NUTRITIONAL STUDY OF SOME ADVANCED MUTANT LINES OF GROUNDNUT (*Arachis hypogaea*)

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This is to certify that the thesis entitled, "**NUTRITIONAL STUDY OF SOME ADVANCED MUTANT LINES OF GROUNDNUT (*Arachis hypogaea*)**" Submitted to the Department of Biochemistry, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN BIOCHEMISTRY** embodies the result of a piece of bona fide research work carried out by **MARIOM MITU, Registration No. 14-05895** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma in any other institutes.

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

Dated: December, 2020
Dhaka, Bangladesh

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Dedicated to

My Beloved

Parents

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ABSTRACT

Groundnut, popularly known as "chinabadam" is the second most important oil seed crop in Bangladesh, after mustard (Brassica spp.) in terms of annual production. It is used as an edible oil in the food industry to produce cake, cookies, and baked goods. It plays an important role in human nutrition containing high protein, calories, minerals, and vitamins. Binachinabadam-6 and four advanced mutant lines derived from it, B6/282/63, B6/282/64, B6/282/80 and RM-Kha-19 were studied to investigate the qualitative properties of the genotypes and to find potential groundnut genotypes which can be released as variety. Among the 5 selected genotypes oil content were observed from 54.6% to 48.5%. Myristoleic acid (2.463-0.009%), oleic acid (55.7-29.977%), linoleic acid (17.129-13.691%), erucic acid (3.035-1.634%) as unsaturated fatty acids and hexanoic acid (23.291-4.232%), lauric acid (4.742-1.302%), palmitic acid (8.660-1.044%), arachidic acid (28.455-16.829%) were estimated as saturated fatty acids. O/L ratio (3.68-1.75) and iodine value (74.12-53.71) were calculated from fatty acid components. Ca (85.54-74.12 mg/100g), Mg (173-148 mg/100g), S (89-56 mg/100g), N (5190-4370 mg/100g), P (490-360 mg/100g), K (730-650 mg/100g) as major minerals and Fe (2.15-1.62 mg/100g), Zn (5.9-3.8 mg/100g), Cu (1.5-0.5 mg/100g), B (3.6-2.4 mg/100g) were estimated as minor minerals. Other properties i.e protein (32.42-27.34%), ash (2.38-2.23%), crude fiber (3.71-3.52%), carbohydrate (11.4- 5.57%) and total energy (628.4- 594.58%) were also observed. Considering all the findings, B6/282/80 was the best performer among all mutants in this study.

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CHAPTER 1

INTRODUCTION

Groundnut, often called as “The King of Oilseeds”, is botanically known as *Arachis hypogaea* and belongs to family Leguminosae. It is a self-pollinating, indeterminate, an allotetraploid (2n = 2x = 40), annual herbaceous legume crop (Adinya *et al.*, 2010). Groundnut is one of the world's most important economic crops, ranking 13th among food crops, fourth most important source of edible oil and the third most important source of vegetable protein (Sorrensen et al., 2004; Taru et al., 2008). The history of groundnuts dates back to the times of the ancient Incas of Peru. They were the first to cultivate wild groundnuts and offered them to the sun God as part of their religious ceremonials. They used to call groundnuts as ynchic. The modern history of groundnut popularization began with the civil war of the 1860s in America. George Washington Carver is known as the "Father of the Groundnut Industry" since he developed over 300 products derived from groundnuts (Carver 1925).

Groundnut is the second most important oil seed crop in Bangladesh, after mustard (Brassica spp.) in terms of annual production, and third in terms of acreage, behind sesame (Sesamum indicum L.) (Kabir *et al*, 2013). It has a higher oil content than soybean (Glycine max) and mustard. Earthnuts, peanuts, goobers, goober peas, pindas, jack nuts, pinders, manila nuts, g-nuts, and monkey nuts are some of the other names for groundnuts; the last of these is often used to refer to the whole pod (Annadurai and Palaniappan, 2009). It's known as "chinabadam" in Bangladesh. As a major oil seed crop as well as a food source, groundnut is grown on 31769 hectares in Bangladesh, with 53664 metric tons produced during the Rabi and Kharif seasons (BBS, 2011). It is currently cultivated on approximately 35,000 ha, with 40,000 metric tons of groundnuts produced annually in the Gangetic delta districts of viz. Noakhali, Faridpur, Kishoreganj, Patuakhali and Rangpur districts. Groundnut is a very important crop in Bangladesh. It is used as an edible oil in the food industry to produce cake, cookies, and baked goods. Oil cake is used as cattle feed and for human is traditionally eaten as fried ‘badam'.

The nutritional quality of groundnut (*Arachis hypogaea* L.) products depends on the protein content, oil content, and composition of oil. Groundnut kernel contains 48-50% oil, 25-28% protein, 8-14% soluble sugar, vitamin B, and vitamin E, as well as more than 30 essential nutrients. The oil contains about 30% linoleic acid, which is an essential fatty acid for humans. Except for leucine and methionine, groundnut oil contains all of the essential amino acids. Groundnuts' monounsaturated fats play an important role in a heart-healthy diet. Moreover, an adult human requires 55 grams of protein per day, and groundnuts alone can provide 5-6 grams (10%) of that requirement. Groundnuts are high in niacin, foliate, fiber, magnesium, manganese, phosphate, flavonoids, and isoflavones, as well as flavonoids and isoflavones (Janila et al. 2013). Each 100 gm of groundnut contain 600 kcal, 50gm fat, 800mg sodium and 10mg fiber and no cholesterol. For this reason, World Health Organization recommends 2 servings of 100 gm of processed nuts as a survival base for African children per day (http://news.bbc.co.uk/2/hi/europe/8610427.stm, Date: November 6, 2012). This useful seed is also used to produce processed foods like delicious dry roasted groundnuts, groundnut butter, oil etc.

Since legumes are high in protein, calories, minerals, and vitamins, they play an important role in human nutrition (Deshpande, 1992). All these components are present in their most beneficial forms. The protein is plant-based and the fat is unsaturated which are all proved to be the best for human nutrition. Despite the fact that legumes are low in S-containing amino acids (Farzana & Khalil, 1999), they increase the protein content of cereal-based diets and can boost the nutritional status of cereal-based diets that are low in lysine (Amjad *et al*., 2003). Nuts are a good source of oil among legumes since they contain more unsaturated fatty acids than saturated fatty acids (Sabate, 2003). Groundnut contains more protein than any other nut, with levels equal to or better than a serving of beans. The protein content of the cake can exceed 50% after the extraction of the groundnut oil, which contains all 20 amino acids in varying amounts (Zhao *et al*. 2011).

The components in groundnut are highly digestible. The true protein digestibility of groundnut is comparable with that of animal protein (Singh and Singh 1991). Groundnut products (raw, butter and oil) are more beneficial to heart health when compared to the low fat diets. The high monounsaturated fat groundnut diets lowered their total body cholesterol by 11 % and bad LDL cholesterol by 14 %, while their

good HDL cholesterol was maintained with reduction in triglycerides (Pelkman 2004). The benefits of the groundnut diets on cholesterol were comparable to the olive oil diet. There is strong evidence supporting an association between monounsaturated fat as well as overall nut intake and reduction in the risk of coronary heart disease (Matilsky *et al*. 2009). As they are highly nutritious, groundnut and products based on groundnut can be promoted as nutritional foods to fight energy, protein, and micronutrient malnutrition among the poor (Janila *et al*. 2013).

The soil and climate of Bangladesh are ideal for growing groundnuts. It is grown mainly in sandy soils and riverbeds (Nath and Alam, 2002). Groundnut is a major crop in Bangladesh's char lands, but farmers only make a small profit from it due to low yields. The productivity of groundnut depends on proper selection of variety, fertilizer management, environmental factors, metal contents in soil and other management practices (Uddin *et al*., 2016). To make the country self-sufficient in edible oil, the potential production of oil seed crops, including groundnut, must be increased, either by increasing yield per hectare, increasing cultivation acreage, or a combination of both. Groundnut productivity is highly influenced by proper selection of variety. The aim of this study was to investigate the qualitative properties of some advanced mutant genotypes and to find potential groundnut genotypes which can be released as variety.

The specific objectives of the study are as follows:

1. To study the chemical composition and mineral profiles of five groundnut genotypes.
2. To evaluate the quality and potentiality of 4 mutant lines of groundnut with reference of a promising variety ‘Binachinabadam-6’.

CHAPTER 2

REVIEW OF LITERATURE

Shad *et al.* (2009) conducted an experiment in which the biochemical composition and some phytochemicals in the seeds of 4 groundnut (*Arachis hypogaea* L.) varieties viz., Golden, Barri 2000, Mongphalla and Mongphalli 334 cultivated in arid zones of Pakistan, were determined. The biochemical analysis included ash, crude fat, total nitrogen, proteins and sugar contents. A statistically significant difference ($p<0.05$) was observed among the varieties regarding the ash, crude fat, water soluble proteins, salt soluble proteins and sugar contents. The four groundnut varieties were also found to be significantly different ($p<0.05$) on the basis of phytochemicals analyzed including tannins (822±3.78 to 903±4.45 mg/100g), saponins (438±2.12 to 480±2.30 mg/100g), non-protein nitrogen (1.33±0.03 to 1.56±0.02 mg/100g), hydrogen cyanide (40.80±0.32 to 42.82±0.75 mg/100g), total phenolic acids (218±2.11 to 256±2.02 mg/100g), total phosphorus (700±3.62 to 889±3.84 mg/100g) and phytic acid (572±4.37 to 714±3.74 mg/100g).

Ibrahim (2009) used groundnut seed cake of Barberton and Ashford cultivars in his study to investigate the nutritional and functional properties of the cake and defatted flour. Significant ($P\leq0.05$) differences were obtained in the seed cake between the two cultivars in protein, fat, ash, fiber and carbohydrates. Barberton was high in Ca content (0.38%), while Ashford was high in Fe content (0.31%) and there was no significant ($P\geq0.05$) difference in P content between the two cultivars. Defatted flours were analyzed for functional properties (protein solubility, water and oil absorption capacity, emulsifying and foaming properties, bulk density, dispersibility, wettability and gelation), and there was no significant difference between the two cultivars in these functional properties. Solubility of groundnut protein was determined at different pH values; minimum solubility was recorded at pH 4 and maximum solubility at pH 10 for both cultivars. In both cultivars, the flours had good functional characteristics with high water and oil absorption capacities. The emulsifying capacity was 28.10 and 22.90 ml/g for Barberton and Ashford, respectively. The emulsifying

activity was 28.33% and 22.90% for the two cultivars, respectively. The emulsion stability was 13.86% for Barberton and 11.36% for Ashford and the foaming capacity was 4.2% and 4.0%, respectively, with high stability for both cultivars. The flours of both cultivars had high dispersibility in alkaline and acidic media than the neutral. The bulk density was 0.71g/ml and had good wettability and gelation property.

