure. (Sharif *et al.*, 2017).

3.4.7 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 Least Significance Difference (LSD) test (Gomez & Gomez, 1984).

CHAPTER IV

RESULTS AND DISCUSSION

Five varieties of Soybean (*Glycine max*) were taken for the determination of physical and chemical characteristics. The seed were stored in the store house under a suitable storage condition. The proximate composition and some nutrient compositions of soybean seeds are also reported.

4.1 Physical characteristics of different varieties of Soybean (*Glycine max*)

4.1.1 1000 Grain weight

The weight of thousand grains of five different varieties of soybean has been compared in Table 1. It was found that seed weight varied with their size and shape. The highest thousand grain weight was found in BINA Soybean-2(159.00±1.0%) followed by BINA Soybean-1 (151.67±1.65%), BARI Soybean-5 (122.13±2.15%). The lowest weight was found in Shohag (117.33±2.65%) followed by BARI Soybean-6 (119.27±0.75). The observed values were more or less similar to the reported values of Krishna *et al.* (2003), Sultan *et al.* (2015), Deshpande *et al.* (1993). Sharma *et al.* (2014), Rani *et al.* (2008) showed lower value than present value.

4.1.2 Moisture

Seed quality is decreased as moisture content is increased. When seeds contain moisture above 18% then confronted by molds and insects. The moisture content of different released varieties of soybean had been presented in Table 1. The significant variations were observed among the different varieties in terms of effect on moisture content. The moisture

content of soybean varieties was ranged from 9.29 to 10.49%. The highest value of moisture content was found in BINA Soybean-2 ($10.49 \pm 0.28\%$) followed by BARI Soybean-5 ($9.79 \pm 0.01\%$). The lowest value of moisture content was found in Shohag ($9.29 \pm 0.12\%$) followed by BARI Soybean-6 ($9.53 \pm 0.06\%$). Present data is supported by Rani *et al.* (2008), Anwar *et al.* (2016), Kulkarni (2019), Deshpande *et al.* (1993), Sharma & Miglani (2016), Ashaolu *et al.* (2013). Etiosa *et al.* (2018) reported lower value than present value.

4.1.3 Ash

Ash content of five different varieties of soybean were variable and ranged from 4.32% to 5.18% (Table 1). The highest value was found in Shohag ($5.18 \pm 0.12\%$), which is statistically identical to BARI Soybean-6 ($5.07 \pm 0.03\%$). The lowest value was found in BINA Soybean-2 ($4.32 \pm 0.12\%$), followed by BARI Soybean-5 ($4.65 \pm 0.01\%$). Present data is supported by Anwar *et al.* (2016), Rani *et al.* (2008), Krishna *et al.* (2003), and Gupta *et al.* (2013), and Kuzniar *et al.* (2018).

Table 1. Weight of 1000 seed, Moisture percentage and Dry matter percentage of different varieties of soybean (*Glycine max*).

Name of the released cultivars (Treatments)	Wt. of 1000 seeds (gm)	Moisture %	Ash%
	mean \pm SD (n=3)		
BINA Soybean-1	151.67 ^b \pm 1.65	9.78 ^{bc} \pm 0.04	4.80 ^b \pm 0.03
BINA Soybean-2	159.00 ^a \pm 1.00	10.49 ^a \pm 0.28	4.32 ^d \pm 0.12
BARI Soybean-5	122.13 ^c \pm 2.15	9.79 ^b \pm 0.01	4.65 ^c \pm 0.01
BARI Soybean-6	119.27 ^{cd} \pm 0.75	9.53 ^{cd} \pm 0.06	5.07 ^a \pm 0.03
Shohag	117.33 ^d \pm 2.65	9.29 ^d \pm 0.12	5.18 ^a \pm 0.12
CV%	1.33	1.42	1.48
LSD _(0.05)	3.24	0.25	0.13

Mean values in columns marked with the same letter(s) do not differ significantly by LSD at 5%, where SD: Standard deviation

4.2 Chemical characteristics of different varieties of Soybean (*Glycine max*)

4.2.1 Oil content

The oil content of the soybean depends on many factors like genetic factors, agro-ecological conditions including cultivation sites and crop management system etc. The oil content of different varieties was extracted by petroleum ether (40-60°C) varied from 12.67% to 16.73% (Table 2). BINA Soybean-1 contained the highest percentage of oil (16.73 \pm 0.64%), followed by Shohag (15.83 \pm 0.29%) and BINA Soybean-2 (15.21 \pm 0.36%). BARI Soybean-5 has the lowest amount of oil percentage (12.67 \pm 1.15%); which is statistically identical to BARI

Soybean-6 ($13.12 \pm 1.02\%$). Anwar *et al.* (2016), Sharma *et al.* (2013), Sultan *et al.* (2015) reported higher oil content of soybean than present data. More or less similar data about 14.51% was reported by Amos-Tautua and Onigbinde (2013) which is similar to present data. These variations might be due to biological factor, environment factor, soil and also crop management practices.

4.2.2 Oil cake

Oil cake/meals are used for various purposes. Soybean oilcake is used for high-grade animal feed and feed mixture. The highest oil cake percentage was obtained from BARI Soybean-5 ($87.33 \pm 1.15\%$) which is statically identical to BARI Soybean-6 ($86.88 \pm 1.02\%$). The lowest value was from BINA Soybean-1 ($83.27 \pm 0.64\%$) followed by Shohag ($84.16 \pm 0.29\%$) and BINA Soybean-2 ($84.79 \pm 0.36\%$).

More or less similar result was reported by Sharma *et al.* (2014) and Anwar *et al.* (2016).

