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Characteristics of Functional Components and Antioxidant Activity of 28 Common Beans

Chengyu Jiang[1,2], Zhaohong Ci[1,2], Shuo Feng[1,2], Shan Wu[1,2], Michiyuki Kojima[1,*]

[1]Department of Food Science, Graduate School of Animal Husbandry, Obihiro University of Agriculture and Veterinary Medicine, 080-8555, 11, Nishi-2-Sen, Inada-Cho, Obihiro, Hokkaido, Japan.
[2]Department of Bioresources Science, United Graduate School of Agricultural Sciences, Iwate University, 3-18-8, Ueda, Morioka, Iwate 020-8550, Japan
*Corresponding author: kojima@obihiro.ac.jp

Abstract Common beans are either white or colored. They contain large amounts of phenolic compounds and other phytochemicals. Polyphenols exhibit high antioxidant activity that promote health by reducing oxidative stress. The objective of this study was to compare the content and composition of polyphenols in 28 common beans and determine the relation between their antioxidant activities and seed coat color. Here, we measured seed coat color by the International Commission in Illumination method, estimated polyphenol content and antioxidant activity by colorimetry, and identified polyphenol compositions by RP-HPLC. The results showed that polyphenol content and antioxidant activity were higher in colored beans than in the white beans. Taisho-Kintoki (red kidney) had the highest polyphenol content (6.12 mg / g seed) and antioxidant activity (21.98 μmol / g seed; 3.75 mg / g seed). There was a high correlation between the total polyphenol content and seed coat redness. There were also high equilateral correlations between antioxidant activity and polyphenol content. Twelve phenolic compounds were identified. Based on their polyphenol compositions synthesized by various enzymes, the 28 common beans were divided into three groups. White beans and several half-spotted beans contained a large amount of catechin-7-O-glucoside with low antioxidant activity. Anthocyanin, procyanidin, and kaempferol-3-O-glucoside constituted significant proportions of the total polyphenol compounds and were positively correlated with antioxidant activity in the colored common beans. These findings are expected to help guide consumers and breeders in the selection of common bean varieties with high antioxidant activity.

Keywords: *common bean, polyphenols, antioxidant activity, principal component analysis*

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1. Introduction

In Japan, the common bean is mostly cultivated in Hokkaido. The beans are categorized as either white or colored types. They contain large amounts of carbohydrates and proteins, but relatively little lipids. In general, they are widely consumed after boiling or used in desserts. In recent years, the polyphenols such as anthocyanins, catechins, and phenolic acids in beans, vegetables, and fruits have received much attention worldwide [1]. It is reported that common beans contain various polyphenols [2]. Phenolic compounds are synthesized by various enzymes in the biosynthetic pathways of common beans. For example, dihydro-flavonols can be converted to flavonols by flavonol synthase and to leucoanthocyanins by dihydroflavonol 4-reductase. Furthermore, leucoanthocyanins can be converted to anthocyanidins by anthocyanidin synthase or to flavonols by leucoanthocyanidin reductase. Phenolic glycosides are produced by glucosyltransferase. However, the key enzyme responsible for procyanidin condensation has not yet been identified [3,4,5]. Much has already been reported about the polyphenol content and the antioxidant and enzyme inhibition activity of common beans [6]. Nevertheless, the relative differences in their polyphenol composition and the correlations between their polyphenol compounds and antioxidant activity have seldom been addressed. In the present study, we analyzed the physicochemical characteristics of the polyphenols and antioxidants in common beans and identified the relation between seed coat color and total polyphenol content. We also characterized the traits of common beans known to have high antioxidant activity.

2. Materials and Methods

2.1. Materials

Twenty-eight common beans were harvested at Tokachi Agricultural Experiment Station, Hokkaido, Japan in 2014.

Folin-Ciocalteau reagent was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). DPPH (2, 2-diphenyl-1-picrylhydrazyl) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Catechin, epicatechin,

gallic acid, trolox, potassium ferricyanide and rutin were purchased from Sigma-Aldrich Co., LLC. (Tokyo, Japan). Cyanidin-3-O-glucoside and cyaniding-3-O-rutinoside were purchased from Extrasynthese Co. (Genay, France). Quercetin was purchased from Tokiwa Phytochemical Co., Ltd. (Tokyo, Japan). Ethanol, Na_2CO_3, KH_2PO_4, trichloroacetic acid, ferric chloride, vitamin C were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The other reagents were purchased from Funakoshi Co., Ltd. (Tokyo, Japan).

2.2. Determination of Seed Coat Color

Whole beans were transferred to petri dishes for 30 color determinations as defined by the International Commission on Illumination [7]. The Lab color space index was used to categorize seed color by lightness (L*), redness (a*), and yellowness (b*). These measurements were made with a CR-400 colorimeter. Chroma (C*) was calculated by redness and yellowness.