Ingale and Shrivastava (2011) determined proximate composition, anti-nutritional and nutritional value of seeds of new variety of groundnut (*Arachis hypogaea* L) JL-24. The result showed that the groundnut seed contain moisture (5.529%), crude fibre (1.149%), lipid (46.224%), crude protein (25.20%), carbohydrate (21.26%), ash (2.577%), calcium (0.087%), phosphorus (0.29%) and energy (601.856%). The total fatty acid composition was 10.44 and 33.51% for saturated and unsaturated fatty acid, respectively. The protein solubility at different pH ranging from 0.5 to 13.5, the maximum seeds proteins were extracted at pH 12. The serine has not been reported in the seed protein and the seed was found to contain highest amount of proline (6.412%). The anti-nutritional analysis shows that cyanide content 4.818 HCN/100 g, tannin 0.412/100 g, oxalate 0.180/100g and haemagglutinin activity for goat blood group is 1:8 and no haemagglutinin activity for chicken and human blood group, and no trypsin inhibition was found. The nutritive values were determined in terms of feed utilization 6.552%, nitrogen utilization 0.2957%, protein efficiency ratio (PER) (+) 1.368% and feed efficiency ratio (FPR) (+) 0.345%.

Zahran and Tawfeuk (2019) carried out a study in which some nutrients and characteristics of the seeds oil extracted from four groundnut (Arachis hypogaea L.) varieties: Line 27r (Israel), Line 9 (Malawi), Line 4 (Brazil) and Line 18 (Israel) cultivated, for first time, in Upper Egypt were subjected to the comparative assessment with control NC variety (USA). Groundnut seeds are a rich source of oil content (50.45 to 52.12 g 100 g1 dry weight *"*DW*"*). The physicochemical properties of extracted oil were investigated in this study. The obtained data showed that the ratios of saturated fatty acids ranged from 14.24 to 17.23%, and the amounts of unsaturated fatty acids ranged from 82.77 to 85.76%. Significant variations ($p<0.05$) of oil content, saponification value, oleic/linoleic (O/L), and oil characteristics were found. Line 9

was found to be high in oil content, while Line 27r was said to have a high O/L ratio (3.22%) and proportion of unsaturated fatty acids (85.76%).

Asibuo *et al*. (2008) studied Oil, fatty acids, protein, oleic/linoleic (O/L) acid ratio, iodine value and free soluble sugars in 20 groundnut varieties grown in Ghana to determine their nutritional quality and to inform producers which variety to choose for maximum benefit. Results indicated a significant difference ($p<0.05$) in oil content among the varieties. Oil content ranged from 33.60 to 54.95%. Mean oil content of the subspecies *hypogaea* (49.7%) was higher than in subspecies *fastigiata* (47.3%). The major fatty acids were oleic and linoleic which accounted for 77.89% of the total fatty acids. The subspecies *hypogaea* had significantly higher ($p<0.01$) content of oleic acid (55.9%) than the subspecies *fastigiata* (43.3%). The sum of three fatty acids oleic, linoleic and palmitic acid constitute 89.35% of the total fatty acids of the seeds. The mean O/L ratio ranged from 1.14 to 3.66; the mean for subspecies *hypogaea* was 2.59 as compared to 1.28 for subspecies *fastigiata*. There was high correlation between oleic and O/L acid ratio ($r2=0.983$) and negative correlation between oleic acid and linoleic acid ($r2=-0.996$). The iodine value ranged from 85.77 to 98.43% and total soluble sugars from 9.20 to 13.30%. Protein of defatted portion ranged from 39.65 to 53.45%. Subspecies *fastigiata* had higher mean protein content than subspecies *hypogaea*. Generally, there were significant variations in the parameters measured in the groundnut varieties. Five varieties with O/L ratio more than 2.0 were identified and their oils suggested be further tested for their stability.

Janila *et al*. (2014) conducted a study to facilitate the initiation of a breeding program to improve the concentration of iron (Fe) and zinc (Zn) in groundnut (*Arachis hypogaea* L.) seeds. The experiment was conducted with 64 diverse groundnut genotypes for 2 years in eight different environments at the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India to assess the genetic variation for Fe and Zn concentrations in groundnut seeds and their heritability and correlations with other traits. Significant differences were observed among the genotypes and environments for Fe (33–68 mg/kg), Zn (44–95 mg/kg), protein (150–310 mg/g) and oil (410–610 mg/g) concentration in seeds and their heritability was

high, thus indicating the possibility of improving them through breeding. As seen in other plants, a significant positive association between concentrations of Fe and Zn was observed. Trade-offs between pod yield and Fe and Zn concentrations were not observed and the same was also true for oil content. Besides being high yielding, genotypes ICGV 06099 (57 mg/kg Fe and 81 mg/kg Zn) and ICGV 06040 (56 mg/kg Fe and 80 mg/kg Zn) had stable performance for Fe and Zn concentrations across environments. They suggested that, those were the ideal choices for use as parents in a breeding program and in developing mapping populations.

Sibt-e-Abbas *et al*. (2015) conducted a research in which defatted peanut flour (DPF) of indigenous varieties i.e. Golden and BARI-2011 was used for the extraction of proteins through isoelectric precipitation. The results showed that the protein content ranged from 26.17±0.56% to 27.42±0.61% in GOLDEN and BARI 2011 respectively. The protein isolate recovery was 27.573±0.49% and 29.057±0.30% while protein yield was found to be 79.047±1.95% and 86.840±3.52% respectively for both peanut varieties. Results regarding the functional properties i.e. bulk density, oil & amp; water absorption capacity, foaming & amp; emulsion properties revealed that the values increased by the addition of protein isolates. In the nutshell, protein isolates obtained from defatted peanut flour hold the potential to enrich various baked products. They suggested that, such protein enriched products can be further utilized to control the menace of protein energy malnutrition in developing economies.

Wu *et al*. (2009) studied the effect of different preparations on the functional properties of peanut protein concentrates. Peanut protein concentrates were isolated from defatted peanut flour by isoelectric precipitation, alcohol precipitation, isoelectric precipitation combined with alcohol precipitation, alkali solution with isoelectric precipitation and their functional properties (protein solubility, water holding/oil binding capacity, emulsifying capacity and stability, foaming capacity and rheology) were evaluated. The results showed that the protein solubility, foaming capacity and stability of protein prepared by alkali solution with isoelectric precipitation were the best of all the peanut protein products. But the protein prepared by alcohol precipitation had better water holding/oil binding capacity, which was

significantly different from other protein products. The emulsifying stability of protein concentrate prepared by different methods was significantly lower than that of defatted protein flour. The protein prepared by isoelectric precipitation and isoelectric precipitation combined with alcohol precipitation had better gel properties which indicated that they were a potential food ingredient.

Shibli *et al.* (2019) investigated three indigenous peanut cultivars from Pakistan specifically Local-334, Bard-92 and Bard-479 in the study for compositional quality and peanut butter development. Chemical composition of peanut cultivars indicated 5.53±0.20 to 5.93±0.02 % moisture, 2.00±0.11 to 2.17±0.05% ash, 49.80±3.54 to 50.90±0.93% fats, 23.83±1.71 to 26.43±1.15 % proteins, 13.23±2.20 to 19.42±3.83 % carbohydrates and 4.95±0.06 to 8.53± % fiber. Mineral analysis of peanut cultivars showed 12.60±0.38 to 16.61±1.51 mg/100g Fe , 2.34±0.075 to 3.37±0.040 mg/100g Zn, 38.64±3.50 to 48.24±32.58 mg/100g Ca, 67.81±7.86 to 82.72±9.09 mg/100g Mg, 199.19±33.18 to 342.00±19.03 mg/100g Na and 1220.6±9.045 to 1411.3±1.71 mg/100g P and 841.01±50.41 to 992.98±36.10 mg/100g K. Fatty acid characterization of groundnut cultivars through gas liquid chromatography revealed six fatty acids namely palmitic acid, oleic acid, linoleic acid, arachidic acid, eicosenoic acid and behenic acid. The peanut cultivars Bard-479 and Local-334 were more suitable for oil extraction and peanut butter development because of their high oleic acid to linoleic acid ratio (2.3-2.4). Bard-92 was less preferable cultivar for product development owing to its high linoleic acid (42.56%) and low O/L ratio (0.93) that attributed to its oxidative instability. Sensory evaluation of peanut butter samples showed overall good acceptability of product among the people. Storage study of peanut butter samples demonstrated shelf stability of product up to three months at room temperature.

Upadhyaya *et al*. (2012) conducted a study to identify stable genotypes with better nutritional traits and good agronomic performance for use in future breeding programs. The 184 mini core accessions and four control cultivars were evaluated for nutritional traits for two seasons at two locations and for agronomic traits at one location. Significant genotypic and genotype × environment interactions were observed for all the nutritional and agronomic traits in the entire mini core collection

and within each *A. hypogaea* subspecies of *fastigiata* Waldron and *hypogaea*. Eighteen accessions with higher nutritional traits such as protein content, oil content, oleic acid, and oleic to linoleic acid ratio with superior agronomic traits were identified and their stability analysis resulted in identification of a high oleic acid content (>73%) accession (ICG 2381). On the basis of higher nutritional and agronomic traits 11 subsp. *fastigiata* and 10 subsp. *hypogaea* diverse accessions were identified with more than two trait combinations for use in peanut breeding programs for genetic enhancement of nutritional traits.

Fekria *et al*. (2012) used groundnut seed cake of Barberton and Ashford cultivars in their study to investigate the nutritional and functional properties of the defatted cake flour, and hence possibility of their application in food system. Significant ($P \leq 0.05$) differences were observed in the seed cake between the two cultivars with respect to protein, fat, ash, fiber and carbohydrates contents. Barberton was high in Ca content (0.38%) while Ashford was high in Fe content (0.31%). Minimum solubility was recorded at pH 4.0 and maximum at pH 10.0 for both cultivars. Both cultivars had good functional characteristics with high water and oil absorption capacities. The emulsifying capacity was 28.10 and 22.90 ml/g for Barberton and Ashford defatted seed cake flour, respectively. The emulsifying activity was 28.33% and 22.90% for the two cultivars, respectively. The emulsion stability was 13.86% for Barberton and 11.36% for Ashford and the foaming capacity was 4.2% and 4.0% for the cultivars, respectively. The foam stability for both cultivars was found to be high. Both cultivars cake had high dispersibility in alkaline and acidic media than the neutral with a bulk density of 0.71 g/ml and average wettability and gelation property.

