American Journal of Food Science and Technology, 2019, Vol. 7, No. 1, 1-6
Available online at http://pubs.sciepub.com/ajfst/7/1/1
Published by Science and Education Publishing
DOI:10.12691/ajfst-7-1-1

Soybean (*Glycine Max*): Alternative Sources of Human Nutrition and Bioenergy for the 21st Century

Ishrar Islam[2], Z. Adam[1], Shahidul Islam[1,*]

[1]Department Agriculture, University of Arkansas -Pine Bluff, 1200 North University Drive, Mail Slot 4913,153 Woodard Hall. Pine Bluff, AR 71601, USA
[2]Hendrix College, 1600 Washington Ave, Conway, AR 72032, USA
*Corresponding author: islams@uapb.edu

Received November 12, 2018; Revised December 27, 2018; Accepted January 07, 2019

Abstract Finding the nutritional value in soybeans is essential for keeping commodities at a low price while remaining conscious about people's health. This experiment brings into question whether it would be beneficial to consume soybeans more often and focus on the nutritional aspects of them, as well as the bioenergy benefits it provides. Therefore, the amounts of nutritional elements such as lipids, fatty acids, polyphenols, antioxidants, and soluble fibers tested within different varieties of soybeans. The highest phenolic content was in AS GROW 4754, followed by AS GROW 4632. The antioxidant capacity ranged from 2.35 3.44 µg/g of TEAC per 100g dry sample. The antioxidant activity follows as: AS GROW 4632> AS GROW 4754> AS GROW 4835. AS GROW 14632. AS GROWAG 4934. The Protein content ranges from 34.1 to 44.9 (%). The highest total protein was in AS GROW AG 4934, followed by AS GROW 4632, AS GROW 4754, AS GROW 4835 and AS GROW 14632. The lipid content ranges from 20.8 to 30.8 (%). The highest was in AG GROW 4835, and the lowest was in AS GROW 4632. Eight different fatty acids were found in the soybean**.** The Linoleic acid was found the predominant fatty acid 54%0 followed by Oleic acid (21%) and Palmitic acid (11%). The Behenic and Eicosenic acids found as trace amounts in soybean. Therefore, consumption of soybeans is beneficial but should also be incorporated within an overall healthy lifestyle. The difference in biomass and cell-wall components of five Arkansas grown soybean varieties examined to find out accessions that exhibited quality traits suitable for a potential bioenergy/biofuel crop. The Hemi-cellulose (HCE), cellulose (CE) and ASH contents ranged 14–30 %, 8-18 % and 1-3 % of the DM, respectively. The results showed that the NDF% ranged from 28 to 47, and ADF% ranged from 22-32. The extensive range of distinction in biomass and cell wall components point out that soybean has great potential for use as bioenergy/biofuel crops. The hypothesis was correct according to the results of the experiment, where several varieties showed high contents within all sources of nutritional value. The high amounts of cell-wall components between its species and in comparison to other bioenergy crops as well. The extensive range of distinction in biomass and cell wall components point out that soybean has great potential for use for multiple uses such as human food and nutrition, oil, energy, and biofuel potentials.

Keywords: *soybean, human nutrition, soluble fiber, fatty acids, bioenergy, biofuel*

Cite This Article: Ishrar Islam, Z. Adam, and Shahidul Islam, "Soybean (*Glycine Max*): Alternative Sources of Human Nutrition and Bioenergy for the 21st Century." *American Journal of Food Science and Technology*, vol. 7, no. 1 (2019): 1-4. doi: 10.12691/ajfst-7-1-1.

1. Introduction

In the present world, there are obstacles encountered by people that threaten human existence, such as energy insecurity, the need to moderate climate changes, and investments in the economic development in agriculture. To address these challenges, countries research to discover solutions that will meet the demand for clean energy and meet financial needs. Many different plants are already used for biofuel production, such as sugar crops, natural oils from soybean and algae, and starch, such as corn or maze. However, many of these plants are used to aid in the economy of the agricultural field, and this leads to problems with the distribution of the amount for biofuel production and food sources. Soybean, however, is not a familiar source in energy industries, as well as in biofuel manufacture, but show similar attributes to the plants above.

