



Antioxidant Capacity and Isoflavone Content in Seeds of Five Edamame (*Glycine max* L. Merrill) Cultivars

LUTHER C. CARSON*¹, JOSHUA H. FREEMAN², KEQUAN ZHOU³,
AND GREGORY E. WELBAUM⁴

¹University of Florida, IFAS/SWFREC, 2685 State Road 29 North, Immokalee, FL 34142

²Eastern Shore AREC, Virginia Polytechnic Institute and State University, 33446 Research Drive, Painter, VA 23420

³Department of Nutrition and Food Science, Wayne State University, 410 West Warren Avenue, 3009 Science Hall, Detroit, MI 48202

⁴Department of Horticulture, Virginia Polytechnic Institute and State University, 306-B Saunders Hall, Blacksburg, VA 24061

ADDITIONAL INDEX WORDS. vegetable soybean, ORAC, DPPH

Edamame, immature or vegetable soybeans, has received growing attention in the US largely due to its purported health benefits from antioxidants and isoflavones. However, previous studies predominately focused on antioxidant capacity and isoflavone content of fully mature, dry, grain or agronomic soybeans. The purpose of this study was to measure the antioxidant and isoflavone contents of five commercially available edamame cultivars grown in Painter, VA in 2008 and 2009. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay results ranged from a low of 24.1 $\mu\text{mol/g}$ sample trolox equivalent (TE) for 'Midori Giant' to a high of 46.5 $\mu\text{mol/g}$ for 'Sunrise' in 2008. In 2009, DPPH assay results ranged from 13.0 $\mu\text{mol/g}$ TE for 'Midori Giant' to 18.0 $\mu\text{mol/g}$ TE for 'BeSweet2015'. In 2008, oxygen radical absorption capacity (ORAC) assay results ranged from 20.4 $\mu\text{mol/g}$ TE for 'Midori Giant' to 37.2 $\mu\text{mol/g}$ TE for 'BeSweet2015'; although there were no significant differences. In 2009, ORAC assay results ranged from 26.0 $\mu\text{g/g}$ TE for 'Sunrise' to 40.2 $\mu\text{g/g}$ TE for 'BeSweet292'. Total isoflavone content ranged from 144.6 to 529.2 $\mu\text{g/g}$ and 127.2 to 315.5 $\mu\text{g/g}$ in 2008 and 2009, respectively. Malonyl genistin was the most abundant isoflavone. Antioxidant and isoflavone contents varied by year and cultivar. 'Midori Giant' had relatively low antioxidant capacity both years, but the highest isoflavone content. 'BeSweet2015' and 'BeSweet2001' had relatively high antioxidant capacities, but lower isoflavone contents.

Edamame or vegetable soybeans are specialty cultivars of soybean harvested before physiological maturity when the pods are green, at the R6 stage of soybean development (Mebrahtu and Devine, 2008). They tend to have a green seed coat, but may range from yellow to black with gray or light color hilum, white pubescence on the pods, and large seed size (Konovsky et al., 1994). Edamame flavor has been described as sweet, nutty, flowery, and buttery (Wszelaki et al., 2005).

Edamame has been gaining popularity in the United States, likely due to the nutritional quality of edamame, purported human health benefits, and flavor (Kelly and Sanches, 2005; Wszelaki et al., 2005). Edamame sales in the US increased from \$18 million in 2003 to \$41 million in 2008 (Bernick, 2009; Soyatech, 2010).

Isoflavones, a class of polyphenolic compounds, have been linked to reduced prostate cancer, reduced menopausal symptoms, and decreased risk of heart disease (Messina, 2001). The health benefits from isoflavones may be due to their estrogenic effects and antioxidant capacity (Lee et al., 2004). There are twelve isoflavone conjugates; three aglycones (genistein, glycitein, and daidzein), three 7-O-glucosides (genistin, glycitin, and daidzin), three 6-O-malonyl glucosides, and three 6-O-acetyl glucosides (Wang and Murphy, 1994).

Soybean antioxidant capacity is provided by different com-

pounds including flavonoids, phenolics, tannins, proanthocyanidins, and other polyphenolics (Malencic et al., 2007). Soybean antioxidant capacity varies by cultivar, postharvest storage, growing environment, and type (Lee et al., 2003). Many studies have measured the antioxidant capacity of agronomic and food grade soybeans using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) quench and the Oxygen Radical Absorbing Capacity (ORAC) assays, but few have investigated edamame (Chung et al., 2008; Lee et al., 2004; Xu and Chang, 2007). The purpose of this study was to determine isoflavone contents and antioxidant capacity of five commercially available edamame cultivars.