4.2.3 Protein

Protein is the major nutrient of different soybean variety. 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 varieties of soybean have been presented in Table 2. Statistically highest amount of protein was found in BINA Soybean-1 ($41.50 \pm 0.18\%$), followed by BARI Soybean-5 ($40.01 \pm 0.42\%$) which is statistically identical to Shohag ($39.86 \pm 0.05\%$). The lowest protein content was recorded in BARI Soybean-6 ($37.99 \pm 0.36\%$) which is statistically identical to BINA Soybean-2 ($38.29 \pm 0.10\%$). The observed values were more or less similar

to the values of Rani *et al.* (2008), Sharma *et al.* (2014), Kulkarni (2019), Rao *et al.* (1998), Sharma *et al.* (2013), Krishna *et al.* (2003), Sharma & Miglani (2016), Zhu *et al.* (2018), Jahan *et al.* (2013), Kim (1988), and Etiosa *et al.* (2018).

Table 2. Oil content (%), oil cake (%), and protein (%) of different varieties soybean (*Glycine max*).

Name of the released cultivars (Treatments)	Oil %	Oil cake (%)	Protein%
	mean \pm SD (n=3)		
BINA Soybean-1	16.73 ^a \pm 0.64	83.27 ^c \pm 0.64	41.50 ^a \pm 0.18
BINA Soybean-2	15.21 ^b \pm 0.36	84.79 ^b \pm 0.36	38.29 ^c \pm 0.10
BARI Soybean-5	12.67 ^c \pm 1.15	87.33 ^a \pm 1.15	40.01 ^b \pm 0.42
BARI Soybean-6	13.12 ^c \pm 1.02	86.88 ^a \pm 1.02	37.99 ^c \pm 0.36
Shohag	15.83 ^{ab} \pm 0.29	84.16 ^{bc} \pm 0.29	39.86 ^b \pm 0.05
CV%	5.27	0.91	0.68
LSD (0.05)	1.41	1.41	0.49

Mean values in columns marked with the same letter(s) do not differ significantly by LSD at 5%, where SD: Standard deviation

4.3 Carbohydrate

Carbohydrate content of different soybean varieties were determined moisture free basis. The data had been presented in Table 3. The highest amount of carbohydrate found in BARI Soybean-6 (34.72 \pm 0.58%), followed by BARI Soybean-5 (32.67 \pm 0.74%). The lowest value was observed in BINA Soybean1 (27.19 \pm 0.45%), followed by Shohag (30.69 \pm 0.10%) which is statistically identical to BINA Soybean-2 (30.99 \pm 0.41%). Krishna *et al.* (2003), Rani *et al.* (2008), Middelbos &

Fahey Jr. (2008) reported more or less similar value to the present value. Lower carbohydrate value was reported by Etiosa *et al.* (2018) and Gupta *et al.* (2013) and Krishna *et al.* (2003).

Table 3. Carbohydrate content (%) of different varieties of soybean (*Glycine max*).

Name of the released cultivars (Treatments)	Carbohydrate% mean \pm SD (n=3)
BINA Soybean-1	27.19 ^d \pm 0.45
BINA Soybean-2	30.99 ^c \pm 0.41
BARI Soybean-5	32.67 ^b \pm 0.74
BARI Soybean-6	34.72 ^a \pm 0.58
Shohag	30.69 ^c \pm 0.10
CV%	1.62
LSD (0.05)	0.92

Mean values in columns marked with the same letter(s) do not differ significantly by LSD at 5%, where SD: Standard deviation

4.4 Chemical constant

4.4.1 Saponification Value

Saponification value ranged from 187.70-191.79 (Table 4). The highest and the lowest saponification value were observed in BINA Soybean-1 (191.79 \pm 0.21), followed by BARI Soybean-5 (189.87 \pm 0.03) and BARI Soybean-6 (188.90 \pm 0.08). The lowest amount of saponification value observed in Shohag (187.70 \pm 0.3) which is statistically identical to the value of BINA Soybean-2 (187.71 \pm 0.17). The observed values were more or less similar to the reported values of Anwar *et al.* (2016), Belsare &

Badne (2017). Higher saponification value than presented value was presented by AmosTautua & Onigbinde (2013) and Prodhan *et al.* (2015).

4.4.2 Iodine value

Iodine values of soybean varieties are presented in Table 4. Significantly highest amount of iodine value was observed in BINA Soybean-1 (95.69 ± 0.01), followed by BINA Soybean-2 (79.89 ± 0.01). The lowest amount of iodine value was found in BARI Soybean-6 (73.66 ± 0.03). The present values are supported by the reported values of Amos-Tautua & Onigbinde (2013) which is about 73.02 g/100g oil. But the present values are lower than the results investigated by Prodhan *et al.* (2015), Anwar *et al.* (2016) and Abitogun *et al.* (2008).

Iodine value is directly correlate to the fatty acid present in oils and fats. Oils rich in saturated fatty acids have low iodine numbers, on the flip side oils rich in unsaturated fatty acids have high iodine numbers. As BINA Soybean-1 showed maximum iodine value (Appendix 1) thus it showed the highest amount of unsaturated fatty acid namely oleic acid ($C_{18:1}$) and linolenic acid ($C_{18:3}$).

4.4.3 Acid Value

Acid value of different varieties of soybean seed was ranged from 0.91 to 1.04 (Table 4). The highest amount of acid value was observed in BARI Soybean6 (1.04 ± 0.06). The lowest value was observed in BINA Soybean-1 (0.91 ± 0.01) which is statistically identical to BINA Soybean-2 (0.93 ± 0.02), Shohag (0.95 ± 0.04), and BARI Soybean-5 (0.95 ± 0.02). Present value is supported by the report of Belsare and Badne (2017) where the acid value ranged from 0.56 to 1.12 mg KOH/g. But the data is higher than the reported values of Prodhan *et al.* (2015) and lower than

the values reported by AmosTautua & Onigbinde (2013) and Abitogun *et al.* (2008).

