American Journal of Plant Sciences, 2018, 9, 1455-1466
http://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Screening of Seed Soluble Sugar Content in Cowpea (*Vigna unguiculata* (L.) Walp)

Yuejin Weng[1], Waltram Second Ravelombola[1], Wei Yang[1], Jun Qin[1], Wei Zhou[1], Y.-J. Wang[2], Beiquan Mou[3*], Ainong Shi[1*]

[1]Department of Horticulture, University of Arkansas, Fayetteville, AR, USA
[2]Department of Food Science, University of Arkansas, Fayetteville, AR, USA
[3]US Department of Agriculture, Agricultural Research Service (USDA-ARS), Salinas, CA, USA
Email: *ashi@uark.edu, *Beiquan.Mou@ARS.USDA.GOV

How to cite this paper: Weng, Y.J., Ravelombola, W.S., Yang, W., Qin, J., Zhou, W., Wang, Y.-J., Mou, B.Q. and Shi, A.N. (2018) Screening of Seed Soluble Sugar Content in Cowpea (*Vigna unguiculata* (L.) Walp). *American Journal of Plant Sciences*, **9**, 1455-1466.
https://doi.org/10.4236/ajps.2018.97106

Received: May 1, 2018
Accepted: June 18, 2018
Published: June 21, 2018

Abstract

Cowpea (*Vigna unguiculata* (L.) Walp) is a legume crop grown worldwide to provide protein, starch, soluble sugar, amino acids, fatty acids, minerals, and vitamins for human consumption and animal feed. Soluble sugar is an important physiological trait in cowpea seeds. It not only plays an important role in storability and abiotic stress tolerance in seeds, but also provides energy to the human body and improves cooking quality by giving a desirable taste and mouth feel. Therefore, this research aimed to evaluate variation of cowpea seed soluble sugar content, and assess the soluble sugar content of cowpea varieties with different seed coat colors for cowpea consumers. A total of 113 cowpea genotypes were used, including 89 USDA GRIN germplasm accessions and 24 advanced breeding lines developed from University of Arkansas, AR, USA. Seed soluble sugar content was measured using a Spectrophotometer by phenol-sulphuric acid method. The results showed that the seed soluble sugar contents among 113 cowpea genotypes exhibited a wide range varying from 32.6 mg/g to 86.1 mg/g with an average of 54.5 mg/g. The five highest soluble sugar contents in seeds were found in the Arkansas cultivar Empire (86.1 mg/g), USDA germplasm accession PI583202 (84.5 mg/g), Arkansas advanced breeding line 09-655 (82.1 mg/g), USDA accession PI601085 (81.6 mg/g), and Arkansas advanced breeding line 09-529 (80.9 mg/g), and they can be used in cowpea breeding programs to develop new cowpea cultivars with higher seed soluble sugar content. It was also observed that the soluble sugar contents in Arkansas commercial cultivars and advanced breeding lines were higher than those in USDA germplasm and the seeds with colorful coat may have soluble sugar levels desirable by consumers.

Keywords

Seeds Storage, Soluble Sugar, Germplasm, Seed Color

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp), a legume crop that is grown worldwide, is beneficial for human consumption, animal feed and the environment [1]-[6]. It was estimated that, the total area under production amounts to about 14 million hectares with an annual production of over 5.4 million tones worldwide [7] [8]. Cowpea is widely distributed throughout the tropics, but Central and West Africa amounts to 64 percent of the area with about 8 million hectares followed by about 2.4 million hectares in Central and South America, 1.3 million hectares in Asia and about 0.8 million hectares in East and Southern Africa [9]. Cowpea is also grown as a vegetable (both for the leafy greens and the green peas). It primarily provides protein, starch, soluble sugar, amino acids, fatty acids, minerals and vitamins which benefit human health.