2.3. Extraction and Determination of Total Polyphenols

Polyphenols in the beans were extracted by the method of Saito [8]. Ground seeds (5 g) were placed in a falcon tube and mixed with 20 mL of 80% v/v ethanol, vortexed, and ultrasonicated for 30 min. The suspension was then centrifuged at 1,006 ×*g* for 10 min. The supernatant was transferred to another falcon tube to which 20 mL of 80% v/v ethanol was added. Vortex-mixing, ultrasonication, and centrifugation were repeated twice. The final ethanol extract was mixed with 20 mL of 70% v/v acetone and the aforementioned process was repeated thrice to obtain an acetone extract. The total polyphenol content was determined by the Folin-Ciocalteau method [9] modified by using catechin as the standard at a concentration range of 0-0.25 mg mL^{-1}. The extract (100 µL) was placed in a micro-tube and mixed with 300 µL distilled water, 400 µL Folin-Ciocalteau reagent, and 400 of 10% Na_2CO_3. The solution was then placed in a 30°C water bath for 30 min. It was then centrifuged at 1,006 ×*g* for 10 min and its absorbance was read at 760 nm. The results were expressed as mg catechin equivalents (CE) per gram seed ($y = 10.953x - 0.0548$, $R^2 = 0.9965$).

2.4. Determination of Antioxidant Activity

The DPPH radical scavenging activity was determined by the method of Brand-Williams [10]. The extract (50µL) was added to a microplate and mixed with 100 µL of 99.5% v/v ethanol and 150 µL DPPH solution. The solution was kept in the dark for 15 min after which its absorbance was determined at 520 nm by a microplate reader. Ten-fold diluted 2 mM Trolox was used as the standard and the results were expressed as µmol Trolox equivalents (TE) per gram seed ($y = -15.755x + 26.858$, $R^2 = 0.9987$).

The reducing power was determined by the method of Oyaizu [11]. The extract (250µL) was added to a microtube and mixed with 250 µL of KH_2PO_4 buffer (pH 7.5) and 250 µL of 1% (w/v) potassium ferricyanide, then incubated at 50°C in a water bath for 20 min. Then, 250 µL of 10% (w/v) trichloroacetic acid was added and the mixture was centrifuged at 1,006 ×*g* for 10 min. Then, 500 µL supernatant was transferred to another microtube and mixed with 500 µL distilled water and 100 µL of 0.1% (w/v) ferric chloride. After placing the solution in the dark for 15 min, its absorbance was measured at 700 nm. Vitamin C (1 mg mL^{-1}) was used as the standard and the results were expressed as mg vitamin C equivalents (VE) per gram seed ($y = 8.7561x + 0.2194$, $R^2 = 0.9982$).

2.5. Identification of Polyphenols by RP-HPLC

The polyphenol extracts were vacuum-dried and 20µg was re-dissolved in 200 µL distilled water. The solution was filtered with a Minisart RC4 (0.45 µm pore diameter; Sartorius AG, Göttingen, Germany). The filtered sample (20 µL) was injected into an RP-HPLC with a Shimadzu LC-6A HPLC system (LC-6A quaternary pump, SLC-6B system controller, CTO-6A column oven, SPD-10ADvp detector; Shimadzu Corporation, Kyoto, Japan), fitted with a Luna® RC18(2) 4.6 × 250 mm, 5 µm column (Phenomenex, Torrance, CA, USA). The temperature of the column oven was 40°C and the UV detection wavelength was 280 nm. The initial column conditions were as follows: developing solvent A (0.1% v/v trifluoroacetic acid) and eluent B (0.1% v/v trifluoroacetic acid-acetonitrile). The gradient program followed was 0.01 min, 8% B; 30 min, 30% B; 40 min, 30% B; 55 min, 8% B at a flow rate of 1 mL min^{-1}.

2.6. Data Analysis

The experiments were repeated at least three times. Data were expressed as means ± standard deviation. Significant differences were determined by one-way ANOVA and Fisher's test (SAS v. 7.1, SAS Institute Inc., Cary, NC, USA). Differences were considered to be significant at $P < 0.05$. Principal component analysis was performed with Ekuseru-Toukei v. 2008.

3. Results and Discussion

3.1. Physical Properties of Common Beans

In this study, we determined the weight and color of seeds of 28 common beans. The results are shown in Table 1. Lightness tended to decrease with increasing redness. There was a high positive correlation between total polyphenol content and redness (a*) ($r = 0.8753$; Figure 1). Similar correlations between polyphenol content and seed coat color are also reported for sweet potatoes and sorghum [12].