Material and Methods

Five edamame cultivars [BeSweet 292 (BS292), BeSweet 2015 (BS2015), BeSweet 2001 (BS2001), Midori Giant (MG), and Sunrise (SR)] were grown in Painter, VA in 2008 and 2009 (Carson et al., 2011). Bean samples were collected from each plot and frozen at -20°C after harvest. Beans were then lyophilized and ground to pass through a 20-mesh sieve using a Mini-Mill (Thomas Wiley, Swedesboro, NJ).

To extract antioxidants, 2 g of dry, defatted beans and 20 mL of 50% acetone were placed in a 30-mL test tube. Test tubes were vortexed for 1 min every 30 min for 2 h and incubated in the refrigerator for 24 h.

*Corresponding author; phone: (239) 658-3400; email: lutherc@ufl.edu

The DPPH radical scavenger assay measures a sample's antioxidant capacity compared to Trolox™ (6-hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid). A serial dilution was prepared as 0, 5, 10, 20, 40, 80, and 100 µM Trolox in 50% acetone (Chung et al., 2008). In a clear well plate, 100 µL of 0.2 mM DPPH was mixed with 100 µL of the Trolox serial dilution or bean extract diluted 3-fold, in triplicate. The absorbance was measured at 515 nm by a plate reader (Victor3, Perkin Elmer, Waltham, MA). The reading was taken every 5 min for 30 min. Results were reported as Trolox equivalence (TE) (µmoles/g sample).

The ORAC assay was also used to assess antioxidants (Chung et al., 2008). Trolox was prepared as 0, 5, 10, 20, 40, 80, and 100 µM in 50% acetone. Briefly, 40 µL of the Trolox standard or bean extract (after 100-fold dilution) was added to 200 µL of 158 µM fluorescein in a 96-well black plate. The plate was incubated for 20 min at 37 °C and 35 µL of 0.36 M 2,2'-azobis(2-amidinopropane) dihydrochloride solution was added to each well. The plate was read every minute for 30 min using an excitation wavelength of 485 nm and emission wavelength of 535 nm. The results were expressed as TE (µmoles/g sample).

Isoflavones were extracted from dry-milled beans using 0.1 N hydrochloric acid, acetonitrile, and distilled water (2:7:3, v/v/v) (Chung et al., 2008). A 0.5-g sample of ground beans was added to 12 mL of extraction solution and vortexed for 1 min and shaken overnight. The effluent was filtered using Whatman #6 filter paper. In a rotary evaporator, 4 mL of bean solution was dried in a 10-mL test tube. The residue was reconstituted with 1 mL of methanol and filtered with a 0.45-µm Whatman disk filter. An isoflavone profile of the soybean extract was performed by HPLC using a C18 column (250 mm × 4.6 mm, particle size 5 µm) with a mobile phase of solvent A (0.1% glacial acetic acid in H₂O) and B (0.1% glacial acetic acid in acetonitrile). The mobile phase flow rate of 1.0 mL/min with a linear gradient was programmed

from 15 to 35% solvent B in 50 min. The individual isoflavones and their concentrations were determined by comparing their retention time and area under curve to the isoflavone standards.

Data were analyzed using analysis of variance. Year, cultivar, and respective nutritional constituent were main effects. The fit model platform in JMP (version 8.0; SAS Institute, Cary, NC) was used for statistical analysis. Means were separated using Tukey's HSD ($P \leq 0.05$)

Results

In 2008, the DPPH radical scavenging activities of 'SR', 'BS2015', and 'BS2001' were not different and greater than 'MG' at 24.1 µmol/g TE (Fig. 1); 'BS292' was not different from any other cultivar in 2008. In 2009, 'BS2015' and 'BS2001' had greater DPPH radical scavenging ability than 'BS292', 'MG', and 'SR'.

In 2008, there were no significant differences among the cultivars in their ability to quench peroxy radicals, which ranged from 20.4 to 37.2 µmol/g TE (Fig. 2). In 2009, 'BS292' was significantly greater than 'SR' with ORAC values of 40.2 and 26.0 µmol/g TE, and neither were different from 'MG', 'BS2015', and 'BS2001'.