The content of soluble sugar is an important physiological trait which influences cooking quality in seeds and seed production [1] [10] [11] [12]. In fact, cowpea breeding has to feature traits desired by consumers in the market as well as farmers. The "sweet" trait, characterized by a sweeter and milder taste could help broaden cowpea consumption in the market. It not only provides calories as energy to human body, but also improves the cooking quality by producing a desirable taste and mouth feel. Additionally, soluble sugar has been proposed to play an important role in storability and abiotic stress tolerance in seeds, which are highly sensitive to environmental stresses [13]. Increases in levels of sugar in seeds have been correlated with the onset of desiccation tolerance during seed development [14]. Similarly, the loss of these soluble sugars in seeds at the beginning of germination has been found to coincide with the re-acquisition of desiccation sensitivity [15]. Seed longevity and development have also been positively correlated with the level of soluble sugars in seed [16].

However, this seed soluble sugar content characteristic has not received much attention in the cowpea breeding program, which has been primarily concerned with increasing yield, pest resistance, draught tolerance, protein content and other quality characteristics [3] [4] [17]. A few articles have reported on variable soluble sugar content in different genotypes in cowpea. Baptiste *et al*. (2011) reported three methods measuring soluble sugar content in cowpea seeds [1]. Nassourou *et al*. (2017) assessed the amount of total soluble sugar among 15 fully homozygous cowpea cultivars using the phenol sulfuric acid reagent method [3]. The objective of the present work was to evaluate the soluble sugar content in cowpea seeds and investigate elite germplasm with desired soluble sugar contents in order to propose a suitable breeding strategy for improving sweetness of cowpea seeds.

2. Materials and Methods

2.1. Plant Materials

A total of 113 cowpea genotypes (**Table 1**) were used in this study, including 89 USDA GRIN cowpea accessions and 24 advanced breeding lines from the cowpea

Table 1. List of cowpea genotypes and their mean soluble sugar content.

Genotypes	Genetic background	Seed coat color	Content (mg/g)
01-1781	AR breeding_line	Cream	60.60
07-303	AR breeding_line	Red	74.02
09-105	AR breeding_line	Cream	68.08
09-1090	AR breeding_line	Pinkeye	77.20
09-204	AR breeding_line	Browneye	71.17
09-208	AR breeding_line	Pinkeye	78.68
09-211	AR breeding_line	Pinkeye	69.51
09-393	AR breeding_line	Pinkeye	62.20
09-455	AR breeding_line	Blackeye	73.99
09-462	AR breeding_line	Pinkeye	71.58
09-529	AR breeding_line	Blackeye	80.94
09-655	AR breeding_line	Pinkeye	82.05
09-671	AR breeding_line	Blackeye	72.02
09-686	AR breeding_line	Pinkeye	75.42
09-692	AR breeding_line	Pinkeye	74.34
09-697	AR breeding_line	Pinkeye	61.67
09-714	AR breeding_line	Pinkeye	70.21
09-741	AR breeding_line	Red Holstein	77.20
AR Blackeye #1	AR cultivar	Blackeye	70.24
AR95-348	AR cultivar	Red	60.74
Early Acre	AR cultivar	Cream	54.50
Early Scarlet	AR cultivar	Pinkeye	65.69
Ebony	AR cultivar	Black	69.10
Empire	AR cultivar	Pinkeye	86.05
PI152195	Germplasm	Red	41.66
PI152197	Germplasm	Red	41.02
PI201498	Germplasm	Blackeye	70.16
PI218123	Germplasm	Browneye	48.10
PI220851	Germplasm	Browneye	47.83
PI223023	Germplasm	Browneye	44.75
PI229734	Germplasm	Blackeye	65.39
PI250587	Germplasm	Blackeye	74.66
PI253428	Germplasm	Black	38.89
PI255765	Germplasm	Black	43.49
PI255774	Germplasm	Browneye	37.15
PI262179	Germplasm	Black	44.43
PI291140	Germplasm	Black	49.29
PI292898	Germplasm	Black	40.77
PI293477	Germplasm	Black	38.81