In China, meat is a vast market commodity that can benefit through the use of soybeans for biodiesel to keep meat prices low and benefitting the farmers by allowing them to keep more of their money [1]. Products containing soybeans are known for antioxidants through their source of isoflavones, which protect various cancers, such as multiple myeloma and breast cancer [2] again. Through its high-fat percentage, many consumers may receive their intake of daily fat amounts through soybeans rather than meat or other more harmful sources of fat. Soybean products benefit lung health, cholesterol, and reduce levels of depression as well, proving to help health issues in a variety of ways. As for outside appearances, it can protect against skin wrinkles [2]. Most of the soybeans present within the United States are utilized for feed grade rather than for nutritional value as of now. Along with corn and other choices of grain feeds, soy is known to replace many animal feeds for a low price, specifically as Genetically Modified Organisms [1,2].

Biomass, the total mass of a plant/organisms, generally has been taken as the only primary renewable energy resource, as fuel, adept of replacing huge extents of solid, liquid, and gaseous fossil fuels [3]. As a widely dispersed, naturally occurring carbon resource, biomass is a logical choice as a raw material for the production of a broad range of fossil fuel substitutes [3,4,5]. Soybeans are distinguished by a significantly high dry matter/weight yield potential, which is essential to knowing how much energy can be generated, and lower quality in comparison with plant species with the C3 photosynthetic pathway [5]. The unprocessed biomass is used primarily as fodder in many countries but especially by Central American Indians, who were the original cultivators [6]. The utilization of biomass as a renewable energy source for some advanced bio-energy systems indicates, however, that biomass can be a tremendous and prospective biofuel. Using cellulose from plants would be a better alternative, where the glucose would be released and could be converted into liquid fuels. The biofuel, fuel obtained from a biological source, from the cellulose and hemicellulose generate ethanol when subjected to enzymatic processes, in this instance, that calculate the fiber weight and bacterial decomposition. The higher the percentage composition of these plants, the higher the energy yield potential. Also, it would boost the economy and keep the atmosphere clean because the carbon plants receive are from the atmosphere itself [7]. It has become clear that current knowledge of biomass physical properties, specifically of new energy crops or plants cultivated in polluted fields where unfavorable chemicals are existing which prevents normal crop growth from happening, and is not sufficient for further optimization of industrial energy plants or domestic bio-energy units [8]. The problem being addressed is the need for new sources of energy in the environment, relating to changing the sources already being used in biofuel production today. The purpose is to determine whether or not soybean could be used as biofuel, as well as aid in the reduction and replacement of conventional fuel resources from other plants, such as corn and sugar cane. The procedures are used to estimate how much of the desirable cell wall components are present in the various soybean varieties. The overall impact of this experiment is improving the environment by discovering an alternative source of biofuel by retrieving the surplus waste soybeans.

Finding the nutritional value in soybeans is essential for keeping commodities at a low price while remaining conscious about people's health. While soybean consumption is not as valued as its use for feed grade, this experiment brings into question whether it would be beneficial to consume soybeans more often and focus on the nutritional aspects of them rather than just the bioenergy benefits. This way money can be saved within both the food and energy industry with a cheaper alternative than many options today. Through the use of soybeans as a subsidy for animal feed, it lessens the cost of feed and improves our economy as a result. As for the food industry, meat and other commodities with high protein or lipid contents will compete with many soybeans' contents of both as well as other dietary essentials. Therefore, if amounts of nutritional elements such as lipids, fatty acids, polyphenols, antioxidants, and soluble fibers are being tested for within different varieties of soybeans, then the results will display high amounts within each category for all of the variations.

2. Materials and Methods

2.1. Soybean Varieties

AS GROW 4835, AS GROW AG 4934, AS GROW 4754, AS GROW 4632 and AS GROW 14632 were collected from Jefferson County, AR extension office, and an AR soybean grower Mr. Steven Walker (Pine Bluff, AR). The features of the varieties are presented in Table 1.