In all cultivars except 'SR', the total isoflavone concentration was greater in 2008 than in 2009, ranging from 144.6 to 529.2 µg/g in 2008 and 127.2 to 315.5 µg/g in 2009 (Table 1). In 2008 and 2009, 'MG' had the greatest total isoflavone content at 529.2 µg/g and 344.4 µg/g, respectively. In 2008, 'SR', 'BS2015', and 'BS2001' had the lowest total isoflavone content, while 'BS292' was intermediate. In 2009, 'SR' and 'BS292' had greater total isoflavone contents than both 'BS2015' and 'BS2001'.

The concentration of glucosides was greater than aglycones in all but three samples ('BS292' in both years and 'SR' in 2009). In 2008, 'SR' had the greatest daidzin content with 77.8 µg/g, and 'MG' and 'BS292' had the lowest daidzin contents (Table

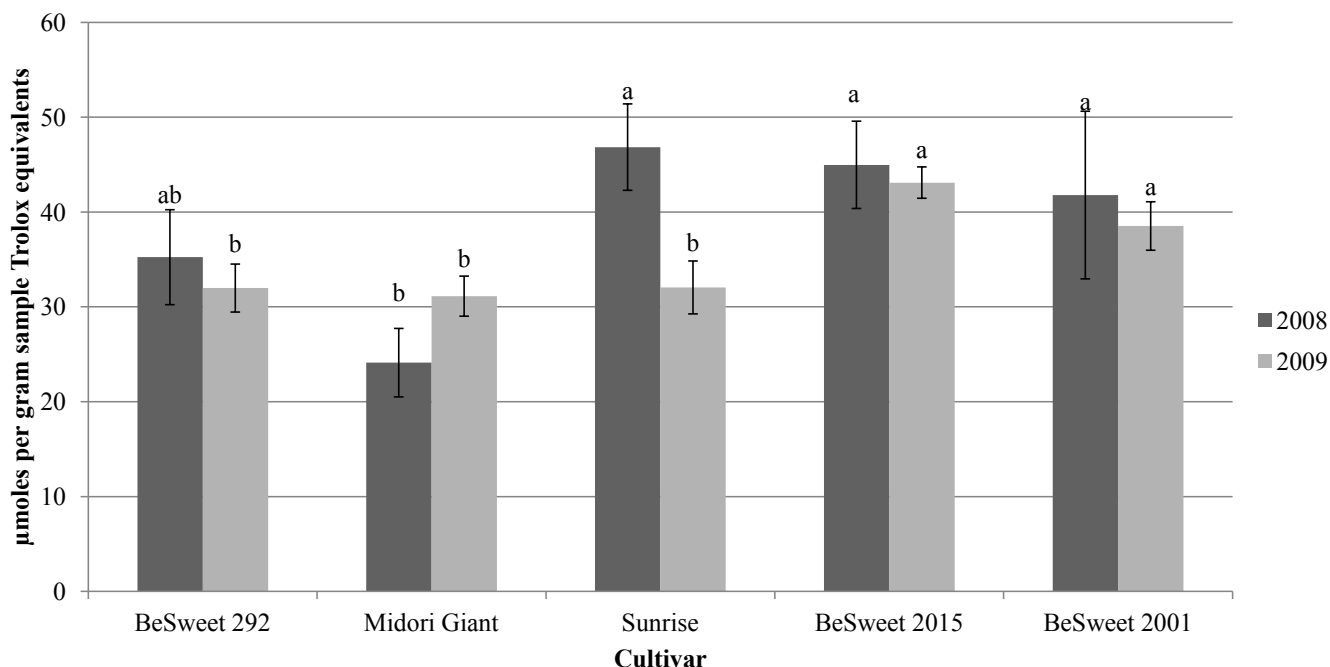


Fig. 1. DPPH radical quench assay was used to determine radical scavenging ability of five edamame cultivars grown during 2008 and 2009 in Painter, VA. The values are given in µmoles Trolox equivalence per gram sample. Columns are the average of four samples (n=4) and are to be compared within year. Columns denoted by the same letter are not different ($P \leq 0.05$) according to Tukey's HSD.