Table 4. Saponification value, Iodine value and Acid value of different varieties of soybean (*Glycine max*).

Name of the released cultivars (Treatments)	Saponification value (mg of KOH/gm)	Iodine value	Acid value
		(gm of I/100gm)	(mg of KOH/gm)
mean \pm SD (n=3)			
BINA Soybean-1	191.79 ^a \pm 0.21	95.69 ^a \pm 0.01	0.91 ^b \pm 0.01
BINA Soybean-2	187.71 ^d \pm 0.17	79.89 ^b \pm 0.01	0.93 ^b \pm 0.02
BARI Soybean-5	189.87 ^b \pm 0.03	79.49 ^c \pm 0.01	0.95 ^b \pm 0.02
BARI Soybean-6	188.90 ^c \pm 0.08	73.66 ^e \pm 0.03	1.04 ^a \pm 0.06
Shohag	187.70 ^d \pm 0.3	75.78 ^d \pm 0.03	0.95 ^b \pm 0.04
CV%	0.10	0.09	3.54
LSD (0.05)	0.34	0.14	0.06

Mean values in columns marked with the same letter(s) do not differ significantly by LSD at 5%, where SD: Standard deviation

4.5 Fatty acid composition

Comparison of Gas chromatography results are demonstrated in Figure 8 and Figure 9. According to the results, there was a significant difference among the varieties of Soybean in terms of their fatty acid compounds. Oleic (C_{18:1}), linoleic (C_{18:2}), palmitic (C_{16:0}), stearic (C_{18:0}), and linolenic (C_{18:3}) acids are present in soybean oil along with traces (less than 1%) of palmitoleic (C_{16:1}), arachidic (C_{20:0}), and behenic (C_{22:0}) acids. The fatty acid compositions of saturated and unsaturated are given below:

4.5.1 Saturated fatty acid composition

Saturated fatty acid values of soybean varieties are presented in Figure 8. Significantly highest amount of palmitic acid ($C_{16:0}$) was observed in BARI Soybean-6 ($11.49\pm 0.07\%$), followed by BARI Soybean-5 ($10.94\pm 0.03\%$), BINA Soybean-1 ($10.74\pm 0.04\%$). Lowest amount of palmitic acid ($C_{16:0}$) content was found in Shohag ($9.74\pm 0.03\%$). The concentration of stearic acid ($C_{18:0}$) varied from $3.18\pm 0.03\%$ (Shohag) to $4.53\pm 0.04\%$ (BARI Soybean-6), whereas arachide acid ($C_{20:0}$) contents ranged from 0.13% to 0.23% in BINA Soybean-2 ($0.13\pm 0.04\%$) and BARI Soybean-6 ($0.23\pm 0.02\%$) respectively. BARI Soybean-6 variety contained the highest amount of behenic acid ($C_{22:0}$) ($0.85\pm 0.01\%$), followed by BINA Soybean-1 ($0.62\pm 0.03\%$), and the lowest value was in BARI Soybean-5 ($0.37\pm 0.02\%$). Present data is more or less similar to the data presented by Gunstone (1996), Amos-Tautua & Onigbinde (2013) and Martin *et al.* (2008). Lower value was presented by Aboitogun *et al.* (2008).

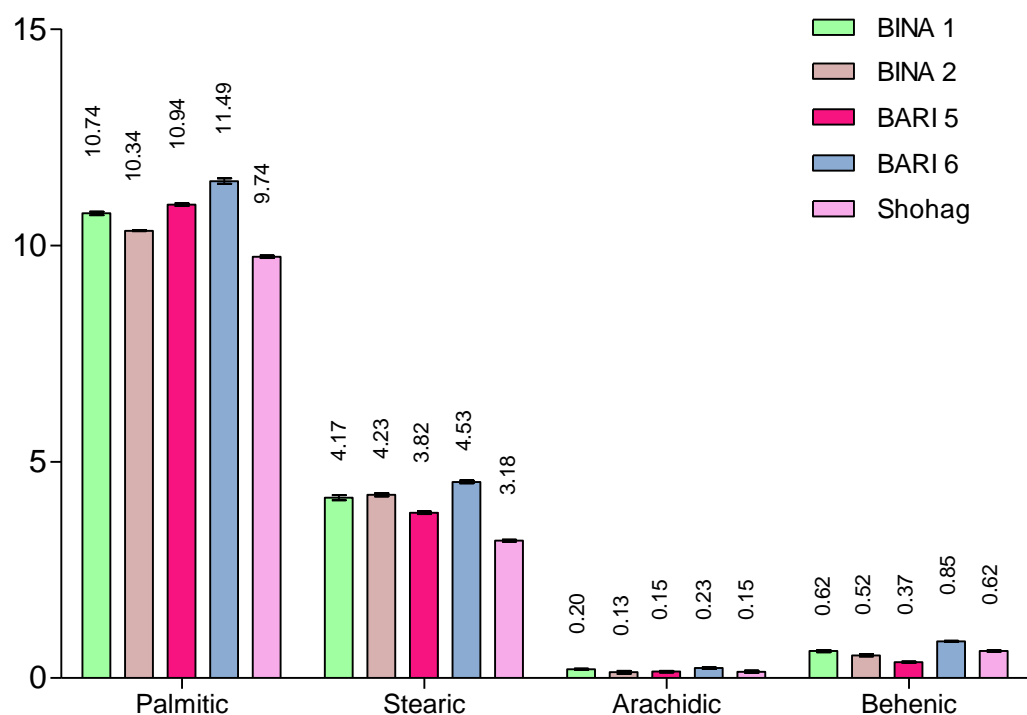


Fig 8: Saturated fatty acid comparison of five soybean varieties.