2.2. Determination of Cell Wall Polymers

The dry samples were ground to fine powder for cell wall component analyses using ANKOM 200 Fiber Analyzer (ANKOM Technology, Fairport, NY). All the cell wall polymers contents namely neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) materials were determined following the protocol provided by ANKOM Technology [9]. Hemicellulose (HCE) and cellulose (CE) contents were determined by subtracting the values of ADF from NDF, and ADL from ADF, respectively [9,10]. Ash content was determined by combustion of samples in a muffle furnace at 930F for 12 hours [10].

Table 1. Features of the soybean varieties used in this study.

Name of the variety	Plant height	Maturity group	Herbicide tolerant	Flower color	Chloride sensitivity
AS GROW 4835	MT	4.8	RR2Y/SR	purple	6 Exc
AS GROW AG 4934	T	4.9	RR2Y/SR	purple	5 Inc
AS GROW 4756	T	4.7	RR2X/SR	purple	5 Inc
AS GROW 4632	T	4.6	RR2Y/SR	purple	4 Inc
AS GROW 14632	M	5.5	RR2Y	purple	5 Exc

M = Medium, T= Tall.

2.3. Measured Antioxidant Activity

The radical-scavenging activity of the extracts was measured by following the procedure reported by Islam et al. [11]. Stock solutions of ABTS (5.00×10^{-4} M) and sodium persulfate (6.89×10^{-3} M) in PBS (pH = 8.0) were prepared. The solution was stored for 16 hours. This solved ABTS$^{+\cdot}$ which gave an absorbance of approximately 0.85 at 734 nm. A 10 mM stock solution of Trolox was prepared for every sample tested. For each dilution, 20 µL was added to 2.5 mL of ABTS.$^{+}$ solution and incubated in a dry bath at 37°C for 30 minutes. Absorbances were measured at 734 nm on an ASYS UVM 340 plate reader. TEAC values were measured by comparing the slopes of sample plots compared to that of Trolox.

2.4. Extraction and Analysis of Lipids and Fatty Acids

The lipid contents were analyzed according to Folch [12]. The fatty acids were analyzed by using a Gas Chromatograph (Model CP-3380). 1 ml of the sample, which was kept in the freezer for fatty acid analysis, was placed in a capped vial. One ml 0.5 N KOH in methanol was added; placed in 70 degrees Celsius water bath for 10 minutes. Then 1 ml of Boron trifluoride methanol solution(12% w/w) was added and flushed with nitrogen and placed in 70 degrees Celius water bath for 30 minutes. After it is cooled off, 2 ml of hexane and 2 ml of saturated NaCl solution were added and vortexed for one minute. The sample is transferred to a disposable glass tube with sodium sulfate powder in it (to remove residual water). The hexane layer is pipetted into a 4 ml screw-top vial with Teflon-line. The cap, labeled FAME (Fatty Acid Methyl Ester). The FAME is flushed with nitrogen and stored frozen (-70 degrees Celsius) until analysis.

2.5. Extraction and Analysis of Protein Contents

The residues left after an 80 % acetone extraction are hydrolyzed in 5 ml of 1 N NaOH for overnight and centrifuged at 5000 rpm for 20 minutes. The supernatant is kept aside, and then the residue is extracted again with 5 ml of 1N NaOH for one hour, being centrifuged afterward. Both the supernatants were pooled, making the volume 10 ml. A 0.5 ml aliquot is taken in a test tube and mixed with 5 ml of reagent alkaline copper solution, allowed to stand for 10 minutes. After that, 0.5 ml of 1N Folin-Ciocalteau reagent (Commercial reagent) is added with instant mixing. After 30 minutes the absorbance is recorded at 570 nm through a spectrophotometer against reagent blank. The standard curve is prepared with a graded concentration of bovine serum albumin [13].

2.6. Determination of Total Phenols

Total phenolic content of each extract was measured using a slightly modified method reported by Islam et al. [14]. Tannic acid was used as a standard. Total phenolic content was expressed as milligrams Tannic acid equivalents per gram dry weight sample (mg TAE g^{-1} DW). TAE was measured by comparing the slope of samples to the slope tannic acid.

2.7. Data Analysis

Trait values were the mean of three replications. Evaluation of variance ($p < 0.01$) was accomplished using Microsoft Excel statistics package.

