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Isoflavones and soyasaponins in the germ of Korean soybean [*Glycine max* (L.) Merr.] cultivars and their compound-enhanced BMP-2-induced bone formation

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Abstract

Soybeans are used worldwide as food and as a healthy ingredient. Specifically, soy germ (SG) has received considerable attention owing to its abundant nutritional and biological components. This study aimed to elucidate the contents of isoflavone and soyasaponin of SG in 24 Korean soybean cultivars and the osteogenic activity of individual compounds. The isoflavone content in the SG ranged from 1110.9 to 3131.1 mg/100 g, and the soyasaponin content in SG ranged from 1173.5 to 3582.3 mg/100 g. The isoflavone and soyasaponin content depended on soybean cultivars. All isoflavone and soyasaponin compounds enhanced bone morphogenetic protein-2-mediated osteoblast differentiation in a dose-dependent manner, especially soyasaponin Ab. In conclusion, our results suggest that Seonpung cultivar with high soyasaponin Ab is beneficial for developing functional materials.

Keywords: Soy germ, Isoflavone, Soyasaponin, Osteoblast, BMP-2

Introduction

Soybeans [*Glycine max* (L.) Merr.] are cultivated worldwide because they are rich in primary metabolites such as proteins and oils. In addition, soybeans contain many secondary metabolites such as isoflavones, soyasaponins and tocopherols [6]. Soybean seeds structurally consist of the seed coat, cotyledon, and germ [14]. The isoflavone and soyasaponin content of the germ is higher than that of the seed coat and cotyledon [1, 5, 21].

Isoflavones are divided into aglycones (daidzein, glycitein, and genistein), β-glycoside (daidzin, glycitin, and genistin), acetyl-glycoside (acetyl-daidzin, acetyl-glycitin, and acetyl-genistin), and malonyl-glycosides (malonyl-daidzin, malonyl-glycitin, and malonyl-genistin) [12]. Isoflavones are known to exhibit biological activities such as anti-oxidant, anti-cancer, anti-diabetic, and bone health [4, 16, 20, 22].

Soyasaponins are oleanane-type triterpenoid saponins. Soyasaponins are divided into soyasaponin A group, B group, E group and DDMP group [10, 17, 18]. The compounds of the soyasaponin A group are known to exhibit various biological activities such as bone health, anti-obesity, and anti-oxidant activities [3, 7, 15]; the compounds of the soyasaponin B group are known to exhibit various biological activities such as bone health, anti-inflammatory, anti-cancer, hepatoprotective and renin inhibitory activities [8, 11, 13, 19, 23].

However, until now, the effect of individual isoflavone and soyasaponin compounds on osteoblast differentiation has not been simultaneously studied. Therefore, we determined isoflavone and soyasaponin contents in soy germ (SG) and investigated the effect of isoflavones

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and soyasaponins on BMP-2-dependent osteoblast differentiation.

Materials and methods

Chemicals and reagents

Water, acetonitrile and methanol (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Aglycones (daidzein, glycitein, and genistein) and β-glycoside (daidzin, glycitin, and genistin) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Acetyl-glycosides (acetyl-daidzin, acetyl-glycitin, and acetyl-genistin) and malonyl-glycosides (malonyl-daidzin, malonyl-glycitin, and malonyl-genistin) were purchased from Nacalai tesque (Nijo Karasuman Nakagyo, Kyoto, Japan). Soyasaponin Aa, soyasaponin Ab, soyasaponin Ac, soyasaponin Ba, soyasaponin Bb, soyasaponin Bc, soyasaponin Bc`, soyasaponin Bd, and soyasaponin Be were purchased from ChemFaces (Wuhan, Hubei, China). Soyasaponin Bb` was purchased from Chroma-Dex (Irvine, CA, USA). Recombinant human bone morphogenetic protein-2 (rhBMP-2) was purchased from R&D Systems (Minneapolis, MN, USA). Penicillin, streptomycin, cell culture medium and fetal bovine serum (FBS) were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). All other chemicals and solvents used in the current study were of analytical grade.

Preparation of soybean cultivars, SG and SG extract

Twenty-four soybean [*Glycine max* (L.) Merr.] cultivars were grown on the experimental field at the National Institute of Crop Science, Jeonbuk, Korea, and harvested in 2018. The separation of SG was conducted using the previously published method [9] with some modifications. Soybean seeds were crushed using a grinder and cotyledon, and the seed coat was removed using a sieve to separate the SG. To make the SG extract, each SG was dried in a freeze-dryer and then ground. Ground SG was defatted using hexane, and the defatted sample (1 g) was extracted using MeOH (40 mL) for 24 h at room temperature. The extract was centrifuged at 5000 rpm for 10 min at 4 °C, and the supernatant was filtered through a regenerated cellulose syringe filter (0.2 μm). The filtered solution was transferred into a 2 mL vial for the analysis of isoflavones and soyasaponins.