3.2. Total Polyphenol and Antioxidant Activities in Common Beans

The total polyphenol content and antioxidant activity of 28 common beans are shown in Table 2. In general, the polyphenol content in the white beans was significantly lower than that in colored beans. In this study, the red kidney bean had the highest polyphenol content (6.12 mg/

g seed) and antioxidant activity (21.98 μmol / g seed; 3.75 mg / g seed). It is reported that white beans have no antioxidant activity, whereas it is the highest in red and black beans [13, 14]. In another study, red kidney beans had the highest antioxidant activity, while white beans had the lowest [15]. In the present study, there were strong correlations between total polyphenol content and antioxidant activity (r = 0.9484, r = 0.9815 in Figure 2A and Figure2B). These results were similar to those reported for 29 common beans in the United States (r = 0.86, $P < 0.05$) [16], and 15 fruits ($R^2 = 0.99$, $R > 0.5$) [17], and 6 cultivars of Iranian olive ($R^2 = 0.976$, $P < 0.05$) [18]. In this study, Taisho-Kintoki (red kidney) bean had the highest procyanidin content of all 28 common beans tested (5.7 mg CE / g seed). Nevertheless, it is still lower than that in chokeberry and grape [19,20].

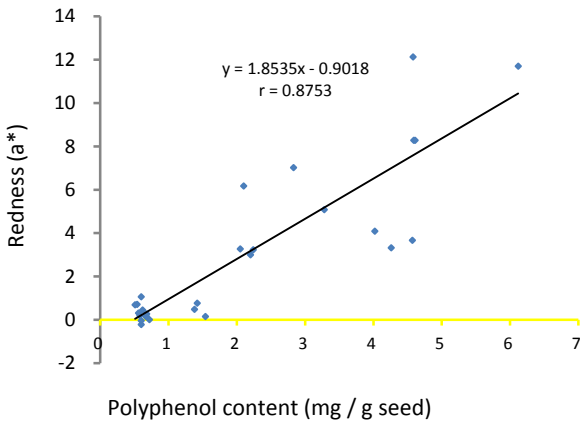


Figure 1. Correlation between polyphenol and redness (a*)