3. Results and Discussion

3.1. Polyphenol and Antioxidant Capacity

The total phenol ranges from 32.8 to 50.1 µg/100mg dry sample (Table 2). The highest phenolic content was in AS GROW 4754, followed by AS GROW 4632. The phenolic content results suggest that most of the soybean varieties have higher amounts of phenolic contents.

The antioxidant capacity ranged from 2.35 3.44 µg/g of TEAC per 100g dry sample (Figure 1). The findings of this study indicate that the soybean varieties had different amounts of antioxidant activity. The antioxidant activity follows as: AS GROW 4632> AS GROW 4754> AS GROW 4835. AS GROW 14632. AS GROWAG 4934. The results indicate that all the soybean varieties have higher antioxidant capacity that are comparable with various other crops [14,15,16,17,18].

3.2. Protein and Lipidscontents

The Protein content ranges from 34.1 to 44.9(%) (Table 2). The highest total protein was in AS GROW AG 4934, followed by AS GROW 4632, AS GROW 4754, AS GROW 4835 and AS GROW 14632. The results suggest that soybean varieties have very high contents of total protein. The lipid content ranges from 20.8 to 30.8 (%) (Table 2). The highest was in AG GROW 4835, and the lowest was in AS GROW 4632.

Table 2. Nutritional quality characteristics of soybean varieties studied

Name of the variety	Phenolic contents (µg/g TAE/100g DW)	Protein contents (%)	Lipid contents (%)
AS GROW 4835	33.7 ± 4.81ab*	37.7 ± 0.72b	30.8 ± 1.16a
AS GROW AG 4934	35.6 ± 2018ab	44.9 ± 1.57a	21.7 ± 1.15 b
AS GROW 4754	50.1 ± 6.82a	38.7 ± 0.89b	20.8 ± 1.17b
AS GROW 4632	45.5 ± 5.80ab	41.4 ± 1.0 ab	21.0 ± 1.15b
AS GROW 14632	32.8 ± 3.34b	34.1 ± 1.10c	22.0 ± 1.18b
LSD(5%)	15.39	4.41	3.67

Mean of three replications± standard error. *Mean followed by common letter(s) are not significantly different from each other according to DMRT (Duncan's multiple range test).

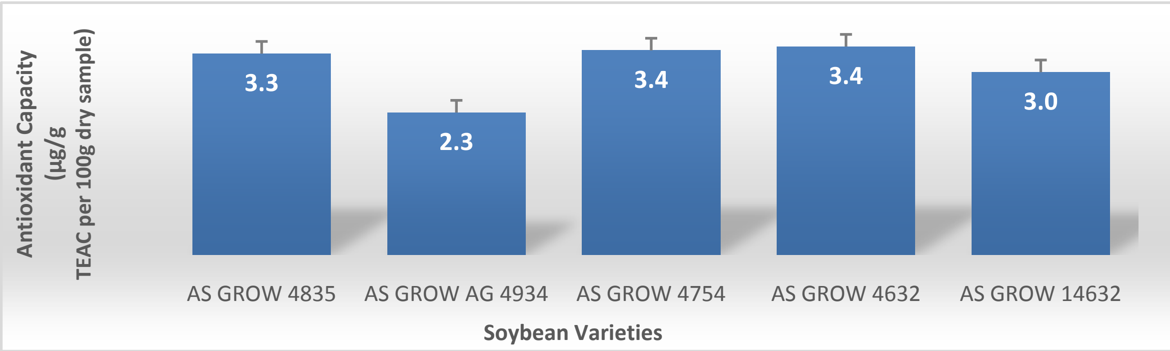

Figure 1. Antioxidant capacity (µg/g of TEAC per 100g DW) the soybean varieties studied. Each bar represents the mean of 3 replications. The vertical bars indicate the SE

3.3. Fatty Acids Contents

Eight different fatty acids were found in the soybean (Figure 2). The Linoleic acid was found the predominant fatty acid 54%0 followed by Oleic acid (21%) and Palmitic acid (11%). The similar pattern of distribution of fatty acids was found in all the soybean varieties studied (Figure 3). The Behenic and Eicosenic acids found as trace amounts in soybean. Oleic acid is a beneficial fatty acid to improve daily health due to its ability to lower cholesterol and harmful low-density lipoproteins in the bloodstream. Linoleic acid is a good source of energy and is necessary to consume within consumer's diets, but consumers should also be cautious of consuming large amounts of this fatty acid if they follow a typical Western diet. Therefore, consumption of soybeans is beneficial but should also be incorporated within an overall healthy lifestyle.

