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Nutrient composition and anti-nutritional factors in vegetable soybean: II. Oil, fatty acids, sterols, and lipoxygenase activity‡

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Seventeen vegetable soybean genotypes were analyzed for oil, fatty acids, sterols and lipoxygenase activity. The mean oil content was 18·44% and ranging from 15·65% for Wilson-5 to 23·36% for Sango. The lipoxygenase activity ranged from 829·8 units/min/mg meal for PI 417310 to 4750·4 units/min/mg meal for PI 423759, with a mean of 2176·3 units/min/mg meal. β-Sitosterol (45·95%) was the major plant sterol present, followed by stigmasterol (16·48%), campsterol (16.06%) and dihydroxybrassicasterol (5·62%). Palmitic, oleic and linoleic acids comprised over 90% of the total fatty acids. The means of linoleic (53·34%) and linolenic acids (9·19%) were higher than that reported for the grain-type soybean. The concentrations of oleic were inversely related to the concentrations of linoleic ($r = -0{\cdot}820$) and linolenic ($r = -0{\cdot}663$) acids.

The oils of cultivars sango and sooty, and PIs 423759, 417310, 222397 and 171437 possess superior nutritional quality. In general, the high oil, linoleic, linolenic and sterol content of vegetable-type soybeans make them a food with high nutritional quality.

INTRODUCTION

Consumers across the United States concerned with their health and physical fitness are exploring new vegetable crops that can be incorporated into their diets. Vegetable-type cultivars of soybean which are already popular as a food in the Orient, could be used as an alternative new vegetable in American diets.

Vegetable soybean (*Glycine max* (L.) Merr.) genotypes generally fall into two categories: large-seeded and small-seeded. The large-seeded garden types are used for the fresh market in urban areas with large Oriental populations, whereas small-seeded types are used to prepare soybean sprouts (Anonymous, 1987). Vegetable soybean is served in the pod as snack food, frozen in the pod, and used as a vegetable mix. Green vegetable soybean is imported into the United States to meet the demand for Oriental speciality food products. Certainly, no other vegetable crop can match soybean for high nutritional value. Vegetable soybean, for example, has an average of 12% protein (on a wet basis) compared with 7·6% for lima beans (*Phaseolus vulgaris* L.) and 5·4% for peas (*Pisum sativum* L.) (Anonymous, 1987). Vegetable soybean is also a good source of vitamins and minerals (Mohamed *et al.*, 1991). It has been reported that vegetable-type soybean cultivars are superior to grain-type cultivars in flavor, texture and cooking (Morse, 1950; Kapoor *et al.*, 1977) and are relatively low in trypsin inhibitor activity (Deodhar *et al.*, 1973; Gupta & Deodhar, 1975; Mohamed *et al.*, 1991). Little is known about the nutritional quality of vegetable-type soybeans and no studies have been reported on genetic variations in lipoxygenase, fatty acids and sterol composition in vegetable-type soybeans.

The present study was conducted to evaluate the nutritional quality of the oil fraction of the selected vegetable soybeans. Variations in total oil, lipoxygenase activity, fatty acid and sterol compositions among selected genotypes were determined.

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MATERIALS AND METHODS

Seventeen vegetable soybean genotypes, six cultivars (Ware, Emperor, Sango, Kingston, Sooty and Wilson-5) and 11 plant introductions (PI genotypes 416982, 416771, 417288, 417322, 417052, 417213, 417310, 423759, 423852, 222397 and 171437) were selected, based on the seed size. The seed samples were ground in a centrifugal grinding mill, and passed through a 0·5 mm pore size screen. The oil was extracted with hexane using a Goldfisch apparatus and the percent oil was calculated (AOAC, 1980). The defatted meal samples were used to determine lipoxygenase activity as described below.

Determination of fatty acids and sterols

The total lipid was extracted from the soybean seeds using hexane–isopropanol (3:2 v/v) according to St John and Bell (1989). The extracted lipid was used for analysis of fatty acids and sterols. The fatty acid methyl esters (FAME) were prepared from extracted lipid by the procedure of Kinsella *et al.* (1977). A GC (model HP 5890A) equipped with a flame ionization detector (FID), mass spectrometer detector (MSD, Model HP 5971A), and capillary column (Supelco 2330, 30 m × 0·25 mm i.d. and 0·25 μm film) was used. The GC was connected to an HP 3396A integrator for FID and a HP 59970 ChemStation for MSD. Fatty acid standards were purchased from Sigma Chemical Co., St Louis, Missouri, USA, and methylated using diazomethane, as described by Osman *et al.* (1978).