Table 1. Physical Properties of 28 Common Beans

No.	Sample	Seed Color	Grain Weight (g)	Lightness (L*)	Redness (a*)	Yellowness (b*)	Chroma (C*)
1	Yuki Tebou	White	0.29 ± 0.02lm	71.40 ± 1.54bc	0.71 ± 0.24ij	8.36 ± 0.58fg	8.40 ± 0.58g
2	Gin Tebou	White	0.31 ± 0.01l	70.95 ± 1.96bc	0.19 ± 0.22klmn	7.62 ± 0.45hi	7.63 ± 0.45h
3	Syo Tebou	White	0.32 ± 0.01kl	69.65 ± 0.90c	0.69 ± 0.16ijk	8.38 ± 0.20fg	8.41 ± 0.20g
4	Siro Nagauzura	White	0.61 ± 0.00ef	55.22 ± 2.80fg	0.15 ± 0.20lmn	6.58 ± 0.46kl	6.59 ± 0.46jk
5	Siro Maruuzura	White	0.58 ± 0.01fg	51.93 ± 2.26hi	0.31 ± 0.21jklm	6.85 ± 0.30jk	6.86 ± 0.30ij
6	Fukusiro Kintoki	White	0.85 ± 0.08ab	66.26 ± 3.25d	0.17 ± 0.30lmn	8.79 ± 0.37f	8.79 ± 0.38fg
7	Toya Daifuku	White	0.87 ± 0.02a	73.68 ± 4.32a	0.22 ± 0.18n	7.71 ± 0.42h	7.72 ± 0.42h
8	Kumamoto Ingen	White	0.30 ± 0.03lm	72.51 ± 2.07ab	0.04 ± 0.41mn	13.70 ± 1.08a	13.70 ± 1.08bc
9	Navy Bean	White	0.19 ± 0.02n	60.42 ± 1.52e	1.06 ± 0.17i	6.53 ± 0.29kl	6.62 ± 0.28jk
10	White Kidney	White	0.54 ± 0.05gh	50.08 ± 2.53ij	0.45 ± 0.17jklm	5.00 ± 0.38mn	5.02 ± 0.38n
11	Canellini	White	0.55 ± 0.01gh	50.66 ± 1.42ij	0.27 ± 0.10jklmn	6.26 ± 0.37l	6.26 ± 0.37kl
12	Umakei No.5	Half-spotted	0.55 ± 0.04gf	69.86 ± 2.84c	0.01 ± 0.25lmn	11.77 ± 0.58c	11.78 ± 0.58d
13	Sihoro Ingen	Half-spotted	0.66 ± 0.02cd	55.23 ± 3.29fg	0.48 ± 0.31jkl	6.75 ± 0.51jkl	6.78 ± 0.49ijk
14	Taoca Bean	Half-spotted	0.53 ± 0.01h	53.77 ± 7.23gh	0.77 ± 0.38ij	7.14 ± 1.68ij	7.20 ± 1.63hi
15	Fuku Tora	Half-spotted	0.81 ± 0.04b	55.82 ± 7.64f	3.23 ± 2.37gh	9.45 ± 2.28e	10.13 ± 2.80e
16	Beni Sibori	Half-spotted	0.63 ± 0.04cde	41.93 ± 5.57k	3.00 ± 1.08h	4.77 ± 1.94no	5.88 ± 1.42l
17	Hakusai Bean	Half-spotted	0.42 ± 0.02j	38.06 ± 2.21lm	4.09 ± 0.72f	5.51 ± 0.84m	6.93 ± 0.52ij
18	Siroji Biruma	Spotted	0.26 ± 0.01m	49.10 ± 2.32j	6.17 ± 0.54d	12.85 ± 0.64b	14.26 ± 0.80ab
19	Shell Bean	Spotted	0.62 ± 0.04def	35.05 ± 1.85no	3.27 ± 1.07gh	4.11 ± 1.62pq	5.26 ± 1.92mn
20	Futsu Biruma	Spotted	0.32 ± 0.02kl	35.95 ± 1.49n	7.02 ± 1.06c	6.76 ± 1.16jkl	9.77 ± 1.43e
21	Kiji Bean	Spotted	0.52 ± 0.03h	39.14 ± 2.28l	12.13 ± 0.76a	8.24 ± 1.27g	14.71 ± 0.85a
22	Triumph De Francy	Spotted	0.33 ± 0.01kl	35.31 ± 1.81no	3.67 ± 0.93fg	4.35 ± 1.52op	5.69 ± 1.75lm
23	Karasu Ingen	Black	0.36 ± 0.01k	33.42 ± 0.68o	0.15 ± 0.08lmn	-0.12 ± 0.11s	0.22 ± 0.08o
24	Gokuwase Murasaki Saya	Brown	0.46 ± 0.01i	59.63 ± 1.63e	5.08 ± 0.33e	14.15 ± 0.47a	15.04 ± 0.51a
25	Sutton's Premier	Brown	0.62 ± 0.02ef	42.16 ± 1.52k	3.32 ± 0.58gh	9.64 ± 0.85e	10.21 ± 0.85e
26	Jaula	Brown	0.55 ± 0.01gh	42.68 ± 0.82k	8.28 ± 0.37b	10.39 ± 0.48d	13.29 ± 0.48c
27	Roxo	Red	0.25 ± 0.00m	38.14 ± 0.73lm	8.28 ± 0.26b	3.76 ± 0.25q	9.10 ± 0.27f
28	Taisho Kintoki	Red	0.66 ± 0.01c	36.56 ± 1.13mn	11.70 ± 0.68a	2.87 ± 0.23r	12.05 ± 0.70d

Data are means ± SD from at least three independent studies. Values with different letters within the same column are significantly different at $P < 0.05$.

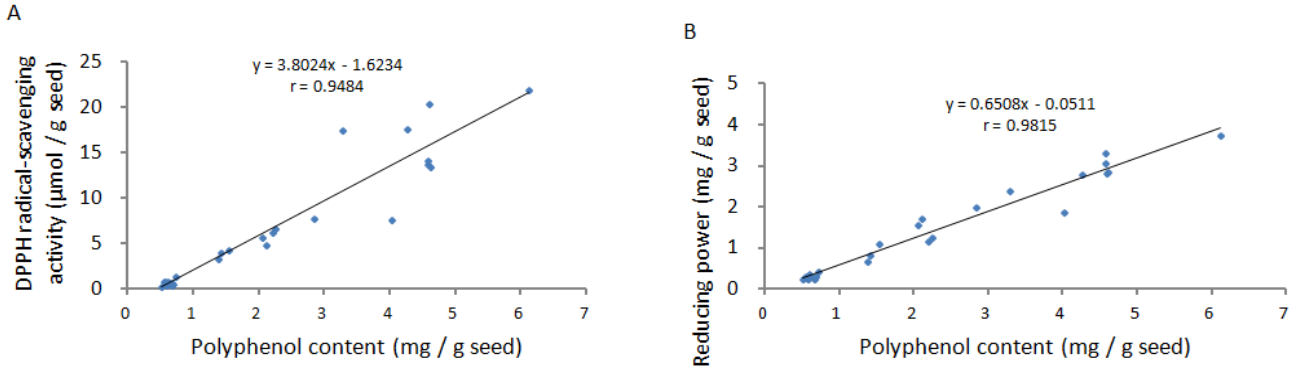


Figure 2. Correlation between polyphenol and antioxidant activity. A. Correlation between polyphenol and DPPH radical-scavenging activity. B. Correlation between polyphenol and reducing power