The highest average protein content was also in sample B, being 44.93 πg/g. While sample C and D showed higher amounts in antioxidant/phenolic activity, sample B includes higher sources of protein; therefore it can be considered a valuable choice concerning consumers buying soybean products of this variety for the protein content, rather than just from other sources such as meat production. Oleic acid is a beneficial fatty acid to improve daily health due to its ability to lower cholesterol and harmful low-density lipoproteins in the bloodstream. Linoleic acid is a good source of energy and is necessary to consume within consumer's diets, but consumers should also be cautious of consuming large amounts of this fatty acid if they follow a typical Western diet. Therefore, consumption of soybeans is beneficial but should also be incorporated within an overall healthy lifestyle.

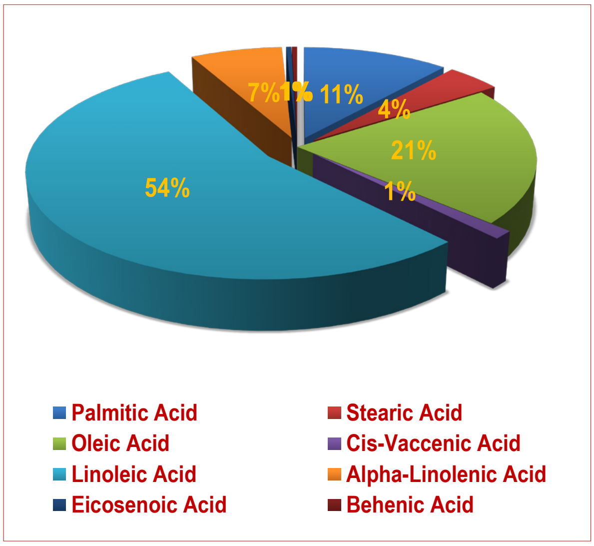

Figure 2. Distribution of different fatty acids in the Soybean varieties studied

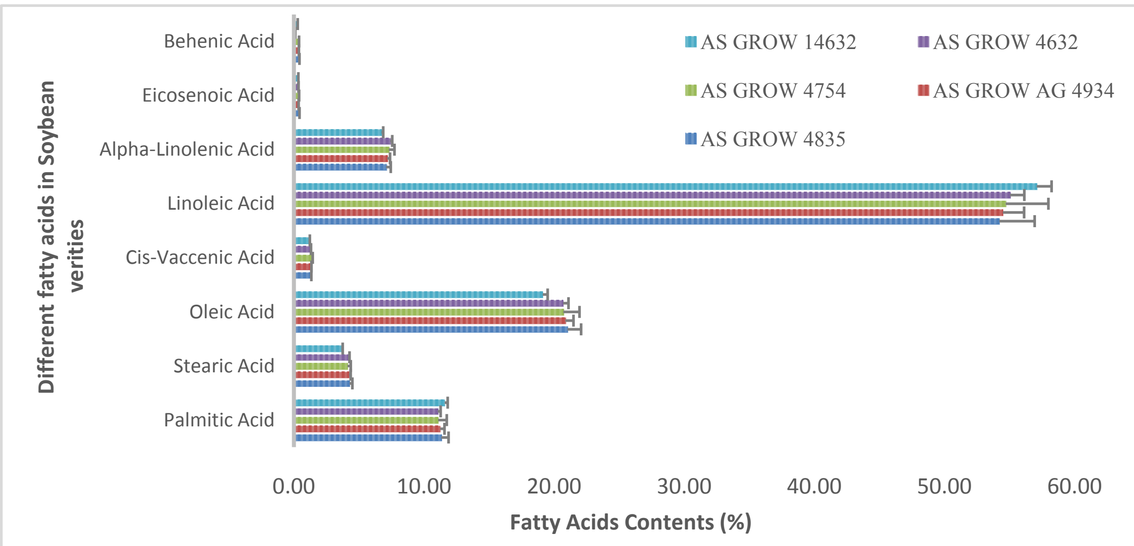

Figure 3. Fatty acid contents of different soybean varieties studied. Each bar represents the mean of three replications. The vertical bars indicate the standard errors (SE)

3.4. Soybean as a Raw Material for Bioenergy/Bio-fuel

The difference in biomass and cell-wall components of five Arkansas grown soybean varieties examined to find out accessions that exhibited quality traits suitable for a potential bioenergy/biofuel crop. The Hemi-cellulose (HCE), cellulose (CE) and ASH contents ranged 14-30 %, 8-18 % and 1-3 % of the DM, respectively (Figure 4).