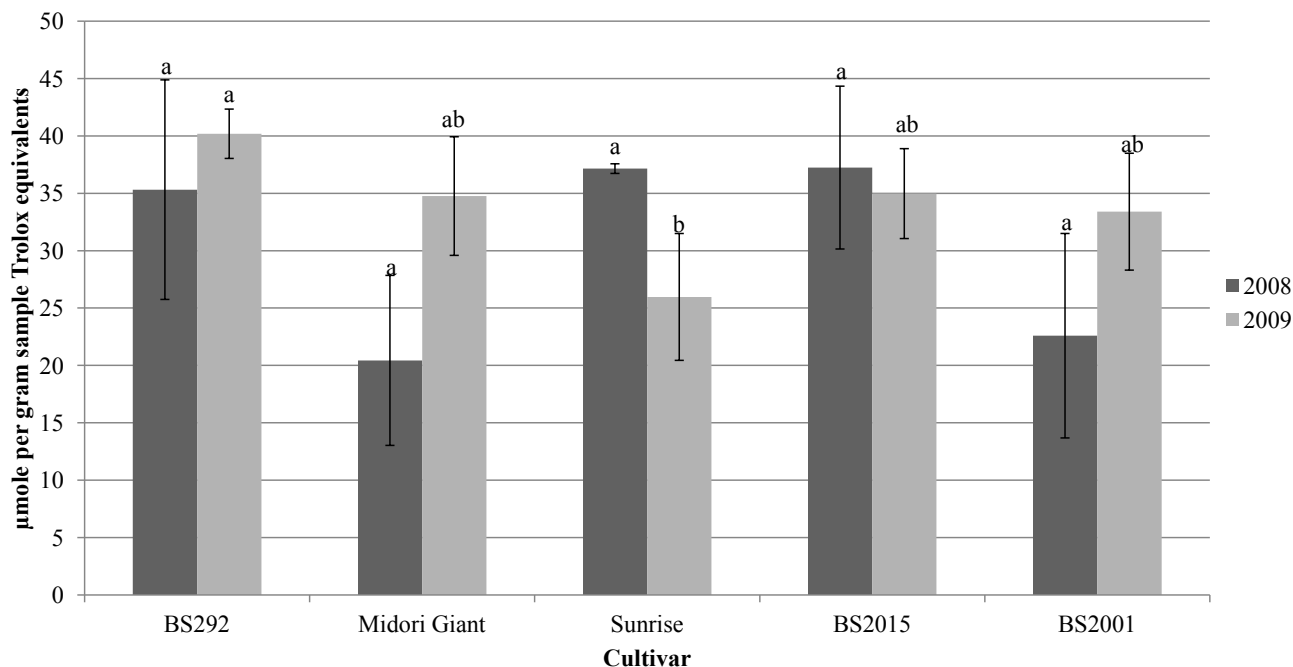


Fig. 2. Oxygen radical absorbing capacity (ORAC) assay was performed on five edamame cultivars grown in Painter, VA during 2008 and 2009. The values are given as μ moles of Trolox equivalent per gram sample. Columns are the average of three samples ($n=3$) and are to be compared by year. Columns denoted by the same letter are not different ($P \leq 0.05$) according to Tukey's HSD.

1). In 2009, daidzin was not detected in 'BS292' and the remaining cultivars were not statistically different and daidzin content ranged from 2.1 to 3.7 μ g/g.

In 2008, 'SR' had the greatest genistin content followed in descending order by 'BS2015', 'BS2001', 'MG', and 'BS292' (Table 1). In 2009, 'MG' had the greatest genistin content followed by 'SR', which was greater than both 'BS2015' and 'BS2001'. Genistin was not detected in 'BS292' in 2009.

In 2008, daidzein was only detected in 'MG', which contained 3.0 μ g/g. In 2009, the highest daidzein concentrations were found in 'BS2001', followed by 'BS292', 'SR', 'MG', and 'BS2015' in descending order.

'MG' and 'BS2001' had the greatest concentration of glycitein in 2008 and 'SR' and 'BS2015' had the lowest concentration of

glycitein (Table 1). In 2009, glycitein was detected only in 'SR' with 28.1 μ g/g.

Genistein was not detected in 2008 (Table 1). In 2009, 'MG' had greater genistein concentration than 'BS292', and genistein was not detected in the remaining cultivars.