Table 2. Polyphenol and Antioxidant Activities of 28 Common Beans

No.	Sample	Seed Color	Polyphenol (mg / g seed)	DPPH radical-scavenging activity (μmol / g seed)	Reducing power (mg / g seed)
1	Yuki Tebou	White	0.54 ± 0.01t	0.83 ± 0.04s	0.31 ± 0.01rst
2	Gin Tebou	White	0.58 ± 0.01s	0.44 ± 0.02w	0.26 ± 0.01u
3	Syo Tebou	White	0.51 ± 0.01u	0.28 ± 0.07x	0.26 ± 0.01u
4	Siro Nagauzura	White	0.67 ± 0.01p	0.53 ± 0.05vw	0.26 ± 0.01u
5	Siro Maruuzura	White	0.56 ± 0.01t	0.88 ± 0.03rs	0.29 ± 0.01tu
6	Fukusiro Kintoki	White	0.67 ± 0.01p	0.73 ± 0.05t	0.29 ± 0.01stu
7	Toya Daifuku	White	0.60 ± 0.01rs	0.63 ± 0.08u	0.33 ± 0.01r
8	Kumamoto Ingen	White	0.60 ± 0.01rs	0.78 ± 0.02st	0.34 ± 0.01qr
9	Navy Bean	White	0.60 ± 0.01qr	0.96 ± 0.06r	0.37 ± 0.01q
10	White Kidney	White	0.62 ± 0.01q	0.81 ± 0.04st	0.33 ± 0.01rs
11	Canellini	White	0.68 ± 0.01p	0.58 ± 0.04uv	0.33 ± 0.01r
12	Umakei No.5	Half-spotted	0.72 ± 0.01o	1.38 ± 0.07q	0.43 ± 0.01p
13	Sihoro Ingen	Half-spotted	1.38 ± 0.02n	3.43 ± 0.03p	0.69 ± 0.01o
14	Taoca Bean	Half-spotted	1.42 ± 0.01m	4.03 ± 0.03o	0.83 ± 0.01n
15	Fuku Tora	Half-spotted	2.24 ± 0.01h	6.70 ± 0.04j	1.26 ± 0.01k
16	Beni Sibori	Half-spotted	2.20 ± 0.02i	6.32 ± 0.05k	1.17 ± 0.01l
17	Hakusai Bean	Half-spotted	4.02 ± 0.02e	7.74 ± 0.04i	1.87 ± 0.01h
18	Siroji Biruma	Spotted	2.10 ± 0.02j	4.96 ± 0.04m	1.72 ± 0.04i
19	Shell Bean	Spotted	2.05 ± 0.02k	5.79 ± 0.08l	1.57 ± 0.04j
20	Futsu Biruma	Spotted	2.83 ± 0.02g	7.85 ± 0.04h	2.01 ± 0.02g
21	Kiji Bean	Spotted	4.58 ± 0.02bc	13.80 ± 0.05f	3.08 ± 0.05c
22	Triumph De Francy	Spotted	4.57 ± 0.01c	14.27 ± 0.01e	3.33 ± 0.06b
23	Karasu Ingen	Black	1.54 ± 0.02l	4.43 ± 0.03n	1.12 ± 0.02m
24	Gokuwase Murasaki Saya	Brown	3.28 ± 0.13f	17.53 ± 0.22d	2.39 ± 0.01f
25	Sutton's Premier	Brown	4.26 ± 0.07d	17.67 ± 0.06c	2.79 ± 0.02e
26	Jaula	Brown	4.59 ± 0.08bc	20.51 ± 0.04b	2.82 ± 0.06e
27	Roxo	Red	4.61 ± 0.11b	13.58 ± 0.32g	2.86 ± 0.04d
28	Taisho Kintoki	Red	6.12 ± 0.19a	21.98 ± 0.30a	3.75 ± 0.05a

Data are means ± SD from at least three independent studies. Values with different letters within the same column are significantly different at $P < 0.05$.