Continued

PI293505	Germplasm	Black	39.01
PI293586	Germplasm	Black	44.00
PI293587	Germplasm	Red	41.34
PI293588	Germplasm	Black	41.44
PI311119	Germplasm	Red	42.65
PI312210	Germplasm	Browneye	47.30
PI339588	Germplasm	Black	42.65
PI339599	Germplasm	Browneye	46.79
PI339605	Germplasm	Red	41.64
PI349674	Germplasm	Black	46.08
PI354673	Germplasm	Black	50.52
PI354805	Germplasm	Red	41.19
PI354832	Germplasm	Black	32.55
PI354835	Germplasm	Black	39.08
PI354843	Germplasm	Black	41.29
PI354854	Germplasm	Red	46.80
PI354860	Germplasm	Black	39.43
PI354864	Germplasm	Black	39.19
PI354865	Germplasm	Black	36.26
PI406285	Germplasm	Blackeye	45.63
PI406290	Germplasm	Browneye	48.28
PI406292	Germplasm	Browneye	56.79
PI427093	Germplasm	Black	46.75
PI430687	Germplasm	Red	50.16
PI548785	Germplasm	Blackeye	62.69
PI578902	Germplasm	Red	49.77
PI578911	Germplasm	Red	45.94
PI582352	Germplasm	Black	47.62
PI582353	Germplasm	Black	47.20
PI582422	Germplasm	Blackeye	67.06
PI582428	Germplasm	Blackeye	64.28
PI582466	Germplasm	Blackeye	58.37
PI582474	Germplasm	Black	38.95
PI582551	Germplasm	Blackeye	52.42
PI582573	Germplasm	Browneye	71.07
PI582575	Germplasm	Black	38.52
PI582702	Germplasm	Red	51.58
PI582815	Germplasm	Red	42.11
PI582863	Germplasm	Browneye	74.92
PI582868	Germplasm	Red	40.42
PI582923	Germplasm	Blackeye	51.60

Continued

PI582926	Germplasm	Browneye	63.10
PI582942	Germplasm	Cream	46.86
PI583195	Germplasm	Red	46.04
PI583197	Germplasm	Black	46.26
PI583200	Germplasm	Blackeye	42.86
PI583201	Germplasm	Browneye	63.17
PI583202	Germplasm	Browneye	84.53
PI583232	Germplasm	Black	47.61
PI583248	Germplasm	Browneye	64.35
PI583250	Germplasm	Browneye	62.67
PI583513	Germplasm	Blackeye	43.70
PI583551	Germplasm	Browneye	62.37
PI592369	Germplasm	Cream	46.69
PI592374	Germplasm	Cream	46.51
PI601085	Germplasm	Browneye	81.57
PI601682	Germplasm	Browneye	79.31
PI608035	Germplasm	Blackeye	51.00
PI610520	Germplasm	Black	47.92
PI610533	Germplasm	Blackeye	45.79
PI610620	Germplasm	Black	43.14
PI642160	Germplasm	Blackeye	55.56
PI662992	Germplasm	Cream	46.33
PI663101	Germplasm	Blackeye	61.23
PI663140	Germplasm	Cream	45.98
PI663148	Germplasm	Blackeye	45.80
PI663151	Germplasm	Cream	45.80
PI664515	Germplasm	Cream	45.62
PI664517	Germplasm	Blackeye	47.80
PI664518	Germplasm	Cream	45.45
PI664519	Germplasm	Cream	45.27
PI664524	Germplasm	Blackeye	62.49
PI666253	Germplasm	Cream	46.15
PI666262	Germplasm	Pinkeye	66.85

breeding program at the University of Arkansas, AR, USA. The field experiment was conducted in three different locations within Arkansas State (Fayetteville, Alma, and Hope) in 2016. During the growing season, no pesticides or herbicides were sprayed to control pests, diseases, and weeds. Cowpea seeds were harvested when the color of pods changed and became mature. Seeds were dried and stored at room temperature until the soluble sugar measurement at laboratory.

Cowpea seed coat color is an important commercial trait to breeders in nursery and consumers in the market because cowpea has a diversity of colorful seed coats (Figure 1). Six seed color patterns were included in this study: black, blackeye, browneye, cream, pinkeye, and red color.