Isoflavone analysis

Isoflavone analysis was conducted using an ultra-high performance liquid chromatography (UHPLC, Dionex Ultimate 3000, Thermo Scientific) instrument equipped with a HALO C18 (2.7 μm, 2.1 mm × 100 mm) column. The mobile phases A and B were 0.1% acetic acid in water and 0.1% acetic acid in acetonitrile, respectively. The solvent flow rate was 0.3 mL/min, and the column temperature was set to 35 °C. The gradient was programmed as 0–2 min, 10% B; 35 min, 30% B; 36 min, 90% B; 36–39 min, 90% B; and 40 min, 10% B, held for 5 min before returning to the initial condition. After the injection of 1.3 μL of the sample, eluted isoflavones were detected at 254 nm using a diode array detector (DAD, Thermo Scientific). The calibration curve was plotted by peak area versus the concentration of isoflavones. To prepare the standard stock solution, 12 isoflavones were dissolved in DMSO at the concentration of 1 mg/mL. The stock solution was serially diluted to make the standard solution (3.125, 6.25, 12.5, 25, and 50 μg/mL).

Soyasaponin analysis

Soyasaponins analysis was conducted using a UHPLC (Dionex Ultimate 3000, Thermo Scientific) instrument equipped with an Acclaim^{TM} RSLC Polar Advantage II (2.2 μm, 2.1 mm × 150 mm) column. The mobile phases A and B were 0.1% acetic acid in water and 0.1% acetic acid in acetonitrile, respectively. The solvent flow rate was 0.5 mL/min, and the column temperature was set to 40 °C. The gradient was programmed as 0–1 min, 20% B; 5 min, 30% B; 35 min, 45% B; 40–42 min, 90% B; and 43 min, 20% B, held for 7 min before returning to the initial conditions. After the injection of 1.3 μL of the sample, eluted soyasaponins were detected using a charged aerosol detector (CAD, Corona Veo, Thermo Scientific). The setting for CAD were as follows: gas, nitrogen; power function, 1.3; pressure, 61 psi; filter, 10 s; gain, 100 pA; evaporation temperature, 50 °C; and data collection rate, 10 Hz. The calibration curve was plotted as the peak area versus the concentration of soyasaponins. To prepare the standard stock solution, 10 soyasaponins were dissolved in DMSO at the concentration of 1 mg/mL. The stock solution was serially diluted to make the standard solution (6.25, 12.5, 25, 50, and 100 μg/mL).

Osteoblast cell Culture and differentiation

All cell experiments were performed as previously described [3] with some modifications. Mouse mesenchymal precursor C2C12 cells were purchased from the American Type Collection (Manassas, VA, USA). C2C12 cells were maintained in an alpha minimum essential medium (α-MEM) containing 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% FBS. To differentiate C2C12 into osteoblasts, the cells were seeded and allowed to attach and grow for 1 d; then which the medium was replaced with a differentiation medium (α-MEM containing 5% FBS and 100 ng/mL rhBMP-2). The medium was changed every 3 d.