The results showed that the NDF% ranged from 28 to 47, and ADF% ranged from 22 to 32 (Figure 5). The extensive range of distinction in biomass and cell wall components point out that soybean has excellent potential for use as bioenergy/biofuel crops. The biofuel production from soybean has also the advantages due to high and relatively stable harvests compared to most of the other bioenergy crops, and in small demands for intensification factors, resulting in low production costs. But the success depends on the choice of appropriate species, genotypes having higher biomass and higher cell wall polymer contents [11,19]. The procedures are used to measure the major structural components of the plants. One use of the components is as a source of biofuel, a fuel obtained from s biological source. Cellulose and hemicellulose are the plant components that generate ethanol where subjected to an enzymatic process or bacterial decomposition. The higher the percentage composition of these components in a plant, the higher the energy yield potential of the plant. Therefore soybean has excellent potential as bioenergy crops.

Usually for improvement of new bioenergy crops depends on identifying variation in critical morphological and physiological traits. The present study found a substantial variation for all the biomass traits in all the accessions studied. The cell wall polymer components among higher plants can differ significantly in quality and quantity [10,20,21] and is an essential consideration for bioenergy production.

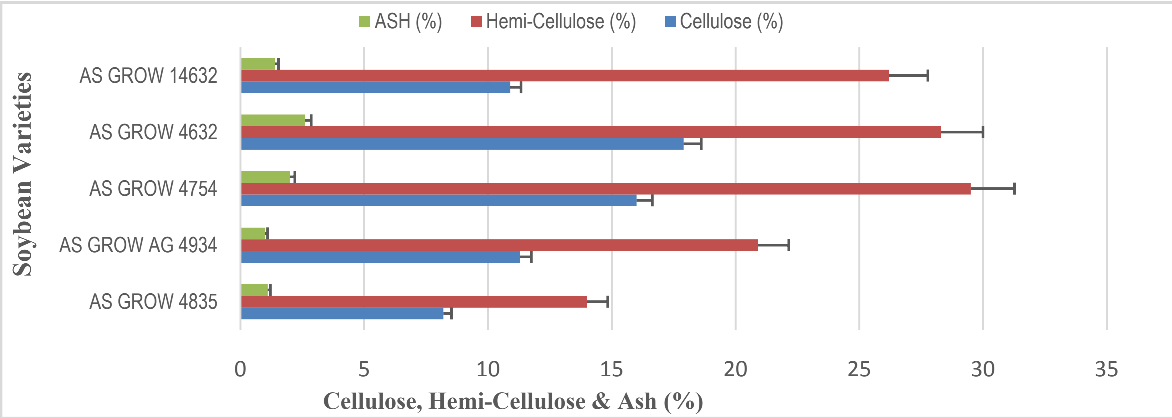

Figure 4. Cellulose, Hemicellulose & Ash contents (%) of soybean varieties. Each bar represents the mean of three replications. The vertical bars indicate the SE