Bishi *et al*. (2015) analyzed kernels of forty-one Indian peanut cultivars for their oil, fatty acid profiles, sucrose, raffinose family oligosaccharides (RFOs); phenolics, and free amino acids contents along with antioxidant capacity. The range and the mean value (given in parenthesis) for each of the traits analysed were, oil: 44.1–53.8% (50.1%), O/L ratio: 0.9–2.8 (1.4), sucrose: 2.61–6.5% (4.63%), RFOs: 0.12–0.76% (0.47%), phenolics: 0.14–0.39% (0.23%), free amino acids: 0.052–0.19% (0.12%) and antioxidant capacity: 1.05–6.97 (3.40) μmol TE g^{-1}. The significant correlation

between phenol content and antioxidant capacity suggests phenol content as an easy marker for rapid screening of genotypes for their antioxidant capacity. A few cultivars with desirable traits and their prospective utility were identified which would be useful for future breeding program to develop nutritional superior peanuts.

Chowdhury *et al*. (2015) conducted an experiment on five varieties growing in large scale in Bangladesh which were evolved by BARI and subjected to the comparative evaluation of physicochemical properties. Among those varieties, the highest seed weight was found in BARI Chinabadam-7 (128.3g) and lowest seed weight was found in Dhaka-1(66.76g). The variety Dhaka-1 was contained highest amount of moisture (5.120%) while lowest amount was found BARI Chinabadam-9(1.230%). The variety BARI Chinabadam-8 contained significantly highest amount of ash (9.6%) and lowest amount of ash contained was found in BARI Chinabadam-9(7.8%). In this analysis, significantly highest amount of carbohydrate found in BARI Chinabadam-6 (6.275%) and was lowest amount of carbohydrate found in BARI Chinabadam-8 (1.218%). Highest amount of protein was obtained from BARI Chinabadam-9 (38.88%) and lowest protein was found in BARI Chinabadam-7 (36.60%). The variety Dhaka-1 had the lowest amount of oil contained (49.20%) while the variety BARI Chinabadam-9 contained significantly highest amount of oil (50.76%). The amounts of total energy contained in these varieties were ranged from 290.3 Kcal/gm to 317.7 Kcal/gm. The amount of saturated fatty acids (10.92 to 17.47%) and the amount of unsaturated fatty acids (81.13-94.81%) were found to be present in each variety. Substantial genetic variability existed for chemical composition and nutritional traits which could be utilized for various food preparation and selection for breeding purpose.

Rodrigues *et al*. (2011) studied the impact of two factors, genotype (G) and treatment (raw or roasted peanut) (T), on the chemical composition of peanuts using a chemometric method and Tukey's test. The peanut genotypes evaluated were cultivar cavalo vermelho (CCV), cultivar cavalo rosa (CCR) and cultivar tatu (CTA), in both raw and roasted states. The total lipid contents in the CTA and CCR peanuts were 40 and 45%, respectively. Those values did not vary significantly (Pb0.05) after roasting. CCV had the greatest total lipid content, but that decreased significantly after roasting

(from 50% to 45%). The variation in the percentage of lipids in the CCV and CCR genotypes was not significant, in contrast to the CTA genotype. The fatty acid (FA) 18:1n−9 predominated in the CCR and CCV samples (50%), without any difference between their raw genotypes. The values for FA 18:1n−9 were lower in the CTA peanut (40%). The second most abundant FA was 18:2n−6(CCV=28%, CTA=38% and CCR=25%), followed by 16:0 (CCV and CCR=16% and CTA=11%). The other FAs found in the peanuts were 18:0, 20:0, 22:0, 24:0, 20:1n−9 and 18:3n−3. The contents of FAs 18:1n−9, 16:0,20:0, and 20:1n−9 suffered significant reduction after roasting in all genotypes. ANOVA analysis of the influence of the main factors indicated that the contribution of the T variable for the majority of responses was low, being between 0.2 and 13%, except for FAs 16:0 and 18:3n−3 and for the saturated FA summations, which were 38, 60 and 22%, respectively. There was a significant contribution from the G factor for all responses, with values between 17 and 99%. The contribution of the interaction between the T and G factors was greater for the responses n6/n3 (56.6%) and for the FA 16:0 (23%). The other responses had values between 0.02 and 14%.

Asibuo *et al*. (2008) initiated a study to examine the nutritional quality of 20 groundnut varieties grown in Ghana. Dry samples were examined for oil content, crude protein, total carbohydrate, calcium, potassium, magnesium, sodium, zinc, copper, iron and manganese. Results from these analyses showed significant variation between the parameters measured. Virginia cultivars which belong to subspecies hypogaea had higher oil content (49.7%) than the Spanish and Valencia market types, which belong to subspecies fastigiata (47.3%). The mean protein content of subspecies fastigiata was however higher (25.69%) than subspecies hypogaea (22.78%). The mineral elements examined were substantial in reducing malnutrition especially in young and growing children. Broni fufuo, a Spanish market type had the highest crude protein content (30.53%) and the least oil content (33.60%).

Dwivedi *et al*. (1993) investigated the effects of environments on oil content and fatty acid composition in peanut. The correlation between oil content and oil quality parameters was also studied. Thirteen peanut (*Arachis hypogaea* L.) genotypes were

grown in 12 environments for the study. Soils at experiment locations differed significantly for pH, EC, and N, P, Zn, Mn, and Fe contents. Significant genotype, environment, and genotype x environment interaction effects were observed for oil content, individual fatty acid contents, and derived oil quality parameters. The original range of 34-54% of oil content based on one season/ location evaluation in these lines was not repeatable, and ranged from 45-50% in multi-location evaluation. Oil content was positively correlated with soil pH and Fe content. The correlation of oleic and linoleic acid content with soil pH and Fe content was positive in the former and negative in the latter. The oil content was positively correlated with O/L ratio. Oleic and linoleic acid contents were negatively correlated. Selection for reduced linoleic acid level in genotypes would also reduce levels of total long chain saturated fatty (TLCSF) acids. Of the thirteen genotypes tested, ICG 5856, ICG 5369, and ICGV 87124 suggested to be used in breeding for improved oil quality.

Gulluoglu *et al.* (2016) conducted a study at the Cukurova University Farm, Turkey as a main and double cropped to determinate oil quality and fatty acid compositions of some peanut varieties in different growing seasons. The results of study indicated that, the oil content (two years average) of peanut varieties ranged from 47.55-51.55% in main cropped and 43.71-50.48% in double cropped growing seasons. Further, the oleic acid content was also varied between 39.80-81.13% and 39.42-81.51% in main and double cropped growing seasons, respectively and the linoleic acid percentage values of peanut varieties ranged from 1.73 to 36.38% in main cropped and from 2.66 to 37.72% in double cropped growing season. Oleic acid vs linoleic acid ratio (O/L) was higher in main cropped than in double cropped growing season and iodine value was higher in double cropped than in main cropped growing season.

Mondragon *et al*. (2009) studied six peanut (*Arachis hypogaea* L.) cultivars (Col-24-Gro, Col-61-Gto, VA-81-B, Ranferi Díaz, NC-2 and Florunner) for agricultural yield, chemical composition (protein, fat, carbohydrates, fiber and ash), amino acid profile, digestibility, fatty acid profile, tocopherol and sterol contents. Results indicated that Ranferi Díaz and Col-61-Gto presented the highest yield (6.3 Ton/ha). Protein content was from 23.5-26.6% and fat content ranged from 49.8-53.4%. Mean digestibility was

86%. Lysine and threonine levels in all cultivars were sufficient to meet human requirements. Total saturated fatty acids ranged from 15-18%. The oleic/linoleic ratio was estimated 1.3-1.4. Tocopherol levels varied from 390 to 706 ppm. The highest tocopherol levels corresponded to the cultivars with the lowest yield. The alpha tocopherol content was estimated at 90-150 ppm, while gamma tocopherol was 270-570 ppm. The main sterol present was β-sitosterol (approx. 65%). Ranferi Diaz variety presented the highest agronomic yield and the highest protein content but low oleic acid, low sterols and low total tocopherols. The differences among cultivars suggested differences in their applications.

Yao *et al*. (2015) evaluated the nutritional properties of the Bambara groundnut Ci12 landrace (*Vigna subterranea* (L.) *Verdc*.) seeds produced in Cote d'Ivoire showed a 19% content of protein, containing all the essential amino acids with tryptophan as the limiting amino acid, a total dietary fiber level of 10%, with a low soluble fraction content, and a fat content of 1.4%, with a high proportion of total unsaturated fatty acids (61%) of which 36% were n-6 fatty acids. The legume groundnut contained phosphorus, as the major mineral, followed by magnesium and calcium, and trace elements (iron, copper and zinc). It was characterized by the same amount of α-tocopherol and antioxidant capacity as common legumes. The high concentration of essential amino acids, n-6 fatty acids and minerals, mainly Fe, in the Ci12 landrace of Bambara groundnut indicates that that local legume has the potentiality to improve the nutritional status in Cote d'Ivoire and could be regarded as a nutrient dense food.

CHAPTER 3

MATERIALS AND METHODS

This research work was conducted at the laboratory of Plant Breeding Division, Soil Science Division and Central Laboratory under Electronics Division of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh during July to November 2020 to study the nutritional properties of groundnut with five genotypes.

3.1 Groundnut genotypes

To carry out this study, the parent Binachinabadam-6 and four advanced mutant lines derived from it, B6/282/63, B6/282/64, B6/282/80 and RM-Kha-19 were used. The seeds of five groundnut genotypes were collected from Plant Breeding Division of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh.

3.2 Sample preparation

100 g groundnut seeds of each genotype was oven dried overnight at 60^0 C. Then the seeds were grinded well. After that, the powdered samples were placed in a desiccator for the further procedures.

3.3 Chemical Analysis

3.3.1 Oil content (%)

Oil contents of five genotypes were determined by Soxhlet method (Jambunathan, 1993) with some modifications. Two gram of groundnut seeds of each genotype were weighed and pulverized into fine powder with a grinder. Then the groundnut meal was extracted with petroleum benzene for 17 hours in Soxhlet apparatus. After that, petroleum benzene was evaporated. Powder weight before and after extraction was taken, the difference between the two was expressed as oil percentage.

Procedure:

1. A thimble was filled with two (2) grams of oven dry powder (T). Each thimble's weight and thimble sample were weighed.
2. Then the thimble was put in the Soxhlet chamber.
3. The Soxhlet flask was filled with 70 mL petroleum benzene and attached to the holder and condenser.
4. It was then put on a hot plate and distilled for 17 hours at a low temperature.
5. The hot plate was switched off after extraction and allowed to air dry for 30-40 minutes.
6. Finally, the thimble and the extracted sample were weighted. The loss of weight was measured as oil (crude fat).
7. The mean value of three sample was the actual oil percentage.