Table 1 Isoflavone content of soy germ in 24 soybean cultivars

Cultivar	Aglycone			β-glycoside			Acetyl-glycoside			Malonyl-glycoside			Total (mg/100 g)[a]
	Daidzein	Glycitein	Genistein	Daidzin	Glycitin	Genistin	Avetyl-daidzin	Acetyl-glycitin	Acetyl-genistin	Malonyl-daidzin	Malonyl-glycitin	Malonyl-geistin	
Daepung2ho	7.5 ± 0.1bc	4.4 ± 0.1a	4.0 ± 0.0 l	241.4 ± 8.0a	537.0 ± 15.6a	115.4 ± 2.9a	22.4 ± 1.3bc	24.5 ± 1.7a	7.7 ± 0.1c	1052.4 ± 26.9 cd	941.7 ± 25.6ab	172.9 ± 5.1b	3131.1 ± 82.7a
Saegeum	4.5 ± 0.3fgh	nd[b]	4.9 ± 0.1j	114.9 ± 23.5 h	334.2 ± 65.3c	65.8 ± 12.4ef	15.2 ± 2.7hi	19.7 ± 5.4bcd	6.7 ± 0.7efg	912.9 ± 199.9defg	1028.7 ± 225.0a	147.9 ± 28.8 cd	2655.4 ± 564.2b
Jungmo3012	6.2 ± 0.2de	nd	5.2 ± 0.0f	134.2 ± 8.7efg	319.2 ± 18.6 cd	66.9 ± 4.1ef	18.6 ± 0.4ef	22.6 ± 1.1b	7.3 ± 0.2 cd	920.6 ± 59.2def	893.0 ± 62.6b	144.0 ± 10.0de	2537.7 ± 165.0bc
Daepung	4.4 ± 0.2gh	0.2 ± 0.2 fg	5.1 ± 0.1 g	124.2 ± 2.1fgh	380.9 ± 5.9b	72.6 ± 1.4de	19.7 ± 0.2def	27.1 ± 0.4a	8.8 ± 0.1a	794.4 ± 12.5gh	884.5 ± 17.9bc	143.7 ± 2.3de	2465.5 ± 43.3bcd
Taeseon	9.5 ± 0.0a	1.9 ± 0.1c	5.5 ± 0.0d	141.9 ± 1.5de	212.2 ± 1.5hijk	57.0 ± 0.6ghij	24.1 ± 0.3b	21.4 ± 0.3bc	7.6 ± 0.2c	994.6 ± 5.0cde	667.2 ± 3.2de	120.9 ± 0.3gh	2263.9 ± 3.7cde
Miso	5.4 ± 0.1ef	nd	4.7 ± 0.1 k	169.1 ± 2.0c	118.7 ± 1.3 m	44.1 ± 0.2kl	24.3 ± 0.4b	9.2 ± 0.1kl	6.3 ± 0.1 fg	1338.4 ± 9.8a	414.5 ± 5.1hijk	123.7 ± 0.6fgh	2258.4 ± 19.7cde
Seonpung	4.4 ± 0.0gh	nd	5.1 ± 0.0 h	111.1 ± 2.4hi	280.3 ± 4.9ef	62.4 ± 0.9fghi	17.8 ± 0.5 fg	19.0 ± 2.2 cd	7.5 ± 0.1c	828.4 ± 14.3fgh	765.5 ± 13.5d	142.1 ± 0.6def	2243.5 ± 38.0cdef
Pungsanna-mul	6.6 ± 0.3 cd	nd	5.0 ± 0.0i	151.3 ± 0.3d	187.9 ± 0.9kl	54.4 ± 0.1ij	20.8 ± 0.1 cd	12.4 ± 0.0hij	6.5 ± 0.2 fg	1113.5 ± 6.1bc	537.9 ± 2.3 fg	123.3 ± 0.1fgh	2219.5 ± 9.6cdefg
Socheongja	7.4 ± 0.9bc	nd	5.3 ± 0.0ef	176.3 ± 0.8bc	167.5 ± 0.9 l	63.3 ± 0.2fgh	28.7 ± 0.6a	13.3 ± 0.1ghi	8.6 ± 0.0ab	1159.7 ± 17.2b	445.5 ± 7.9ghij	140.6 ± 4.2def	2216.1 ± 32.8cdefg
Cheong-ja4ho	6.6 ± 0.4 cd	nd	nd	149.5 ± 21.2de	204.2 ± 27.2ijk	62.4 ± 8.9fghi	20.3 ± 2.1de	10.7 ± 0.8ijk	6.9 ± 0.6def	1113.0 ± 140.6bc	475.6 ± 59.2ghi	137.7 ± 20.3defg	2186.8 ± 281.3defg
Cheong-ja3ho	6.8 ± 0.0 cd	nd	5.3 ± 0.0e	185.9 ± 3.2b	165.2 ± 2.7 l	78.7 ± 2.3 cd	16.5 ± 0.2gh	8.3 ± 0.3kl	5.8 ± 0.1hi	1206.7 ± 17.1b	370.3 ± 4.7ijk	135.5 ± 2.1defg	2185.0 ± 32.0defg