2.2. Sample Preparation

Within each location, seeds from each row were bulk-harvested. Seed soluble sugar content referred to the total sugar content was extracted using the following protocol. An 20 g of cowpea seeds of each sample was ground using a coffee grinder (Hamilton Beach) for 1 min. 2 or 3 g portion of the ground flours was sieved through 100# (nominal wire diameter 0.1 mm, Seedburo Equipment Company). 1.0 g of each sample was weighed and then transferred to a 50 ml microfuge tube. And then 40 ml 80% ethanol was added to each tube, homogenized for 1 min in a vortex and placed in a water bath at 80˚C for 20 min. After this treatment, the tubes were centrifuged at 6000 rpm for 10 min. 2 ml of the supernatant was then transferred into a 100 ml fresh flask to be diluted 50 times.

2.3. Equipment

GENESYS^{TM} 20 Visible Spectrophotometry (Thermo Scientific USA), an instrument which determines the amount of light absorbed while passing through a sample, was used to measure soluble sugar in seeds at laboratories, Department of Food Science, University of Arkansas, Fayetteville, Arkansas, USA.

Figure 1. Six seed coat color patterns in cowpea: (A) Black color (ex. PI354832, 32.55 mg/g); (B) Blackeye color (breeding line 09-925, 80.94 mg/g); (C) Browneye color (PI583202, 84.53 mg/g); (D) Cream color (PI663140, 45.98 mg/g); (E) Pinkeye color (cultivar Empire, 86.05 mg/g); and (F) Red color (PI582868, 40.42 mg/g).

2.4. Standard Curve Establishment

The amount of total soluble sugar was measured by the phenol-sulfuric acid reagent method [18]. A total of 50 ml of glucose (AR, Sigam-Alorich) stock solutions were prepared at the following concentrations: 0, 10, 30, 50, 70, and 100 μg/ml standard curve for glucose. Then 0 ml; 0.5 ml; 1.5 ml; 3.5 ml; and 5.0 ml of glucose stock solution were each poured into a 10 ml recipient. 1 ml was taken from each solution and poured into a test tube for measurement of soluble sugar content with colorimetric phenol-sulfuric acid by Spectrophotometry.

After many repetitions, a standard glucose curve with concentration 0, 10%, 30%, 50%, 70% and 100% was set up in this study (**Figure 2**). Six points look smooth and straight without double to be determined, known as the smooth curve. The best standard curves having the equation Y = 0.0039X + 0.0235 (R^2 = 0.99878) was achieved and accomplished consummation, where X is absorbance coefficient at 490 nm wavelength on Spectrophotometer and Y is soluble sugar content (%) in cowpea seed storage. Daily repeats allowed a soluble sugar standard curve to be constructed which was used to determine the soluble sugar concentration in each cowpea sample.

2.5. Quality Assessment

The use of phenol-sulfuric acid with a phenol concentration of 2% provided a relatively simple and reliable colorimetric method to quantify the total soluble-sugar concentration. Spectrophotometer measures the absorbance with wavelength at 490 nm. Absorbance was estimated using Beer's law:

$$abc = \log\left(I_0 / I\right)$$

I_0: incident radiation or radiation transmitted.
I: radiation transmitted by the sample.
abc: absorbance of the sample.
a: absorbancy coefficient.
b: path length of the sample.
c: concentration of the sample.

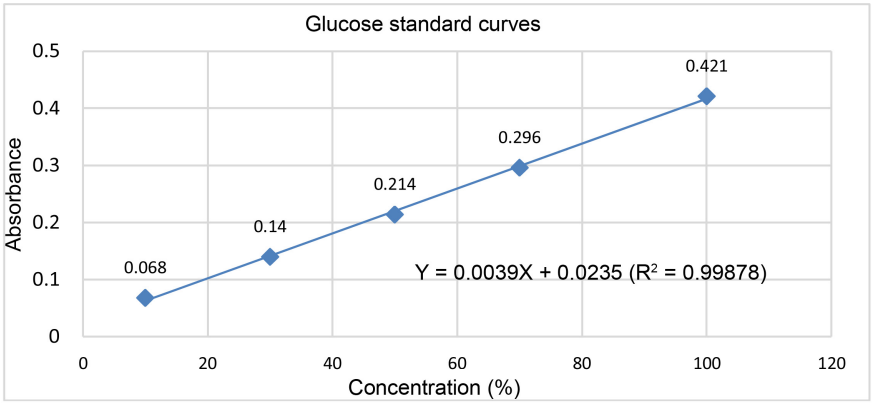

Figure 2. Glucose standard curves was computer generated based on the five standard concentrations.