Weight of thimble and sample before extract = $(T+SW_1)$

Weight of thimble and sample after extract = $(T+SW_2)$

Weight of sample before extract = SW_1

$$\text{Oil (\%)} = \frac{(T+SW_1)-(T+WS_2)}{SW_1} \times 100$$

3.3.2 Fatty acid determination

The fatty acid content of oil was measured using gas chromatography (GC) and a flame ionization detector (FID). Groundnut oil was first converted to fatty acid methyl ester (FAME), which was then injected into a GC system equipped with a FID, where various types of peaks were observed as retention time increased. To confirm the presence of a particular fatty acid in the oil, the observed retention time was compared to the standard FAME (Supelco 37 component FAME mix). The fatty acid analysis protocol was modified from Danish *et al* (2019).

3.3.2.1 FAME preparation from groundnut oil

The dried groundnut seeds were crushed in a small paper bag until they were powdered. Then using an electric balance, 375 mg of powder was weighed and transferred into a test tube for extraction and transesterification. After shaking, eight (8) mL of ethyl reagent Petroleum Ether was added and the tube was left overnight. Thereafter 5 mL of salt solution (NaCl 80g and 3g NaHSO4 in 1-liter water) was added. At the top of the tube, a clear benzene phase was visible. The benzene phase was separated from the test tube carefully and stored that in a 2 mL airtight tube. Then the sample was ready for GC analysis.

Supelco 37 Component FAME Mix was used as the FAME standard. Before use, the FAME was dissolved in methylene chloride at various concentrations (1 µl/ml, 0.6 µl/ml, 0.4 µl/ml, and 0.2 µl/ml) to obtain a prominent peak for fatty acid identification. Concentrations of fatty acids were recorded (percentage as related to total fatty acids).

3.3.2.2 Gas Chromatography (GC) acquisition method

On a VARIAN (CP-3800) Gas Chromatograph, reconstituted FAMEs were analyzed by gas chromatography (GC). Using a split injector set at 250^0 C with a 1:25 split ratio, the above samples were inserted onto a DB-225 column (30 m 0.25 mm with 0.15 µm film thickness) (Jand W Scientific). At 1ml min^{-1}, ultrapure hydrogen was used as the carrier gas. The GC procedure was carried out at 100^0 C for 2 minutes, 25^0 C $minutes^{-1}$ to 180^0 C, 15^0 C $minutes^{-1}$ to 200^0 C, 4^0 C $minutes^{-1}$ to 225^0 C held at 6 minutes. Galaxie™ Chromatography Data System software was used to record peak areas using a flame ionization FID detector set to 300^0 C. The retention periods of FAME peaks were compared to those of standard fatty acid methyl esters to identify them. The samples were analyzed using the retention time (RT) of standard mix FAME. The region of fatty acid concentration in each sample is indicated by the length of the peak. The percentage of fatty acid present in the oil was calculated by dividing the concentration area by the percentage of fatty acid present in the oil.

3.3.4 Iodine value

The iodine values of selected five genotypes were calculated from fatty acid composition by using the following formula (Hashim *et al.*, 1993):

$$\text{Iodine value} = (\%\ \text{Oleic acid} \times 0.8601) + (\%\ \text{Linoleic acid} \times 1.7321)$$

3.3.5 Minerals

3.3.5.1 Reagents preparation

i. Indicator mixture (17 mL)

0.1g bromocresol green was dissolved in 100 mL ethanol and 0.1g methyl red was dissolved in 100 mL ethanol separately. Then 10 mL bromocresol green from 100 mL solution and 7 mL methyl red from 100 mL solution were mixed together in a 100 mL conical flask. Thus 17 mL indicator mixture was prepared.

ii. Nitric-perchloric acid solution (7500mL)

500 mL HNO_3 was mixed with 250 mL $HClO_4$ in a 1L beaker (H_2SO_4: $HClO_4$ = 2:1) to prepare 750 mL nitric-perchloric acid solution.

iii. 4% Boric acid solution (1000 mL)

At first, 40g boric acid powder and 500 mL distilled water were mixed in a 1.0 L beaker. Then dissolved the solution with heat and volume to 1000 mL. Finally, 17 mL indicator mix added to the solution.

iv. 32% NaOH solution (1000 mL)

320g NaOH tablet was put in a 1.0 L beaker with distilled water and dissolved it with heat on a hot plate. The solution was then volume to 1000 mL.

v. 1% Lanthanum solution (5L)

With about 500mL of distilled water, 59 g lanthanum oxide (La_2O_3) was added. To dissolve the La_2O_3 slowly and carefully, 250mL conc. H2SO4 was applied. Then it was filled with distilled water to make it 5L.

vi. Acid seed solution (1L)

65 mL HNO_3 and 250 mL glacial acetic acid were mixed with 500 mL distilled water. Then 3 mL 1000 ppm sulphur standard solution was added and made volume to 1L with distilled water.

vii. Turbidimetric reagent (1L)

10 g polyvinylpyrolidone (PVP) was dissolved in 200 mL warm distilled water. In another beaker, 150 g $BaCl_2$. $2H_2O$ was dissolved in 500 mL distilled water. After cooling, both solutions were transferred to a 1L volumetric flask and made volume with distilled water.

viii. Barton's solution (2L)

50 g ammonium molybdate $\{(NH_4)_6MO_7O_{24}. 4H_2O\}$ was dissolved in about 800 mL distilled water. 2.50 g ammonium meta-vandate (NH_4VO_3) was dissolved in 600 mL hot distilled water and cooled in room temperature. Then 500 mL concentrated HNO_3 was added to meta-vandate solution and cooled. Finally, molybdate solution was poured into meta-vandate solution and diluted to 2L.

ix. Ammonium molybdate-ascorbic acid solution

4.1 g ascorbic acid was dissolved in 1L ammonium molybdate solution and mixed it well.

x. 1:100 diluted HNO_3

20 mL 68% HNO_3 was transferred to a 2L volumetric flask and made volume with distilled water.

xi. 1:20 diluted HNO_3

100 mL 68% HNO_3 was transferred to a 2L volumetric flask and made volume with distilled water.

3.3.5.2 Standard solutions preparation

i. Calcium standard solutions

0,5,10,15,20 mL calcium stock solutions were pipetted into 200 mL volumetric flasks. 20 mL $LaCl_3$ solution and 80 mL 1:100 diluted HNO_3 were added to each flask and made volume with distilled water. The solutions contained 0.00, 5.01, 10.02, 15.03,20.04 mg/L Ca.

ii. Magnesium standard solutions

0,5,10,15,20 mL magnesium stock solutions were pipetted into 200 mL volumetric flasks. 20 mL $LaCl_3$ solution and 20 mL 1:100 diluted HNO_3 were added to each flask and made volume with distilled water. The solutions contained 0.00, 0.61, 1.22, 1.52,2.43 mg/L Mg.

iii. Potassium standard solutions

0,5,10,15,20 mL magnesium stock solutions were pipetted into 200 mL volumetric flasks. 40 mL 1:100 diluted HNO_3 was added to each flask and made volume with distilled water. The solutions contained 0.00, 10.0, 20.0, 30.0, 40.0mg/L K.

iv. Phosphorous standard solutions

5 mL 1:100 diluted HNO_3 was transferred into 550 mL volumetric flasks. 0. 5, 10, 15, 20 mL P stock solution and 30 mL distilled water were added to flasks. 10 mL ammonium molybdate-ascorbic acid solution was added to each flask and made volume with distilled water. After 15 minutes, the absorbance was measured on a spectrophotometer at 890 nm.

v. Iron standard solutions

0, 5, 10, 15, 20 mL Fe stock solutions were pipetted into 5 200 mL volumetric flasks. Made volume with 1: 20 diluted HNO_3 and mixed well. The solutions contained 0.00, 2.5, 5.0, 7.5, 10.0 mg/L Fe.

vi. Zinc standard solutions

0, 5, 10, 15, 20 mL Zn stock solutions were pipetted into 5 200 mL volumetric flasks. Made volume with 1: 20 diluted HNO_3 and mixed well. The solutions contained 0.00, 1.0, 2.0, 3.0, 4.0 mg/L Zn.

vii. Copper standard solutions

0, 5, 10, 15, 20 mL Cu stock solutions were pipetted into 5 200 mL volumetric flasks. Made volume with 1: 20 diluted HNO_3 and mixed well. The solutions contained 0.00, 1.0, 2.0, 3.0, 4.0 mg/L Cu.

viii. Boron standard solutions

0, 5, 10, 15, 20 mL B stock solutions were pipetted into 5 100 mL volumetric flasks. Made volume with 1: 20 diluted HNO_3 and mixed well. The solutions contained 0.00, 0.5, 1.0, 1.5, 2.0 mg/L B.

3.3.5.3 Digestion

0.5g each groundnut sample was added with 12 mL Nitric-perchloric acid mixture in 100 mL conical flask separately. The mixture containing conical flasks were transferred in a fume hood and kept there overnight. The mixture containing conical flasks were placed on a hot plate in another big fume hood at 350℃ and heat 3 hours for complete digestion. Then the conical flasks were kept at room temperature to become cool. The solutions were volume to 50 mL with distilled water. Each sample solution was transferred in a bottle as stock solution for further operations.

3.3.5.4 Determination of Ca, Mg, N, P, K, S, Fe, Zn, Cu and B

i. Measurement of Ca

20 mL diluted filtrate of each sample was transferred into 50 ml volumetric flask using a pipette. Then 5 mL $LaCl_3$ solution was added and made volume with distilled water. The Content of Ca was measured by Atomic Absorption Spectrophotometer (SHIMADZU, AA-7000).

ii. Measurement of Mg

5 mL diluted filtrate of each sample was transferred into 50 mL volumetric flasks using a pipette. 5 mL $LaCl_3$ solution was added and made volume with distilled water. The content of Mg was measured by Atomic Absorption Spectrophotometer (SHIMADZU, AA-7000).

iii. Measurement of N

This process was carried out by Kjeldahl method invented by Johann Kjeldahl in 1883. The method can conveniently be divided into three steps: digestion, distillation and titration. In this study, digestion process was done manually and a machine "UKD 159 Automatic Distillation & Titration System" was used that could perform distillation and titration automatically. The solution for each sample then measured in UKD 159 Automatic Distillation & Titration System. Total N% in each sample were found from the reading of UKD 159 Automatic Distillation & Titration System.

iv. Measurement of P

2 mL diluted filtrate of each sample was transferred into 50 mL volumetric flasks using a pipette. 10 mL Barton's solution was added and made volume with distilled water. The content of P was measured by Atomic Absorption Spectrophotometer (SHIMADZU, AA-7000).

v. Measurement of K

2 mL diluted filtrate of each sample was transferred into 50 mL volumetric flasks using a pipette and volume with distilled water. The content of K was measured by Atomic Absorption Spectrophotometer (SHIMADZU, AA-7000).

vi. Measurement of S

2 mL diluted filtrate of each sample was transferred into 50 mL volumetric flasks using a pipette. Then 10 mL acid seed solution and 5 mL turbidimetric reagent were added and volume with distilled water. The content of S was measured by Atomic Absorption Spectrophotometer (SHIMADZU, AA-7000).

vi. Measurement of Fe, Zn, Cu and B

The content of these elements were measured directly by Atomic Absorption Spectrophotometer (SHIMADZU, AA-7000).