Malonyl genistin was the most abundant isoflavone in all the soybean cultivars except SR in 2008 (Table 1). 'MG' had the highest malonyl genistin concentration in both 2008 and 2009 with 469.7 and 334.3 μ g/g, respectively. In 2008, 'BS292' contained 265.5 μ g/g malonyl genistin, which was greater than both 'BS2015' and 'BS2001'. 'SR' had the lowest malonyl genistin concentration with 26.5 μ g/g in 2008. In 2009, 'BS292' and 'SR' had the second highest malonyl genistin concentration, although 'SR' was not different from 'BS2015' and 'BS2001'. Standards

Table 1. Isoflavone concentration (μ g/g) of an edamame cultivar evaluation trial by year and cultivar. The cultivar evaluation trial was grown in Painter, VA during 2008 and 2009.

	Glucoside		Aglycone			Malonyl	Total
	Daidzin	Genistin	Daidzein	Glycitein	Genistein	Genistin	
Cultivar 2008							
BeSweet 292	4.2 c ^z	4.0 d	ND ^y	16.6 b	ND	265.5 b	290.3 b
Midori Giant	17.9 c	12.4 c	3.0 a	26.3 a	ND	469.7 a	529.2 a
Sunrise	77.8 a	31.4 a	ND	8.9 c	ND	26.5 d	144.6 c
BeSweet 2015	50.6 b	24.9 b	ND	13.9 bc	ND	73.5 c	162.8 c
BeSweet 2001	44.4 b	16.2 c	ND	19.7 ab	ND	76.2 c	156.4 c
Cultivar 2009							
BeSweet 292	ND	ND	3.1 b	ND	1.4 b	200.3 b	204.8 b
Midori Giant	2.7 NS ^y	2.7 a	2.5 cd	ND	2.1 a	334.3 a	344.3 a
Sunrise	2.4 NS	2.3 b	2.9 bc	28.1 a	ND	177.9 bc	213.6 b
BeSweet 2015	2.1 NS	1.5 c	2.5 d	ND	ND	119.3 c	125.4 c
BeSweet 2001	3.7 NS	1.7 c	3.6 a	ND	ND	126.6 c	135.5 c

^zData in columns denoted by the same letter are not different according to Tukey's HSD ($P=0.05$). Means are to be compared within column and year.

^yNS= nonsignificant; ND= not detected.

for the acetyl and other malonyl and glucoside species could not be obtained to quantify results.

For the DPPH and ORAC assays, there were significant interactions between year and cultivar ($P = 0.0012$ and $P = 0.013$, respectively). There were also significant three-way interactions between year, cultivar, and individual isoflavone ($P < 0.0001$), and two-way interactions between cultivar and year ($P < 0.0001$), cultivar and isoflavone ($P < 0.0001$) and year and isoflavone ($P < 0.0001$). Interactions occurred in the DPPH assay, because 'MG' and 'Sunrise' did not perform similarly to the other genotypes. 'MG' increase in TE when all the others decreased, and 'SR' decreased by a much larger percentage than the other three cultivars that decreased. The ORAC assay performed much differently among cultivars, with three cultivars' TE increasing in various amounts and two cultivars' TE decreasing by different amounts, leading to interactions. The three-way interactions between year, cultivar, and isoflavone were expected because year was significant ($P < 0.0001$) and the individual isoflavones differed among years but not in a consistent pattern.

Discussion

All cultivars except 'MG' had lower TE in the DPPH assay in 2009 than in 2008. Compounds associated with antioxidant content change in concentration with harvest maturity, growing environment, and location (Dolde et al., 1999). Edamame showed a higher DPPH radical scavenging activity than previously reported for fully mature agronomic grain soybeans (Chung et al., 2008; Xu and Chang, 2007). Antioxidant activity decreased as the soybeans matured past the R5 stage of development (Kumar et al., 2009).

The ORAC and DPPH assays involve different radicals and interaction mechanisms, so different antioxidant capacities were expected (Chung et al., 2008). Whent et al. (2009) found for agronomic soybeans that environment was the most important main effect for ORAC values, describing 55.8% of the variation. The ORAC values of edamame samples in this study were in line with the ORAC range for agronomic soybeans reported by Whent et al. (2009) but lower than ORAC values for agronomic soybeans and similar to the values for food grade soybeans reported by Xu and Chang (2008).