Based on each sample's absorbent value, the concentration of total soluble sugar in each cowpea sample was estimated using the regression equation form the standard curve. Individual extractions were replicated two times for each sample.

2.6. Date Collection and Analysis

Descriptive statistics including mean, minimum, maximum, variance and standard error were computed using the "Tabulate" option of JMP Genomics 7 (SAS Institute, Cary, NC). The distribution graph of the cowpea soluble contents was drawn using the "Distribution". Analysis of variance (ANOVA) was performed using the general linear model (GLM) of JMP Genomics 7. Student T-test at $\alpha = 0.05$ was used for multiple comparisons of the least square mean (LSM) among the genotypes and the different seed colors.

3. Results and Discussion

3.1. Soluble Sugar Quantification in Cowpea Seeds

The soluble sugar content of cowpea seeds affects the final flavor of cowpea food and cowpea-derived products. This work was to use a simple, low-cost, spectrophotometric method for soluble sugar quantification in cowpea seeds. Significant variations were observed for soluble sugar contents in 113 diverse cowpea genotypes tested in this study (Table 1). The range in soluble sugar contents among tested samples was from 32.6 mg/g to 86.1 mg/g with an average of 54.5 mg/g, based on the standard cures, indicating seed soluble sugar contents having wide variations among the 113 cowpea genotypes. Soluble sugar content has a bimodal distribution (Table 1 and Figure 3).

The result of soluble sugar content in this experiment was apparently higher than previous report from other studies. Akpapunam *et al*. (1979) reported that the average of sucrose contents was 22 mg/g from 13 American cowpea (*Vigna sinensis*) varieties extracted by 70% ethanol [19]. Onigbinde and Akinyele (1983)

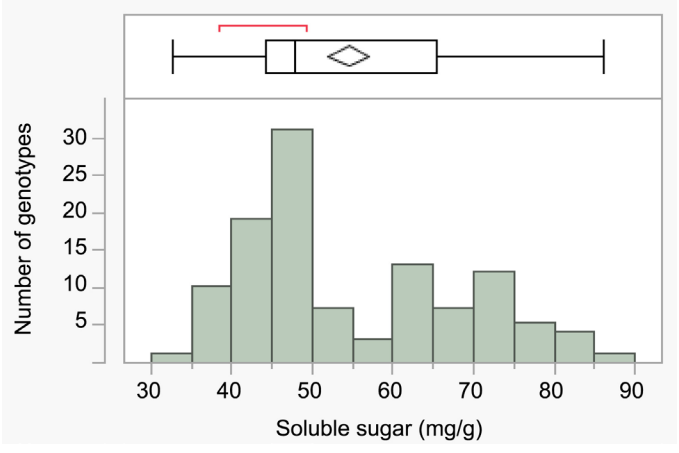

Figure 3. Distribution of soluble sugar content in cowpea seeds among 113 genotypes.

reported that the average content of the sucrose was 8 mg/g in mature dry seeds of 20 varieties of cowpea grown in Nigeria [20]. Nassourou *et al*. (2017) reported the total soluble sugar content in cowpea seeds using the phenol sulfuric acid reagent method among 15 cowpea cultivars and observed that the highest values were 40.79 mg/g (IT93K-693-2) and 40.64 mg/g (BR1) with a mean of 22.31 mg/g [17]. The content of soluble sugar in cowpea seeds in this study was obviously higher than what Onigbinde *et al*. (1983) and Nassourou *et al*. (2017) reported, which may be caused by different cowpea genotypes and extraction method. The result also was shown higher than that Akpapunam *et al*. (1979) reported due to their different species.