3.3.6 Estimation of Protein

Protein content was calculated by multiplying %N with a conversion factor. To convert measured nitrogen concentration to protein concentration, a conversion factor (CF) 6.25 (equivalent to 0.16 g nitrogen per gram of protein) was used (Chowdhury *et al.*, 2015).

3.3.7 Moisture content

Moisture contents of the total five genotypes were determined directly by Kett Grain Moisture Tester (PM-450). The code for groundnut moisture measurement is 21.

3.3.8 Ash content

The following methodology was used to determine the ash content of the sample (Fekria, 2009):

At first 2 g of sample were placed in a clean, dry, pre-weighed crucible. Then it was burnt in a muffle furnace at 550℃ for 3 hours until light gray ash was appeared. The crucible was taken from the furnace and placed in a desiccator to cool. Then weight was carefully. The crucible was re-ignited in the furnace and allowed to cool until it reached at a constant weight. The following equation was used to calculate the ash in the sample-

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

Where,

W1= weight of empty crucible.

W2= weight of crucible with ash.

W3= weight of sample.

3.3.9 Crude fiber

Two grams of defatted material were treated with boiling H_2SO_4 and KOH solutions in that order 0.26 N and 0.23 N, respectively. The resulting residues were filtered, rinsed, and placed into a crucible.

Then it was baked for 18–24 hours at 105°C in a preheated oven. The crucible was then weighed and ached in a muffle furnace at 500°C with the sample inside (Fekria, 2009).

The following equation was used to calculate crude fiber in the sample:

$$CF\ \% = \frac{W1 - W2}{Ws} \times 100$$

Where:
CF = Crude fiber
W1 = Weight of crucible with sample before ashing
W2 = Weight of crucible with sample after ashing
Ws = weight of sample

3.3.10 Carbohydrates

Carbohydrates were determined by the following equation (Raghuramulu *et al*.,2003):

Carbohydrates % = 100 – (moisture + ash + fat/oil + fiber + protein)

3.3.11 Energy

The total energy content generated from the five selected genotypes of groundnut were determined by multiplying the percentages of crude protein, crude fat and carbohydrates by factors of 4, 9 and 4 respectively (Osborne and Voogt, 1987).

3.4 Statistical analysis

The recorded data of the study for all characters was analyzed statistically using MSTAT package program. The mean for all treatments were calculated and analysis of variance was performed by F variance test. The mean differences were evaluated by least significant different (LSD) test (Gomez and Gomez, 1984).

CHAPTER 4

RESULTS AND DISCUSSION

This chapter comprises the presentation of the results of the study. The present study was undertaken to analyze nutritional and functional properties of groundnut seeds. Total five genotypes (1 released variety and 4 advanced mutant lines) of groundnut were collected from Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh.

4.1 Oil content

The oil content of 5 groundnut genotypes varied from 54.6% to 48.5 %. The highest oil content (54.6%) was found in B6/282/80; followed by RM-Kha-19 (50.8%), B6/282/64 (50.6%), B6/282/63 (50.1%) and Binachinabadam-6 (48.5%). Asibuo *et al.* (2008) stated that significant differences were observed in 20 cultivars for oil. Oil content ranged from 33.6 to 54.95%. Five varieties had oil content higher than 50%. Broni fufuo, a Spanish variety had unusually low oil content compared with the other varieties. Groundnut varieties belonging to subspecies *hypogaea* had slightly more oil than the *fastigiata* varieties.

Table 1. Oil contents of 5 groundnut genotypes

Sl. No.	Name of the genotypes	Oil %
1	B6/282/63	50.1 bc
2	B6/282/64	50.6 b
3	B6/282/80	54.6 a
4	RM-Kha-19	50.8 b
5	Binachinabadam-6	48.5 c
	CV (%)	1.54

4.2 Fatty acid composition

According to the Gas Chromatography results there was significant difference in fatty acid composition among the selected genotypes of groundnut. The saturated and unsaturated fatty acids are demonstrated in tabular (Table 2 & 3) and graphical form (Fig. 1-5) separately.

4.2.1 Unsaturated fatty acid composition

The highest amount of Myristoleic acid was found in Binachinabadam-6 (2.463%); followed by B6/282/64 (1.907%), B6/282/80 (1.566%), RM-Kha-19 (1.356%) and B6/282/63 (0.009%). The amount of Oleic acid ranged from 55.7% to 29.977%. The highest percentage found in B6/282/80 (55.7%); followed by B6/282/63 (45.628%), Binachinabadam-6 (42.167%), RM-Kha-19 (34.71%) and B6/282/64 (29.977%). Linoleic acid among the five genotypes were varied from 17.129% to 13.691%. The highest amount was found in B6/282/64 (17.129%); followed by B6/282/63 (15.898%), B6/282/80 (15.136%), RM-Kha-19 (13.774%) and Binachinabadam-6 (13.691%). The highest amount of Erucic acid was observed in B6/282/64 (3.035%); followed by B6/282/80 (2.445%), RM-Kha-19 (2.19%), Binachinabadam-6 (2.04%) and B6/282/63 (1.634%). Gulluoglu *et al.* (2016) found the oleic acid content in groundnut varying between 39.80-81.13% and 39.42-81.51% in main and double cropped growing seasons, respectively. The linoleic acid percentage values of groundnut varieties ranged from 1.73 to 36.38% in main cropped and from 2.66 to 37.72% in double cropped growing season. Onemli (2012) found 0.01-0.18% erucic acid in Virginia, Valencia, and Spanish variety in different years. There is no available reference of myristoleic acid but my study reveals that it was important findings.

Table 2. Unsaturated fatty acid composition

Genotypes	Unsaturated fatty acid			
	Myristoleic acid (%) ($C_{14:1}$)	Oleic acid (%) ($C_{18:1}$)	Linoleic acid (%) ($C_{18:2}$)	Erucic acid (%) ($C_{22:1}$)
B6/282/63	0.009 e	45.628 b	15.898 b	1.634 e
B6/282/64	1.907 b	29.977 e	17.129 a	3.035 a
B6/282/80	1.566 c	55.700 a	15.136 c	2.445 b
RM-Kha-19	1.356 d	34.710 d	13.774 d	2.190 c
Binachinabadam-6	2.463 a	42.167 c	13.691 e	2.040 d
CV (%)	0.36	0.11	0.05	0.30

4.2.2 Saturated fatty acid composition

Among the five selected genotypes the highest amount of Hexanoic acid was found in RM-Kha-19 (23.291%); followed by B6/282/64 (9.254%), Binachinabadam-6 (6.737%), B6/282/63 (4.326%) and B6/282/80 (4.232%). The percentage of Lauric acid varied from 4.742 to 1.302. The highest percentage found in B6/282/64 (4.742%); followed by Binachinabadam-6 (4.13%), B6/282/80 (3.048%), B6/282/63 (2.745%) and RM-Kha-19 (1.302%). Palmitic acid ranged from 8.660% to 1.044%. The highest amount was found in B6/282/64 (8.66%); followed by Binachinabadam-6 (1.523%), B6/282/63 (1.305%), RM-Kha-19 (1.14%) and B6/282/80 (1.044%). The highest amount of Arachidic acid was observed in B6/282/63 (28.455%); followed by Binachinabadam-6 (27.249%), B6/282/64 (25.296%), RM-Kha-19 (22.307%), and B6/282/80 (16.829%). Anyasor *et al.* (2009) found lauric acid ranged from 5.57% to 8.1% and in six different varieties of groundnut collected from northern, eastern and western Nigeria. Gulluoglu *et al.* (2016) found palmitic acid varied from 5.86% to 12.25% and arachidic acid varied from 1.35% to 1.78% among 12 genotypes. Zahran *et al*. (2019) observed palmitic acid ranging from 12.01% to 15.25% among 5

groundnut genotypes. Higher percentage of Arachidic acid of B6/282/64 mutant line creates interest on my study. It may be used for further study to find the cause of higher quantity of Arachidic acid.

Table 3. Saturated fatty acid composition

Genotypes	Saturated fatty acid			
	Hexanoic acid (%) ($C_{6:0}$)	Lauric acid (%) ($C_{12:0}$)	Arachidic acid (%) ($C_{20:0}$)	Palmitic acid (%) ($C_{16:0}$)
B6/282/63	4.326 d	2.745 d	1.305 a	28.455 c
B6/282/64	9.254 b	4.742 a	8.660 c	25.296 a
B6/282/80	4.232 e	3.048 c	1.044 e	16.829 e
RM-Kha-19	23.291 a	1.302 e	1.140 d	22.307 d
Binachinabadam-6	6.737 c	4.130 b	1.523 b	27.249 b
CV (%)	0.06	0.21	0.34	0.03