4.5.2 Unsaturated fatty acid composition

Unsaturated fatty acid values of soybean varieties are presented in Figure 9. The highest palmitoleic acid ($C_{16:1}$) content was observed in BINA Soybean-1 ($0.19 \pm 0.01\%$); followed by BINA Soybean-2 ($0.13 \pm 0.01\%$) and BARI Soybean-5 ($0.11 \pm 0.00\%$). The lowest palmitoleic acid ($C_{16:1}$) was found in BARI Soybean-6 ($0.08 \pm 0.02\%$). Highest amount of oleic acid ($C_{18:1}$) was found in BINA Soybean-1 ($27.72 \pm 0.03\%$) and the lowest amount of oleic acid found in BARI Soybean-6 ($16.99 \pm 0.12\%$). The linoleic acid ($C_{18:2}$) content varied 30.61% to 35.63%. The maximum amount of linoleic acid ($C_{18:2}$) was found in BINA Soybean-1 ($45.63 \pm 0.03\%$) and minimum in BARI Soybean-6 ($30.61 \pm 0.02\%$). The highest amount of linolenic acid ($C_{18:3}$) observed in BINA Soybean-1

($9.24 \pm 0.03\%$) and the lowest was found in BARI Soybean-6 ($5.28 \pm 0.03\%$). Present data is more or less similar to the data presented by Rao *et al.* (1998), Özcan & Al Juhaimi (2014), Aboitogun *et al.* (2008), Amos-Tautua & Onigbinde (2013).

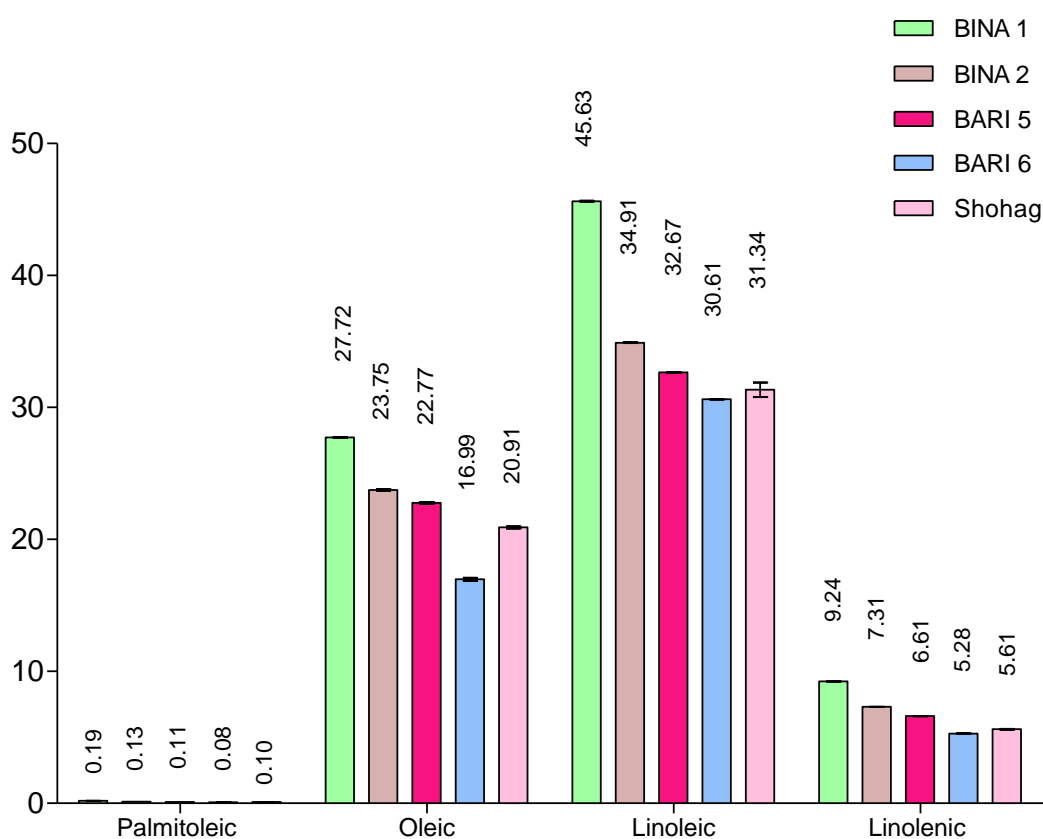


Fig 9: Unsaturated fatty acid comparison of five soybean varieties.

4.6 Mineral composition

Different major and minor minerals were analyzed in this work. The amounts of major minerals content of soybean have been shown in Table 5.

Phosphorus (P)

In case of Phosphorus content of different varieties of soybean was ranged from 0.50% to 0.82% (Table 5). Significantly highest amount of Phosphorus (P) content was observed in Shohag ($0.82 \pm 0.82\%$) which was followed by BINA Soybean-1 ($0.79 \pm 0.79\%$) and BARI Soybean-6 ($0.72 \pm 0.72\%$). Lowest amount of phosphorus content was obtained from BARI Soybean-5 ($0.50 \pm 0.50\%$) which was followed by BINA Soybean-2 ($0.68 \pm 0.68\%$). Present data is more or less similar to the reported value of Krishna *et al.* (2003), Özcan & Al Juhaimi (2014), Rani *et al.* (2008) and Etiosa *et al.* (2018).