Table 3. Polyphenols Compositions of 28 Common Beans

Peak	Compound	tR min	YuT	GiT	SyT	SiN	SiM	FuK	ToD	KuI	NaB	WKB	Cal	SiI	UmN	TaB
1	Gallic acid	5.29	1.6	0.6	0.6	0.8	1.1	0.7	0.8	1.1	1.0	0.7	0.8	0.5	1.1	1.4
2	Catechin-7-O-glucoside	6.17	8.8	14.1	8.0	9.0	9.4	16.1	17.0	7.3	9.7	9.7	14.4	9.7	6.2	8.7
3	Procyanidin dimer	8.56	4.1	2.5	2.7	4.5	5.1	4.4	3.2	3.8	4.4	4.5	4.7	4.7	4.2	3.0
4	Catechin	9.23	7.6	5.5	5.9	10.4	9.2	8.8	7.5	8.9	8.5	9.7	8.7	7.7	8.2	5.2
5	3-caffeoylquinic acid	10.24	3.4	1.9	1.7	2.1	2.3	2.1	1.9	2.1	2.3	2.0	1.6	3.2	1.9	2.4
6	5-caffeoylquinic acid	11.39	5.3	3.5	3.9	3.8	2.7	3.3	3.5	3.0	3.1	3.9	3.0	3.3	3.6	2.4
7	Epicatechin	12.47	3.6	2.7	2.6	2.2	2.4	1.9	3.1	2.8	2.7	2.1	2.1	2.5	2.7	2.6
8	Cyanidin-3-O-glucoside	14.36	-	-	-	-	0.4	-	-	0.7	0.5	0.5	0.6	0.4	0.5	1.1
9	Cyanidin-3-O-rutinoside	15.29	-	-	0.5	0.6	0.5	0.6	0.8	0.6	0.5	0.8	0.6	0.9	0.9	0.7
10	Rutin	20.25	-	-	-	-	-	-	-	-	-	-	-	0.8	-	0.6
11	Kaempferol-3-O-glucoside	22.99	-	-	-	-	-	-	-	-	-	-	-	0.5	-	0.9
12	Quercetin	24.94	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-
13	Others	-	65.6	69.3	73.4	66.6	67.0	62.1	62.3	69.8	67.3	66.1	63.5	65.9	70.8	71.2

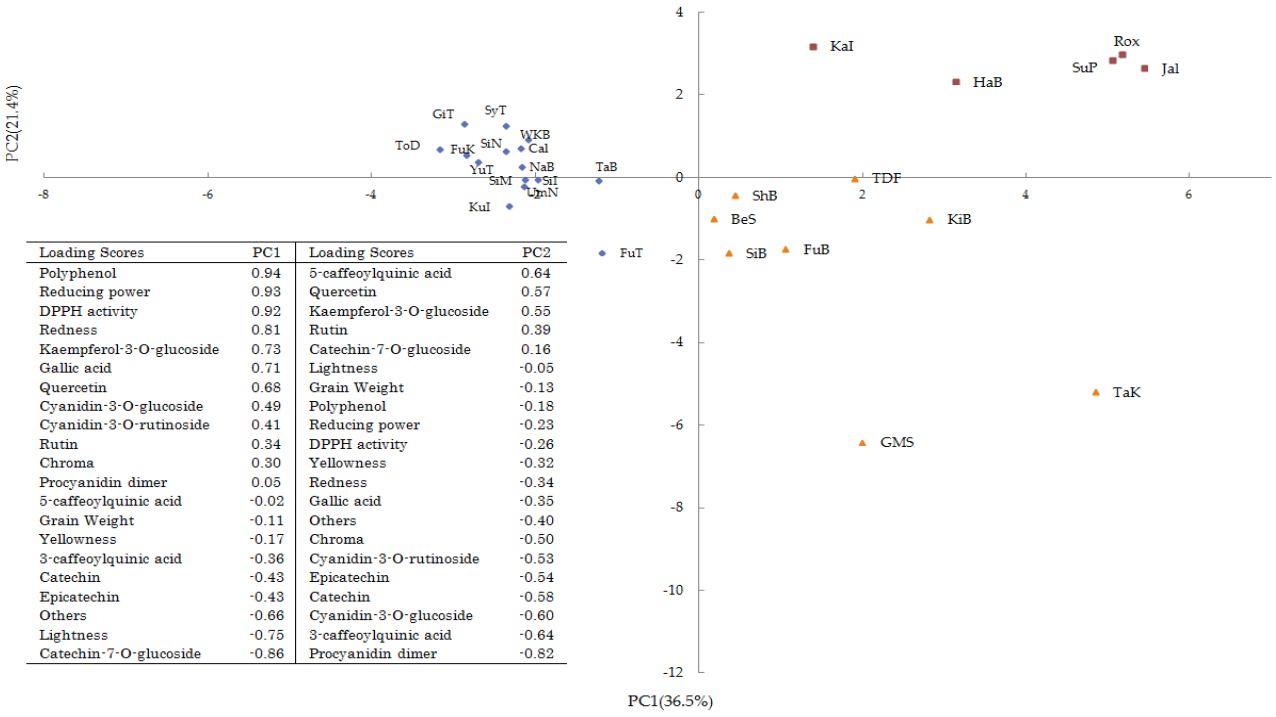
Peak	Compound	FuT	BeS	FuB	SiB	KiB	TDF	ShB	GMS	TaK	HaB	CaI	Jal	SuP	Rox
1	Gallic acid	0.6	1.9	1.0	0.7	1.4	1.4	1.7	4.3	3.5	2.5	0.6	2.7	3.4	1.7
2	Catechin-7-O-glucoside	7.6	6.2	2.0	2.0	1.6	2.6	2.8	-	-	3.7	-	-	-	-
3	Procyanidin dimer	5.0	4.3	9.2	6.3	3.0	3.1	6.6	15.5	7.0	3.6	1.9	3.5	2.0	1.3
4	Catechin	7.4	5.8	3.2	3.7	3.4	3.0	3.5	16.7	10.5	3.2	6.0	2.8	1.3	2.5
5	3-caffeoylquinic acid	3.3	3.0	2.4	2.1	1.6	1.2	3.0	2.7	3.6	2.0	1.7	0.6	0.7	0.8
6	5-caffeoylquinic acid	2.8	2.9	1.9	1.9	2.4	2.2	3.0	2.3	2.7	4.0	4.2	4.2	3.4	4.9
7	Epicatechin	3.8	2.2	2.2	2.3	0.9	2.2	3.0	4.5	2.9	1.6	3.1	0.7	0.6	2.0
8	Cyanidin-3-O-glucoside	1.4	3.2	1.4	1.8	1.1	1.4	1.3	3.9	3.1	1.1	2.4	-	0.5	1.7
9	Cyanidin-3-O-rutinoside	1.2	0.9	1.0	1.1	0.7	-	0.8	2.0	9.3	0.5	1.0	-	0.4	2.7
10	Rutin	-	0.5	-	-	-	-	1.5	0.9	-	1.7	8.5	1.0	1.0	3.0
11	Kaempferol-3-O-glucoside	-	-	-	-	1.4	1.1	1.1	1.8	4.2	36.9	31.5	72.6	70.0	51.2
12	Quercetin	-	-	-	-	-	-	1.1	-	-	4.5	1.4	3.1	3.3	4.4
13	Others	67.0	69.2	75.8	78.1	82.4	81.8	70.8	45.5	53.3	34.7	37.7	8.7	13.3	23.9

