DF-512
[1-6]

RESEARCH ARTICLE Agricultural Science Digest

Relationship of Phytochemical and Seed Characteristics of Indonesian Soybean Varieties

H.A. Arifin, A.G. Arifin, M.J. Mejaya

10.18805/ag.DF-512

ABSTRACT

Background: Soybean phytochemicals possess antioxidants that are associated with numerous benefits to human health, therefore, the seed quality and the beneficial content must be considered. This study aimed to assess the relationship between phytochemicals and seed characteristics of Indonesian soybean varieties.

Methods: A total of 20 soybean varieties were studied on the seed characteristics (eccentricity index, flatness index and weight). This study was conducted in 2021 at the University of Brawijaya, Malang, East Java, Indonesia. The whole soybean seeds were extracted to determine the values of crude protein, oil content, total phenol, total flavonoid and antioxidant activity.

Result: Four soybean varieties, namely Detam 1, Detam 2, Dering 1 and Grobogan, contain a moderately high amount of crude protein and oil and are prominent in phytochemicals and antioxidant activity. In addition, the correlation analysis indicated that the level of antioxidant activity is correlated with total phenol, total flavonoid and seed size.

Key words: Antioxidant activity, *Glycine max*, Oil, Protein, Seed size.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is one of the subtropical legumes native to southeastern Asia cultivated throughout the world in tropical, subtropical and temperature climates (Alghamdi *et al.* 2018). It has been consumed widely as a source of protein-rich foods and supplements like tempeh (Indonesian yeast fermented soybean), soy sauce, tofu, natto (bacterially fermented soybean) and edamame (boiled immature seeds). Particularly, in Indonesia, the consumption of tempeh and tofu is approximately 7.61 kg and 8.23 kg/capita/year, respectively (Harsono *et al.* 2021).

One of the primary soybean seed qualities is its size and color (Rehal *et al.* 2019). Soybean seeds contain phytochemicals such as phenolic, flavonoids, isoflavones, soyasaponins and triterpenoids and have excellent immune-active effects on preventing chronic degenerative diseases (Kim *et al.* 2021). These phytochemicals possess pharmacological properties such as antioxidant, antimutagenic, anticancer, antidiabetic, antiobesity, antiviral, antimicrobial and antiinflammatory and can protect cell against free radicals and oxidative damage primarily by donating a hydrogen atom or an electron (Dhungana *et al.* 2021; Kuchlan *et al.* 2017).

Different varieties exhibit distinct properties even within the same origin. Previous study has shown that the Japanese soybean accessions exhibited significant varieties of antioxidant activities in a cell-based assay (Arifin *et al.* 2021). Indonesian soybean varieties, in particular, also have variations in agronomical and morphological characteristics (Sulistyo *et al.* 2019). Therefore, it is necessary to investigate their phytochemicals among varieties. This study aimed to determine a relationship between phytochemicals, antioxidant activity and seed characteristics of soybean varieties. The result of this study will be helpful for morpho-chemical-based selection in soybean breeding programs.

[1]Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, Miyazaki 889-2192, Japan.
[2]Faculty of Agriculture, Brawijaya University, Malang 65145, East Java, Indonesia.
[3]Indonesian Legumes and Tuber Crops Research Institute (ILETRI), Malang 65101, East Java, Indonesia.

Corresponding Author: M.J. Mejaya, Indonesian Legumes and Tuber Crops Research Institute (ILETRI), Malang 65101, East Java, Indonesia. Email: mmejaya@yahoo.com

How to cite this article: Arifin, H.A., Arifin, A.G. and Mejaya, M.J. (2022). Relationship of Phytochemical and Seed Characteristics of Indonesian Soybean Varieties. Agricultural Science Digest. DOI: 10.18805/ag.DF-512.

Submitted: 30-09-2022 **Accepted:** 21-12-2022 **Online:** 27-12-2022

MATERIALS AND METHODS

Plant materials

This study used 20 soybean varieties which comprised 19 Indonesian soybean varieties and 1 Korean soybean variety. The experiment was conducted in 2021 at the University of Brawijaya, Malang, East Java, Indonesia. The seeds of these soybean varieties were obtained from the germplasm collection of the Indonesian Legumes and Tuber Crops Research Institute (ILETRI), Malang, East Java, Indonesia. The information on soybean varieties used in this study is presented in Table 1.