Growing seasons accounted for from 16.6 to 400% differences in isoflavone concentrations in one study (Eldridge and Kwolek, 1983). Isoflavone content of soybeans may be reduced by increasing temperature and reduced UV light interception, and increased by drought (Sakthivelu et al., 2008; Swinny and Ryan, 2005; Tsukamoto et al., 1995). The difference in isoflavone content year-to-year was likely due to yearly variations in temperature, which was 0.47 °C warmer during the growing season in 2009 than 2008, and shading, due to weed competition.

Isoflavone content of the edamame was lower than previously reported for agronomic soybean (Chung et al., 2008; Yin and Vyn, 2005). The lower isoflavone content of edamame when compared to agronomic soybeans is likely due to its earlier harvest. Berger et al. (2008) and Kim and Chung (2007) have shown that isoflavone accumulation in developing soybeans peaks after the edamame harvest maturity stage.

Wang and Murphy (1994) reported that malonyl genistin, genistin, malonyl daidzin, and daidzin were the most abundant isoflavones in Iowa-grown grain soybeans. Similarly, malonyl genistin was the predominate isoflavone found in this study. Malonyl genistin made up as much as 97.8% of the total isoflavones. However, malonyl genistin was not the most abundant

isoflavone in 2008 for 'SR' (18.3%), 'BS2015' (45.1%), and 'BS2001' (48.7%). In the other cultivars, malonyl genistin made up more than 83.3% of total isoflavones. In cultivars in which malonyl genistin did not predominate, daidzin and genistin made up between 28.4% to 53.8% and between 10.4% and 21.7% of the total isoflavones, respectively. Outside of those three cultivars, daidzin, genistin, daidzein, and genistein made up fewer than 3.4% of the total isoflavones. When acetyl glucosides were measured, they contributed less than 2.6% to total isoflavones (Kim and Chung, 2007; Wang and Murphy, 1994). Often, acetyl glucosides were not measured (Berger et al., 2008; Chung et al., 2008; Kumar et al., 2009; Lee et al., 2003; Yin and Vyn, 2005). Kim and Chung (2007) measured all 12 isoflavone conjugates and found that, from the R6 to R7 stages of development, the concentration of each isoflavone changed. Thus, relative harvest maturity affects isoflavone content.

Cultivars and environmental factors can significantly affect the amount of antioxidants, including isoflavones, in developing soybean seeds. 'Midori Giant' had relatively low antioxidant capacity both years, but the highest isoflavone content. 'BeSweet2015' and 'BeSweet2001' had relatively high antioxidant capacities, but lower isoflavone contents. Relative to reported isoflavone contents of agronomic grain soybeans, edamame contains fewer isoflavones, likely because it is harvested when immature.

Literature Cited

- Berger, M., C.A. Rasolohery, R. Cazalis, and J. Dayde. 2008. Isoflavone accumulation kinetics in soybean seed cotyledons and hypocotyls: Distinct pathways and genetic controls. *Crop Sci.* 48:700–708.
- Bernick, K. 2009. Edamame takes root in U.S. *Corn and Soybean Dig.* Penton Media Inc., Overland Park, KS.
- Carson, L.C., J.H. Freeman, K. Zhou, G.E. Welbaum, and M. Reiter. 2011. Cultivar evaluation and lipid and protein contents of Virginia-grown edamame. *HortTechnology* 21:131–135.
- Chung, H., S. Hogan, L. Zhang, K. Rainey, and K. Zhou. 2008. Characterization and comparison of antioxidant properties and bioactive components of Virginia soybeans. *J. Agr. Food Chem.* 56:11515–11519.
- Dolde, D., C. Vlahakis, and J. Hazebroek. 1999. Tocopherols in breeding lines and effects of planting location, fatty acid composition, and temperature during development. *J. Amer. Oil Chem. Soc.* 76:349–355.
- Eldridge, A.C. and W.F. Kwolek. 1983. Soybean isoflavones: Effect of environment and variety on composition. *J. Agr. Food Chem.* 31:934–936.
- Kelley, K.M. and E.S. Sanchez. 2005. Accessing and understanding consumer awareness of and potential demand for edamame. *HortScience* 40:1347–1353.
- Kim, J.-A. and I.-M. Chung. 2007. Change in isoflavone concentration of soybean (*Glycine max* L.) seeds at different growth stages. *J. Sci. Food Agr.* 87:496–503.
- Konovsky, J., T.A. Lumpkin, and D. McClary. 1994. Edamame: The vegetable soybean, p. 173–181. In: A.D. O'Rourke (ed.). *Understanding the Japanese food and agrimarket: A multifaceted opportunity.* Binghamton, Hayworth, UK.
- Kumar, V., A. Rani, A.K. Dixit, D. Bhatnagar, and G.S. Chauhan. 2009. Relative changes in tocopherols, isoflavones, total phenolic content, and antioxidative activity in soybean seeds at different reproductive stages. *J. Agr. Food Chem.* 57:2705–2710.
- Lee, S.J., J.K. Ahn, S.H. Kim, J.T. Kim, S.J. Han, M.Y. Jung, and I.M. Chung. 2003. Variation in isoflavone of soybean cultivars with location and storage duration. *J. Agr. Food Chem.* 51:3382–3389.
- Lee, J., M. Renita, R.J. Fioritto, S.K. St. Martin, S.J. Schwartz, and Y. Vonovotz. 2004. Isoflavone characterization and antioxidant activity of Ohio soybeans. *J. Agr. Food Chem.* 52:2647–2651.
- Malencic, D., M. Popovic, and J. Miladinovic. 2007. Phenolic content and antioxidant properties of soybean (*Glycine max* L. Merr.) seeds. *Molecules* 12:576–581.

