

Determination of flavonoid and anthocyanin contents, and antioxidant activity of selected Philippine soybean (*Glycine max*) genotypes

¹Esguerra, C.J., ^{2,*}de los Reyes, A.M. and ¹Ocampo, E.T.M.

¹Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines, Los Baños, College, Laguna, Philippines, 4031

²Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines, Los Baños, College, Laguna, Philippines, 4031

Article history:

Received: 6 May 2022

Received in revised form: 9

July 2023

Accepted: 15 March 2023

Available Online: 5 June

2024

Keywords:

Anthocyanin,

Antioxidant activity,

Flavonoid,

Soybean

DOI:

[https://doi.org/10.26656/fr.2017.8\(3\).245](https://doi.org/10.26656/fr.2017.8(3).245)

Abstract

Soybeans contain flavonoids and anthocyanins that are associated with health benefits related to antioxidant activities. In this study, the total flavonoid content (TFC), total anthocyanin content (TAC), and the relative antioxidant activities (AOA) of selected Philippine soybean genotypes were screened in order to select and recommend promising genotypes that may be used for further breeding and varietal development. Using the aluminum trichloride method, the genotypes POP 1-44 (TFC = 0.619 g/100 g), PHL 29272 (TFC = 0.616 g/100 g), and Tiwala 6 (TFC = 0.608 g/100 g) were determined to have the highest calculated TFC values. pH differential and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays distinguished all three black-seeded soybean genotypes PHL 29272 (TAC = 177.010 mg/100 g; AOA = 24.476%), PHL 29502 (TAC = 143.750 mg/100 g; AOA = 25.745%), and PHL 29552 (TAC = 123.572 mg/100 g; AOA = 29.297%) with the highest anthocyanin contents and relative antioxidant activities. Genotype PHL 29272 was consistently found among those with the highest results for the three tests. Significant correlations ($\alpha = 0.05$) were found between flavonoid content and antioxidant activity ($p < 0.0001$), and between anthocyanin content and antioxidant activity ($p < 0.0001$). In conclusion, the study was able to identify genotypes that can be recommended as the most suitable candidates for soybean breeding activities.

1. Introduction

Soybean is deemed one of the world's most valuable crops, known for its substantial protein and oil content (Liu, 1997; Hartman *et al.*, 2011; Gupta, 2016). In fact, around 40-42% of protein and 18-22% of fat may be extracted from soybean, providing a good protein feed source for animals and aquaculture; vegetable oil and protein source for humans; and a resource for industrial products (Wynstra, 1986; Gaonkar and Rosentrater, 2019).

Beyond its notability as a quality source of plant-based protein and oil, soybean has also gained interest for its beneficial health effects in humans. Much of this interest is attributed to the favorable contribution of soyfoods in reducing the risks and/or treatment of diseases (Liu, 1997; Messina, 2016), such as cardiovascular disease, diabetes, osteoporosis and cancer (Messina, 1995; Omoni and Aluko, 2005; Weaver *et al.*, 2012). A number of publications have associated the lowered risk or improvement in examined markers or

health profiles with soy isoflavones (Onozawa *et al.*, 1998; Sarkar and Li, 2003; Allen *et al.*, 2007; Shu *et al.*, 2009; Mahmoud *et al.*, 2014; Sathyapalan *et al.*, 2018; Sahin *et al.*, 2019). These isoflavones are polyphenolic compounds that belong to a group of ubiquitous plant secondary metabolites called flavonoids.

Flavonoids have a general structure composed of three rings, where the two phenyl rings are connected by a heterocyclic three-carbon ring. Various chemical reactions such as methylation, hydroxylation, and substitution give rise to the different classes of flavonoids, namely: the flavones, flavonols, flavanones, isoflavonoids, anthocyanins, and others. Currently, over 4,000 types of flavonoids have been identified to occur naturally (Iwashina, 2000). Even though flavonoids occur in different plant groups, isoflavones are largely produced by the Leguminosae plant family (Veitch, 2007). Soybean stands out with having the highest isoflavone quantity of up to 3 mg/g dry weight (Liu, 1997; Rostagno *et al.*, 2004). According to a study by

*Corresponding author.

Email: amdelosreyes4@up.edu.ph

Han *et al.* (2009), isoflavones have the capability to interact with nucleotides, influence intercellular redox status, and play a role in intracellular signaling among several specific proteins. Isoflavones have been reported to have both antioxidant and phytoestrogen properties that allow them to prevent and contribute to treatment of associated chronic diseases (Yao *et al.*, 2004; Wang *et al.*, 2013).

Another prominent sub-classification of flavonoids is the anthocyanins. Generally, anthocyanins are water-soluble colored pigments that are usually stored within plant vacuoles. Although several types of anthocyanin have been identified, only cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin are considered to be the major types (Tanaka *et al.*, 2008). These pigments are partly responsible for the antioxidant properties of soybeans, which have been proposed to arise from the capability of anthocyanins to scavenge O_2^- generated by chloroplasts (Neill and Gould, 2003; Kwon *et al.*, 2007).

This study was done as a pre-breeding procedure for the improvement of Philippine soybean varieties. Since local varieties and newly developed breeding lines were used, the study will provide insight into the relative antioxidant activity, total flavonoid and anthocyanin content of genotypes that are currently being utilized for breeding (lines and accessions) and soybean production (varieties) in the country.

The objectives of this study were (1) to screen soybean genotypes for total flavonoid and anthocyanin content, and relative antioxidant activity; (2) to determine the level of correlation between flavonoid content and antioxidant activity, as well as that of anthocyanin content and antioxidant activity; and finally, (3) to identify genotypes that have promising levels of the aforementioned compounds for further breeding studies.

2. Materials and methods

2.1 Plant materials

Genotypes from the National Plant Genetic Resource Laboratory (NPGRL) and local varieties were planted in October 2018 while the rest of the genotypes were planted in December 2018 at the Legumes Section of the Institute of Plant Breeding (IPB), University of the Philippines, Los Baños, Laguna. Genotypes from NPGRL included three black-seeded varieties while eight were yellow-seeded. One farmer's variety and three established local varieties developed by IPB were also used, representing the current varieties being cultivated in the country. Lastly, 63 fifth-generation stable lines developed by crossing commercial variety Tiwala 10

with IPB SY-96-27-30 were also included in the screening (Table 1). Although the genotypes had different dates of planting and harvesting, the plants were subjected to the same cultural management practices.

2.2 Sample preparations

2.2.1 Preparation of soybean seeds

Approximately 10 g of seeds for each soybean genotype were weighed and dried at 40°C for 24 hrs; after which, the dried seeds were ground and stored in a desiccator until subsequent extractions.

2.2.2 Preparation of the methanolic extract

In the determination of total flavonoid content and relative scavenging activity (antioxidant capacity), methanolic extracts of the samples were used. The methanolic extract was prepared by mixing 100 mg of the sample with 5.0 mL of 50% methanol. The resulting mixture was then vortexed for 2 min and then centrifuged for 5 min at 3,000 rpm before the supernatant was collected and transferred into a vial. A second round of extraction was done on the remaining centrifugate using 5.0 mL of 50% methanol. Again, the resulting mixture was vortexed for 2 min and then subjected to a 5-min centrifugation at 3,000 rpm. The supernatant was collected and pooled together with that of the first extraction. Extracts were stored in the refrigerator until analysis.