Shinhwa	5.2 ± 0.6fgh	nd	nd	116.7 ± 3.0 h	305.9 ± 7.6cde	76.1 ± 1.4 cd	16.5 ± 0.6gh	17.4 ± 0.2def	8.6 ± 0.2ab	759.8 ± 28.4hij	710.4 ± 23.1de	163.7 ± 4.9bc	2180.3 ± 70.0defg
Taekwang	8.0 ± 0.1b	1.1 ± 0.0d	nd	121.8 ± 2.3gh	196.3 ± 3.2jkl	51.7 ± 1.1jk	18.3 ± 0.4efg	17.9 ± 0.4def	6.4 ± 0.0 fg	874.0 ± 19.7efgh	634.5 ± 10.4ef	110.7 ± 2.3hi	2040.8 ± 39.7efgh
Haewon	5.3 ± 0.1fgh	0.6 ± 0.1e	nd	77.1 ± 0.3kl	291.0 ± 0.7de	84.0 ± 0.2c	11.2 ± 0.0 k	15.8 ± 0.0efg	7.2 ± 0.0cde	586.4 ± 3.7 k	781.7 ± 4.1 cd	149.0 ± 0.6 cd	2009.2 ± 9.6efghi
Haepum	5.3 ± 0.1 fg	0.3 ± 0.2f	5.7 ± 0.0c	88.6 ± 2.0jk	254.0 ± 5.0 fg	78.2 ± 1.7 cd	12.1 ± 0.3jk	15.0 ± 0.3fgh	8.2 ± 0.1b	615.5 ± 8.3 k	691.5 ± 8.4de	175.8 ± 0.6b	1950.2 ± 27.0efghi
Jinpung	4.3 ± 0.3 h	nd	nd	74.7 ± 5.6kl	278.6 ± 14.4ef	56.0 ± 2.4hij	15.2 ± 1.0hi	19.9 ± 1.2bcd	7.6 ± 0.4c	645.8 ± 41.9kj	686.9 ± 43.8de	127.3 ± 6.6efgh	1916.3 ± 117.7fghi
Seonyu	8.1 ± 0.7b	nd	6.9 ± 0.0a	135.0 ± 3.9defg	109.5 ± 2.8 m	102.3 ± 4.1b	12.0 ± 0.2jk	9.4 ± 0.3jkl	8.2 ± 0.1b	925.1 ± 29.3def	346.6 ± 12.1jk	249.8 ± 5.4a	1913.0 ± 48.8fghi
Daechan	4.4 ± 0.1gh	nd	nd	97.7 ± 0.2ij	239.5 ± 0.8ghi	55.9 ± 0.0hij	15.4 ± 0.0hi	18.6 ± 0.1 cde	7.3 ± 0.1cd	673.2 ± 2.8ijk	663.4 ± 2.0de	126.4 ± 0.3efgh	1901.8 ± 6.1ghi
Soyeon	7.9 ± 0.6b	2.4 ± 0.1b	6.0 ± 0.0b	85.3 ± 2.6jk	247.7 ± 6.7fgh	65.0 ± 1.3efg	12.1 ± 0.3jk	13.5 ± 0.2ghi	6.9 ± 0.1def	632.8 ± 19.6 k	669.1 ± 23.2de	148.5 ± 3.5 cd	1897.3 ± 58.0ghi
Daewon	nd	nd	nd	80.8 ± 3.9kl	238.0 ± 11.0ghi	55.9 ± 2.3hij	13.6 ± 1.0ij	20.0 ± 1.5bcd	7.5 ± 0.0c	573.1 ± 21.3 k	689.8 ± 22.6de	124.2 ± 5.4fgh	1803.1 ± 69.1 hij
Cheongmiin	5.2 ± 0.2fgh	nd	nd	115.0 ± 2.1h	97.6 ± 0.0 m	40.1 ± 0.3 l	21.6 ± 0.5 cd	9.8 ± 0.2jk	6.2 ± 0.1gh	948.9 ± 16.3def	346.4 ± 4.1jk	95.0 ± 1.4ij	1685.9 ± 24.7ij
Hwangkeu-mol	6.6 ± 1.1 cd	nd	nd	138.6 ± 6.5def	129.6 ± 3.8 m	64.7 ± 2.9efg	10.6 ± 0.4 k	6.7 ± 0.1 l	5.1 ± 0.0j	777.4 ± 11.2hi	302.4 ± 5.0 k	112.6 ± 2.0hi	1554.3 ± 32.9jk
Chamol	nd	1.0 ± 0.2d	nd	54.5 ± 0.3 m	224.7 ± 5.2ghij	50.1 ± 0.2jk	6.7 ± 0.1 l	11.2 ± 0.1ijk	5.3 ± 0.0ij	325.8 ± 5.7 m	505.8 ± 10.8gh	94.9 ± 3.9ij	1280.0 ± 26.5kl
Saedanbaek	4.8 ± 0.0fgh	nd	nd	68.4 ± 0.2 lm	116.9 ± 2.1 m	37.4 ± 0.5 l	11.2 ± 0.4 k	9.5 ± 0.1jkl	5.6 ± 0.1ij	455.3 ± 6.6 l	322.1 ± 4.8 k	79.6 ± 2.0j	1110.9 ± 16.4 l