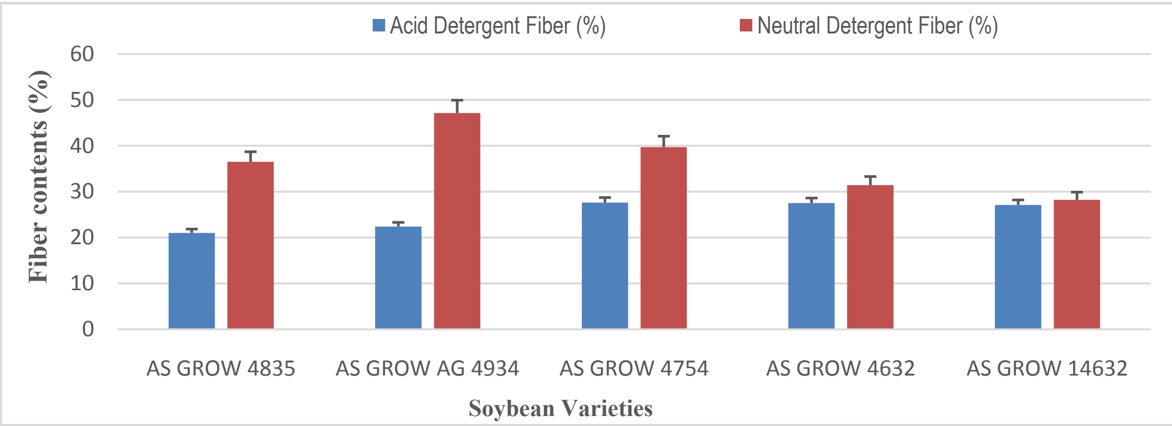

Figure 5. Fiber contents (%) of soybean varieties. Each bar represents the mean of three replications. The vertical bars indicate the SE

Table 3. Percentage of cell wall polymers of different bioenergy crops [22,23]

Bioenergy crops	Cellulose (%)	Hemi-cellulose	Ash (%)
Kenaf (whole)	42	14	3.8
Reed	34	18	4.9
Miscanthus	40	29	2.0
Switchgrass	41	33	4.1
Cotton	42	15	3.4
Olive tree	41	17	1.8
Almond tree	40	25	2.0
Corn	38	19	6.0
Sweet Sorghum	23	11	5.0
Soybeans studied	14 - 30	8-18	1.0- 3.0

Table 3 showed the percentage of different cell wall polymers of various bioenergy crops. From Table 3, it is apparent that Amaranths accessions have similar and higher contents of some cell wall polymers.

The biofuel production from amaranth also has the advantages due to high and relatively stable harvests compared to most of the other bioenergy crops, and in small demands for intensification factors, resulting in low production costs. But the success depends on the choice of appropriate species, genotypes having higher biomass and higher cell wall polymer contents [10,19,24]. The third table displays previous research on different bioenergy crops and their percentages of cell wall polymers. These percentages prove to show that soybeans can surpass the proportions of the plants used in the experiment shown. Therefore all the cell wall parameters in soybean varieties studied are comparable and higher as relate to other commercial bioenergy crops. Therefore the outputs from this study on an extensive range of distinction in biomass and cell wall components point out that soybeans provide an opportunity to use it as a new alternative bioenergy/biofuel crop.

4. Conclusion

The results show that several varieties showed high contents within all sources of nutritional value, individually sample ASGROW 4756 and ASGROW 4632. Also, many varieties displayed specific benefits towards one type of nutritional value and are, therefore, another option for consumers to consider. Therefore considering their nutritional, health benefits, oil, energy/biofuel, and other potentiality Soybean have a high potential of becoming an alternative source of biofuel/biodiesel as well as solving human nutrition issues for the 21st Century.

Money can be saved within both the food and energy industry with soybeans, a cheaper alternative than many options today. Through the use of soybeans as a subsidy for animal feed, it lessens the cost of feed and improves our economy as a result. As for the food industry, meat and other commodities with high protein or lipid contents will compete with many soybeans' contents of both as well as other dietary essentials. Also, the soybean varieties contain the higher contents of biomass and cell wall polymers, would be tested for further studies to determine which varieties have more potential for use in the production of biofuel.

References

[1] Solecki, M. (2015, September 30). Soybeans for Biodiesel. Retrieved from *http://www.fuelinggrowth.org/soybeans*-for-biodiesel. (2015).

[2] Greger, M. 2018. Medical Nutrition Blog. How to Block Breast Cancer's Estrogen-Producing Enzymes/ Is Soy Healthy for Breast Cancer Survivors? *https://nutritionfacts.org/blog/* (2018).

[3] Huska, J. Some agronomical aspect of amaranth production. *In Amaranth- plant for the future.* Fifth international symposium of the European amaranth association. Nitra, Slovak Republic. Nov 9-14, 2008. p. 27-28 (2008).