Phytochemical analysis

The crude protein content was analyzed according to the standard by AOAC (2005) and by using a Kjeldahl system

(Buchi K-350 and K-426, Switzerland). The powdered soybean seeds (0.5 g) were used to detect each variety for triplicates. The crude protein content was calculated as a percentage of nitrogen multiplied by 6.25 (the standard Kjeldahl factor).

The soybean seed powder (6 g) was extracted in 60 mL of 70% ethanol (containing 0.1% acetic acid) (Carryo-Panizzi *et al.* 2002). Samples were placed on an orbital shaker at 150 rpm (Protechâ, type 722) for 48 h at room temperature. All extraction for each variety was conducted in triplicate and stored at -20°C before assay. The amount of total phenol in the extract was analyzed using the Folin-ciocalteau assay (Magalhães *et al.* 2010). The total phenol content was expressed as mg g^{-1} gallic acid equivalents (GAE). To determine the total flavonoid content, the soybean extract was subjected to aluminum nitrate nonahydrate colorimetric assay with slight modification (Sembiring *et al.* 2018). The experiments were performed in 96-well microtiter plates with triplicates of each soybean accession. The mixture was then incubated for 40 min and the absorbance was measured at 415 nm. The flavonoid content was expressed as mg g^{-1} quercetin equivalents (QE). The antioxidant activity of each soybean variety was assessed by a 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay according to a previously described method (Zahratunnisa *et al.* 2017). The results were plotted as absorbance value vs. sample concentration (mg/assay). The results were expressed as the half-inhibitory concentration (IC_{50}) in mg mL^{-1} reaction mixture.

Statistical analysis

The results of this study were presented as means ± standard deviation (SD). The analysis variance (ANOVA) was used to determine the significant differences between group means with probabilities of $P \leq .01$ and .05. Correlation analyses were obtained using Pearson's product-moment correlation (with normality). Statistical and clustering analysis was performed using the R software package (Version 4.1.2).

RESULTS AND DISCUSSION

Crude protein and oil content

The values of crude protein content ranged ranged from 28.03% in Argopuro to 44.72% in Detam 1 varieties, with a mean value of 38.60% (Table 2). The six soybean varieties containing more than 40% crude protein were Detam 1, Detam 2, Grobogan, Mahameru, Anjasmoro and Detap 1. Our result showed that the crude protein content diversity is more significant than the previous studies, which the crude protein values of different soybean genotypes ranged from 35.35 to 43.13% (Alghamdi *et al.* 2018). The oil content in the present study showed a significant difference among varieties from 14.50% to 33.12%, with a mean value of 18.76%. In particular, the Detam 1 variety is superior in terms of crude protein and oil content, while Daewon has the lowest oil content.

Total phenol and total flavonoid content

Subsequent Folin-Ciocalteau assay revealed that the total phenol content ranged from 3.02 to 9.54 mg GAE g^{-1} with a mean of 5.66 mg GAE g^{-1} (Table 2). The highest total phenol content was present in Detam 2, while the lowest total phenol content was present in Anjasmoro. The total phenol content of seeds in all varieties was significantly different from each other. The total flavonoid content of 20 soybean varieties ranged from 0.21 to 2.56 mg QE g^{-1}. Daewon variety contained the lowest total flavonoid content (0.21 mg QE g^{-1}), while Detam 2 had the highest value of 2.56 mg QE g^{-1}. The overall result, Detam 2 showed prominent total phenol and flavonoid content.

Antioxidant activity

The antioxidant activity was expressed by the IC_{50} values, which are defined as the concentration of the sample (mg mL^{-1} reaction mixture) required to scavenge 50% of the DPPH free radicals. The lower IC_{50} value indicates that the sample has higher antioxidant activity and is more efficient in extinguishing free radicals. The result showed that IC_{50} of 20 varieties ranged from 0.11 to 2.45 mg mL^{-1} with a mean of 1.03 mg mL^{-1} (Table 2). The lowest value of IC_{50} founded in Dering 1, while the highest was Dena 1. Varieties that recorded lower than 0.5 mg mL^{-1} IC_{50} were Dering 1, Detam 2, Demas 1 and Devon 2.