The top five genotypes with highest soluble sugar content in cowpea seeds were determined to be "Empire" (86.05 mg/g), PI583202 (84.53 mg/g), 09-655 (82.05 mg/g), PI601085 (81.57 mg/g), and 09-529 (80.94 mg/g). In contrast, the USDA germplasm accession PI354832 (32.55 mg/g) and PI354865 (36.26 mg/g) had the lowest soluble sugar content among the 113 cowpea genotypes tested. Overall, the soluble sugar content in Arkansas cultivars and advanced breeding lines was higher than the USDA germplasm accessions. However, higher soluble sugar content was also observed in some USDA accessions such as PI583202 (84.50 mg/g) and PI601085 (81.60 mg/g). These results indicated that high level of soluble sugar content not only can be discovered from released cowpea cultivars, but also from cowpea germplasm. These cowpea genotypes with higher soluble sugar content will be beneficial to the future breeding program and genetic improvement for meeting the needs of the market and consumers.

3.2. Compare Seed Soluble Sugar Content in Genetic Background

A highly significant difference ($P < 0.001$) was observed between Arkansas cowpea cultivars/advanced breeding lines and USDA cowpea germplasm accessions (**Table 2**). Most of the highest soluble sugar contents are found in Arkansas cowpea cultivars and advanced breeding lines. The average soluble sugar content of Arkansas cowpea cultivars/advanced breeding lines was 71.13 mg/g, significantly higher than the average soluble sugar content of cowpea germplasm accessions (50.04 mg/g). Cowpea breeding program in University of Arkansas have had more than 60 years of history since the first variety "Monarch" was released in 1956. After selection and enhancement from generation to generation, the Arkansas cowpea cultivars/breeding lines have developed more closely to

Table 2. Descriptive statistic for soluble sugar (mg/g) by genetic background.

Genetic background	N	Mean	Std Dev	Min	Max	Variance	Std Err	Prob < t = 0.05
AR cultivar/ Breeding lines	24	71.13	7.60	54.50	86.05	57.69	1.55	A
USDA Germplasm	89	50.04	11.05	32.55	84.53	121.99	1.17	B

consumer's needs, including quality improvement and increasing breeder's expectation in seeds. Regardless of intentional or unintentional selection, the seed soluble sugar content of cowpea cultivars and advanced breeding lines has been also improved and increased after breeding and genetic improvement in Arkansas State and the Southern region of US (Table 2). These cultivars and advanced breeding lines exhibited more soluble sugar content and better adaptation to growing conditions in Arkansas State and the Southern region than USDA germplasm accessions.

3.3. Soluble Sugar Content Evaluation in Different Seed Color

Cowpea seed coat color is an important morphological character and commercial trait to costumers because of diversity of cowpea seed coat (Figure 4), which provides various products with colorful seed coat for consumers to choose in supermarket or grocery stores. In order to represent detailed cowpea seed coat color description in markets, ix major types of cowpea seed colors were used for this study: black, blackeye, browneye, cream, pinkeye, and red color. The variance of soluble sugar content in color coat seed was measured using GENESYS™ 20 Visible Spectrophotometer in this study (Table 1 and Table 3). The results showed that the pinkeye color cowpea seeds had the highest soluble sugar content averaging 72.42 mg/g, and the black color cowpea contained the

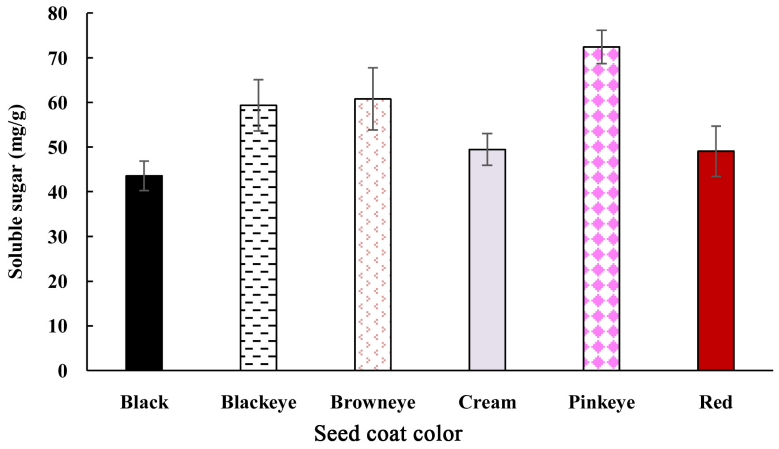

Figure 4. Average soluble sugar content (mg/g) per seed coat color.