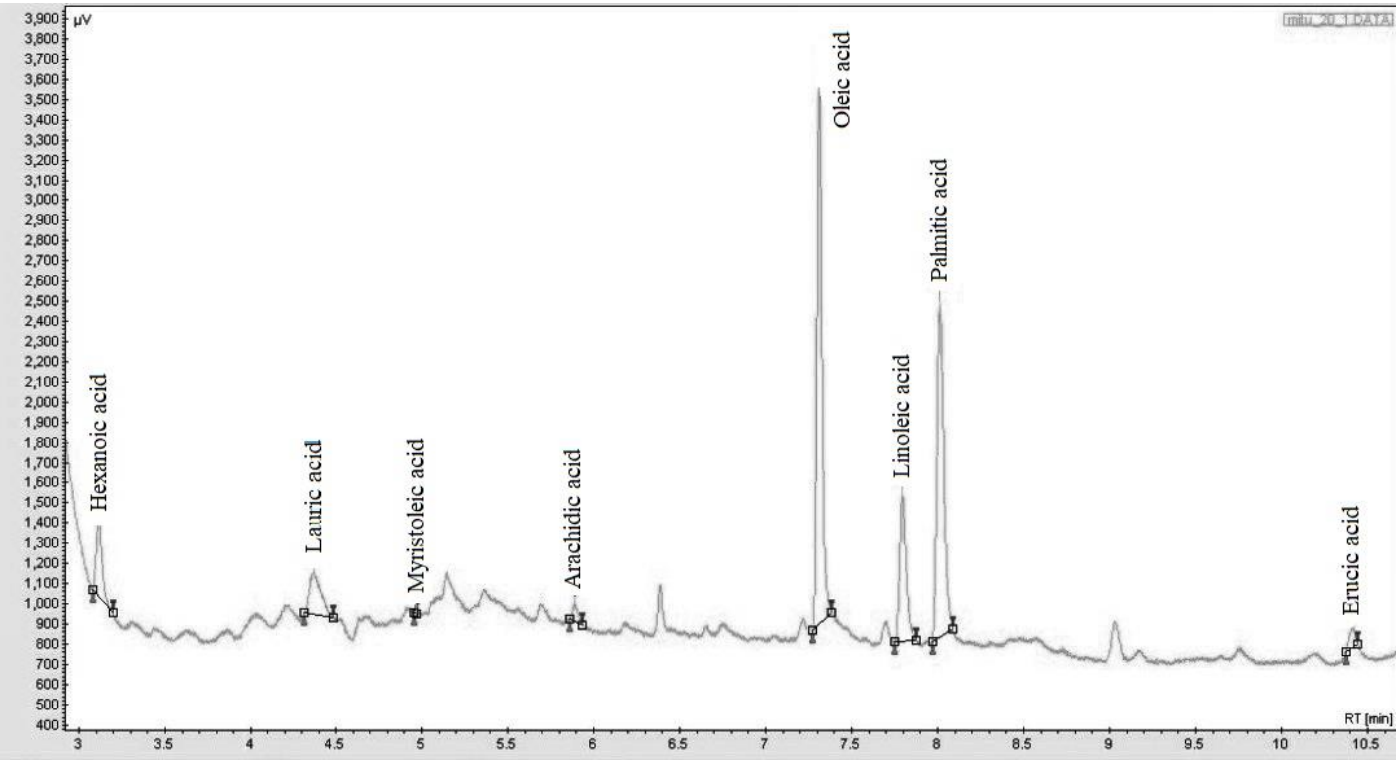

Figure 1. Fatty acid profile of B6/282/63 (*Arachis hypogaea L.)*

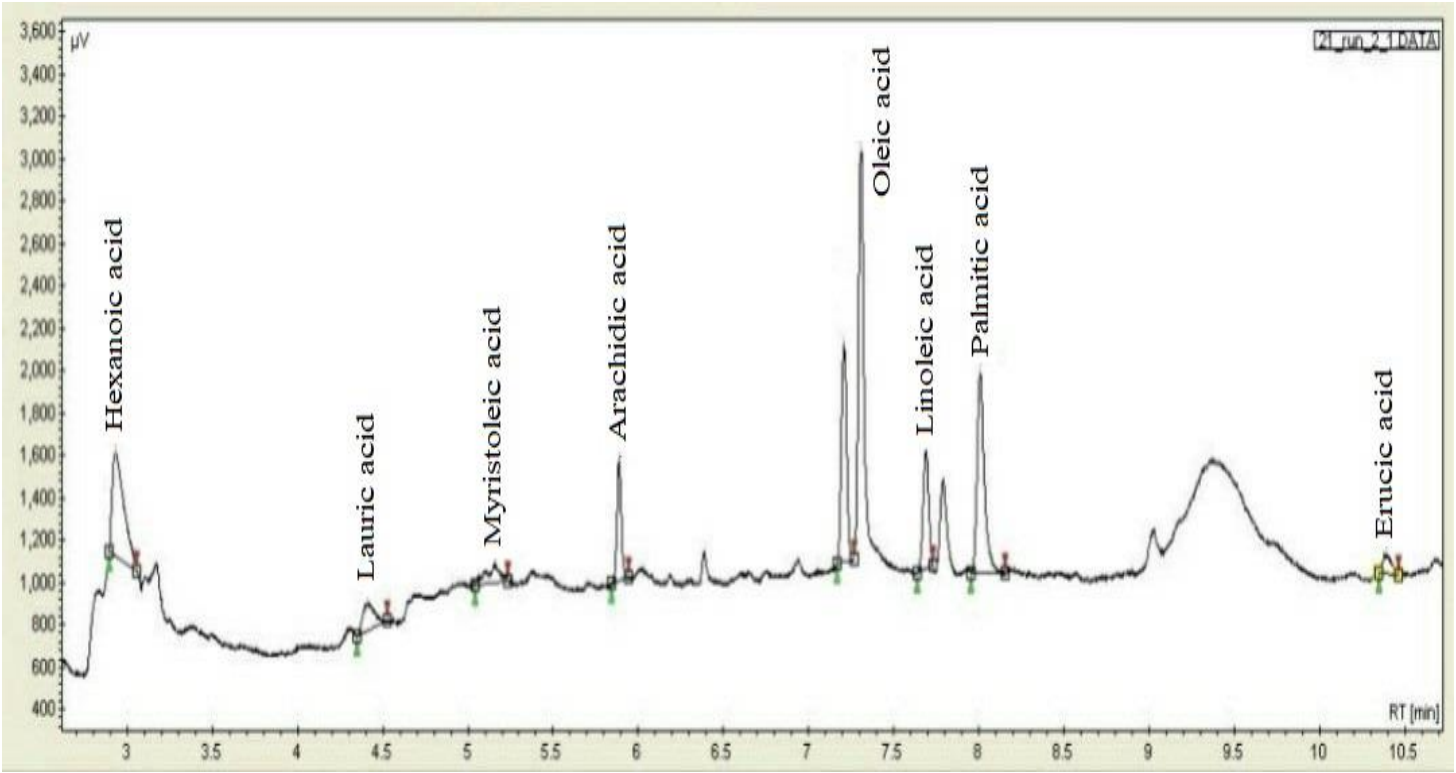

Figure 2. Fatty acid profile of B6/282/64 (*Arachis hypogaea L.*)

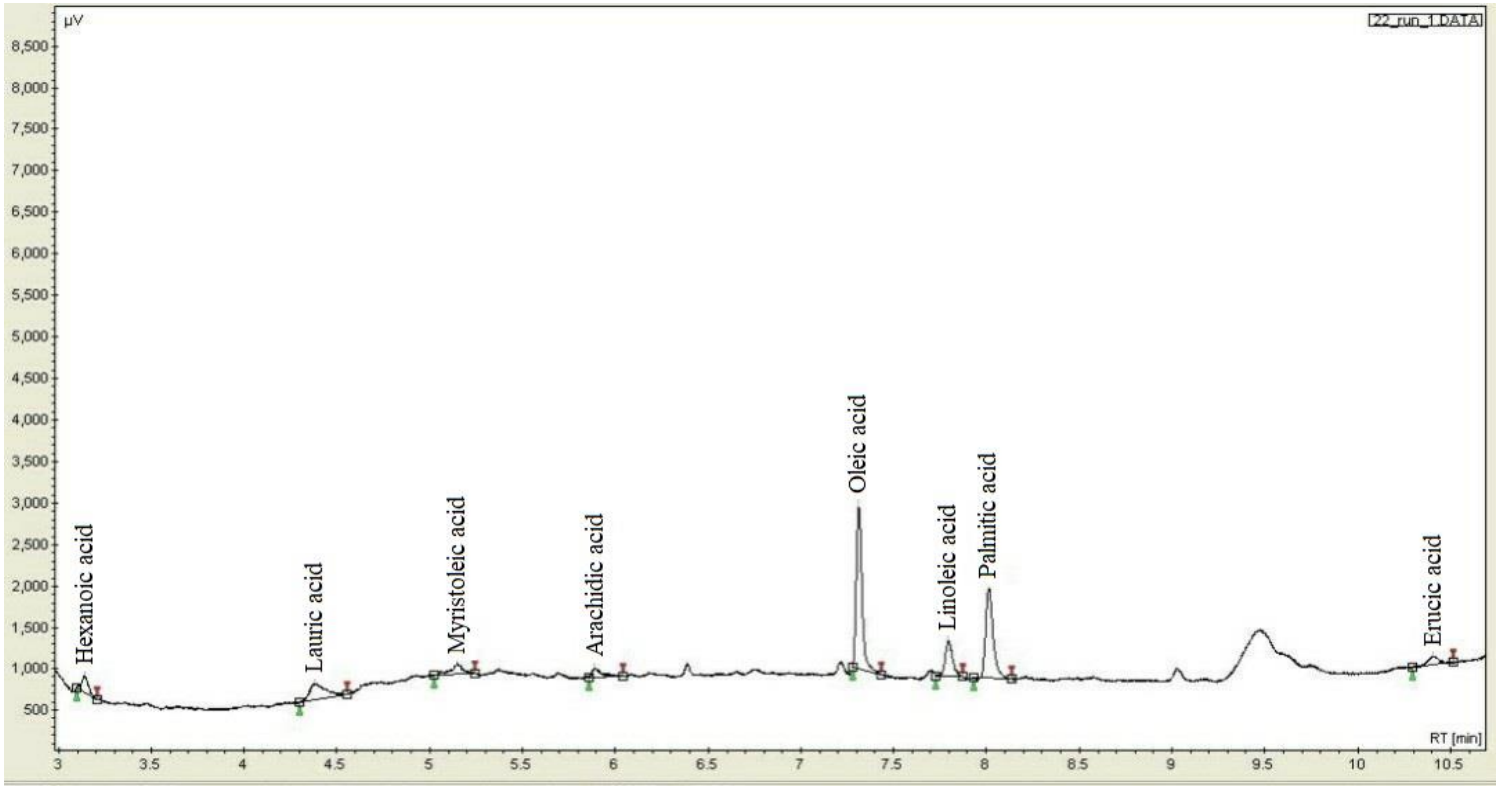

Figure 3. Fatty acid profile of B6/282/80 (*Arachis hypogaea L.*)