Sterols were extracted from saponified lipids and methylated using diazomethane, as described by Ibrahim *et al.* (1990). Sterol standards — β-sitosterol, stigmasterol, campsterol and dihydroxybrassicasterol (Sigma Chemical Co.) — were dissolved in hexane and methylated as above. The GC system described above was used with a HP1 capillary column (12·5 m × 0·2 mm i.d., 0·1 μm film thickness). Helium was the carrier gas, and the oven temperature was programmed as follows: initial temperature, 250°C for 2 min; then temperature raised from 250 to 295°C at the rate of 6°C/min; the final temperature was held for 12 min. For further confirmation, standards and samples were run on an MSD.

Determination of lipoxygenase activity

The lipoxygenase activity in vegetable-type soybeans was assayed by a modified method described in details by Hafez *et al.* (1985), using linoleic acid as the substrate. One unit of lipoxygenase activity is defined as that quantity of enzyme which causes an increase of 0·001 absorbance unit/min at 234 nm.

Two samples from each of three replicates were assayed for each genotype. The data from the chemical analyses were statistically analyzed, and the means were separated using the least significant differences (LSD) test at the 5% level of significance.

RESULTS AND DISCUSSION

The oil content of 17 vegetable-type soybean genotypes was determined and wide variations were found among the genotypes tested (Table 1). The mean oil content for the selected genotypes was 18·44%, and ranged from 15·65% for Wilson-5 to 23·36% for Sango. The mean percent oil of cultivars (18·16%) was significantly lower than that of the PI genotypes (19·30%). In general, the mean oil content of vegetable soybeans reported in this study was not significantly lower than that reported by Deodhar *et al.* (1973). The variation may be due to varietal differences and/or environmental effects. However, the mean percent oil for vegetable soybeans is similar to values reported for grain-type soybean (Mohamed & Rangappa, 1992).

Lipoxygenase is an anti-nutritional factor prevalent in soybean. Lipoxygenase activity is primarily responsible for the development of off-flavor in soybean foods and beverages, and it also reduces the amount of essential fatty acids in soy oil (Nelson *et al.*, 1976; Rackis *et al.*, 1979; Sessa 1979). Lipoxygenase activity in soybean (Table 1) ranged from 829·8 units/min/mg meal for PI 417310 to 4750·4 units/min/mg meal for PI 423759, with a mean of 2176·3 units/min/mg meal. This study also reveals that the mean of lipoxygenase activity in cultivars (2530·46 units/min/mg meal) was significantly higher than the mean activity of PI genotypes (1956·7 units/min/mg meal). The values of lipoxygenase activity among vegetable soybeans were lower than those reported for grain-type soybean (Hafez, 1983). The wide variations in lipoxygenase activity reported here indicate that it may be possible to breed low lipoxygenase in vegetable soybean.

Lipoxygenase activity is affected by several factors, such as oil content, temperature, pH and moisture content (Borhan & Synder, 1979; Wei *et al.*, 1981; Hafez *et al.*, 1985). Earlier reports indicated that, in whole seed, lipoxygenase activity is drastically reduced by food processing techniques, such as conventional heating, microwave heating, γ-irradiation and soaking in acidic solutions (Johnson *et al.*, 1978; Hafez *et al.*, 1985; Chen Man *et al.*, 1991). Therefore, the importance of lipoxygenase as an anti-nutritional factor in vegetable-type soybean will depend on the method of food processing.

Considering total unsaturated fatty acids in the selected vegetable-type soybeans (Table 1), Ware had the lowest total unsaturated fatty acids (78·82%), while Sooty had the highest (88·26%). The mean of total unsaturated fatty acids was 84·43% for the selected vegetable genotypes.

The percent total sterols was determined in the unsaponifiable fraction of selected vegetable-type soybeans (Table 1). Wide variation in the percent sterols was observed among the vegetable-type soybeans, with a mean of 20·91% and a range from 6·30% for Sango to 50·96% for PI 417310. The mean sterol for PI genotypes (20·21%) was significantly higher than the mean for cultivars (11·18%). Given that some plant sterols reduce serum cholesterol (Mattson *et al.*, 1977), a higher amount of sterols in vegetable soybean is desirable. No study has investigated the genetic variation in sterols of vegetable- and grain-type soybeans, but such information is necessary for food design efforts.