2.2.3 Preparation of anthocyanin extracts

An 80:20 methanol/water extraction buffer acidified with 1% acetic acid was used for extraction in the assessment of total anthocyanin content. Extracts were prepared by the addition of 10 mL extraction buffer to 1.0 g of the samples. The mixtures were placed in a 40°C water bath for 2 hrs before filtering and storing in the refrigerator until analysis.

2.3 Measurement of total flavonoid content

2.3.1 Preparation of standard curve

A modified protocol by Zhishen *et al.* (1999) was used in the measurement of the flavonoid content. Catechin, a commonly used standard for flavonoids, was used in the preparation of the standard curve. Five milligrams of catechin were initially dissolved in 5 mL absolute methanol and a final volume of 50 mL was achieved by adding distilled water. Six tubes with different concentrations of catechin were prepared as shown in Table 2. In each tube, the specific volumes of catechin and water were the first ones to be mixed together. After which, 300 μ L of 5.0% $NaNO_2$ was added to the solution. The solution was mixed and left to stand for 5 mins before the addition of 300 μ L 10%

Table 1. Soybean genotypes used in screening.

Code	Genotype	Seed Color	Source	Code	Genotype	Seed Color	Source
1	IPB SY-96-27-30	Yellow	NPGRL	40	POP 1-19	Yellow	IPB
2	PHL 29008	Yellow	NPGRL	41	POP 1-17	Yellow	IPB
3	PHL 29389	Yellow	NPGRL	42	POP 1-20	Yellow	IPB
4	PHL 29527	Yellow	NPGRL	43	POP 1-23	Yellow	IPB
5	PHL 29528	Yellow	NPGRL	44	POP 1-24	Yellow	IPB
6	PHL 29826	Yellow	NPGRL	45	POP 5-5-1	Yellow	IPB
7	PHL 28970	Yellow	NPGRL	46	POP 1-25	Yellow	IPB
8	PHL 6923	Yellow	NPGRL	47	POP 1-26	Yellow	IPB
9	PHL 29272	Black	NPGRL	48	POP 1-27	Yellow	IPB
10	PHL 29502	Black	NPGRL	49	POP 1-29	Yellow	IPB
11	PHL 29552	Black	NPGRL	50	POP 5-7-1	Yellow	IPB
12	Manchuria	Yellow	Farmer's Variety	51	POP 1-28	Yellow	IPB
13	Tiwala 6	Yellow	IPB Variety	52	POP 1-28-1	Yellow	IPB
14	Tiwala 8	Yellow	IPB Variety	53	POP 1-29-1	Yellow	IPB
15	Tiwala 10	Yellow	IPB Variety	54	POP 1-27	Yellow	IPB
16	POP 1-3	Yellow	IPB	55	POP 1-21	Yellow	IPB
17	POP 5-2-1	Yellow	IPB	56	POP 1-34	Yellow	IPB
18	POP 1-2	Yellow	IPB	57	POP 1-32	Yellow	IPB
19	POP 1-1	Yellow	IPB	58	POP 5-6-1	Yellow	IPB
20	POP 1	Yellow	IPB	59	POP 1-33	Yellow	IPB
21	POP 1-53	Yellow	IPB	60	POP 1-30	Yellow	IPB
22	POP 1-4	Yellow	IPB	61	POP 1-35	Yellow	IPB
23	POP 1-10	Yellow	IPB	62	POP 1-37-1	Yellow	IPB
24	POP 1-9	Yellow	IPB	63	POP 1-36	Yellow	IPB
25	POP 5-3	Yellow	IPB	64	POP 5-8-1	Yellow	IPB
26	POP 1-7	Yellow	IPB	65	POP 1-37-1	Yellow	IPB
27	POP 5-4-1	Yellow	IPB	66	POP 1-34-1	Yellow	IPB
28	POP 1-5	Yellow	IPB	67	POP 1-42	Yellow	IPB
29	POP 1-8	Yellow	IPB	68	POP 1-43	Yellow	IPB
30	POP 1-11	Yellow	IPB	69	POP 1-41	Yellow	IPB
31	POP 1-12	Yellow	IPB	70	POP 1-39	Yellow	IPB
32	POP 5-4	Yellow	IPB	71	POP 1-38	Yellow	IPB
33	POP 1-13	Yellow	IPB	72	POP 5-7-1	Yellow	IPB
34	POP 1-16	Yellow	IPB	73	POP 1-49	Yellow	IPB
35	POP 1-15	Yellow	IPB	74	POP 1-44	Yellow	IPB
36	POP 5-6	Yellow	IPB	75	POP 1-45	Yellow	IPB
37	POP 1-17-1	Yellow	IPB	76	POP 1-52	Yellow	IPB
38	POP 5-5	Yellow	IPB	77	POP 5-8	Yellow	IPB
39	POP 1-16-1	Yellow	IPB	78	POP 1-45-1	Yellow	IPB

Table 2. Summary of the reagents used in the preparation of the standard curve.

Tube	Catechin	dH ₂ O	NaNO ₂	AlCl ₃	NaOH
1	0 µL	3.0 mL	300 µL	300 µL	1.0 mL
2	200 µL	2.8 mL	300 µL	300 µL	1.0 mL
3	400 µL	2.6 mL	300 µL	300 µL	1.0 mL
4	600 µL	2.4 mL	300 µL	300 µL	1.0 mL
5	800 µL	2.2 mL	300 µL	300 µL	1.0 mL
6	1000 µL	2.0 mL	300 µL	300 µL	1.0 mL

AlCl₃. A minute after mixing the solution, 1.0 mL of 1.0 N NaOH was added. The final solution was mixed before its absorbance was read at 510 nm using a Shimadzu UV-1280 UV-VIS Spectrophotometer.

2.3.2 Assessment of total flavonoid content of the samples

For each sample, 0.50 mL of the methanolic extract was mixed with 2.0 mL of distilled water. Similar to the preparation of the standard curve, a 5-min resting period was allotted after the addition of 300 µL 5.0% NaNO₂. Afterwards, 300 µL of 10% AlCl₃ was also added into the

solution, which was mixed and left to stand for 1 min before the addition of 1.0 mL 1.0 N NaOH. The absorbance of the final solution was read at 510 nm within 20-30 min of preparation using a Shimadzu UV-1280 UV-VIS Spectrophotometer (Japan). The assay was done in triplicates per sample.

TFC of the samples were computed through the generation of a linear equation $y = mx + b$ from the standard curve; where y is the absorbance, m is the slope of the line, and b is the y-intercept. The absorbance of the samples was corrected by subtracting the y-intercept from the obtained values of the samples. After this, the amount of flavonoids per sample (grams of flavonoids per 100 g of sample) was computed through the following equation:

$$\text{Total Flavonoid Content} = \frac{\text{absorbance of sample}}{\text{slope from standard curve}} \times \frac{100}{\text{DF}}$$

where DF is the dilution factor, computed through the following equation:

$$\text{DF} = \frac{\text{amount of sample (g)}}{\text{volume of extracting solvent (mL)}} \times \text{aliquot of methanolic extract (mL)}$$

2.4 Measurement of total antioxidant activity

2.4.1 Preparation of the DPPH reagent

The study used the DPPH assay (Malenčić et al., 2008; Yang et al., 2014) with modifications in the measurement of total AOA. Initially, 4.0 mg of DPPH powder was dissolved in a small amount of absolute methanol and additional methanol was added to a final volume of 100 mL. The solution was kept in a container covered with foil to prevent degradation of the reagent.