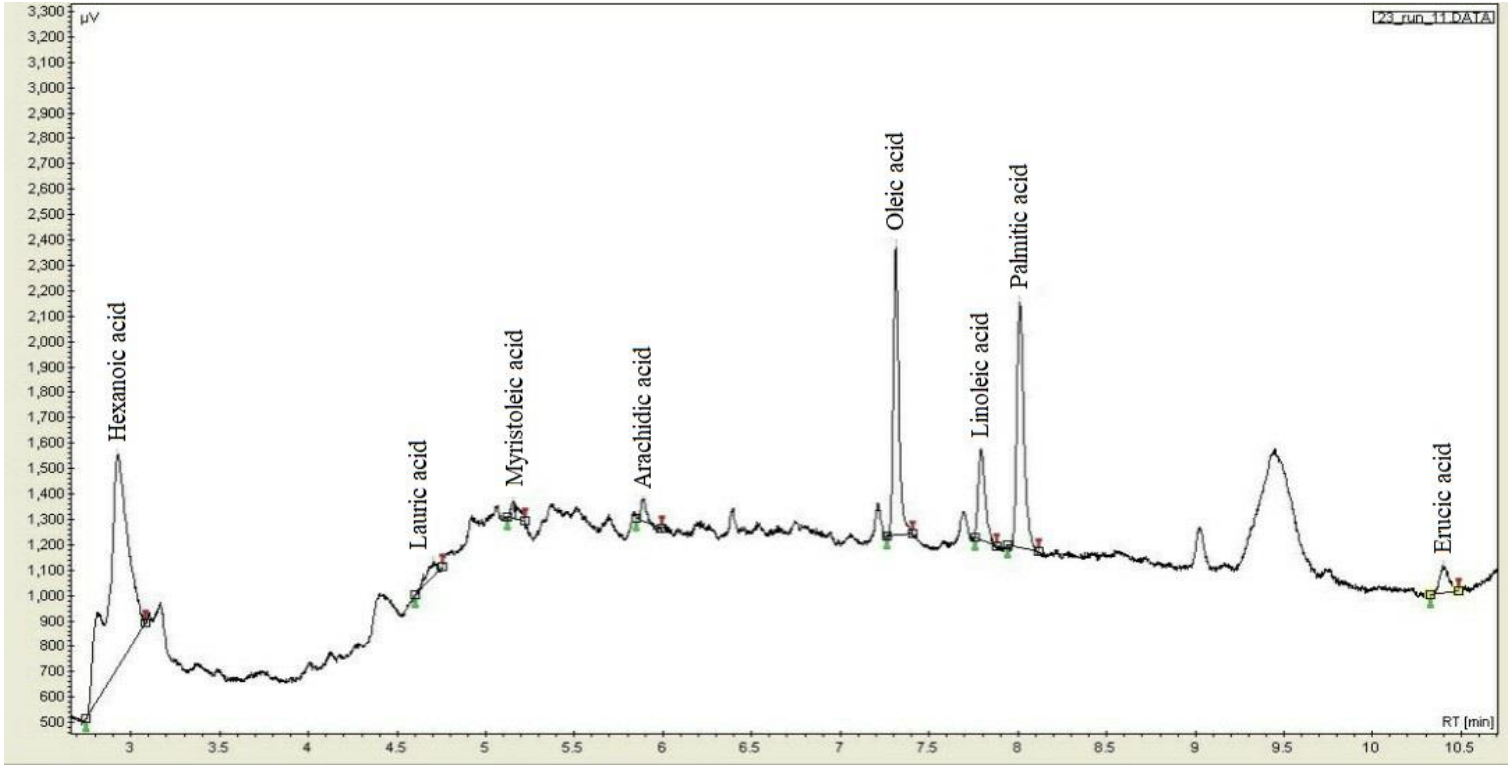

Figure 4. Fatty acid profile of RM-Kha-19 (*Arachis hypogaea L.*)

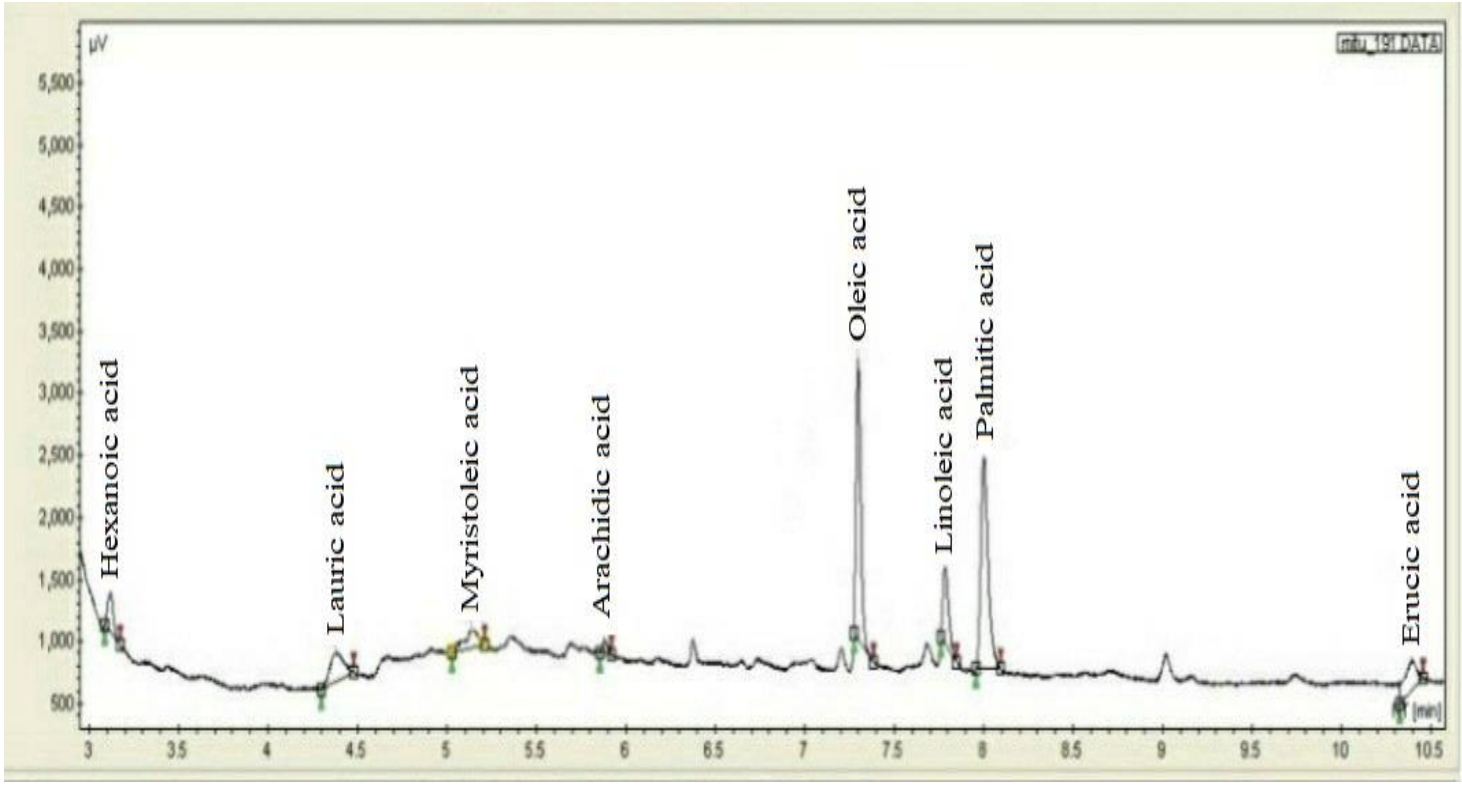

Figure 5. Fatty acid profile of Binachinabadam-6 (*Arachis hypogaea L.*)

4.3 O/L ratio

The O/L ratio of the genotypes were ranged from 3.68 to 1.75. The highest ratio 3.68 was found in B6/282/80; followed by Binachinabadam-6 (3.08), B6/282/63 (2.87), RM-Kha-19 (2.52) and B6/282/64 (1.75). Gulluoglu *et al.* (2016) observed that, the O/L ratio of groundnut varieties varied between 1.09-3.78 and 1.05-2.47 in main and double cropped growing season, respectively.

4.4 Iodine value

The iodine value of the genotypes varied from 74.12 to 53.71. The highest iodine value 74.12 was found in B6/282/80; followed by B6/282/63 (66.78), Binachinabadam-6 (59.98), B6/282/64 (55.45) and RM-Kha-19 (53.71). Gulluoglu *et al.* (2016) found the iodine value of peanut varieties ranged from 73.58 to 98.24 in main cropped and from 75.41 to 99.93 in double cropped growing seasons.

Table 4. The ratio of Oleic acid versus Linoleic acid and Iodine value of the genotypes

Genotypes	O/L ratio	Iodine value
B6/282/63	2.87 c	66.78 b
B6/282/64	1.75 e	55.45 d
B6/282/80	3.68 a	74.12 a
RM-Kha-19	2.52 d	53.71 e
Binachinabadam-6	3.08 b	59.98 c
CV (%)	0.64	0.29

4.5 Minerals

4.5.1 Calcium (Ca)

The Ca contents of 5 groundnut genotypes were ranged from 85.54 to 74.12 mg/100g. The highest Ca content 85.54 mg/100g was found in B6/282/80; followed by B6/282/64 (83.43 mg/100g), Binachinabadam-6 (80.36 mg/100g), RM-Kha-19 (78.29 mg/100g), and B6/282/63 (74.12 mg/100g).

4.5.2 Magnesium (Mg)

The Mg contents of 5 groundnut genotypes were ranged from 173 to 148 mg/100g. The highest Mg content 173 mg/100g was measured in RM-Kha-19; followed by B6/282/64 (162 mg/100g), B6/282/80 (157 mg/100g), Binachinabadam-6 (154 mg/100g) and B6/282/63 (148 mg/100g).

4.5.3 Sulphur (S)

The S contents of selected 5 groundnut genotypes were ranged from 89 to 56 mg/100g. The highest S content 89 mg/100g was measured in B6/282/63; followed by Binachinabadam-6 (77 mg/100g), B6/282/64 (68 mg/100g), B6/282/80 (61 mg/100g) and RM-Kha-19 (56 mg/100g).

Table 5. Ca, Mg and S contents of 5 groundnut genotypes

Name of the genotypes	**Ca (mg/100g)**	**Mg (mg/100g)**	**S (mg/100g)**
B6/282/63	74.12 c	148 e	89 a
B6/282/64	83.43 a	162 b	68 c
B6/282/80	85.54 a	157 c	61 d
RM-Kha-19	78.29 b	173 a	56 e
Binachinabadam-6	80.36 b	154 d	77 b
CV (%)	1.72	0.72	1.31

4.5.4 Nitrogen (N)

The N contents of 5 groundnut genotypes were varied from 5190 to 4370 mg/100g. The highest N content 5190 mg/100g was measured in RM-Kha-19; followed by B6/282/64 (4710 mg/100g), B6/282/80 (4580 mg/100g), Binachinabadam-6 (4490 mg/100g) and B6/282/63 (4370 mg/100g).

4.5.5 Phosphorous (P)

The P contents of 5 groundnut genotypes were ranged from 490 to 360 mg/100g. The highest P content 490 mg/100g was found in B6/282/80; followed by RM-Kha-19 (450 mg/100g), Binachinabadam-6 (440 mg/100g), B6/282/64 (380 mg/100g) and B6/282/63 (360 mg/100g).