Table 3. Descriptive statistics for soluble sugar (mg/g) by seed coat color.

Seed Coat Color	N	Mean	Std Dev	Min	Max	Variance	Std Err	Prob < t = 0.05
Pinkeye	13	72.42	7.44	61.67	86.05	55.30	2.06	A
Browneye	19	60.80	13.90	37.15	84.53	193.15	3.19	B
Blackeye	23	59.38	11.39	42.86	80.94	129.76	2.38	B
Cream	13	49.53	7.15	45.27	68.08	51.16	1.98	C
Red	17	49.08	11.30	40.42	77.20	127.71	2.74	C
Black	28	43.58	6.62	32.55	69.10	43.87	1.25	C

lowest soluble sugar, averaging 43.58 mg/g in cowpea seeds. The browneye color (60.80 mg/g) contained almost similar amount of soluble sugar compared with that of blackeye color (59.38 mg/g). Overall, the soluble sugar content in pinkeye color (72.42 mg/g) > browneye (60.80 mg/g) ≥ blackeye (59.39 mg/g) > cream (49.53 mg/g) ≥ red (49.08 mg/g) ≥ black (43.58 mg/g). This study will inform consumers to buy which type of the seed color cowpea that fits their needs in terms of cooking quality and sweetness.

4. Conclusion

A total of 113 cowpea genotypes were measured for their soluble sugar contents in seeds using Spectrophotometer by phenol-sulphuric acid method in this study. A wide range of solublesugar content were observed from 86.05 mg/g to 32.55 mg/g with an average of 54.52 mg/g. Commercial cultivars and advanced breeding lines from Arkansas region, USA contained higher soluble sugar content than USDA germplasm did. The soluble sugar content changed with the seed color coat: pinkeye (72.42 mg/g) > browneye (60.80 mg/g) ≥ blackeye (59.38 mg/g) > cream (49.53 mg/g) ≥ red (49.08 mg/g) ≥ black (43.58 mg/g).

References

[1] Baptiste, N.T.J., Youmbi, E., Njintang, N.Y. and Youmbi, E. (2011) Generation Means Analysis of Seed Sucrose Content in Cowpea (*Vigna unguiculata* L. Walp.). *Asian Journal of Agricultural Sciences*, **3**, 4795-4805.

[2] Namasaka, R.W., Tusiime, G., Orawu, M., Gibson, P., Nyiramugisha, J. and Edema, R. (2017) Evaluation of Cowpea Genotypes for Resistance to *Fusarium redolens* in Uganda. *American Journal of Political Science*, **8**, 2296-2314.

[3] Nassourou, M.A., Noubissié, T.J.B., Njintang, Y.N. and Bell, J.M. (2017) Diallel Analyses of Soluble Sugar Content in Cowpea (*Vigna unguiculata* L. Walp.). *The Crop Journal*, **5**, 556-559. https://doi.org/10.1016/j.cj.2017.05.005

[4] Ravelombola, W.S, Shi, A., Weng, Y., Motes, D., Chen, P., Srivastava, V. and Wingfield, C. (2016) Evaluation of Total Seed Protein Content in Eleven Arkansas Cowpea (*Vigna unguiculata* (L.) Walp.) Lines. *American Journal of Political Science*, **7**, 2288-2296.

[5] Roberts, P.A., Cisse, N., Drabo, I., Dovala, A.C. and Ehlers, J. (2008) Modern Cowpea Breeding to Overcome Critical Production Constraints in Africa and the US. http://www.canr.msu.edu/legumelab/uploads/files/UCR-1%20Tech%20Prog%20Rpt%20FY08%20Final.pdf

[6] Weng, Y., Shi, A., Ravelombola, W.S., Yang, W., Qin, J., Motes, D., Moseley, D.O. and Chen, P. (2017) A Rapid Method for Measuring Seed Protein Content in Cowpea (*Vigna unguiculata* (L.) Walp.). *American Journal of Political Science*, **8**, 2387-2396.