2.4.2 Assessment of average relative scavenging activity

The antioxidant activity per sample was tested using three technical replicates. For each replicate, 100 μ L of the methanolic extract was mixed with 2.9 mL of the DPPH solution. The final solution was mixed before being incubated in the dark for 30 mins at 30°C. The absorbance of the samples was read at 517 nm with a Shimadzu UV-1280 UV-VIS Spectrophotometer right after incubation.

The relative scavenging activity was computed based on the following formula:

$$\% \text{ Relative Scavenging Activity} = \frac{\text{DPPH} - \text{A}_{517}}{\text{DPPH}} \times 100$$

where DPPH is the absorbance of the DPPH solution at 517 nm and A_{517} is the absorbance of the sample at 517 nm.

2.5 Measurement of total anthocyanin content

2.5.1 pH differential method

The pH differential method described by Lee et al. (2005) was used in the assessment of TAC. Similar to the previous assays, the procedure was done in triplicates for each sample. For every replicate, two tubes containing 1.0 mL of the extract were prepared. In the first tube, the extract was diluted with 4.0 mL of potassium chloride (KCl), pH 1.0, buffer; while the extract in the second tube was mixed with sodium acetate ($\text{CH}_3\text{CO}_2\text{Na} \cdot 2\text{H}_2\text{O}$), pH 4.5, buffer. The absorbances at 520 nm and 700 nm were read for both the pH 1.0 and pH 4.5 solutions.

2.5.2 Assessment of total anthocyanin content

Before converting to cyanidin-3 glucoside equivalents (CyE) in mg per 100 g of sample, the anthocyanin content was initially computed in terms of mg/L using the following formula:

$$\text{Total Anthocyanin} = \frac{(A) \times (MW) \times (DF) \times (10^3)}{\epsilon \times l}$$

where $A = [\text{pH } 1.0 (A_{520} - A_{700}) - \text{pH } 4.5 (A_{520} - A_{700})]$; $MW = 449.2$ g/mol, the molecular weight of the cyanidin-3-glucoside; DF is the dilution factor established during the sample preparation; 10^3 is the conversion from gram to milligram; $\epsilon = 26,900$ L/mol-cm, the molar extinction coefficient of cyanidin-3-glucoside; and l = path length in centimeters (Lee et al., 2005).

2.6 Statistical analysis

SAS version 9.1 was used for the analysis of variance (ANOVA) of flavonoid content, anthocyanin content, and antioxidant activity (SAS Institute Inc., 2004). Furthermore, the same software was used for a post-hoc test – Tukey's Honest Significant Difference (HSD) – on variables that showed significant differences based on the ANOVA results, and for correlation analysis of flavonoid and anthocyanin contents with antioxidant activity.

3. Results and discussion

3.1 Total flavonoid content

The mean total flavonoid content (TFC) of the 78 soybean genotypes was 0.2098 g/100 g obtained from a wide range of TFC values of 0.0240 g/100 g to 0.6190 g/100 g. Comparison of the mean TFC between black-seeded and yellow-seeded genotypes showed that the former had a higher (0.600 g/100 g) content than the latter (0.194 g/100 g). The mean TFC content of the black-seeded genotypes was higher than the published result of 0.343 g/100 g obtained by Xu and Chang

(2008a). Similarly, the current average flavonoid content of the yellow soybeans observed was comparably higher than the reported values of 0.068 g/100 g (Xu and Chang, 2008a), 0.041 g/100 g (Lee *et al.*, 2011), and 0.091 g/100 g (Wang *et al.*, 2008) for yellow soybeans from other studies.

The distribution of samples among the different levels of flavonoid contents in Figure 1A showed that there was an evident variation in the flavonoid contents among the genotypes. Thirty-three (42.31%) of the samples had flavonoid contents that fell within the range of 0.1 to 0.2 g/100 g. It may also be seen in Figure 1B that within that range, there was a scattered distribution in mean TFC. Thus, analysis of variance was applied to further determine whether there are significant differences among the flavonoid contents.

At $\alpha = 0.05$, significant differences ($P < 0.0001$) in TFC were observed among the genotypes. Tukey's test (Table 3) further showed the classification of samples into 15 groups although many of the genotypes belonged to several groups at a time (e.g., two to 11) indicating

non-significant differences. Genotypes POP 1-44 and PHL 29272 had the highest mean TFC of 0.619 g/100 g and 0.616 g/100 g, respectively. The two aforementioned genotypes have significantly higher total flavonoid content in comparison with the rest of the samples (Table 3); nevertheless, their flavonoid contents are still comparable to Tiwala 6 (0.608 g/100 g), PHL 29552 (0.606 g/100 g), PHL 29502 (0.578 g/100 g), POP 1-49 (0.528 g/100 g), and POP 1-37-1 (0.451 g/100 g). On the other hand, POP 1-25 had the lowest mean TFC (0.024 g/100 g).

The variation in flavonoid content may have arisen from genetic differences in the samples. Apart from this, environmental differences and circumstances, such as nutrient availability, temperature, drought, carbon dioxide availability, crop management, and even dates and seasons of sowing during the growing periods of the plants may also have effects on the flavonoid content (Aussenac *et al.*, 1998; Caldwell *et al.*, 2005; Mitchell *et al.*, 2007; Lillo *et al.*, 2008), which may have contributed to the observed variation in TFC.