Data are expressed as % of total area and represent the relative abundance of a compound relative to the sum of areas of all peaks in the chromatogram for each cultivar. Identification was based on pure standards. YuT: Yuki-Tebou; GiT: Gin-Tebou; SyT: Syo-Tebou; SiN: Siro-Nagauzura; SiM: Siro-Maruuzura; FuK: Fukusiro-Kintoki; ToD: Toya-Daifuku; KuI: Kumamoto-Ingen; NaB: Navy-Bean; WKB: White Kidney Bean; Cal: Canellini; SiI: Sihoro-Ingen; UmN: Umakei-No.5; TaB: Taoca-Bean; FuT: Fuku-Tora; BeS: Beni-Sibori; FuB: Futsu-Biruma; SiB: Siroji-Biruma KiB: Kiji-Bean; TDF: Triumph De Francy; ShB: Shell Bean; GMS: Gokuwase-Murasaki-Saya; TaK: Taisho-Kintoki; HaB: Hakusai-Bean; CaI: Carasu-Ingen; Jal: Jaula; SuP: Sutton's Premier; Rox: Roxo.

3.3. Polyphenol Compounds in Common Beans

A total of 12 phenolic compounds were identified in the 28 common beans (Table 3). The relative abundance of a compound is expressed as the ratio of the sum of areas of all peaks in the chromatogram for each cultivar. Gallic acid (0.50-4.26%), procyanidin dimer (1.31-15.47%), catechin (1.34-16.72%), 3-caffeoylquinic acid (0.64-3.55%), 5-caffeoylquinic acid (1.88-5.34%), and epicatechin (0.63-4.50%) were found in all samples and have been previously identified in common beans [21]. Catechin-7-O-glucoside (1.55-16.96%) was found in 22 samples. It was observed that white colored common beans tend to have relatively high levels of catechin-7-O-glucoside. Cyanidin-3-O-glucoside (0.41-3.89%) was found in 21 cultivars and cyanidin-3-O-rutinoside (0.40-9.34%) was found 24 samples. Colored common beans have high relative abundances of anthocyanins. These compounds have been reported elsewhere for common beans [22]. Rutin (0.51-8.51%), quercetin (0.52-4.48%), and kaempferol-3-O-glucoside (0.48-72.63%) were identified in ten, seven, and twelve common beans, respectively. Quercetin and kaempferol were identified in 10 common beans at levels of 14.1-24.2 μg g^{-1} and 11.4-61.0 μg g^{-1}, respectively [23].