Potassium (K)

The Potassium content of different soybean varieties were ranges from 0.60% to 0.80% in (Table 5). Significantly highest amount of K contained was found in Shohag ($0.80 \pm 0.04\%$) which was statistically identical to BINA Soybean1 ($0.79 \pm 0.01\%$). The lowest amount was found in BARI Soybean-5 ($0.60 \pm 0.17\%$) which was followed by BINA Soybean-2 ($0.69 \pm 0.02\%$) which is statistically identical to BARI Soybean-6 (0.73 ± 0.02). According to Özcan & Al Juhaimi (2014) the potassium contents of soybean seeds ranged between 16,375 mg/kg (raw Adasoy) and 20,357 mg/kg (sprouted A3127) which is higher than that of present data.

Table 5. P (%), K (%) content of different varieties of soybean (*Glycine max*).

Name of the released cultivars (Treatments)	P (%)	K (%)
	mean \pm SD (n=3)	
BINA Soybean-1	0.79 ^b \pm 0.02	0.79 ^a \pm 0.01
BINA Soybean-2	0.68 ^d \pm 0.01	0.69 ^{ab} \pm 0.02
BARI Soybean-5	0.50 ^e \pm 0.01	0.60 ^b \pm 0.17
BARI Soybean-6	0.72 ^c \pm 0.02	0.73 ^{ab} \pm 0.02
Shohag	0.82 ^a \pm 0.01	0.80 ^a \pm 0.04
CV%	1.76	10.90
LSD (0.05)	0.02	0.02

Mean values in columns marked with the same letter(s) do not differ significantly by LSD at 5%, where SD: Standard deviation

Sulphur (S)

The Sulphur content of different soybean varieties were ranges from 0.04% to 0.09% in (Table 6). Significantly highest amount of S contained was found in BARI Soybean-5 (0.09 \pm 0.0%) which was followed by BARI Soybean-6 (0.09 \pm 0.0%). The lowest amount was found in Shohag (0.04 \pm 0.0%), followed by BINA Soybean-1 (0.06 \pm 0.0%).

Magnesium (Mg)

Magnesium is the major minerals for human nutrition. Magnesium content of different varieties of soybean had been presented in Table 6. Magnesium content of different varieties were ranged from 0.31% to 0.41%. The highest amount of Magnesium content was found in BINA Soybean-2 (0.41 \pm 0.01%); followed by BINA Soybean-1 (0.37 \pm 0.01%)

and the lowest amount in BARI Soybean-6 ($0.31\pm 0.01\%$). Present value is higher than the data reported by Etiosa *et al.* (2018) and Uwem *et al.* (2017).

Calcium (Ca)

In case of calcium content of different varieties of soybean was ranged from 0.15% to 0.22%. Highest Calcium value found in BINA Soybean-2 ($0.22\pm 0.01\%$), second highest was from Shohag ($0.20\pm 0.01\%$) which is statistically identical to BARI Soybean-5 (0.20 ± 0.01). The lowest value was observed in BARI Soybean-6 ($0.16\pm 0.01\%$) which is statistically identical to BINA Soybean-1 ($0.16\pm 0.0\%$). Present data is more or less similar to the data of Krishna *et al.* (2003), Rani *et al.* (2008), and Etiosa *et al.* (2018) and Uwem *et al.* (2017).

Table 6. S (%), Mg (%), and Ca (%) content of different varieties of soybean (*Glycine max*).

Name of the released cultivars (Treatments)	S (%)	Mg (%)	Ca (%)
	mean \pm SD (n=3)		
BINA Soybean-1	$0.06^d \pm 0.0$	$0.37^b \pm 0.01$	$0.16^c \pm 0.0$
BINA Soybean-2	$0.07^c \pm 0.0$	$0.41^a \pm 0.01$	$0.22^a \pm 0.01$
BARI Soybean-5	$0.09^a \pm 0.0$	$0.32^{cd} \pm 0.03$	$0.20^b \pm 0.01$
BARI Soybean-6	$0.09^b \pm 0.0$	$0.31^d \pm 0.01$	$0.16^c \pm 0.01$
Shohag	$0.04^e \pm 0.0$	$0.35^{bc} \pm 0.0$	$0.20^b \pm 0.01$
CV%	1.34	5.09	3.87
LSD (0.05)	0.002	0.02	0.02

Mean values in columns marked with the same letter(s) do not differ significantly by LSD at 5%, where SD: Standard deviation

Iron (Fe)

Iron contained of different varieties of soybean from 55.03 ppm to 86.50 ppm (Table 7). Significantly highest amount of Fe contained was observed in BINA Soybean-1 (86.50 ± 0.45 ppm), followed by Shohag (72.50 ± 0.0 ppm). The variety BINA Soybean-2 showed lowest amount of Fe value (55.03 ± 0.05 ppm), followed by BARI Soybean-6 (56.03 ± 0.06 ppm). Uwem *et al.* (2017) and Rani *et al.* (2008) reported more or less similar iron content. Etiosa *et al.* (2018) and Krishna *et al.* (2003) reported higher iron content than present data.

Manganese (Mn)

The Manganese content of different soybean varieties were ranges from 25.46 ppm to 32.03 ppm in (Table 7). Significantly highest amount of Mn contained was found in Shohag (32.03 ± 0.06 ppm) which was followed by BINA Soybean-2 (31.69 ± 0.01 ppm). The lowest amount was found in BINA Soybean-1 (25.46 ± 0.79 ppm), followed by BARI Soybean-5 (25.51 ± 0.50 ppm). Uwem *et al.* (2017) stated that the Mn content in soybean variety is about 0.651 (mg/100g).

Copper (Cu)

Copper contained of different soybean varieties were ranged from 21.50 to 29.0 ppm (Table 7). Significantly highest amount of Cu observed in Shohag (29.0 ± 0.0 ppm). Lowest amount of Cu observed in BARI Soybean-6 (21.50 ± 0.0 ppm) followed by BARI Soybean-5 (21.50 ± 0.01 ppm). According to Garcia *et al.* (1998) copper contents are minimal for all soybean products (≤ 2.00 mg/100 g).