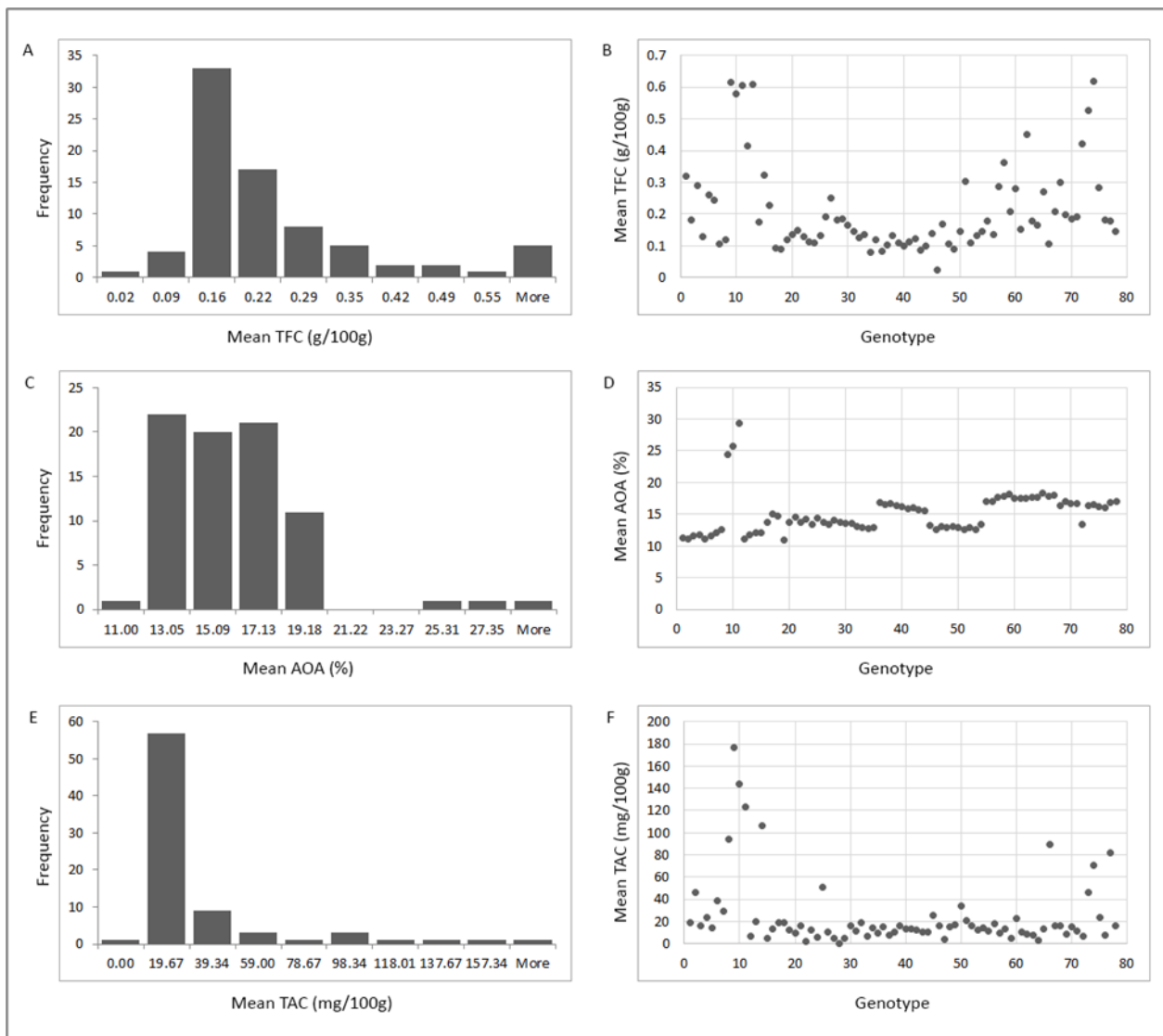


Figure 1. Respective frequency distribution and scatter plot for mean TFC (1A, 1B), mean AOA (1C, 1D), and mean TAC (1E, 1F) of the 78 soybean genotypes.

Table 3. Summary table of Tukey's Honest Significance Difference Test for total flavonoid content.

Genotype	Mean	Tukey Grouping	Genotype	Mean	Tukey Grouping
74	0.61900	A	64	0.16433	G, H, I, J, K, L, M, N, O, P
9	0.61600	A	61	0.15133	G, H, I, J, K, L, M, N, O, P
13	0.60767	A, B	21	0.15000	G, H, I, J, K, L, M, N, O, P
11	0.60633	A, B	31	0.14633	G, H, I, J, K, L, M, N, O, P
10	0.57767	A, B	50	0.14600	G, H, I, J, K, L, M, N, O, P
73	0.52767	A, B, C	54	0.14500	G, H, I, J, K, L, M, N, O, P
62	0.45067	A, B, C, D	78	0.14433	G, H, I, J, K, L, M, N, O, P
72	0.42067	B, C, D, E	45	0.13800	G, H, I, J, K, L, M, N, O, P
12	0.41567	B, C, D, E	33	0.13700	G, H, I, J, K, L, M, N, O, P
58	0.36167	C, D, E, F	20	0.13667	G, H, I, J, K, L, M, N, O, P
15	0.32333	D, E, F, G	56	0.13433	G, H, I, J, K, L, M, N, O, P
1	0.31867	D, E, F, G, H	38	0.13233	G, H, I, J, K, L, M, N, O, P
51	0.30167	D, E, F, G, H, I	53	0.13233	G, H, I, J, K, L, M, N, O, P
68	0.29967	D, E, F, G, H, I, J	25	0.13133	G, H, I, J, K, L, M, N, O, P
3	0.29100	D, E, F, G, H, I, J, K	4	0.12933	H, I, J, K, L, M, N, O, P
57	0.28700	D, E, F, G, H, I, J, K, L	22	0.12933	H, I, J, K, L, M, N, O, P
75	0.28400	D, E, F, G, H, I, J, K, L, M	32	0.12533	H, I, J, K, L, M, N, O, P
60	0.28100	D, E, F, G, H, I, J, K, L, M, N	42	0.12200	I, J, K, L, M, N, O, P
65	0.27000	D, E, F, G, H, I, J, K, L, M, N, O	8	0.11800	I, J, K, L, M, N, O, P
5	0.25867	D, E, F, G, H, I, J, K, L, M, N, O	19	0.11800	I, J, K, L, M, N, O, P
27	0.25167	E, F, G, H, I, J, K, L, M, N, O	35	0.11800	I, J, K, L, M, N, O, P
6	0.24500	E, F, G, H, I, J, K, L, M, N, O	23	0.11400	I, J, K, L, M, N, O, P
16	0.22933	E, F, G, H, I, J, K, L, M, N, O	41	0.11200	I, J, K, L, M, N, O, P
67	0.20967	F, G, H, I, J, K, L, M, N, O, P	24	0.11033	I, J, K, L, M, N, O, P
59	0.20900	F, G, H, I, J, K, L, M, N, O, P	39	0.11000	I, J, K, L, M, N, O, P
69	0.19800	F, G, H, I, J, K, L, M, N, O, P	52	0.10800	I, J, K, L, M, N, O, P
26	0.18967	F, G, H, I, J, K, L, M, N, O, P	48	0.10700	J, K, L, M, N, O, P
71	0.18967	F, G, H, I, J, K, L, M, N, O, P	66	0.10667	J, K, L, M, N, O, P
29	0.18600	F, G, H, I, J, K, L, M, N, O, P	7	0.10533	K, L, M, N, O, P
70	0.18400	F, G, H, I, J, K, L, M, N, O, P	37	0.10200	K, L, M, N, O, P
2	0.18267	F, G, H, I, J, K, L, M, N, O, P	44	0.10000	K, L, M, N, O, P
28	0.18200	F, G, H, I, J, K, L, M, N, O, P	40	0.09933	K, L, M, N, O, P
76	0.18100	F, G, H, I, J, K, L, M, N, O, P	17	0.09333	L, M, N, O, P
77	0.17800	F, G, H, I, J, K, L, M, N, O, P	49	0.09133	M, N, O, P
55	0.17700	F, G, H, I, J, K, L, M, N, O, P	18	0.09000	N, O, P
63	0.17700	F, G, H, I, J, K, L, M, N, O, P	43	0.08567	O, P
14	0.17633	F, G, H, I, J, K, L, M, N, O, P	36	0.08167	O, P
47	0.16800	F, G, H, I, J, K, L, M, N, O, P	34	0.08033	O, P
30	0.16533	G, H, I, J, K, L, M, N, O, P	46	0.02400	P

3.2 Total antioxidant activity

Obtained relative antioxidant activity values range from 11.034% to 29.397%. Among the black soybeans alone, the average antioxidant activity was 26.539%, while an average of 15.114% was obtained among the yellow soybeans. The mean antioxidant activities of all 78 genotypes was 15.553%. These findings are relatively low compared to those tested by Malenčić *et al.* (2008), in which the activities ranged from 21.9% to 52.7%; however, the deviation in the results obtained may have come from the amount of sample aliquot and DPPH solution used in the procedure. Unlike the protocol used in this experiment, where 0.1 mL aliquot was allowed to react with 2.9 mL DPPH, Malenčić *et al.* (2008) mixed

2.0 mL of the extract with only 1.0 mL of DPPH. The differences in the results due to the protocols used may be attested by the closer values obtained for the yellow genotypes in this study (15.114%) and in the 2014 study by Yang *et al.* (16.7%), as the amount of aliquot and DPPH solution used was more similar to the amounts used in this experiment.