Correlation analysis among characters observed

Correlation analyses between seed traits, crude protein, oil, total phenol content, total flavonoid content and IC_{50} were conducted (Table 3). The seed size indicated with the seed eccentricity index has significantly and negatively correlated with oil content (r= -0.47, P<.05). This result reflects that the lower eccentricity index values mean that seeds with more roundish shapes might contain the highest oil content. The relationship between the seed size and oil content was also reported by Liu *et al.* (2020). In particular, the authors claimed that essential fatty acids such as linolenic acid and oleic acid contained in soybean oil were significantly correlated with the seed width, thickness, length and weight of 100 seeds (P<.0001).

Another parameter of seed size in the present study was the seed flatness index, which is shown to be significantly and positively correlated to seed weight (r = 0.77, P<.01) and IC_{50} (r= 0.47, P<.05). These results indicated that seeds with a small flatness index and low seed weight have low IC_{50} values, which means high antioxidant activity. The varieties in this study that show this particular trend (of having a small flatness index and seed weight) are Dering 1, Detam 2, Demas 1 and Devon 2, which indeed were reported to have high antioxidant activity (Table 1 and Table 2). In a previous study, Choi *et al.* (2020) also conducted a similar investigation and found that small seeds showed the maximum ferric reducing antioxidant power (FRAP) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS) scavenging activities. They also reported that the

Table 1: The information on seed characteristics.

Varieties	Seed size		Weight	Color						Flowering time[c]	Pod maturity[c]
	EI (mm)[a]	FI (mm)[b]	100 seeds (g)	Seed coat	Hilum	Hypocotyl	Epicotyl	Cotyledon	Pubescence[c]	(DAP)[d]	(DAP)[d]
Anjasmoro	1.22±0.08	0.35±0.02	14.8±0.45	Yellow	Buff	Purple	Purple	Green	Gray	36	82
Argomulyo	1.20±0.04	0.30±0.01	14.8±0.45	Yellow	White	Purple	Green	Yellow	Brown	35	80
Argopuro	1.15±0.09	0.38±0.03	17.4±0.55	Yellow	Buff	Green	Green	Green	Gray	32	84
Baluran	1.20±0.03	0.28±0.03	10.80±0.45	Yellow	Buff	Purple	Green	White	Brown	33	80
Daewon	1.25±0.12	0.35±0.03	17.80±0.45	Yellow	Yellow	Green	Green	Yellow	Gray	35	84
Dega 1	1.21±0.04	0.39±0.04	19.80±0.45	Yellow	Brown	Purple	Purple	Purple	Brown	29	69
Deja 1	1.27±0.06	0.22±0.04	11.20±0.45	Yellow	Buff	Purple	Purple	Yellow	Brown	39	89
Demas 1	1.20±0.01	0.20±0.04	8.80±0.45	Yellow	Dark brown	Purple	Green	White	Brown	37	84
Dena 1	1.30±0.06	0.30±0.06	16.00±0.00	Yellow	Brown	Purple	Green	Green	Brown	33	78
Derap 1	1.22±0.10	0.29±0.04	15.60±0.89	Yellow	Buff	Purple	Green	White	Gray	34	76
Dering 1	1.26±0.11	0.22±0.03	8.80±0.45	Yellow	Dark brown	Purple	Purple	White	Brown	35	81
Detap 1	1.22±0.07	0.26±0.07	14.80±0.45	Yellow	Yellow	Purple	Green	White	Gray	35	78
Detam 1	1.15±0.08	0.24±0.05	15.20±0.45	Black	White	Purple	Green	Yellow	Light brown	35	84
Detam 2	1.32±0.11	0.23±0.02	12.20±0.45	Black	Brown	Purple	Green	Yellow	Dark brown	34	82
Devon 1	1.27±0.16	0.24±0.03	14.80±0.45	Yellow	Buff	Purple	Green	White	Brown	34	83
Devon 2	1.22±0.09	0.25±0.06	17.20±0.45	Yellow	Yellow	Purple	Green	White	Gray	33	77
Gepak kuning	1.11±0.09	0.18±0.04	7.20±0.45	Yellow green	Brown	Purple	Green	Green	Brown	28	73
Grobogan	1.25±0.07	0.29±0.06	18.00±0.71	Light yellow	Brown	Purple	Purple	Green	Brown	30	76
Mahameru	1.31±0.04	0.29±0.03	17.40±0.89	Yellow	Buff	Purple	Purple	Green	Gray	36	83
Raja Basa	1.24±0.28	0.30±0.04	14.20±0.45	Yellow	Brown	Purple	Purple	Green	Brown	35	82