Zinc (Zn)

The Zinc content of different soybean varieties were ranges from 23.44 ppm to 54.70 ppm in (Table 7). Significantly highest amount of Zn was found in Shohag (54.70±00 ppm) which was followed by BINA Soybean-2 (36.98±00 ppm). The lowest amount was found in BINA Soybean-1 (23.44±0.01 ppm), followed by BARI Soybean-5 (27.00±0.1 ppm). Rani *et al.* (2008), Etiosa *et al.* (2018) reported that the zinc value ranges from 7.16-7.89 and 2.7 mg/100 g respectively.

Table 7. Fe (ppm), Mn (ppm), Cu (ppm), and Zn (ppm) content of different varieties of soybean (*Glycine max*).

Name of the released cultivars (Treatments)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)
	mean ± SD (n=3)			
BINA Soybean-1	86.50 ^a ± 0.45	25.46 ^e ± 0.79	28.75 ^b ± 0.02	23.44 ^e ± 0.01
BINA Soybean-2	55.03 ^e ± 0.05	31.69 ^b ± 0.01	27.55 ^c ± 0.01	36.98 ^b ± 0.0
BARI Soybean-5	61.24 ^c ± 0.01	25.51 ^d ± 0.50	22.25 ^d ± 0.01	27.00 ^d ± 0.1
BARI Soybean-6	56.03 ^d ± 0.06	31.25 ^c ± 0.01	21.50 ^e ± 0.00	34.87 ^c ± 0.01
Shohag	72.50 ^b ± 0.0	32.03 ^a ± 0.06	29.00 ^a ± 0.00	54.70 ^a ± 0.0
CV%	0.31	0.09	0.04	0.13
LSD (0.05)	0.02	0.02	0.02	0.08

Mean values in columns marked with the same letter(s) do not differ significantly by LSD at 5%, where SD: Standard deviation

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was carried out at the Biochemistry laboratory of Sher-e-Bangla Agricultural University and also in the laboratory of Soil resource development institute (SRDI), Khamar Bari Rd, Dhaka 1215, Bangladesh during October 2019 to February 2020 to find out thousand seed weight, moisture%, ash%, fat%, protein%, chemical constant, fatty acid composition, carbohydrate and mineral composition of five soybean genotypes and their cakes. Significant ($P < 0.05$) variations in the composition of examined seeds and cakes, as well as in their mineral content, were found.

Among the varieties the highest thousand grain weight was found in BINA Soybean-2 (159.00 ± 1.0 g) and the lowest thousand grain weight was found in Shohag (117.33 ± 2.65 g). BINA Soybean-2 contained highest amount of moisture content ($10.49 \pm 0.28\%$) whereas Shohag contained the lowest ($9.29 \pm 0.12\%$). Significantly highest amount of ash contained was recorded in Shohag ($5.18 \pm 0.12\%$), whereas BINA Soybean-2 contained the lowest ($4.32 \pm 0.12\%$). The highest amount of oil content was found in BINA Soybean-1 ($16.73 \pm 0.64\%$), whereas the lowest amount of oil content was found in BARI Soybean-5 ($12.67 \pm 1.15\%$). Highest amount of oil cake content found in BARI Soybean-5 (87.33 ± 1.1 %) and the lowest found in BINA Soybean-1 ($83.27 \pm 0.64\%$). Significantly highest amount of protein contained was found in BINA Soybean-1 ($41.50 \pm 0.18\%$), whereas in BARI Soybean-6 (37.99 ± 0.36 %) was found the lowest. Significantly highest amount of Carbohydrate contained was recorded in BARI Soybean-6 ($34.72 \pm 0.58\%$), whereas in BINA Soybean-1 ($27.19 \pm 0.45\%$) contained lowest.

Significantly highest amount of Iodine value was obtained from BINA Soybean-1 (95.69 ± 0.10), and the lowest value was obtained from BARI Soybean-6 (73.66 ± 0.03). Significantly highest amount of Saponification value was obtained from BINA Soybean-1 (191.79 ± 0.21) and the lowest value was obtained from Shohag (187.70 ± 0.3). Significantly highest amount of Acid value was obtained from BARI Soybean-6 (1.04 ± 0.06), and the lowest value obtained from BINA Soybean-1 (0.91 ± 0.01). BINA Soybean-1 content highest amount of Palmitoleic acid ($0.19 \pm 0.01\%$), Oleic acid ($27.72 \pm 0.03\%$), Linoleic acid ($45.63 \pm 0.03\%$) and Linolenic acid ($9.24 \pm 0.03\%$). BARI Soybean-6 contained the highest amount of Palmitic ($11.49 \pm 0.07\%$), Stearic ($4.53 \pm 0.04\%$), Arachidic ($0.23 \pm 0.02\%$), and Behenic acid ($0.85 \pm 0.01\%$). Highest amount of Phosphorus, Potassium, Sulphur, Magnesium, Calcium content found in Shohag ($0.82 \pm 0.82\%$), Shohag ($0.80 \pm 0.04\%$), BARI Soybean-5 ($0.09 \pm 0.0\%$), BINA Soybean-2 ($0.41 \pm 0.01\%$), BINA Soybean-2 ($0.22 \pm 0.01\%$) respectively; the lowest values were from BARI Soybean-5 ($0.50 \pm 0.50\%$), BARI Soybean-5 ($0.60 \pm 0.17\%$), Shohag ($0.04 \pm 0.0\%$), BARI Soybean-6 ($0.31 \pm 0.01\%$), BARI Soybean-6 ($0.16 \pm 0.01\%$) respectively. Highest Iron, Manganese, Copper, Zinc content was found in BINA Soybean-1 (86.50 ± 0.45 ppm), Shohag (32.03 ± 0.06 ppm), Shohag (29.0 ± 00 ppm), Shohag (54.70 ± 00 ppm) respectively whereas the lowest values were observed in BINA Soybean-2 (55.03 ± 0.05 ppm), BINA Soybean-1 (25.46 ± 0.79 ppm), BARI Soybean-6 (21.50 ± 00 ppm) respectively.