The mean values in the same column indicated by the same letter are not significantly different at the level of 0.05 according to Duncan's multiple range test

[a] All values are shown as the mean ± standard deviation of three independent experiments

[b] nd: not detected

Table 2 Soyasaponin content of soy germ in 24 soybean cultivars

Cultivar	Ac	Bc′	Bd	Aa	Be	Ab	Bc	Ba	Bb	Bb′	Total (mg/100 g)[a]
Seonpung	nd[b]	nd	nd	nd	nd	3478.1 ± 81.3a	nd	35.6 ± 0.0b	68.6 ± 0.9def	nd	3582.3 ± 82.2a
Daepung	nd	nd	nd	nd	nd	2467.3 ± 42.8b	nd	36.1 ± 0.3b	73.4 ± 1.9 cd	nd	2576.9 ± 44.9b
Taeseon	nd	nd	nd	2195.2 ± 30.1a	nd	129.9 ± 2.2i	nd	45.4 ± 0.7a	136.5 ± 8.1a	nd	2507.0 ± 41.1bc
Pungsannamul	nd	nd	nd	nd	nd	2398.8 ± 11.7bc	nd	26.7 ± 0.5efgh	44.2 ± 0.3ij	nd	2469.7 ± 12.5bc
Daepung2ho	nd	nd	nd	nd	nd	2283.6 ± 57.9bc	nd	32.2 ± 0.3c	72.0 ± 1.3cde	nd	2387.8 ± 59.5bc
Socheongja	nd	nd	nd	2174.2 ± 23.9b	nd	102.4 ± 1.1i	nd	28.1 ± 0.3defgh	49.5 ± 1.1hi	nd	2354.2 ± 26.3c
Jinpung	nd	nd	nd	nd	nd	2254.8 ± 120.7c	nd	30.2 ± 0.6 cd	61.6 ± 1.3defgh	nd	2346.6 ± 118.7c
Cheongja4ho	nd	nd	nd	nd	nd	2020.4 ± 258.3d	nd	28.7 ± 2.4de	59.9 ± 8.6efgh	nd	2109.0 ± 269.2d
Taekwang	nd	nd	nd	1831.6 ± 30.7c	nd	111.0 ± 2.4i	nd	31.6 ± 1.1c	81.5 ± 0.6c	nd	2055.7 ± 34.8de
Daechan	nd	nd	nd	nd	nd	1822.2 ± 0.1e	nd	26.8 ± 0.2efgh	56.2 ± 0.8fghi	nd	1905.2 ± 0.7def
Chamol	nd	nd	nd	nd	nd	1794.5 ± 115.2e	nd	32.0 ± 1.4c	60.0 ± 4.5efgh	nd	1886.5 ± 121.1ef
Miso	nd	nd	nd	nd	nd	1731.4 ± 4.2e	nd	30.3 ± 0.5 cd	59.8 ± 0.9efgh	nd	1821.5 ± 2.8f
Cheongmiin	nd	nd	nd	nd	nd	1481.2 ± 10.5f	nd	29.8 ± 0.0 cd	61.6 ± 1.0defgh	nd	1572.6 ± 11.5 g
Haepum	nd	nd	nd	nd	nd	1509.2 ± 57.4f	nd	23.1 ± 0.2i	36.3 ± 0.9j	nd	1568.6 ± 58.5 g
Jungmo3012	nd	nd	nd	nd	nd	1442.0 ± 43.4 fg	nd	25.6 ± 0.7fghi	53.2 ± 1.5ghi	nd	1520.8 ± 45.6gh
Hwangkeumol	nd	nd	nd	nd	nd	1430.0 ± 67.0 fg	nd	28.4 ± 1.0defg	59.4 ± 8.2efgh	nd	1517.8 ± 76.3gh
Seonyu	nd	nd	nd	nd	nd	1407.4 ± 13.9 fg	nd	32.3 ± 0.2c	71.0 ± 1.5cde	nd	1510.6 ± 15.2gh
Saegeum	nd	nd	nd	nd	nd	1364.2 ± 260.8 fg	nd	35.4 ± 5.0b	99.9 ± 20.1b	nd	1499.5 ± 285.9gh
Haewon	nd	nd	nd	nd	nd	1418.8 ± 25.0 fg	nd	25.5 ± 0.3ghi	49.9 ± 0.7hi	nd	1494.2 ± 26.0gh
Soyeon	nd	nd	nd	nd	nd	1367.2 ± 8.6 fg	nd	25.8 ± 0.1efghi	55.8 ± 0.2ghi	nd	1448.9 ± 8.9gh
Daewon	nd	nd	nd	nd	nd	1326.7 ± 41.7 fg	nd	28.5 ± 0.4def	64.7 ± 2.0defg	nd	1420.0 ± 44.1gh
Cheongja3ho	nd	nd	nd	nd	nd	1264.0 ± 23.0gh	nd	25.9 ± 0.1efghi	49.1 ± 2.9hi	nd	1339.0 ± 20.0hi
Shinhwa	nd	nd	nd	nd	nd	1237.9 ± 31.6gh	nd	27.6 ± 0.2defgh	62.7 ± 2.0defg	nd	1328.2 ± 33.8hi
Saedanbaek	nd	nd	nd	nd	nd	1095.3 ± 20.1 h	nd	25.4 ± 0.1hi	52.8 ± 1.6ghi	nd	1173.5 ± 21.6i

The mean values in the same column indicated by the same letter are not significantly different at the level of 0.05 according to Duncan's multiple range test

[a] All values are shown as the mean ± standard deviation of three independent experiments

[b] nd: not detected

Alkaline phosphatases (ALP) staining and activity assay

The ALP activity of C2C12 cells was assessed using ALP staining and an ALP activity detection kit (Sigma-Aldrich, St. Louis, MO, USA). Briefly, C2C12 cells were cultured under osteogenic differentiation conditions in the presence of the vehicle, isoflavones, or soyasaponins. After differentiation for 3 d, the cells were washed twice with PBS, fixed with 10% formalin in PBS for 5 min, rinsed with deionized water, and stained with the ALP staining kit or measured using the one-step PNPP substrate solution (Thermo Scientific, Waltham, MA, USA).