Seed size: (a): eccentricity index; (b): flatness index; (c): data accessed from Indonesian Legumes and Tuber Crops Research Institute (2021); (d): days after planting.

small seeds contained higher total phenolic than the large and medium seed sizes.

The total phenol content of 20 soybean varieties was significantly high and positively correlated with total flavonoid content (*r*= 0.63**, *P*<.01). Moreover, the IC_{50} value was significantly high and negatively correlated with the total phenol (*r*= -0.58, *P<.01*). In addition, the IC_{50} value was also significantly and negatively correlated with the total flavonoid content (*r*= -0.46, *P*<.05). This finding is consistent with a previous study that reported that the phenol content is correlated with antioxidant activity, which is expressed by the linear relationship between IC_{50} values and total phenol content (Malenčić *et al.* 2012). It has been reported that phenolic compounds are responsible for the level of antioxidant activity (Arifin *et al.* 2021; Govindaraj *et al.* 2017). As represented in Table 2, the varieties Dering 1, Detam 2, Demas 1 and Devon 2 possessed moderately high total phenol and flavonoid content, contributing to scavenging DPPH free radicals.

Another critical parameter that correlates with phytochemicals in soybean and other legumes is seed colors (Dhungana *et al.* 2021). Varieties Dering 1, Demas 1 and Devon 2 have yellow soybean seed coats, while Detam 2 has black soybean seed coats (Table 1). The comparison between the colors of the seeds and their phytochemicals has been discussed and it has been found that both yellow and black soybeans were predominant in certain phenolic compounds (Pawar *et al.* 2019). Black seed coats soybean had the highest content of total phenols, flavonoids and anthocyanins. Anthocyanins belong to the flavonoid family and accumulate in the epidermis palisade layer of the seed coat (Choi *et al.* 2020), acting as pigments. On the other hand, anthocyanins have not been detected in the cotyledon and seed coats of yellow soybean (Yusnawan 2016). The reason is that the pigmentation of the seed coat is inhibited in yellow soybeans, causing low levels of anthocyanins and proanthocyanidins (Zhu *et al.* 2018). Although yellow soybeans lack anthocyanins, they are abundant in isoflavones which are responsible for the quantity of flavonoid content. Therefore, black and yellow soybeans have their respective roles based on their dominant content as natural ingredients that may play a crucial role in human health.

Table 2: Phytochemical properties.