Four plant sterols were identified in selected vegetable-type soybean: β-sitosterol, stigmasterol, campsterol and dihydroxybrassicasterol. The ratios of identified sterols are listed in Table 2. The ratio of the mean for these sterols was found to be 8·2:2·9:2·9:1. These results are in agreement with reported data on grain-type soybeans (Thompson *et al.*, 1963; Ibrahim *et*

Table 1. Total oil, lipoxygenase activity, unsaturated fatty acids and percent sterol in unsaponifiables of selected vegetable genotypes

VSB genotypes	Seed size	Oil (%)	Lipoxygenase (unit/min/mg meal)	Total unsaturated fatty acid (%)	Sterols (%)
Ware	Large	18·01	3787	78·82	9·94
Emperor	Large	17·87	2292	86·86	9·44
Sango	Large	23·36	2525	81·45	6·30
PI 416982	Large	19·83	3165	85·18	16·51
PI 417288	Large	18·54	1505	82·01	22·31
PI 417322	Large	17·24	1239	85·21	14·06
PI 417213	Large	19·06	1831	83·65	16·42
PI 417310	Large	18·94	830	84·00	50·96
Kingston	Small	18·42	2424	84·58	6·93
Sooty	Small	19·25	1143	88·26	21·03
Wilson-5	Small	15·65	3012	85·26	13·44
PI 416771	Small	17·64	1271	85·80	22·89
PI 417052	Small	20·95	1559	86·24	10·72
PI 423759	Small	22·40	4750	84·74	14·82
PI 423582	Small	15·15	1689	82·61	17·48
PI 222397	Small	22·26	1603	86·63	14·71
PI 171437	Small	20·25	2374	84·15	24·00
CV (%)		2·64	1·80	5·05	4·84
LSD		0·84	22·64	0·33	2·48

Table 2. Sterol fraction patterns of selected vegetable soybeans in unsaponifiables

VSB genotypes	Seed size	β-Sitosterol	Stigmasterol	Campsterol	Dihydroxy Brassicasterol	Unknown sterol (%)[a]
Ware	Large	47·07	11·97	20·24	5·97	14·76
Emperor	Large	43·43	17·70	13·60	6·48	18·61
Sango	Large	41·63	12·41	10·92	6·47	28·57
PI 416982	Large	47·02	16·00	13·90	5·43	17·65
PI 417288	Large	54·09	18·03	15·92	3·11	8·86
PI 417322	Large	39·61	17·85	15·10	7·33	20·10
PI 417213	Large	44·90	17·77	22·48	7·72	7·14
PI 417310	Large	58·88	19·65	20·18	0·69	0·597
Kingston	Small	35·22	15·48	16·01	12·11	21·18
Sooty	Small	43·51	17·74	16·40	4·69	17·67
Wilson-5	Small	42·26	17·41	6·10	7·52	26·71
PI 416771	Small	49·24	12·36	13·52	4·93	19·94
PI 417052	Small	37·99	18·66	15·91	5·81	21·63
PI 423759	Small	53·71	13·62	17·00	4·60	11·80
PI 423582	Small	47·60	19·51	22·03	1·91	8·96
PI 222397	Small	45·11	18·63	17·10	3·56	15·60
PI 171437	Small	49·91	15·30	16·45	7·17	11·17
CV (%)		3·62	4·89	2·50	5·10	7·69
LSD		1·47	0·62	0·97	0·63	1·75

[a] Percentage of the total unidentified sterol in the unsaponifiable fraction.

Table 3. Fatty acid compositions of selected vegetable soybean genotypes

Soybean Accession	Palmitic (C_{16})	Palmitoleic ($C_{16:1}$)	Stearic ($C_{18:0}$)	Oleic ($C_{18:1}$)	Linoleic ($C_{18:2}$)	Linolenic ($C_{18:3}$)	Arachidic ($C_{20:0}$)	Eicosadienoic ($C_{20:2}$)	Eicosatrienoic ($C_{20:3}$)	$C_{22:0}$	$C_{22:1}$
Ware	12·49	0·1	2·97	28·03	43·72	6·07	0·17	0·25	0·21	0·44	T[a]
Emperor	10·73	0·27	2·53	25·53	52·45	7·49	0·21	0·29	0·33	T	T
Sango	10·09	T	3·16	16·07	55·05	10·17	0·39	0·09	0·08	T	T
Kingston	10·43	T	3·15	17·66	53·94	12·60	0·30	0·21	0·17	T	T
Sooty	8·37	0·691	2·83	24·20	53·13	9·31	0·27	0·67	0·26	T	T
Wilson-5	12·43	T	3·80	17·83	55·96	9·21	0·21	0·28	0·17	0·28	1·53
PI 416982	11·15	0·13	3·32	22·67	53·86	7·70	0·11	0·28	0·24	0·30	T
PI 416771	10·69	T	3·30	25·21	51·57	8·52	0·10	0·29	0·26	T	T
PI 417288	10·63	0·12	3·81	20·36	50·62	9·48	0·14	0·28	0·18	0·97	T
PI 417322	11·08	T	3·43	19·28	56·18	9·51	0·09	0·16	0·03	T	T
PI 417052	10·35	T	3·21	29·80	47·21	8·47	0·15	0·32	0·39	T	T
PI 417213	12·58	0·10	3·37	18·95	54·33	9·77	0·21	0·29	0·21	T	T
PI 417310	11·85	0·13	3·35	17·4	54·97	10·36	0·11	0·32	0·37	0·34	0·11
PI 423759	11·52	0·23	3·14	16·31	56·83	10·93	0·14	0·30	0·17	T	T
PI 423852	11·29	0·21	3·38	17·30	54·57	9·24	0·25	0·25	0·17	0·87	T
PI 222397	10·26	2·5	2·85	18·08	56·03	8·35	0·88	0·31	0·79	0·57	T
PI 171437	12·31	0·15	3·08	16·24	56·14	9·08	0·18	0·24	0·13	0·25	1·92
CV (%)	1·90		5·89	0·85	1·17	1·44	9·23	11·99	9·85		
LSD	0·31		0·32	0·3	1·04	0·22	0·03	0·05	0·05		