4.5.6 Potassium (K)

The K contents of 5 groundnut genotypes were varied from 730 to 650 mg/100g. The highest K content 730 mg/100g was measured in B6/282/63; followed by B6/282/80 (700 mg/100g), Binachinabadam-6 (700 mg/100g), B6/282/64 (690 mg/100g) and RM-Kha-19 (650 mg/100g).

Table 6. N, P and K contents of 5 groundnut genotypes

Name of the genotypes	**N (mg/100g)**	**P (mg/100g)**	**K (mg/100g)**
B6/282/63	4370 e	360 e	730 a
B6/282/64	4710 b	380 d	690 c
B6/282/80	4580 c	490 a	700 b
RM-Kha-19	5190 a	450 b	650 d
Binachinabadam-6	4490 d	440 c	700 b
CV (%)	0.11	0.80	0.54

4.5.7 Iron (Fe)

The Fe contents of 5 groundnut genotypes were ranged from 2.15 to 1.62 mg/100g. The highest Fe content 2.15 mg/100g was found in B6/282/64; followed by B6/282/63 (2.02 mg/100g), Binachinabadam-6 (1.98 mg/100g), B6/282/80 (1.85 mg/100g), and RM-Kha-19 (1.62 mg/100g).

4.5.8 Zinc (Zn)

The Zn contents of 5 groundnut genotypes were ranged from 5.9 to 3.8 mg/100g. The highest Zn content 5.9 mg/100g was observed in RM-Kha-19; followed by B6/282/64 (5.3 mg/100g), B6/282/63 (4.7 mg/100g), B6/282/80 (4.6 mg/100g) and Binachinabadam-6 (3.8 mg/100g).

4.5.9 Copper (Cu)

The Cu contents of 5 groundnut genotypes were ranged from 1.5 to 0.5 mg/100g. The highest Cu content 1.5 mg/100g was found in B6/282/80 and RM-Kha-19; followed by B6/282/64 (0.8 mg/100g), B6/282/63 (0.6 mg/100g) and Binachinabadam-6 (0.5 mg/100g).

4.5.10 Boron (B)

The B contents of 5 groundnut genotypes were ranged from 3.6 to 2.4 mg/100g. The highest B content 3.6 mg/100g was measured in Binachinabadam-6; followed by B6/282/80 (3.1 mg/100g), RM-Kha-19 (2.9), B6/282/63 (2.8 mg/100g) and B6/282/64 (2.4 mg/100g).

Aryal et al. (2015) observed the similar results as above mentioned minerals.

Table 7. Fe, Zn, Cu and B contents of 5 groundnut genotypes

Name of the genotypes	Fe (mg/100g)	Zn (mg/100g)	Cu (mg/100g)	B (mg/100g)
B6/282/63	2.02 b	4.7 c	0.6 b	2.8 c
B6/282/64	2.15 a	5.3 b	0.8 b	2.4 d
B6/282/80	1.85 d	4.6 c	1.5 a	3.1 b
RM-Kha-19	1.62 e	5.9 a	1.5 a	2.9 bc
Binachinabadam-6	1.98 c	3.8 d	0.5 b	3.6 a
CV (%)	1.01	5.76	1.92	4.84

4.6 Protein

The protein contents of 5 groundnut genotypes were ranged from 32.42% to 27.34%. The highest protein content 32.42% was measured in RM-Kha-19; followed by B6/282/64 (29.41%), B6/282/80 (28.68%), Binachinabadam-6 (28.12%) and B6/282/63 (27.34%). Asibuo *et al*. (2008) found 21.15% to 30.53% protein content among 20 varieties of groundnut.

Table 8. Protein contents of 5 groundnut genotypes

Sl. No.	Name of the genotypes	Protein %
1	B6/282/63	27.34 d
2	B6/282/64	29.41 b
3	B6/282/80	28.68 bc
4	RM-Kha-19	32.42 a
5	Binachinabadam-6	28.12 c
	CV (%)	1.72

4.7 Moisture content

The moisture contents of 5 groundnut genotypes were varied from 6.2% to 5.1%. The highest moisture content 6.2% was measured in B6/282/64; followed by Binachinabadam-6 (6.0%), B6/282/63 (5.7%), B6/282/80 (5.2%), and RM-Kha-19 (5.1%).

4.8 Ash

The ash contents of 5 groundnut genotypes were ranged from 2.38% to 2.23%. The highest ash content 2.38% was found in B6/282/63; followed by B6/282/80 (2.35%), Binachinabadam-6 (2.27%), B6/282/64 (2.26%) and RM-Kha-19 (2.23%).

4.9 Crude Fiber

The amount of crude fiber of selected groundnut genotypes were ranged from 3.71% to 3.52%. The highest crude fiber 3.71% was measured in Binachinabadam-6; followed by B6/282/63 (3.68%), RM-Kha-19 (3.64%), B6/282/80 (3.60%) and B6/282/64 (3.52%).

4.10 Carbohydrate

The amounts of carbohydrate in 5 groundnut genotypes were varied from 11.4% to 5.57%. The highest carbohydrate 11.4% was measured in Binachinabadam-6; followed by B6/282/63 (10.8%), B6/282/64 (7.91%), RM-Kha-19 (5.69%) and B6/282/80 (5.57%).

The moisture, ash and carbohydrate contents of the selected mutant genotypes were about to similar with the results found by Shibli *et al.* (2019).

Table 9. Moisture, ash, crude fiber and carbohydrate contents of 5 groundnut genotypes

Name of the genotypes	Moisture (%)	Ash (%)	Crude fiber (%)	Carbohydrate (%)
B6/282/63	5.7 c	2.38 a	3.68 ab	10.8 b
B6/282/64	6.2 a	2.26 bc	3.52 d	7.91 c
B6/282/80	5.2 d	2.35 a	3.60 c	5.57 e
RM-Kha-19	5.1 d	2.23 c	3.64 bc	5.69 d
Binachinabadam-6	6.0 b	2.27 b	3.71 a	11.4 a
CV (%)	1.36	0.91	0.69	0.38

4.11 Total Energy

The total energy evolved from the selected genotypes were ranged from 628.4% to 594.58%. The highest total energy 628.4% was found in B6/282/80; followed by RM-Kha-19 (609.64%), B6/282/64 (604.68%), B6/282/63 (603.46%) and Binachinabadam-6 (594.58%). Aryal *et al*. (2015) stated that, the mean of total energy found from 100 g groundnut was 567 kcal.

Table 10. Total energy of selected 5 groundnut genotypes

Name of the genotypes	Carbohydrate (%)	Energy from Carbohydrate (kcal/100g)	Protein (%)	Energy from Protein (kcal/100g)	Oil (%)	Energy from Oil (kcal/100g)	Total Energy (kcal/100g)
B6/282/63	10.80	43.2	27.34	109.36	50.1	450.9	603.46 c
B6/282/64	7.91	31.64	29.41	117.64	50.6	455.4	604.68 c
B6/282/80	5.57	22.28	28.68	114.72	54.6	491.4	628.4 a
RM-Kha-19	5.69	22.76	32.42	129.68	50.8	457.2	609.64 b
Binachinabadam-6	11.4	45.6	28.12	112.48	48.5	436.5	594.58 d
		CV (%)					0.29

CHAPTER 5

SUMMARY AND CONCLUSION

The whole study was carried out in the laboratory of Soil Science Division, the laboratory of Plant Breeding Division, laboratory of Electronics Division and the laboratory of Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Five groundnut (*Arachis hypogaea*) genotypes (4 advanced mutant lines and 1 released variety) were used in this study for nutritional and functional properties analysis. The objective of this study was to estimate oil, fatty acid, O/L ratio, iodine value, minerals, protein, ash, crude fiber, carbohydrate and total energy of 4 advanced mutant groundnut lines with check variety and to compare with the parent of those lines Binachinabadam-6. The quality aspects, to figure out the best performing mutant compared with its parent, is the main objective of this research work. The findings of this study will help the breeders for better utilization of the selected genotypes in further breeding program.

The oil content among 5 groundnut genotypes varied from 54.6% to 48.5%. The highest oil content (54.6%) was found in B6/282/80. All the mutant lines found higher in oil content than the parent Binachinabadam-6 (48.5%). In the composition of oil 8 fatty acids were detected and measured where 4 were unsaturated and the another 4 were saturated. The unsaturated fatty acids were Myristoleic acid, Oleic acid, Linoleic acid and Erucic acid those were detected as the highest amount in Binachinabadam-6 (2.463%), B6/282/80 (55.7%), B6/282/64 (17.129%) and B6/282/64 (3.035%), respectively. The saturated fatty acids were Hexanoic acid, Lauric acid, Arachidic acid and Palmitic acid those were detected as highest amount in RM-Kha-19 (23.291%), B6/282/64 (4.742%), B6/282/63 (28.455%) and B6/282/64 (8.66%), respectively. The lowest amount of Hexanoic acid, Lauric acid, Arachidic acid and Palmitic acid were found in B6/282/80 (4.232%), RM-Kha-19 (1.302%), B6/282/80 (16.829%) and B6/282/80 (1.044%), respectively. O/L ratio and iodine value both were found highest (3.68 and 74.12, respectively) in B6/282/80. B6/282/80 and B6/282/63 found comparatively better in healthy composition of fatty acid among the 5 genotypes.

The 10 important minerals (Ca, Mg, S, N, P, K, Fe, Zn, Cu, B) were measured among the selected genotypes. The higher amounts of major and minor minerals were found differently. The major minerals found abundantly in RM-Kha-19 and B6/282/63. The minor minerals also found in a good proportion in comparison to major minerals in all genotypes. Zn and Fe was found highest in RM-Kha-19 and B6/282/64, respectively.

The protein contents were ranged from 32.42% to 27.34% and RM-Kha-19 found the highest containing 32.42% protein. All the genotypes were found rich in protein content. Ash were highest in B6/282/63. But crude fiber and carbohydrate both were observed highest in Binachinabadam-6. The total energy estimated from the selected genotypes were ranged from 628.4% to 594.58% and the highest 628.4% was found in B6/282/80.

Finally, it can be concluded that the selected advanced mutants of groundnut genotypes are good in chemical analysis which might be the excellent source of nutrition. Induced mutation through radiation could be the mentionable variations in the nutritional and functional properties of genotypes. Considering oil content, fatty acid composition, O/L ratio, iodine value, ash and total energy, mutant B6/282/80 was the best performer among all mutants used in this study. On the other hand, considering protein and mineral contents, mutant RM-Kha-19 performed best; considering crude fiber and carbohydrate, Binachinabadam-6 performed best among the selected genotypes. The findings presented in this comparative study of biochemical properties will be helpful for the breeders for further breeding program.

CHAPTER 6

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