Varieties	Content				
	Crude protein (g 100 g^{-1})	Oil (g 100 g^{-1})	Total phenol (mg GAE g^{-1})[a]	Total flavonoid (mg QE g^{-1})[b]	IC_{50} (mg mL^{-1})
Anjasmoro	42.27±0.15	18.27±0.67	3.02±0.24	0.22±0.20	1.82±0.08
Argomulyo	39.00±0.36	20.47±0.76	3.69±0.19	0.60±0.16	1.44±0.05
Argopuro	28.03±0.50	24.30±0.98	3.91±0.46	0.39±0.49	1.29±0.13
Baluran	39.10±0.36	22.03±0.35	4.44±0.11	0.41±0.17	1.40±0.19
Daewon	38.50±0.53	14.50±0.53	4.53±0.26	0.21±0.12	1.26±0.02
Dega 1	37.13±0.60	17.03±0.55	3.38±0.23	0.47±0.19	0.67±0.11
Deja 1	39.63±0.15	17.17±0.32	4.60±0.20	0.49±0.37	0.86±0.04
Demas 1	35.97±0.32	19.23±1.24	3.15±0.22	0.37±0.18	0.43±0.02
Dena 1	37.00±0.98	18.33±0.90	3.67±0.07	0.32±0.28	2.45±0.06
Derap 1	38.82±0.39	17.77±0.85	4.54±0.26	0.32±0.22	1.52±0.01
Dering 1	34.37±0.76	16.50±1.68	7.87±0.55	0.54±0.40	0.11±0.09
Detap 1	40.34±0.25	15.55±1.45	6.97±0.17	0.46±0.45	0.96±0.28
Detam 1	44.72±0.75	33.12±0.26	6.82±0.19	0.58±0.40	1.00±0.17
Detam 2	44.19±1.84	14.71±0.37	9.54±0.61	2.56±0.13	0.13±0.10
Devon 1	33.33±2.29	17.15±0.22	7.35±0.36	0.52±0.08	0.76±0.09
Devon 2	37.76±0.24	18.68±0.42	7.80±0.17	0.68±0.38	0.54±0.12
Gepak kuning	35.36±0.55	14.93±0.15	4.79±0.39	0.87±0.33	1.01±0.16
Grobogan	43.80±0.36	18.47±0.31	8.06±0.54	1.89±0.35	0.88±0.15
Mahameru	43.27±0.65	17.47±1.91	8.10±0.25	0.43±0.35	0.97±0.11
Raja Basa	39.37±1.27	19.48±0.40	6.97±0.29	0.78±0.31	1.05±0.19
Min	28.03	14.50	3.02	0.21	0.11
Max	44.72	33.12	9.54	2.56	2.45
Mean	38.60	18.76	5.66	0.66	1.03
Stand. Dev.	4.08	4.15	2.04	0.57	0.55
Coeff. Var.	10.57	22.12	36.07	87.42	53.82

All values are expressed as mean±SD of triplicate experiments; [a]GAE= Gallic acid equivalents; [b]QE= Quercetin equivalents.

Table 3: Correlation value among the characters observed.

	EI [a]	FI [b]	Seed weight	Flowering time	Pod maturity	Crude protein	Oil	Total phenol	Total flavonoid	IC_{50}
EI [a]	1.00									
FI [b]	0.00	1.00								
Seed weight	0.19	0.77**	1.00							
Flowering time	0.40	-0.21	-0.19	1.00						
Pod maturity	0.26	-0.18	-0.21	0.82**	1.00					
Crude protein	0.32	-0.11	0.14	0.23	0.04	1.00				
Oil	-0.47*	0.08	0.13	0.08	0.25	0.08	1.00			
Total phenol	0.43	-0.39	0.02	0.02	0.08	0.37	-0.09	1.00		
Total flavonoid	0.28	-0.27	-0.08	-0.28	-0.12	0.41	-0.17	0.63**	1.00	
IC_{50}	-0.09	0.47*	0.29	-0.04	-0.06	-0.01	0.18	-0.58**	-0.46*	1.00

Seed size: (a): Seed eccentricity index; (b): seed flatness index. **p*-value<.05, ***p*-value<.01.