[4] Akond, M., Islam, S. and Wang, W. Characterization of Biomass trits and cell wall components among diverse accessions of *amaranthaceae* family. *J. Applied Phytochemistry in Environmental Sanitation.* 2: 37-45 (2013).

[5] Viglasky, J., Andrejcak, I., & Suchome, L. In *Agronomy Research*. In Amaranth (*Amarantus* L.) is a potential source of raw material for biofuels production (2nd ed., Vol. 7, pp. 865-873 (2009).

[6] Veresova, A. and Hoffmanova, Z. The evaluation of an experimental growing of Amaranth. In: *Biologization of a plant production* VI.", SAU, Nitra, pp. 172-180 (1995).

[7] Harris, R. Plants: The fuel of the future? Retrieved 2015, from *http://www.npr.org/templates/story/story.php?storyId=95444264* (2008).

[8] Svirskis, A. In Agro Res. *In Investigation of amaranth cultivation and utilization in Lithuania*, vol.1, pp. 253-264 (2003).

[9] Hindirchsen, I., Kreuzer, J., Madsen, J., & Bach, K. Fiber and lignin analysis in concentrate forage, and feces: Detergent versus enzymatic-chemical method. *J. Dairy Science*, 89(6), 2168-2176 (2006).

[10] Islam, I., Z. Adam and Islam, S. A potential sources of raw materials for biofuels manufacturing: Amaranth (Amaranth spp). *J. Agril. Envirn. Consurmer Sci*., 18: 41-46 (2018)

[11] Islam, I., Shaikh, A. and Islam, S. Antimutagenic and antioxidative potential phytochemicals from sweetpotato. *International J. Cancer Research*. 5: 83-94 (2009).

[12] Folch, J. A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues. *Journal of Biological Chemistry*. 226: 497-507 (1957).

[13] Lowry, OH, Rosebrough, NJ. Farr, AL, and Randall, RJ. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193: 265-275 (1951).

[14] Islam, S. Polyphenol contents and caffeic acid derivatives from leaves of *Ipomoea batatas* genotypes. *Acta Horticulturare*, vol. 841: 527-530 (2009).

[15] Islam, S. Medicinal and Nutritional Qualities of Sweetpotato Tips and Leave. Published by *Cooperative Extension Service*. FSA6135. p. 1-4 (2014).

[16] Islam, S. Sweetpotato (*Ipomoea batatas* L.) Leaf: Its Potential Effect on Human Health and Nutrition. *Journal of Food Science*, 71: R13-R21 (2006).

[17] Islam, S. "Potential Chemo-preventative properties isolated from *Ipomoea batatas* leaves". pp. 96-109. In*: Functional Foods and Chronic Diseases.* ISBN 978-0976753544. Publisher: Functional Food Center at D & A Inc., TX, USA. (2008).

[18] Shahidul, I, Adam, Z. and Islam I. Potential anticancer activity of fruits & vegetables. *Arkansas Environmental, Agricultural, and Consumer Sciences Journal*. 15-16: 52-58 (2016).

[19] Sitkey, V., Gadus, J., Klisky, L., & Dudak, A. Biogas production from amaranth biomass. *Acta regionalia et environmentalica*., 2: 59-62 (2013).

[20] Pauly, M., & Keegstra, K. Plant J*., K. 2008. Cell-wall Carbohydrates and Their Modification as a Resources for Biofuel., 54*, 559-568 (2008).

[21] Patil, PD, and Deng, S. Optimization of biofuel production from edible and non-edible vegetable oils. *Fuel,* 88: 1302-1306 (2009).

[22] Ververis, C. Industrial Crops and products. In *Fiber dimensions, lignin and cellulose content of various plant materials and their suitability for paper production* (Vol. 19, p. 254) (2004).

[23] Wiselogel, A. Bioresource Technology. In *Compositional changes during storage of large round switchgrass bales* (Vol. 56, pp. 103-109 (1996).

[24] Singh, SP, and Singh, D. Biodiesel production through the use of different sources & characterization of oils and their esters as the substitute of fuel. *Renewable and Sustainable Energy Reviews.* 14: 200-216 (2010).