This study suggested that Shohag contained notable mineral composition. However, in terms of qualitative aspect, BINA Soybean-1 performed better over other varieties.

RECOMMENDATION

- In the light of this study we can recommend that BINA Soybean-1, and Shohag are better varieties.
- Since the oil content of some given varieties are very poor, we can recommend other high oil content varieties of soybean to achieve better results.
- Due to the potential high nutrient content of soybean, further analysis of different Soybean varieties should be done to know content the nutrients which will help the breeders to evolve more nutrient rich in Soybean varieties.

CHAPTER VI REFERENCES

- Abitogun, A., Jide, A., Arawande, J., Alademeyin, O. & Omosheyin, A. (2008). Effects of phosphoric acid on physico-chemical parameters of soyabean oil. *Internet J. Nutr. Wellness*. **8**(2): 1-5.
- Alam, F. (2020). Rising trend in consumption of oils and fats in Bangladesh. e-paper: <https://thefinancialexpress.com.bd/views/analysis/risingtrend-in-consumption-of-oils-and-fats-in-bangladesh-1582381921>.
- Amos-Tautua, B.M.W. and Onigbinde, A.O (2013). Physicochemical Properties and Fatty Acid Profiles of Crude Oil Extracts from Three Vegetable Seeds, *PJN*. **12**(7): 647-650.
- Anwar, F., Kamal, G.M., Nadeem, F. and Shabir, G. (2016). Variation of quality attributes among oils from different soybean varieties. *J King Saud Univ Sci*. **28**(4): 332-338.
- AOAC. 2010. Official methods of analysis. Association of official analytical chemists, Washington, DC.
- Ashaolu, M.O. & Noibi, A.O. (2013). Effects of moisture content on some mechanical properties of Soybean (*Glycine max*) varieties. *AJFST*. **4**(10): 211-220.
- Ayanwole, J.A. (2007). Field evaluation of soybean varieties at Ilorin in the southern guinea savanna ecology of Nigeria. *Afr. J. Agric. Res*. **2**(8): 356-359.
- Azadbakht, L., Shakerhosseini, R., Atabak, S., Jamshidian, M., Mehrabi Y. and Esmail-Zadeh, A. (2003). Beneficiary effects of dietary soy

protein on lowering plasma levels of lipid and improving kidney function in type II diabetes with nephropathy. *Eur J Clin Nutr.* **57**(10): 1292–1294.

Aziz, S., Siddique Md, A. B. & Begum, M. H. A. (2018). Cotton Seed oil cake as a valuable Source of Plant Nutrients for Sustainable Agriculture. *The Pharmaceutical and Chem. J.* **5**(3): 39-45.

Belsare, G.W. and Badne, S.G. (2017). Study on physico-chemical characterization of edible oils from agencies of Buldhana district. *JRPC.* **7**(4): 525-529.

Circle, H. and Smith, A.H. (1972). Soybeans: Chemistry and Technology. Smith, Allan & Circle, Sidney, (ed.). AVI, Westport CT, USA: pp. 104, 163.

Deol, P., Fahrman, J., Yang, J., Evans, J. R., Rizo, A., Grapov, D. & Hammock, B.D. (2017). Omega-6 and omega-3 oxylipins are implicated in soybean oil-induced obesity in mice. *Sci. Rep.* **7**(1): 1-13.

Deshpande, S.D., Bal, S. and Ojha, T.P. (1993). Physical properties of soybean. *J. of Agric: Engineering Res.* **56**(2): 89-98.

Etiosa, O.R., Chika, N.B. and Benedicta, A. (2018). Mineral and Proximate Composition of Soya Bean. *Asian j. phys. chem. Sci.* **4**(3): 1-6.

Garcia, M., Marina, M., Labordab, F. and Torre, M. (1998). Chemical characterization of products. *Food Chem.* **62**(3): 325-331.

- Gomez, K.A. & Gomez, A.A. (1984). Statistical Procedure for Agricultural Research (2nd Edition). IRRI, Laguna, Philippines. John Wiley & Sons, (ed.). pp. 28-192.
- Gunstone, F.D. (1996). Fatty Acid and Lipid Chemistry. **In:** Lipid Chemistry. F.D. Gunstone, (ed.). Blackie, London, US. pp. 61-86.
- Gupta, R., Sharma, D. & Joshi, I. (2013). Nutrient Analysis of Raw and Processed Soybean and Development of Value-Added Soybean Noodles, *Inventi Rapid: Life Style*. **1**(1): 1-5.
- Islam, S. (2015). Study on the physico-chemical characteristics of the oils and oil cakes of the different released and line cultivars of mustard and rapeseed (*Brassica* spp.). M.S thesis. SAU, Dhaka, Bangladesh.
- Jahan, D.A., Hussain, L., Islam, M.A. and Khan, M. (2013). Comparative Study of Mustard Oil Cake and Soybean Meal Based Artificial Diet for Rohu, *Labeo rohita* (Ham.). *BanglaJOL*. **11**(1): 61-66.
- Kim, C.J. (1988). Physico-chemical, nutritional, and flavor properties of soybean extracts processed by rapid-hydration hydrothermal cooking. Ph.D. thesis, ISU, Ames, Iowa, US.
- Krishna, A., Singh, G., Kumar, D. and Agrawal, K. (2003). Physico-chemical characteristics of some new varieties of soybean. *JFST*. **40**(5): 490-492.
- Kulkarni, S.S.U.N. (2019). Physico-chemical properties of black and yellow soybean (*Glycine max* L.) Genotype. *Pharma Innovation*. **8**(7): 33-37.