Cell viability assay

The C2C12 cells were plated on 96-well plates (three replicate plates) at the density of 2.5×10^3 cells/well (C2C12 cells). After the treatment with the indicated concentrations of isoflavones and soyasaponins, the cells were incubated for 3 d, and cell viability was measured using the Cell Counting Kit 8 (CCK-8) according to the manufacturer's protocol. The CCK-8 assay kit was purchased from Dojindo Molecular Technologies (Rockville, MD, USA).

Statistical analysis

All quantitative values are presented as the mean ± standard deviation. Each experiment was performed three times. Several figures show the results from one representative experiment. Statistical differences were analyzed via Student's t test and Duncan's multiple-range test using the statistical analysis software (SAS) enterprise guide 7.1 (SAS Institute Inc., Cary, NC, USA).

Results and discussion

Isoflavone content in the germ of soybean cultivars

Isoflavone analysis in the germ of 24 Korean soybean cultivars was performed by UHPLC-DAD. Twelve isoflavones were detected in the SG (Additional file 1: Fig. S1). The total isoflavone content ranged from 1110.9 to 3131.1 mg/100 g and the highest total isoflavone content

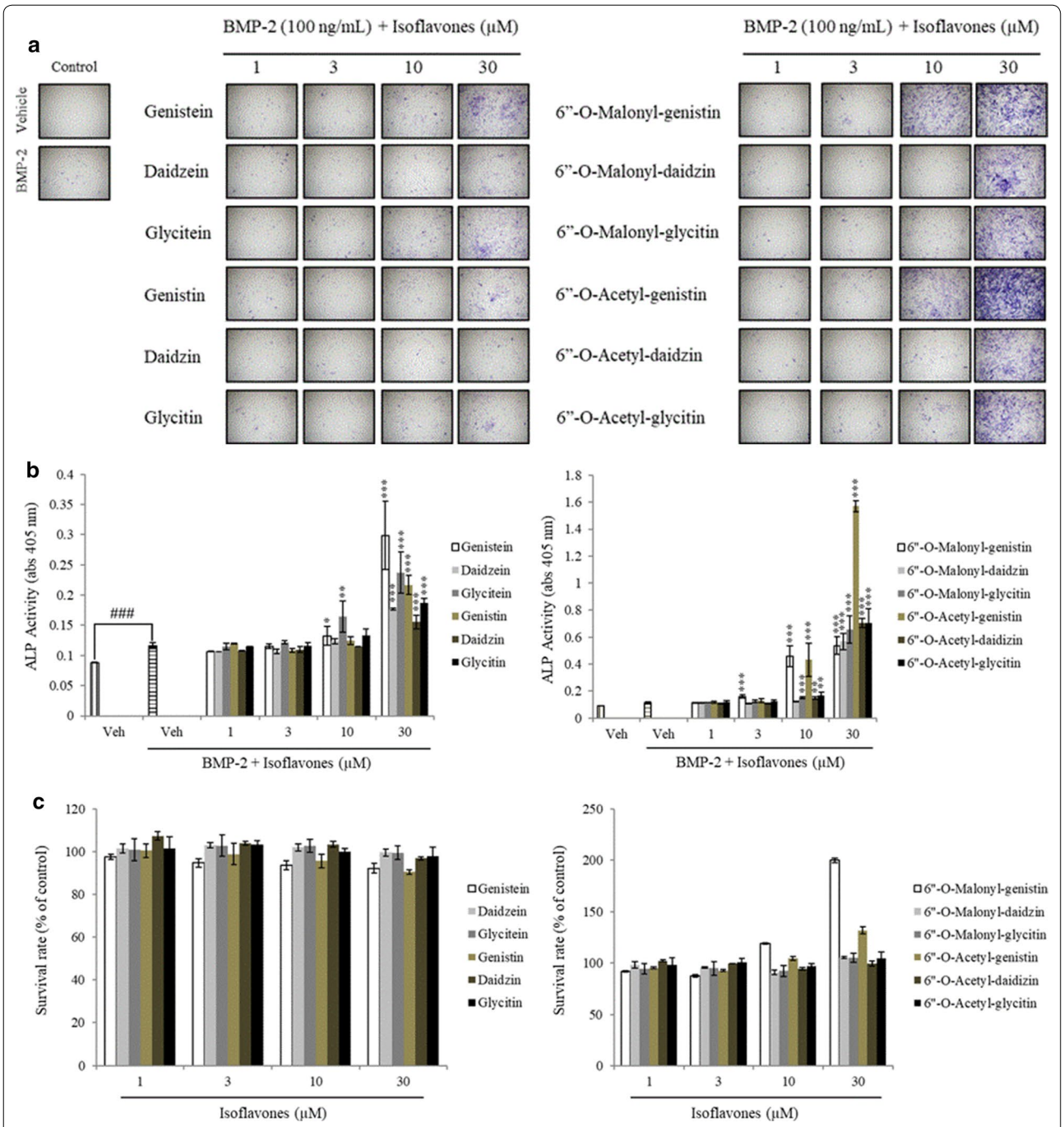

Fig. 1 Isoflavones enhance osteoblast differentiation. **a** C2C12 cells were cultured for 3 d with BMP-2 (100 ng/mL) with either the vehicle (DMSO) or the indicated concentration of isoflavones. Osteoblast differentiation was visualized by ALP staining. **b** The ALP activity was determined by measuring absorbance at 405 nm. ###$p < 0.001$ (versus control); ***$p < 0.001$ (versus BMP-2-treated group). **c** The effects of isoflavones on the C2C12 cell viability were evaluated by the CCK-8 assay. The data are shown as the mean ± SD and are representative of the three experiments