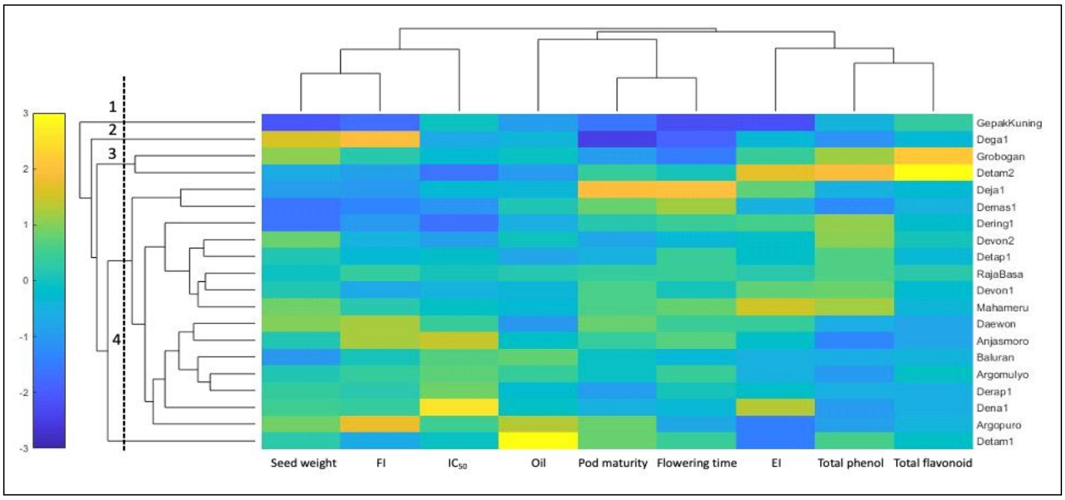

Fig 1: Cluster and heatmap analysis based on significantly correlated variables. FI: seed flatness index, IC_{50}: the 50% inhibitory concentration, EI: seed eccentricity index.

Cluster and heatmap analysis

We used a combined cluster and heatmap analysis to describe the variability among soybean varieties in their seed characteristics, flowering time, pod maturity, seed contents (total phenol, total flavonoid and oil) and antioxidant activity (IC_{50}). In particular, Fig 1 showed the cluster analysis according to the average linkage method.

The color represents the standardized score of the parameters. All varieties were divided into four main groups. Group 1 included only one variety (Gepak Kuning), which has the smallest seed size and fastest flowering time and pod maturity. Group 2 also contained only one variety (Dega 1) that has the highest seed weight and seed size. Group 3 consisted of 2 varieties (Grobogan and Detam 2), which are prominent in the total phenol and flavonoid content and antioxidant activity. Group 4 consisted of the remaining 16 varieties, while the Detam 1 variety was singled out as a specific group. Detam 1 showed the highest oil content with sufficient antioxidant activity, total phenol and total flavonoid content.

CONCLUSION

The 20 soybean varieties showed diversity in morphological characteristics and their phytochemicals. The variability among soybean varieties was also described with cluster analysis, which was divided into four groups. Moreover, the correlation analysis indicated that the antioxidant activity level is correlated with total phenol, total flavonoid and seed size (flatness index). These results could contribute, for example, to enhance soybean breeding programs via morpho-chemical-based selection. In addition, four soybean varieties, namely, Detam 1, Detam 2, Dering 1 and Grobogan, which possess superior traits as an antioxidant, have the potential for the more efficient utilization of soybean varieties to enhance food products.

ACKNOWLEDGEMENT

The authors express their greatest gratitude toward Mrs. Suhartina at the Indonesian Legumes and Tuber Crops Research Institute (ILETRI) for providing the seeds of soybean varieties.

Conflict of interest: None.

REFERENCES

Alghamdi, S.S., Khan, M.A., El-Harty, E.H., Ammar, M.H., Farooq, M. and Migdadi, H.M. (2018). Comparative phytochemical profiling of different soybean [*Glycine max* (L.) Merr] genotypes using GC-MS. Saudi journal of Biological Sciences. 25(1): 15-21.

AOAC. (2005). Microchemical Determination of Nitrogen using Micro Kjeldahl method (12.1.07). Official Methods of Analysis of AOAC International. Vol. I. Agricultural Chemicals, Contaminants, Drugs. Gaithersburg, Maryland, USA: AOAC International.

Arifin, H.A., Hashiguchi, T., Nagahama, K., Hashiguchi, M., Muguerza, M., Sakakibara, Y., Tanaka and H., Akashi, R. (2021). Varietal differences in flavonoid and antioxidant activity in Japanese soybean accessions. Bioscience, Biotechnology and Biochemistry. 85(4): 916-922.

Carrão-Panizzi, M.C., Favoni, S.P.D.G. and Kikuchi, A. (2002). Extraction time for soybean isoflavone determination. Brazilian Archives of Biology and Technology. 45: 515-518.