Although there may have been deviations brought about by the protocols, the relatively low antioxidant activity of the current genotypes studied is still evident when compared to previously published values. For instance, the average value for the black soybean accessions (26.539%) was still significantly lower than the value (93.66%) obtained by Yang *et al.* (2014).

The bulk of samples have antioxidant activity that fell within 12-20% (Figure 1C; Figure 1D). The three black soybean genotypes PHL 29552 (29.397%), PHL 29502 (25.745%), and PHL 29272 (24.476%), were identified as outliers with the highest antioxidant activities. Similar to the results of the total flavonoid content, the ANOVA for total antioxidant activity also showed significant differences among the total antioxidant activities ($P < 0.0001$ at $\alpha = 0.05$). In the post hoc analysis, 27 different Tukey groups were formed (Table 4); the mean AOA of the three black genotypes were found significantly different from each other.

Unlike the black genotypes, the relative AOA of the top five genotypes of the yellow genotypes were not

significantly different from each other (Table 4) POP 1-2, POP 1-53, POP 1-4, POP 1 and POP 1-1 had an average scavenging activity ranging from 21.197% to 20.653%.

On the other hand, the genotypes that had the lowest antioxidant activity are PHL 29528 (11.085%); Manchuria (11.059%), and PHL 29008 (11.034%). It is observable that the genotypes that yielded the lowest in terms of antioxidant activity are the local accessions. Out of the fifteen lowest-scoring genotypes, twelve were local accessions. Furthermore, the local accessions also comprised the bottom 10 genotypes (Table 4). These results point out that the antioxidant activity of the current soybeans being cultivated and produced is low.

Table 4. Summary table of Tukey's Honest Significance Difference Test for total antioxidant activity.

Genotype	Mean	Tukey Grouping	Genotype	Mean	Tukey Grouping
11	29.3967	A	44	15.5637	L, M, N
10	25.7450	B	17	15.0160	M, N, O
9	24.4757	C	25	14.4660	N, O, P
18	21.1973	D	23	14.2073	O, P, Q
21	21.0080	D	28	14.0007	O, P, Q, R
22	20.8187	D	26	13.7840	P, Q, R, S
20	20.7713	D	29	13.6973	P, Q, R, S, T
19	20.6530	D	16	13.6890	P, Q, R, S, T, U
65	18.3610	E	30	13.5240	P, Q, R, S, T, U, V
59	18.2167	E	31	13.5240	P, Q, R, S, T, U, V
67	17.9527	E, F	72	13.4553	P, Q, R, S, T, U, V
58	17.8803	E, F	27	13.4373	P, Q, R, S, T, U, V
66	17.8563	E, F	24	13.3657	P, Q, R, S, T, U, V
57	17.7360	E, F, G	54	13.3333	Q, R, S, T, U, V
63	17.7123	E, F, G	45	13.1897	Q, R, S, T, U, V, W
64	17.6880	E, F, G	32	13.1123	Q, R, S, T, U, V, W, X
60	17.5920	E, F, G, H	47	13.0937	R, S, T, U, V, W, X
61	17.5197	E, F, G, H	49	13.0063	R, S, T, U, V, W, X
62	17.4717	E, F, G, H	33	12.9823	R, S, T, U, V, W, X
55	17.0870	F, G, H, I	48	12.9740	R, S, T, U, V, W, X
78	17.0393	F, G, H, I	50	12.9737	R, S, T, U, V, W, X
56	17.0390	F, G, H, I	52	12.9650	R, S, T, U, V, W, X
69	17.0153	F, G, H, I	35	12.9607	R, S, T, U, V, W, X
36	16.9067	F, G, H, I, J	34	12.7657	S, T, U, V, W, X, Y
77	16.8710	F, G, H, I, J	8	12.6650	T, U, V, W, X, Y
70	16.6787	G, H, I, J, K	46	12.6620	T, U, V, W, X, Y
38	16.6667	G, H, I, J, K	53	12.5970	U, V, W, X, Y
71	16.6547	G, H, I, J, K, L	51	12.5560	V, W, X, Y
37	16.5470	H, I, J, K, L	7	12.1730	V, W, X, Y, Z
74	16.5107	H, I, J, K, L	14	12.1213	W, X, Y, Z, A-2
39	16.3550	I, J, K, L	15	12.0173	X, Y, Z, A-2
68	16.3423	I, J, K, L	13	11.7327	Y, Z, A-2
73	16.3420	I, J, K, L	4	11.7067	Y, Z, A-2
75	16.2463	I, J, K, L	6	11.6810	Y, Z, A-2
40	16.2110	I, J, K, L	3	11.6807	Y, Z, A-2
76	16.0057	I, J, K, L, M	1	11.2407	Z, A-2
42	15.9953	I, J, K, L, M	5	11.0850	Z, A-2
41	15.8273	J, K, L, M	12	11.0593	A-2
43	15.6593	K, L, M	2	11.0337	A-2

Nevertheless, the higher scavenging activities of the genotypes being developed suggest that the antioxidant levels of soybeans may still be increased through breeding.

3.3 Total anthocyanin content

The total anthocyanin contents (TAC) among the soybean genotypes ranged from 0.0 mg/100 g to 177.010 mg/100 g; yielding an overall average of 24.906 mg/100 g. The mean anthocyanin content of the current genotypes is relatively higher compared to previously published studies. For instance, 19.978 mg/100 g CyE was obtained for the yellow genotypes, while no detection was reported for the yellow soybeans tested by Xu and Chang (2008a and 2008b). Similarly, the anthocyanin content of the Philippine black genotypes (148.111 mg/100 g) was higher than that of the black soybeans studied by Xu and Chang (2008a and 2008b) where anthocyanin contents of 36.58 mg/100 g, 4.36 mg/100 g, and 2.63 mg/100 g were detected. The large difference in anthocyanin contents of the soybeans tested by Xu and Chang (2008a and 2008b) in the two separate studies may indicate that the anthocyanin contents of soybeans vary widely among different cultivars and environmental conditions.

A study by Kim *et al.* (2012) found that the planting dates, which may be associated with different temperature and precipitation rates, significantly affected the anthocyanin content quantified in black soybeans. In another crop, anthocyanin content in grapes was significantly changed by the exposure to different temperatures at different growth stages (Yamane *et al.*, 2006), consistently supporting that besides genotype, environmental factors can also influence anthocyanin expression.

