Quality of Lipoxygenase-null Vegetable Soybean Lines at Vegetable and Grain Stages

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Introduction

Soybean (*Glycien max* L.) seed contains a protein content of 40% and oil content of 20% and, in combination with cereals, provides a balanced diet that meets most human nutritional requirements. Increased human consumption of soybean could help to alleviate malnutrition, which occurs in many developing countries. Soybean seed can be consumed at either vegetable (R6) or grain stages (R8), depending upon utilization. However, acceptance of soybean has been limited in areas where people reject the unique soybean flavor often referred to as "beany flavor", which is mainly governed by lipoxygenase isozymes. Lipid peroxidation was also one of the factors limiting soybean consumption. Several lipoxygenase-null grain soybean lines were developed in Japan and were reported to have a reduced beany flavor (Kobayashi et. al., 1995) and increased oil stability (Nishiba and Suda, 1998). AVRDC has developed lipoxygenase-null vegetable soybean lines using AGS 292 as the recurrent parent (Shanmugasundaram et. al., 1998). AGS 292 has been released by Kaohsiung District Agricultural Improvement Station as 'Kaohsiung No. 1' and is widely grown throughout Taiwan and Thailand. The objective of our study was to evaluate the AVRDC lipoxygenase-null lines for contents of major- and micro-constituents, beany flavor and protein recovery rate of soybean milk and to compare them to the AGS 292.

Materials and Methods

Soybean materials

Four lines of the triple null genotype *lx1lx1lx2lx2lx3lx3:* GC96019-6, GC96019-9, GC96019-10, and GC96019-11 (isozyme group L0); five lines with the genotype *lx1lx1lx2lx2Lx3Lx:* GC96019-86, GC96019-96, GC96020-51, GC96020-54 and GC96020-57 (isozyme group L3); and AGS 292, *Lx1Lx1Lx2Lx2Lx3Lx3* (isozyme group L123) were planted in four replications at AVRDC in March 2001. Seeds were harvested at stages R6 and R8 (Fehr et. al., 1971). Two lines each from L0 and L3 isozyme groups were selected for evaluation of beta-carotene, vitamin C, folic acid and isoflavone contents. Eight vegetable and grain soybean lines harvested at AVRDC in the autumn of 2000 were selected to test the relation of soybean milk protein recovery to seed size.

NIRS analysis

Composition of dry matter, protein, oil, sugar, starch, fiber and boiled seed texture (presented as hardness and expressed by the grams of force required to break the seed) were evaluated using Near Infrared Spectroscopy (NIRS). The calibration statistics have shown a high correlation coefficient for prediction of the above constituents and seed hardness (AVRDC, 1989). Vegetable soybeans were blanched, frozen, dried at 45℃ for two days, ground into a fine powder, and subjected to nutritional quality evaluation. Grain soybeans were ground into powder for NIRS analysis.

Statistical analysis

Data were analyzed using the Analysis of Variance (ANOVA) procedure of SAS according to a Randomized Complete Block Design (RCBD). The varietal effect was partitioned using orthogonal contrasts to compare the different isozyme groups.

Micro-constituent analysis

Folic acid content was measured by microbiological assay using *Enterococcus hirae.* Isoflavone and β-carotene analysis was performed by High Pressure Liquid Chromatography (HPLC) method with reversed phase column. Ascorbic acid content was determined by colorimetric method using 2,4-dinitrophenylhydrazine.

Beany flavor and protein recovery

Head-space flavors were analyzed with Gas Chromatography (GC) and the intensity of beany flavors was presented as a value of total peak areas. Soymilk (seeds:water, 1:9, wt/wt) was prepared in a small-scale from mature seeds ranging in weight from about 10 to 40 g/100 seeds. Protein content determination was carried out by the Kjeldahl method. Protein recovery rate was expressed by the protein content of milk extracted against protein content of seeds used.

Results

Nutritional and eating qualities

Except for seed hardness, no statistically significant differences in nutritional qualities were found between L123 and Group L0 and between L123 and Group L3 within harvest stages (Table 1). However, significant differences in protein and oil contents were detected among lines within Group L3 at both vegetable and grain stages.

Folic acid content was over four times greater in seeds at grain stage compared to the vegetable stage for all isozyme groups. The three groups showed similar amounts of folic acid in their grain stage seeds. Soybean has been reported to be rich in folic acid. In spite of a lower concentration at R6 stage, vegetable soybean is a good folic acid source compared with other vegetables ranged from 10 μg/g in cabbage to 130 μg/g in spinach (AVRDC, 2001).

Contents of vitamin C and β-carotene were similar levels among groups at the vegetable stage. Compared to the grain stage, seeds at R6 stage have similar major composition, more vitamin A and C, and without flatulence caused by oligosaccharides normally occurred in grain soybean seeds.

Similar isoflavone contents were found among isozyme groups within stages (Table 2). Isoflavones accumulated continuously during seed maturation, which is coincide the report of Graham (1991). Contents of daidzin and genistin, the glycoside isoflavones, were about two times greater at the fresh grain stage compared to the vegetable stage. After one year of storage, daidzin and genistin in contents were double those of the fresh grain and four times higher than the vegetable stage. The above trend was not detected for daidzein and genistein, which were reported to be associated with inhibition of cancer development (Barnes, 1995). Additional studies are needed to determine ways to process grain soybean seeds to achieve higher contents of daidzein and genistein.