[a] T = traces < 0·08%.

al., 1990). In this study, β-sitosterol was found to be the major sterol in vegetable soybeans, with a mean of 45·95%. The lowest value for β-sitosterol was found for Kingston (35·22%), the highest values was for PI 417310 (58·88%), and the mean was significantly higher for PI genotypes than for cultivars. The means for stigmasterol, campsterol and dihydroxybrassicasterol were 16·48%, 16·06% and 5·62%, respectively. It has been suggested that incorporating vegetable-type soybeans into human diets could have positive health benefits. Mattson *et al.* (1977) found that cholesterol absorption decreased when either free or esterified plant sterols were added to dietary fat and incorporated into human diets. It has also been reported that ingestion of plant sterols and unsaturated oil decreased cholesterol absorption with a consequential decrease in plasma cholesterol (Thompson *et al.*, 1963; Mattson *et al.*, 1977).

Wide variations in fatty acid composition among selected vegetable soybean genotypes were found (Table 3). Furthermore, highly significant variations were observed in oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids (Table 3). Moderate variations were observed in palmitic and stearic acid. Significant differences in $C_{16:0}$ and $C_{18:1}$ were found between large-seeded and small-seeded genotypes; in addition, cultivars showed higher $C_{18:1}$ than PI genotypes. This study indicates that the oil of vegetable-type soybeans has a high concentration of linoleic (53·34%) and a significant amount of linolenic (9·19%) acids. Both of these fatty acids have a significant effect upon oil quality. On the one hand, a high concentration of linoleic and linolenic acids is undesirable in terms of oil stability, because it is readily oxidized and is believed to be the cause of off-flavor in soybean oil, soy food products and beverages (Nelson *et al.*, 1976; Ho *et al.*, 1979; Rackis *et al.*, 1979; Sessa, 1979). On the other hand, these polyunsaturated fatty acids (PUFAs) are essential fatty acids and are required for normal growth of animals, including humans. In addition, several reports indicate the importance of the PUFAs in reducing cholesterol levels in human blood, thus reducing the risk of heart disease (Dryerberg, 1986; Lands, 1986; Hafez *et al.*, 1990).

There was variation in eicosadienoic ($C_{20:2}$) and eicosatrienoic ($C_{20:3}$) acids among selected genotypes and ($C_{20:2}$) was significantly higher in small-seeded than in large-seeded genotypes. The quantities of polyunsaturated fatty acids observed in this study were significantly higher than those reported by Deodhar *et al.* (1973). This study also indicates that the composition of vegetable soybean oil is similar to grain soybean oil, both contain higher amounts of PUFAs, especially linoleic and linolenic, than other oil seed crops.

Highly significant correlations (r = –0·820, –0·663, 0·342, $P \leq 0{\cdot}01$) were documented for $C_{18:1}$ and $C_{18:2}$, $C_{18:1}$ and $C_{18:3}$, and $C_{18:1}$ and $C_{20:2}$. A recent study has provided conclusive evidence that linoleic and linolenic acids are produced by successive desaturation of oleic acid, which serves to explain the direction and the magnitude of correlations among these fatty acids. Several nutritional characteristics, such as high oil content, high plant sterols, high linoleic and linolenic acids and high percent unsaturation are desirable for vegetable soybean. Cultivars Sango and Sooty and PI genotypes 423759, 417310, 222397 and 171438 have the desirable characteristics and possess good nutritional oil quality.

In conclusion, vegetable-type soybean is a nutritious food, its incorporation into the American diet could reduce blood cholesterol and consequently, reduce the risk of heart disease. Studies on the nutritional quality of immature seeds of vegetable-type soybeans at different reproductive stages to determine the best stage that can be used for human consumption are underway.

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