- Kuzniar, P., Szpunar-Krok, E., Findura, P., Bucze, J. and Bobrecka-Jamro, D. (2016). Physical and chemical properties of soybean seeds determine their susceptibility to mechanical damage. *Zemdirbyste*. **103**(2): 183–192.
- Malik, M.F.A., Ashraf, M., Qureshi, A.S. and Ghafoor, A. (2007). Assessment of genetic variability, correlation and path analyses for yield and its components in soybean Pakistan. *J. Bot.* **42**(2): 971-976.
- Martin, C.A., Visentainer, J.V., Oliveira, A.N.D., Oliveira, C.C.D., Matsushita, M. & Souza, N.E.D. (2008). Fatty acid contents of Brazilian soybean oils with emphasis on trans fatty acids. *J. Braz. Chem. Soc.* **19**(1): 117-122.
- Middelbos, I.S. and Fahey, Jr. G.C. (2008). Soybean carbohydrates. **In:** Soybeans Chemistry, Production Processing, and Utilization. L.A. Johnson, P.J. White, and R. Galloway, (eds.). AOCS Press, Urbana, IL. pp. 269-296.
- Murray-Kolb, L.E., Welch, R., Theil, E.C. and Beard, J.L. (2003). Women with low iron stores absorb iron from soybeans. *Am. J. Clin. Nutr.* **77**(1): 180–184.
- Ngalamu, T., Ashraf, M. and Meseke, S. (2013). Soybean (*Glycine max* L) genotype and environment interaction effect on yield and other related traits. *Am J Exp.* **3**(4): 977-987.
- Özcan, M.M. & Al Juhaimi, F. (2014). Effect of sprouting and roasting processes on some physico-chemical properties and mineral contents of soybean seed and oils. *Food Chem.* **154**:337-342.

- Proadhan. U.K., Islam. M.A., Quoser. J., Linkon, M.R., Rahman, M.S. and Rahman, N.M. (2015). Chemical Characteristics of Different Brands of Soybean Oil Available in Bangladesh. *IJERT*. **4**(4): 338-343.
- Raghuramulu, N., Madhavan, N.K. and Kalyanasundaram, S. (2003). A Manual of laboratory Techniques. National Institute of Nutrition. Indian council of Medical Research, Hyderabad-500007. Hyderabad, India. pp: 56-58.
- Ranganna, S. (1986). Handbook of analysis and quality control for fruit and vegetable production. New Delhi, India. pp. 124-125.
- Rani, V., Grewal, R.B. and Khetarpaul, N. (2008). Physical characteristics, proximate and mineral composition of some new varieties of soybean (*Glycine max l.*). *Legume Res.* **31**(1): 31 – 35.
- Rao, M.S.S., Bhagsari, A.S. and Mohamed, A.I. (1998). Yield, protein, and oil quality of soybean genotypes selected for tofu production. *Plant Foods Hum Nutr.* **52**(3): 241–251.
- Sharif, R.H., Paul, R.K., Bhattacharjya, D.K. and Ahmed, K.U. (2017). Physicochemical characters of oilseeds from selected mustard genotypes. *JBAU*. **15**(1): 27-40.
- Sharma, S. and Miglani, H. (2016). Cell wall composition and nutrients in soybean genotypes of varying seed coat colour. *Legume Res.* **40**(3): 444-452.
- Sharma, S., Goyal, R. and Barwal, S. (2013). Domestic processing effects on physicochemical, nutritional and anti-nutritional attributes in soybean (*Glycine max L. Merill*). *Int Food Res J.* **20**(6): 3203-3209.

- Sharma, S., Kaur, M., Goyal, R. & Gill, B.S. (2014). Physical characteristics and nutritional composition of some new soybean (*Glycine max* (L.) Merrill) genotypes. *JFST*. **51**(3): 551–557.
- Singh, V.P., Singh, S.P., Kumar, A., Tripathi, N. & Nainwal, R.C. (2010). Efficacy of Haloxyfop, a post-emergence herbicide on weeds and yield of soybean. *IJWS*. **42**(1&2): 83-86.
- Sultan, S.M., Dikshit, N. and Vaidya, U.J. (2015). Oil content and fatty acid composition of soybean (*Glycine max* L.) genotypes evaluated under rainfed conditions of Kashmir Himalayas in India. *IASET*. **7**(2): 910 – 915.
- Uppstrom, B. and Aa, J.S. (1978). Methods for determination of fatty acids applied to a breeding program. In Proceedings, 5th international rapeseed conference, June. 12-16, Malmo, Sweden, pp. 140-144.
- US Food and Drug Administration. (October, 1999). Food Labeling: Health Claims; Soy & Protein Coronary Heart Disease. <https://www.govinfo.gov/content/pkg/FR-1999-10-26/pdf/99-27693.pdf>.
- Uwem, U.M., Babafemi, A.A. and Sunday, D.M. (2017). Proximate Composition, Phytoconstituents and Mineral Contents of Soybean (*Glycine Max*) Flour Grown and Processed in Northern Nigeria. *AAS*. **2**(4): 48-53.
- Zhu, Z., Chen, S., Wu, X., Xing, C. and Yuan, J. (2018). Determination of soybean routine quality parameters using near-infrared spectroscopy. *Food Sci Nutr*. **6**(4): 1109-1118.

CHAPTER VII APPENDIX

Appendix 1: Relation between Iodine value and unsaturated fatty acid (palmitoleic, oleic, linoleic and linolenic) present in soybean varieties (*Glycine max*).