- Mebrahtu, T. and T.E. Devine. 2008. Combining ability analysis for selected green pod yield components of vegetable soybean genotypes (*Glycine max*). *N.Z. J. Crop Hort. Sci.* 36:97–105.
- Messina, M. 2001. An overview of the health effects of soyfoods and soybean isoflavones, p. 117–122. In: T.A. Lumpkin and S. Shanmugasundaram (eds.). 2nd Intl. Veg. Soybean Conf., Wash. State Univ., Pullman.
- Sakthivelu, G., M.K. Akitha Devi, P. Giridhar, T. Rajasekaran, G.A. Ravishankar, M.T. Nikolova, G.B. Angelov, R.M. Todorova, and G.P. Kosturkova. 2008. Isoflavone composition, phenol content, and antioxidant activity of soybean seeds from India and Bulgaria. *J. Agr. Food Chem.* 56:2090–2095.
- Soyatech. 2010. Soy foods: The US market 2009. Soyatech, Southwest Harbor, ME.
- Swinny, E. and K.G. Ryan. 2005. Red clover *Trifolium pretense* L. phytoestrogens: UV-B radiation increases isoflavone yield, and postharvest drying methods change the glucoside conjugate profiles. *J. Agr. Food Chem.* 53:8273–8278.
- Tsukamoto, C., S. Shimada, K. Igita, S. Kudou, M. Kokubun, K. Okubo, and K. Kitamura. 1995. Factors affecting isoflavone content in soybean seeds: Changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. *J. Agr. Food Chem.* 43:1184–1192.
- Wang, H-J. and P.A. Murphy. 1994. Isoflavone content in commercial soybean foods. *J. Agr. Food Chem.* 42:1666–1673.
- Whent, M., J. Hao, M. Slavin, M. Zhou, J. Song, W. Kenworthy, and L. Yu. 2009. Effects of genotype, environment, and their interaction on chemical composition and antioxidant properties of low-linolenic soybeans grown in Maryland. *J. Agr. Food Chem.* 57:10163–10174.
- Wszelaki, A.L., J.F. Delwiche, S.D. Walker, R.E. Liggett, S.A. Miller, and M.D. Kleinhenz. 2005. Consumer liking and descriptive analysis of six varieties of organically grown edamame-type soybean. *Food Qual. Pref.* 16:651–658.
- Xu, B.J. and S.K.C. Chang. 2007. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *J. Food Sci.* 72:S159–S166.
- Xu, B.J. and S.K.C. Chang. 2008. Characterization of phenolic substances and antioxidant properties of food soybeans grown in the North Dakota–Minnesota region. *J. Agr. Food Chem.* 56:9102–9113.
- Yin, X. and T.J. Vyn. 2005. Relationship of isoflavones, oil, and protein in seed with yield of soybean. *Agron. J.* 97:1314–1321.