Choi, Y.M., Yoon, H., Lee, S., Ko, H.C., Shin, M.J., Lee, M.C., Hur, O.S., Ro, N.Y. and Desta, K.T. (2020). Isoflavones, anthocyanins, phenolic content and antioxidant activities of black soybeans [*Glycine max* (L.) Merril] as affected by seed weight. Scientific Reports. 10(1): 1-13.

Dhungana, S.K., Seo, J.H., Kang, B.K., Park, J.H., Kim, J.H., Sung, J.S., Baek, I.Y., Shin, S.O. and Jung, C.S. (2021). Protein, amino acid, oil, fatty acid, sugar, anthocyanin, isoflavone, lutein and antioxidant variations in colored seed-coated soybeans. Plants. 10(9): 1765.

Govindaraj, M., Masilamani, P., Albert, V.A. and Bhaskaran, M. (2017). Role of antioxidant in seed quality-A review. Agricultural Reviews. 38(3): 180-190.

Harsono, A., Harnowo, D., Ginting, E. and Elisabeth, D.A.A. (2021). Soybean in Indonesia: Current status, challenges and opportunities to achieve self-sufficiency. DOI: 10.5772/intechopen.101264.

Kim, M.Y., Lee, Y.J., Jang, G.Y., Hwang, B.Y., Lee, J., and Jeong, H.S. (2021). Isolation, identification and conversion characteristics of anti-inflammatory active saponin in black soybean sprout treated by High Hydrostatic Pressure (HHP). Legume Research: An International Journal. 1: 7.

Kuchlan, M.K., Kuchlan, P., and Husain, S.M. (2017). Effect of foliar application of growth activator, promoter and antioxidant on seed quality of soybean. Legume Research: An International Journal. 40(2): 313-318.

Liu, J.Y., Zhang, Y.W., Han, X., Zuo, J.F., Zhang, Z., Shang, H., Song, Q. and Zhang, Y.M. (2020). An evolutionary population structure model reveals pleiotropic effects of GmPDAT for traits related to seed size and oil content in soybean. Journal of Experimental Botany. 71(22): 6988-7002.

Magalhães, L.M., Santos, F., Segundo, M.A., Reis, S. and Lima, J.L. (2010). Rapid microplate high-throughput methodology for assessment of Folin-ciocalteu reducing capacity. Talanta. 83(2): 441-447.

Malenèiæ, D., Cvejiæ, J. and Miladinoviæ, J. (2012). Polyphenol content and antioxidant properties of colored soybean seeds from Central Europe. Journal of Medicinal Food. 15(1): 89-95.

Pawar, P.V., Naik, R.M., Deshmukh, M.P., Satbhai, R.D. and Mohite, S.G. (2019). Biochemical and molecular marker based screening of seed longevity in soybean [*Glycine max* (L.) Merill]. Legume Research: An International Journal. 42(5): 575-584.

Rehal, J., Beniwal, V., and Gill, B.S. (2019). Physico-chemical, engineering and functional properties of two soybean cultivars. Legume Research: An International Journal. 42(1): 39-44.

Sembiring, E.N., Elya, B. and Sauriasari, R. (2018). Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of *Caesalpinia bonduc* (L.) Roxb. Pharmacognosy Journal. 10(1): 123-127.

Sulistyo, A., Indriani, F.C., Mejaya, M.J., Sugiharto, A.N. and Agranoff, J. (2019). Genetic diversity of Indonesian Soybean [*Glycine max* (L.) Merrill] Germplasm based on Morphological and Microsatellite Markers. IOP Conference Series: Earth and Environmental Science. 293(1): 012006. IOP Publishing.

Yusnawan, E. (2016). The diversity of secondary metabolites in Indonesian soybean genotypes. Biodiversitas. 17(2): 704-710.

Zahratunnisa, N., Elya, B. and Noviani, A. (2017). Inhibition of Alpha-glucosidase and antioxidant test of stem bark extracts of *Garcinia fruticosa* Lauterb. Pharmacognosy Journal. 9 (2): 273-275.

Zhu, Y.L., Zhang, H.S., Zhao, X.S., Xue, H.H., Xue, J. and Sun, Y.H. (2018). Composition, distribution and antioxidant activity of phenolic compounds in 18 soybean cultivars. Journal of AOAC International. 101(2): 520-528.