was in the Daepung2ho cultivar, whereas the lowest one was in the Saedanback cultivar. Among isoflavones, β-glycoside (daidzin, glycitin, and genistin) and malonyl-glycoside (malonyl-daidzin, malonyl-glycitin, and malonyl-genistin) isoflavones were the major compound in SG (Table 1). The range of isoflavone content has been reported to depend on soybean cultivars [5].

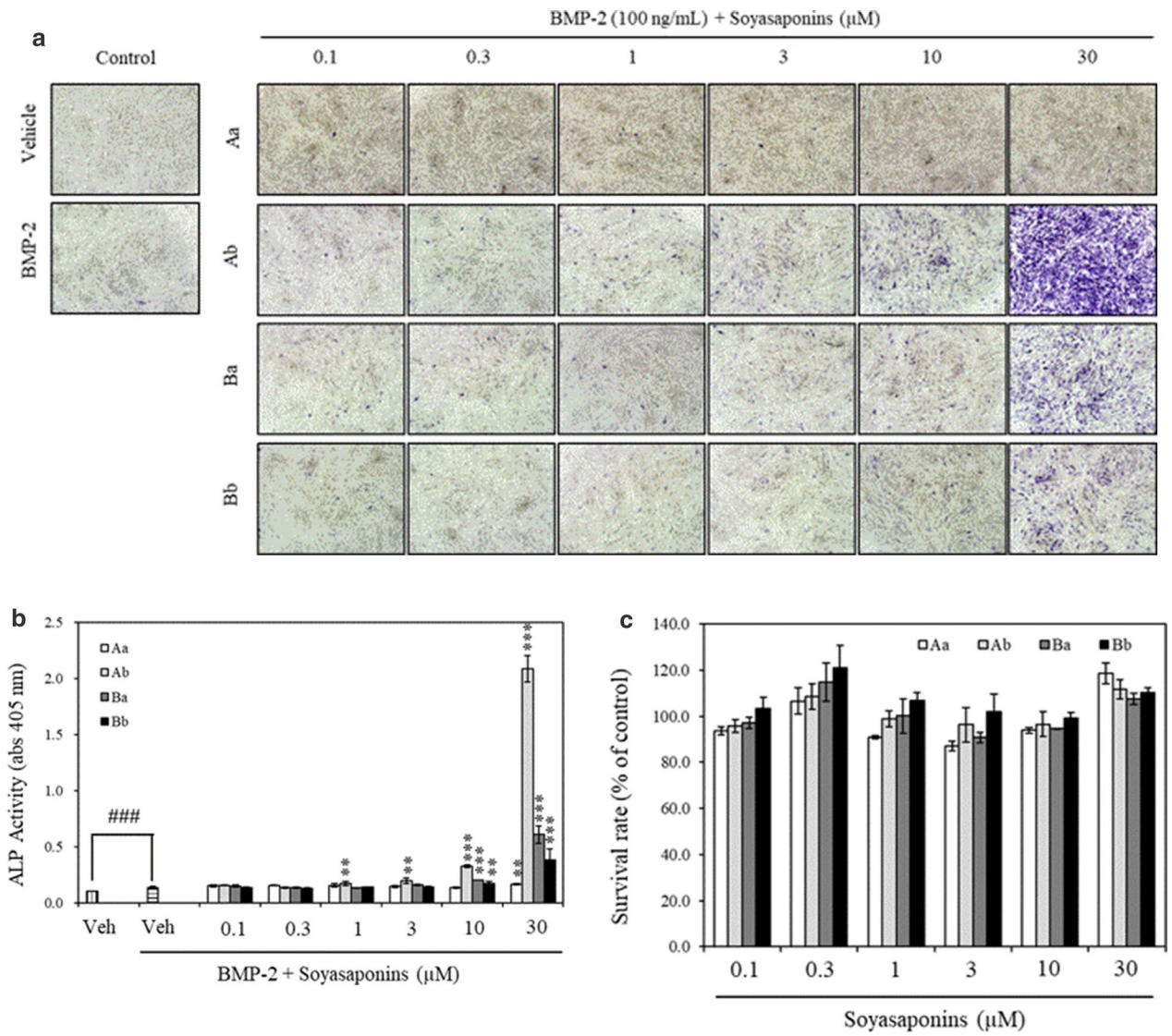

Fig. 2 Soyasaponins enhance osteoblast differentiation. **a** C2C12 cells were cultured for 3 d with BMP-2 (100 ng/mL) with either the vehicle (DMSO) or the indicated concentration of soyasaponins. Osteoblast differentiation was visualized by ALP staining. **b** The ALP activity was determined by measuring absorbance at 405 nm. ###$p < 0.001$ (versus control); ***$p < 0.001$ (versus BMP-2-treated group). **c** The effects of isoflavones on the C2C12 cell viability were evaluated by the CCK-8 assay. The data are shown as the mean ± SD and are representative of the three experiments