Table 1. Comparison of soybean isozyme groups for seed qualities at vegetable and grain stages

	L123		L0		L3	
	Vegetable	Grain	Vegetable	Grain	Vegetable	Grain
NIRS analysis[a] *(%)*						
Dry matter	29.8	93.8	29.6	94.5	29.6	94.4
Protein	39.6	39.1	39.6	39.1	39.5	38.8
Oil	24.1	23.3	23.8	23.4	24.1	23.7
Sugar	12.1	NT	12.2	NT	11.7	NT
Starch	8.5	NT	8.9	NT	9.1	NT
Fiber	5.8	NT	5.8	NT	5.8	NT
Hardness (g)	17.4	NT	17.2	NT	17.1	NT
Folic acid (μg/100g)	89±3	459±26	105±20	481±91	112±26	486±55
b-carotene (mg/100g)	0.22	NT	0.19	NT	0.19	NT
Vitamin C (mg/100g)	29	NT	30	NT	28	NT

[a]: on dry weight basis
NT: not tested.

Table 2. Isoflavone contents (μg/g in dry wt base) at vegetable and grain stages

	Vegetable			Fresh grain			Aged grain[a]		
	L123	L0	L3	L123	L0	L3	L123	L0	L3
Daidzin	121	116±20	115±22	254	265±12	251±14	420	502±45	481±97
Genistin	73	58±12	67±27	207	187±13	179±27	289	306±24	297±64
Daidzein	22	21±1	23±6	20	22±2	20±5	35	29±7	26±4
Genistein	71	8±4	11±8	10	14±7	12±0	22	16±3	17±9

[a]: seeds were harvested in May 2000 and stored for one year under room temperature.

Beany flavor and protein recovery

In taste test, the beany flavors of soymilk made from L0 and L3 lines were less than that made from the L123 line (preliminary data not shown). Flavor intensities of L0 and L3 were weaker than that of L123 according to their lower total peak area, which were about 43% and 53% less than L123, respectively. Beany flavors are mainly produced when making soybean milk. Heat treatment is generally applied during extraction of protein to eliminate the beany flavors, but this treatment reduces protein yield. Therefore lipoxygenase-null lines can be used without heat treatment during protein extraction to improve protein yield.

Furthermore, large-seeded (about 20g /100 seeds) soybean cultivars are favored for tofu-making and receive higher prices due to their higher protein yield (Carter et. al., 1998). The high correlation between protein recovery and seed size of grain soybean has been reported (Hong, 1994). Seeds of vegetable soybean lines usually exceed 25 g/100 seeds. In this study we compared five grain soybean lines for protein recovery of soymilk. Protein recoveries of 78-82% were measured for lines with seed sizes ranging from 13 to 35 g/100 seeds (Fig. 1). NPT2 with a seed size of 40 g per100 seeds had the highest protein recovery. This could suggest that vegetable soybean lines have potential use as grain soybean in processing.

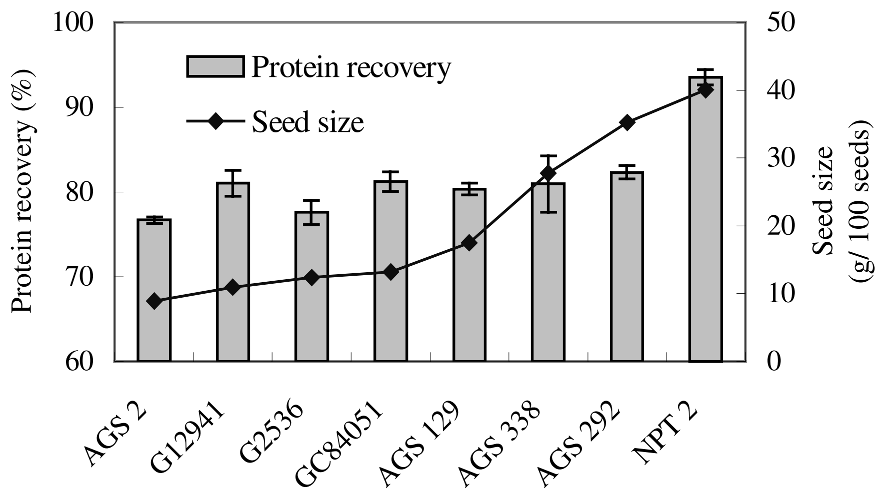

Figure 1. Protein recovery of soymilk made with seeds of varied seed sizes

Conclusions

The lipoxygenase-null vegetable soybean lines produced less beany flavors but similar nutritional qualities and isoflavone contents compared to AGS 292. This indicated that the introduction of lipoxygenase null alleles do not affect other qualities. The lipoxygenase-null lines produce lower beany flavor and also provide the advantages of AGS 292 such as good quality, shorter maturation period and large seed size. The lowering of beany flavors can enhance the consumption of soybean and avoid heat caused protein loss for soybean milk. Larger-sized seeds typical of vegetable soybean varieties also produce promising protein yield in making soymilk. Because soybean seeds at grain stage can provide more folic acid and isoflavone and import better oil stability, the AVRDC lipoxygenase-null lines could be used at both vegetable and grain stages.

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