Figure 1E illustrates that the majority of the soybean samples fell within the range of 20 mg/100 g TAC and below. Several outliers are observed in Figure 1F; application of the outliers' formula determined 12 genotypes with TAC beyond the upper extreme value of 35.49 mg/100g. Among these, the three black soybean genotypes had the highest anthocyanin contents, with PHL 29272, PHL 29502, and PHL 29552 having 177.01 mg/100 g, 143.75 mg/100 g, and 123.57 mg/100 g, respectively. Tiwala 8 (106.038 mg/100 g), PHL 6923 (93.65 mg/100 g), and POP 1-42 (89.76 mg/100 g) were among the yellow genotypes with the highest TAC. In contrast, no anthocyanin was detected in POP 1-5 similar to the findings of Xu and Chang (2008a and 2008b) in yellow soybeans.

ANOVA procedure showed that there were significant differences ($P < 0.0001$) among the TAC of

the 78 samples while Tukey's test (Table 5) revealed 33 different groups. Although more groups were formed in Tukey's HSD for anthocyanin compared to that of flavonoids and antioxidant activity, there was a higher occurrence of overlap of non-significant differences in content among multiple genotypes in the anthocyanin content, compared with that of flavonoids. In fact, a handful of genotypes belonged to as many as 12 different groupings in the Tukey test. Furthermore, there was an observable regression of anthocyanin content among the genotypes; from the ninth-highest anthocyanin content (POP 5-3 with 50.79 mg/100 g) down to those with the least content. A possible explanation for the trend in anthocyanin content is the fact that the 5th-generation stable lines used in the experiment descended from the same parental cross (Tiwala 10 × IPB SY-96-27-30).

3.4 Correlation of flavonoid and anthocyanin content with antioxidant activity

Although there was no direct quantification of the correlation between flavonoids and antioxidant activity, the capability of flavonoids to act as antioxidants has been tested both *in vitro* and *in vivo*. According to a review by Pietta (2000), flavonoids exhibit antioxidant properties by inhibiting the formation of Reactive Oxygen Species (ROS) and scavenging them. Additionally, a study by Burda and Oleszek (2001) demonstrated the high antioxidant activities of certain flavonols that have free hydroxyl groups at the C-3 position. These data suggest that flavonoids are correlated with antioxidant activities, as flavonoids themselves may be the source of antioxidant activities. Current findings showed, and therefore supported, a significant correlation (p -value < 0.0001) between the flavonoid content and antioxidant activity (Table 6).

Several studies in different berries have confirmed the high correlation between antioxidant activity and the anthocyanin content (Kalt *et al.*, 1999; Wang and Lin, 2000; Orak, 2007). Furthermore, tests on black soybeans by Xu *et al.* (2007) showed that the maximum significant correlation existed between anthocyanin and antioxidant activities. In this study, correlation analysis showed that there was a significant correlation between anthocyanin content and antioxidant activity (p -value < 0.0001) in the sampled soybeans (Table 6).

While both flavonoid and anthocyanin contents were concluded to be significantly correlated with antioxidant activity, Pearson correlation coefficients (Table 6) showed that anthocyanin content (0.417) was more highly correlated with antioxidant activity in comparison with flavonoid content (0.294).

Table 5. Summary table of Tukey's Honest Significance Difference Test for total anthocyanin content.

Genotype	Mean	Tukey Grouping	Genotype	Mean	Tukey Grouping
9	177.010	A	5	14.332	O, P, Q, R, S, T, U, V, W, X
10	143.750	B	54	14.193	O, P, Q, R, S, T, U, V, W, X
11	123.572	C	16	13.777	O, P, Q, R, S, T, U, V, W, X, Y
14	106.038	D	40	13.775	O, P, Q, R, S, T, U, V, W, X, Y
8	93.653	E	58	13.638	O, P, Q, R, S, T, U, V, W, X, Y
67	89.757	E	66	13.497	O, P, Q, R, S, T, U, V, W, X, Y
78	82.242	F	41	12.942	P, Q, R, S, T, U, V, W, X, Y, Z
75	70.553	G	53	12.830	P, Q, R, S, T, U, V, W, X, Y, Z
25	50.793	H	19	12.523	Q, R, S, T, U, V, W, X, Y, Z, A-2
74	46.478	H	23	12.107	Q, R, S, T, U, V, W, X, Y, Z, A-2, B-2
2	46.477	H	42	12.107	Q, R, S, T, U, V, W, X, Y, Z, A-2, B-2
6	38.965	I	72	11.828	R, S, T, U, V, W, X, Y, Z, A-2, B-2, C-2
50	33.817	I, J	55	11.272	S, T, U, V, W, X, Y, Z, A-2, B-2, C-2, D-2
7	29.642	J, K	31	11.132	S, T, U, V, W, X, Y, Z, A-2, B-2, C-2, D-2
45	25.188	K, L	43	10.993	S, T, U, V, W, X, Y, Z, A-2, B-2, C-2, D-2
4	23.518	K, L, M	26	10.993	S, T, U, V, W, X, Y, Z, A-2, B-2, C-2, D-2
76	23.378	K, L, M	44	10.853	S, T, U, V, W, X, Y, Z, A-2, B-2, C-2, D-2
61	22.822	K, L, M, N	62	10.575	S, T, U, V, W, X, Y, Z, A-2, B-2, C-2, D-2
51	20.597	L, M, N, O	38	10.437	T, U, V, W, X, Y, Z, A-2, B-2, C-2, D-2
13	20.038	L, M, N, O, P	35	9.742	T, U, V, W, X, Y, Z, A-2, B-2, C-2, D-2, E-2
1	19.343	L, M, N, O, P, Q	20	9.740	T, U, V, W, X, Y, Z, A-2, B-2, C-2, D-2, E-2
18	19.065	L, M, N, O, P, Q, R	57	9.600	T, U, V, W, X, Y, Z, A-2, B-2, C-2, D-2, E-2
32	18.787	L, M, N, O, P, Q, R	70	8.767	U, V, W, X, Y, Z, A-2, B-2, C-2, D-2, E-2, F-2
17	18.785	L, M, N, O, P, Q, R	63	8.627	V, W, X, Y, Z, A-2, B-2, C-2, D-2, E-2, F-2
56	17.812	M, N, O, P, Q, R, S	77	7.792	W, X, Y, Z, A-2, B-2, C-2, D-2, E-2, F-2
49	16.838	M, N, O, P, Q, R, S, T	37	7.375	X, Y, Z, A-2, B-2, C-2, D-2, E-2, F-2
39	16.560	M, N, O, P, Q, R, S, T	64	7.375	X, Y, Z, A-2, B-2, C-2, D-2, E-2, F-2
69	16.422	M, N, O, P, Q, R, S, T	73	7.097	X, Y, Z, A-2, B-2, C-2, D-2, E-2, F-2, G-2
30	16.420	M, N, O, P, Q, R, S, T	33	6.678	Y, Z, A-2, B-2, C-2, D-2, E-2, F-2, G-2
52	16.282	M, N, O, P, Q, R, S, T	12	6.540	Y, Z, A-2, B-2, C-2, D-2, E-2, F-2, G-2
3	16.282	M, N, O, P, Q, R, S, T	24	6.123	Z, A-2, B-2, C-2, D-2, E-2, F-2, G-2
46	16.003	N, O, P, Q, R, S, T, U	27	5.288	A-2, B-2, C-2, D-2, E-2, F-2, G-2
21	16.003	N, O, P, Q, R, S, T, U	60	5.287	A-2, B-2, C-2, D-2, E-2, F-2, G-2
68	16.002	N, O, P, Q, R, S, T, U	29	5.010	B-2, C-2, D-2, E-2, F-2, G-2
71	15.585	N, O, P, Q, R, S, T, U, V	15	4.730	C-2, D-2, E-2, F-2, G-2
36	15.030	O, P, Q, R, S, T, U, V, W	47	4.173	D-2, E-2, F-2, G-2
48	14.890	O, P, Q, R, S, T, U, V, W	65	2.920	E-2, F-2, G-2
59	14.750	O, P, Q, R, S, T, U, V, W	22	2.087	F-2, G-2
34	14.750	O, P, Q, R, S, T, U, V, W	28	0.000	G-2

Table 6. Correlation analysis of flavonoid content, anthocyanin content and antioxidant activity.