Principal component analysis of 12 identified compounds and antioxidant activity of 28 common beans indicated that the first two principal components had eigenvalues explaining 64.8% of the total variance (PC1 = 38.0; PC2 = 26.8) (Figure 3). In this study, 28 common beans were divided into three groups according to their phenolic compound content. Group one contained 14 common beans like white beans and several half-spotted beans. This cluster had positive scores in PC2 and negative scores in PC1. They were positively correlated with caffeoylquinic acid and catechin-7-O-glucoside and negatively correlated with antioxidant activity. Similarly, the cluster containing nine common beans like Taisho-Kintoki and Gokuwase-Murasaki-Saya presented higher values than group 1 based on PC1, and large negative scores in PC2. This group correlated with anthocyanin and procyanidin dimer and the samples had higher antioxidant activity than samples of group 1. Cluster 3 contained five samples like Jaula and Sutton's Premier. It had large positive scores in PC1. They were positively correlated with kaempferol-3-O-glucoside, quercetin, and antioxidant activity. The main polyphenol compounds detected in common beans were phenolic acid, flavonol, anthocyanin, and tannin. Their functions include antioxidant activity and disease resistance. It was reported that kaempferol glycosides are hepatoprotectant and inhibit α-glucosidase [24,25]. Anthocyanins and proanthocyanidins from adzuki bean were found to have strong antioxidant activity [26,27]. Common beans with high antioxidant activity may contain large amounts of kaempferol-3-O-glucoside, which is synthesized by key enzymes flavonol synthase and glycosyltransferase or substantial quantities of cyanidin glycosides synthesized by dihydroflavonol 4-reductase, anthocyanidin synthase, and glycosyltransferase. The content of these enzymes in common beans may determine the strength of their antioxidant activity. In future research, this information could be used to breed new cultivars of common beans with high antioxidant activity.



Loading Scores	PC1	Loading Scores	PC2
Polyphenol	0.94	5-caffeoylquinic acid	0.64
Reducing power	0.93	Quercetin	0.57
DPPH activity	0.92	Kaempferol-3-O-glucoside	0.55
Redness	0.81	Rutin	0.39
Kaempferol-3-O-glucoside	0.73	Catechin-7-O-glucoside	0.16
Gallic acid	0.71	Lightness	-0.05
Quercetin	0.68	Grain Weight	-0.13
Cyanidin-3-O-glucoside	0.49	Polyphenol	-0.18
Cyanidin-3-O-rutinoside	0.41	Reducing power	-0.23
Rutin	0.34	DPPH activity	-0.26
Chroma	0.30	Yellowness	-0.32
Procyanidin dimer	0.05	Redness	-0.34
5-caffeoylquinic acid	-0.02	Gallic acid	-0.35
Grain Weight	-0.11	Others	-0.40
Yellowness	-0.17	Chroma	-0.50
3-caffeoylquinic acid	-0.36	Cyanidin-3-O-rutinoside	-0.53
Catechin	-0.43	Epicatechin	-0.54
Epicatechin	-0.43	Catechin	-0.58
Others	-0.66	Cyanidin-3-O-glucoside	-0.60
Lightness	-0.75	3-caffeoylquinic acid	-0.64
Catechin-7-O-glucoside	-0.86	Procyanidin dimer	-0.82

Figure 3. Cluster and principal component analysis score plot of 28 common beans. Score plots for the first two principal components, PC1 (36.5) and PC2 (21.4). Results are expressed as % of total area and represent the relative abundance of a compound relative to the sum of areas of all peaks in the chromatogram for each cultivar. Identification was based on pure standards. YuT: Yuki-Tebou; GiT: Gin-Tebou; SyT: Syo-Tebou; SiN: Siro-Nagauzura; SiM: Siro-Maruuzura; FuK: Fukusiro-Kintoki; ToD: Toya-Daifuku; KuI: Kumamoto-Ingen; NaB: Navy-Bean; WKB: White Kidney Bean; Cal: Canellini; SiI: Sihoro-Ingen; UmN: Umakei-No.5; TaB: Taoca-Bean; FuT: Fuku-Tora; BeS: Beni-Sibori; FuB: Futsu-Biruma; SiB: Siroji-Biruma KiB: Kiji-Bean; TDF: Triumph De Francy; ShB: Shell Bean; GMS: Gokuwase-Murasaki-Saya; TaK: Taisho-Kintoki; HaB: Hakusai-Bean; CaI: Carasu-Ingen; Jal: Jaula; SuP: Sutton's Premier; Rox: Roxo

4. Conclusion

There is a strong correlation between total polyphenol content and redness (a*) in common beans. Antioxidant activity in colored beans is higher than it is in white beans. There is a high positive correlation between the total polyphenol content and antioxidant activity. In general, common beans are abundant in polyphenols. Common beans with high antioxidant activity not only have high total polyphenol content, but also elevated levels of particular polyphenols. The biosynthetic enzymes flavonol synthase, dihydroflavonol 4-reductase, and anthocyanidin synthase are positively correlated with antioxidant activity in common beans.

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Statement of Competing Interests

The authors have no competing interests.

List of Abbreviations

CE: catechin equivalents; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; TE: Trolox equivalents; VE: vitamin C equivalents; PC: principal component

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