Soyasaponin content in the germ of soybean cultivars

Soyasaponin analysis in the germ of 24 Korean soybean cultivars was performed by UHPLC-CAD. Only four compounds out of 10 soyasaponin standards were detected (Additional file 1: Fig. S2); the total soyasaponin contents ranged from 1173.5 to 3582.3 mg/100 g; soyasaponin Aa content ranged from 1831.6 to 2195.2 mg/100 g; soyasaponin Ab content ranged from 102.4 to 3478.1 mg/100 g; soyasaponin Ba content ranged from 23.1 to 45.4 mg/100 g, and soyasaponin Bb contents ranged from 36.3 to 136.5 mg/100 g. The highest total soyasaponin content was in the Seonpung cultivar, whereas the lowest content was in the Saedanback cultivar (Table 2). These various ranges of soyasaponin content have been reported to depend on soybean cultivars [5]. The content of soyasaponins Ab and Aa was high in the total soyasaponin content and the soyasaponin phenotype in SG was largely divided into Aa and Ab (Table 2). These results were similar to those that have been previously reported [1, 2].

Isoflavone and soyasaponin in SG stimulate BMP-2-induced osteoblast differentiation in C2C12 cells

To study the effects of isoflavone and soyasaponin in SG on BMP-2-mediated osteogenesis, C2C12 cells were incubated with various concentrations of 12 isoflavones and 4 soyasaponins, followed by BMP-2 (100 ng/mL). As shown in Figs. 1a and 2a, isoflavones and soyasaponins induced ALP expression in a dose-dependent manner in the presence of BMP-2. Consistent with this result, isoflavones and soyasaponins considerably enhanced the BMP-2-stimulated ALP activity in a dose-dependent manner (Figs. 1b and 2b), especially soyasaponin Ab. Isoflavones and soyasaponins did not show cytotoxicity (Figs. 1c and 2c). Our study determined that Seonpung cultivar had a higher concentration of soyasaponin Ab than that in other cultivars (Table 2). The results suggest that Seonpung cultivar is promising functional food materials for preventing and improving bone loss disorders including osteoporosis. Further research is needed to examine the soyasaponin Ab content in Seonpung cultivar according to various environmental factors because phytochemical is influenced by the environmental factors [1].

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13765-020-00508-y.

Additional file 1: Figure S1. Chemical structures and representative chromatograms of isoflavones in the germ of 24 soybean cultivars analyzed by UHPLC-DAD. (a) Chemical structures of isoflavones, (b) isoflavone standards, and (c) Daepung2ho cultivar. The number of peaks is as follows: 1, daidzin; 2, glycitin; 3, genistin; 4, 6″-O-malonyl-daidzin; 5, 6″-O-malonyl-glycitin; 6, 6″-O-acetyl-daidzin; 7, 6″-O-malonyl-genistin; 8, 6″-O-acetyl-glycitin; 9, daidzein; 10, glycitein; 11, 6″-O-acetyl-genistin; and 12, genistein. **Figure S2.** Chemical structures and representative chromatograms of soyasaponins in the germ of 24 soybean cultivars analyzed by UHPLC-CAD. (a) Chemical structures of soyasaponins, (b) soyasaponin standards, and (c) Taeseon cultivar in soyasaponin Aa phenotype. (d) Daepung2ho cultivar in soyasaponin Ab phenotype. The number of peaks is as follows: 1, soyasaponin Ac; 2, soyasaponin Bc`; 3, soyasaponin Bd; 4, soyasaponin Aa; 5, soyasaponin Be; 6, soyasaponin Ab; 7, soyasaponin Bc; 8, soyasaponin Ba; 9, soyasaponin Bb; 10, soyasaponin Bb`. **Figure S3.** Calibration curve for isoflavone standards analyzed by UHPLC-DAD. **Figure S4.** Calibration curve for soyasaponin standards analyzed by UHPLC-CAD. **Table S1.** Extraction efficiency of soy germ extract in 24 soybean cultivars

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Authors' contributions

KSL contributed to the writing of the manuscript and performed the majority of data analysis. SYW performed the osteoblast differentiation study. MJL, HYK, and HMH performed minor experiments and prepared raw materials. DJL and SWC contributed to the discussion of experimental results. WDS planned and led this research. All authors read and approved the final manuscript.

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Competing interests

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