	Flavonoid content	Anthocyanin content	Antioxidant activity
Flavonoid content	1	0.41413 < 0.0001	0.29382 < .0001
Anthocyanin content		1	0.41701 < 0.0001
Antioxidant activity			1

4. Conclusion

This study identified soybean genotypes with higher

flavonoid and anthocyanin contents, and antioxidant activity among selected Philippine soybean samples, which included currently grown varieties for commercial use, developed varieties, and breeding lines under research. Findings infer that soybean lines developed via breeding had better flavonoid and anthocyanin contents, and antioxidant activities, in comparison with the varieties presently used in the local market. The positive and significant correlation between flavonoid content and antioxidant activity, and between anthocyanin content and antioxidant activity presents a good prospect in developing TFC, TAC and AOA in soybean through breeding.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The research was supported by the DOST-PCAARRD project “Soybean Variety Development for Large Seed Size, Higher Yields and Enhanced Functional Properties” under the Soybean Program with project fund code: N91592B.

References

- Allen, J.K., Becker, D.M., Kwiterovich, P.O., Lindenstruth, K.A. and Curtis, C. (2007). Effect of soy protein-containing isoflavones on lipoproteins in postmenopausal women. *Menopause*, 14(1), 106-114. <https://doi.org/10.1097/01.gme.0000229572.21635.49>
- Aussenac, T., Lacombe, S. and Dayde, J. (1998). Quantification of isoflavones by capillary zone electrophoresis in soybean seeds: effects of variety and environment. *The American Journal of Clinical Nutrition*, 68(6 Suppl.), 1480S-1485S. <https://doi.org/10.1093/ajcn/68.6.1480S>
- Burda, S. and Oleszek, W. (2001). Antioxidant and antiradical activities of flavonoids. *Journal of Agricultural and Food Chemistry*, 49(6), 2774-2779. <https://doi.org/10.1021/jf001413m>
- Caldwell, C.R., Britz, S.J. and Mirecki, R.M. (2005). Effect of temperature, elevated carbon dioxide, and drought during seed development on the isoflavone content of dwarf soybean [*Glycine max* (L.) Merrill] grown in controlled environments. *Journal of Agricultural and Food Chemistry*, 53(4), 1125-1129. <https://doi.org/10.1021/jf0355351>
- Gaonkar, V. and Rosentrater, K.A. (2019). Soybean. In Pan, Z., Zhang, R. and Zicari, S. (Eds). *Integrated Processing Technologies for Food and Agricultural By-Products*, p. 73-104. USA: Academic Press. <https://doi.org/10.1016/B978-0-12-814138-0.00004-6>
- Gupta, S.K. (Ed). (2016). *Breeding Oilseed Crops for Sustainable Production: Opportunities and Constraints*. USA: Academic Press.
- Han, R.M., Tian, Y.X., Liu, Y., Chen, C.H., Ai, X.C. Zhang, J.P. and Skibsted, L.H. (2009). Comparison of flavonoids and isoflavonoids as antioxidants. *Journal of Agricultural and Food Chemistry*, 57(9), 3780-3785. <https://doi.org/10.1021/jf803850p>
- Hartman, G.L., West, E.D. and Herman, T.K. (2011). *Crops that feed the World 2. Soybean—worldwide production, use, and constraints caused by pathogens and pests*. *Food Security*, 3, 5-17. <https://doi.org/10.1007/s12571-010-0108-x>
- Iwashina, T. (2000). The structure and distribution of the flavonoids in plants. *Journal of Plant Research*, 113, 287-299. <https://doi.org/10.1007/PL00013940>
- Kalt, W., Forney, C.F., Martin, A. and Prior, R.L. (1999). Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *Journal of Agricultural and Food Chemistry*, 47(11), 4638-4644. <https://doi.org/10.1021/jf990266t>
- Kim, E.H., Kim, S.L., Kim, S.H. and Chung, I.M. (2012). Comparison of isoflavones and anthocyanins in soybean [*Glycine max* (L.) Merrill] seeds of different planting dates. *Journal of Agricultural and Food Chemistry*, 60(41), 10196-10202. <https://doi.org/10.1021/jf3031259>
- Kwon, S.H. Ahn, I.S., Kim, S.O., Kong, C.S., Chung, H.Y., Do, M.S. and Park, K.Y. (2007). Anti-obesity and hypolipidemic effects of black soybean anthocyanins. *Journal of Medicinal Food*, 10(3), 552-556. <https://doi.org/10.1089/jmf.2006.147>
- Lee, J., Durst, R.W. and Wrolstad, R.E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. *Journal of AOAC International*, 88(5), 1269-1278. <https://doi.org/10.1093/jaoac/88.5.1269>
- Lee, J.H., Jeon, J.K., Kim, S.G., Kim, S.H., Chun, T. and Imm, J.Y. (2011). Comparative analyses of total phenols, flavonoids, saponins and antioxidant activity in yellow soy beans and mung beans. *International Journal of Food Science and Technology*, 46(12), 2513-2519. <https://doi.org/10.1111/j.1365-2621.2011.02775.x>
- Lillo, C., Lea, U.S. and Ruoff, P. (2008). Nutrient depletion as a key factor for manipulating gene expression and product formation in different branches of the flavonoid pathway. *Plant, Cell and Environment*, 31(5), 587-601. <https://doi.org/10.1111/j.1365-3040.2007.01748.x>
- Liu, K. (1997). Chemistry and Nutritional Value of Soybean Components. In Liu, K. (Ed). *Soybeans*, p. 25-113. Boston, USA: Springer. https://doi.org/10.1007/978-1-4615-1763-4_2
- Mahmoud, A.M., Yang, W. and Bosland, M.C. (2014). Soy isoflavones and prostate cancer: a review of molecular mechanisms. *The Journal of Steroid Biochemistry and Molecular Biology*, 140, 116-132. <https://doi.org/10.1016/j.jsbmb.2013.12.010>
- Malenčić, D., Maksimović, Z., Popović, M. and Miladinović, J. (2008). Polyphenol contents and