[7] Xiong, H., Shi, A., Mou, B., Qin, J., Motes, D., Lu, W., Ma, J., Weng, Y., Yang, W. and Wu, D. (2016) Genetic Diversity and Population Structure of Cowpea (*Vigna unguiculata*L. Walp). *PLoS ONE*, **11**, e0160941. https://doi.org/10.1371/journal.pone.0160941

[8] Singh, B. (2003) Improving the Production and Utilization of Cowpea as Food and Fodder. *Field Crops Research*, **84**, 169-150. https://doi.org/10.1016/S0378-4290(03)00148-5

[9] Olufajo, O.O. (2012) Agronomic Performance of Improved Cowpea Varieties under Natural Infestation with *Alectra vogelii* (Benth.) in the Northern Guinea Savannah of Nigeria. *Agricultura Tropica et Subtropica*, **45**, 66-71. https://doi.org/10.2478/v10295-012-0011-x

[10] Ngalamu, T., Odra, J. and Tongun, N. (2014) Cowpea Production Handbook https://www.researchgate.net/publication/284900187_Cowpea_Production_Handbook

[11] Basha, S.M. (1992) Soluble Sugar Composition of Peanut Seed. *Journal of Agricultural and Food Chemistry*, **40**, 780-783. https://doi.org/10.1021/jf00017a015

[12] Hou, A., Chen, P., Shi, A., Zhang, B. and Wang, Y.-J. (2009) Sugar Variation in Soybean Seed Assessed with a Rapid Extraction and Quantification Method. *International Journal of Agronomy*, **2009**, Article ID: 484571. https://doi.org/10.1155/2009/484571

[13] Teixeira, A.I., Ribeiro, L.F., Rezende, S.T., Barros, E.G. and Moreira, M.A. (2012) Development of a Method to Quantify Sucrose in Soybean Grains. *Food Chemistry*, **130**, 1134-1136. https://doi.org/10.1016/j.foodchem.2011.07.128

[14] Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J.A., Hilal, M. and Prado, F.E. (2009) Soluble Sugars—Metabolism, Sensing and Abiotic Stress: A Complex Network in the Life of Plants. *Plant Signaling & Behavior*, **4**, 388-393. https://doi.org/10.4161/psb.4.5.8294

[15] Pukacka, S. and Pukacki, P.M. (1997) Changes in Soluble Sugars in Relation to Desiccation Tolerance and Effects of Dehydration on Freezing Characteristics of *Acer platanoides* and *Acer pseudoplatanus* Seeds. *Acta Physiologiae Plantarum*, **19**, 147-154. https://doi.org/10.1007/s11738-997-0031-8

[16] Steadman, K.J., Pritchard, H.W. and Dey, P.M. (1996) Tissue-Specific Soluble Sugars in Seeds as Indicators of Storage Category. *Annals of Botany*, **77**, 667-674. https://doi.org/10.1093/aob/77.6.667

[17] Blackman, S.A., Obendorf, R.L. and Leopold, A.C. (1992) Maturation Proteins and Sugars in Desiccation Tolerance of Developing Soybean Seeds. *Plant Physiology*, **100**, 225-230. https://doi.org/10.1104/pp.100.1.225

[18] Michel, D., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Fred, S. (1956) Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry*, **28**, 350-356. https://doi.org/10.1021/ac60111a017

[19] Akpapunam and Markakis (1979) Oligosaccharides of 13 American Cultivars of Cowpea (*Vignasinensis*). *Journal of Food Science*, **44**, 1317-1319. https://doi.org/10.1111/j.1365-2621.1979.tb06428.x

[20] Onigbinde and Akinyele (1983) Oligosaccharide Content of 20 Varieties of Cowpeas in Nigeria. *Journal of Food Science*, **48**, 1250-1251. https://doi.org/10.1111/j.1365-2621.1983.tb09203.x