- antioxidant activity of soybean seed extracts. *Bioresource Technology*, 99(14), 6688-6691. <https://doi.org/10.1016/j.biortech.2007.11.040>
- Messina, M. (1995). Modern applications for ancient bean: soybeans and the prevention and treatment of chronic disease. *The Journal of Nutrition*, 125(3 Suppl.), 567S-569S. <https://doi.org/10.3390/nu8120754>
- Messina, M. (2016). Soy and health update: Evaluation of the clinical and epidemiologic literature. *Nutrients*, 8(12), 754. <https://doi.org/10.3390/nu8120754>
- Mitchell, A.E., Hong, Y.J., Koh, E., Barrett, D.M., Bryant, D.E., Denison, R.F. and Kaffka, S. (2007). Ten-year comparison of the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes. *Journal of Agricultural and Food Chemistry*, 55(15), 6154-6159. <https://doi.org/10.1021/jf070344+>
- Neill, S.O. and Gould, K.S. (2003). Anthocyanins in leaves: light attenuators or antioxidants? *Functional Plant Biology*, 30(8), 865-873. <https://doi.org/10.1071/FP03118>
- Omoni, A.O. and Aluko, R.E. (2005). Soybean foods and their benefits: potential mechanisms of action. *Nutrition Reviews*, 63(8), 272-283. <https://doi.org/10.1111/j.1753-4887.2005.tb00141.x>
- Onozawa, M., Fukuda, K., Ohtani, M., Akaza, H., Sugimura, T. and Wakabayashi, K. (1998). Effects of soybean isoflavones on cell growth and apoptosis of the human prostatic cancer cell line LNCaP. *Japanese Journal of Clinical Oncology*, 28(6), 360-363. <https://doi.org/10.1093/jjco/28.6.360>
- Orak, H.H. (2007). Total antioxidant activities, phenolics, anthocyanins, polyphenoloxidase activities of selected red grape cultivars and their correlations. *Scientia Horticulturae*, 111(3), 235-241. <https://doi.org/10.1016/j.scienta.2006.10.019>
- Pietta, P.G. (2000). Flavonoids as antioxidants. *Journal of Natural Products*, 63(7), 1035-1042. <https://doi.org/10.1021/np9904509>
- Rostagno, M.A., Palma, M. and Barroso, C.G. (2004). Pressurized liquid extraction of isoflavones from soybeans. *Analytica Chimica Acta*, 522(2), 169-177. <https://doi.org/10.1016/j.aca.2004.05.078>
- Sahin, I., Bilir, B., Ali, S., Sahin, K. and Kucuk, O. (2019). Soy isoflavones in integrative oncology: Increased efficacy and decreased toxicity of cancer therapy. *Integrative Cancer Therapies*, 2019, 18. <https://doi.org/10.1177/1534735419835310>
- Sarkar, F.H. and Li, Y. (2003). Soy isoflavones and cancer prevention. *Cancer Investigation*, 21(5), 744-757. <https://doi.org/10.1081/CNV-120023773>
- Sathyapalan, T., Aye, M., Rigby, A.S., Thatcher, N.J., Dargham, S.R., Kilpatrick, E.S. and Atkin, S.L. (2018). Soy isoflavones improve cardiovascular disease risk markers in women during the early menopause. *Nutrition, Metabolism and Cardiovascular Diseases*, 28(7), 691-697. <https://doi.org/10.1016/j.numecd.2018.03.007>
- Shu, X. O., Zheng, Y., Cai, H., Gu, K., Chen, Z., Zheng, W. and Lu, W. (2009). Soy food intake and breast cancer survival. *The Journal of the American Medical Association*, 302(22), 2437-2443. <https://doi.org/10.1001/jama.2009.1783>
- Tanaka, Y., Sasaki, N. and Ohmiya, A. (2008). Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. *The Plant Journal*, 54(4), 733-749. <https://doi.org/10.1111/j.1365-313X.2008.03447.x>
- Veitch, N.C. (2007). Isoflavonoids of the leguminosae. *Natural Product Reports*, 24(2), 417-464. <https://doi.org/10.1039/b511238a>
- Wang, M.L., Gillaspie, A.G., Morris, J.B., Pittman, R.N., Davis, J. and Pederson, G.A. (2008). Flavonoid content in different legume germplasm seeds quantified by HPLC. *Plant Genetic Resources*, 6(1), 62-69. <https://doi.org/10.1017/S1479262108923807>
- Wang, Q., Ge, X., Tian, X., Zhang, Y., Zhang, J. and Zhang, P. (2013). Soy isoflavone: The multipurpose phytochemical (Review). *Biomedical Reports*, 1(5), 697-701. <https://doi.org/10.3892/br.2013.129>
- Wang, S.Y. and Lin, H.S. (2000). Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *Journal of Agricultural and Food Chemistry*, 48(2), 140-146. <https://doi.org/10.1021/jf9908345>
- Weaver, C.M., Alekel, D.L., Ward, W.E. and Ronis, M.J. (2012). Flavonoid intake and bone health. *Journal of Nutrition in Gerontology and Geriatrics*, 31(3), 239-253. <https://doi.org/10.1080/21551197.2012.698220>
- Wynstra, R.J. (1986). The soybean solution: meeting world food needs. Champaign, USA: INTSOY, College of Agriculture, University of Illinois.
- Xu, B. and Chang, S.K.C. (2008a). Total phenolics, phenolic acids, isoflavones, and anthocyanins and antioxidant properties of yellow and black soybeans as affected by thermal processing. *Journal of Agricultural and Food Chemistry*, 56(16), 7165-7175. <https://doi.org/10.1021/jf8012234>
- Xu, B. and Chang, S.K.C. (2008b). Characterization of phenolic substances and antioxidant properties of food soybeans grown in the North Dakota–Minnesota region. *Journal of Agricultural*

- and *Food Chemistry*, 56(19), 9102-9113. <https://doi.org/10.1021/jf801451k>
- Xu, J.R., Zhang, M.W., Liu, X.H., Liu, Z.X., Zhang, R.F., Sun, L. and Qiu, L.J. (2007). Correlation between antioxidation and the content of total phenolics and anthocyanin in black soybean accessions. *Agricultural Sciences in China*, 6(2), 150-158. [https://doi.org/10.1016/S1671-2927\(07\)60029-7](https://doi.org/10.1016/S1671-2927(07)60029-7)
- Yamane, T., Jeong, S.T., Goto-Yamamoto, N., Koshita, Y. and Kobayashi, S. (2006). Effects of temperature on anthocyanin biosynthesis in grape berry skins. *American Journal of Enology and Viticulture*, 57(1), 54-59. <https://doi.org/10.5344/ajev.2006.57.1.54>
- Yang, H.W., Hsu, C.K. and Yang, Y.F. (2014). Effect of thermal treatments on anti-nutritional factors and antioxidant capabilities in yellow soybeans and green-cotyledon small black soybeans. *Journal of the Science of Food and Agriculture*, 94(9), 1794-1801. <https://doi.org/10.1002/jsfa.6494>
- Yao, L.H., Jiang, Y.M., Shi, J., Tomás-Barberán, F.A., Datta, N., Singanusong, R. and Chen, S.S. (2004). Flavonoids in food and their health benefits. *Plant Foods for Human Nutrition*, 59(3), 113-122. <https://doi.org/10.1007/s11130-004-0049-7>
- Zhishen, J., Mengcheng, T. and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4), 555-559. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2)