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Short Communication

Effect of Different Cultivars and Preparation Methods on Vitamin E, Total Fat and Fatty Acid Content of Soybean (*Glycine max* (L.) Merr.) Seeds

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ABSTRACT. *Soybean (Glycine max (L.) Merr.) has been considered an important crop in the world because of its unique nutritional composition. On an average dry matter basis, soybean contains about 20% fat. Soy fat contains a high proportion of essential fatty acids such as linoleic and linolenic and vitamin E.*

In this study, vitamin E and linoleic and linolenic acid contents of four Sri Lankan soybean cultivars were estimated under different preparation methods. Soybean cultivars differed significantly ($p<0.05$) in fat content (17.9-21.1%), but not in vitamin E content (5.7-7.4 µg/g). These cultivars also differ significantly ($p<0.05$) in essential fatty acids like linoleic acid (91.2-107.6 mg/g of seed) and linolenic acid (13.9-17.9 mg/g of seed) contents. Soybean cultivars showed a significant correlation between vitamin E and linoleic acid and linolenic acid content.

Among the 3 different preparation methods, the mean fat content of boiled (18.2%) and baked (18.3%) samples was significantly lower ($p<0.05$) than the control (PM 25 variety). Vitamin E content of pressure-cooked (1.5 µg/g of seed), boiled samples (0.3 µg/g of seed) was significantly lower when compared to the control. Vitamin E was completely lost in baked sample. During pressure-cooking, boiling and baking, the Vitamin E loss was around 76, 95 and 100%, respectively. The linoleic and linolenic acid contents of the control were significantly ($P<0.05$) higher than all processed samples. The linoleic acid content of the processed samples ranged from 83.9-99.5 mg/g of seed and linolenic acid from 10.9-17.6 mg/g of seed. The percentage loss of essential fatty acids in pressure cooking, baking and boiling was 9, 15 and 15%, respectively. Different preparation methods showed a significant positive correlation between vitamin E and essential fatty acids.

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) has been considered an important world crop because of it's unique nutritional composition. On an average dry matter basis, soybean contains about 40 % protein and 20 % fat. Soy fat contains a high proportion of vitamin E (α-Tocopherol) and essential fatty acids such as linoleic and linolenic acids. Tocopherols

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are natural antioxidants with heart/vascular, and cancer protective properties. The amounts of alpha, gamma and delta Tocopherol in soybean seed are 10.9-28.4, 150-191 and 24.6-72.5 μg/g, respectively (Liu, 2000). Essential fatty acids are necessary for the biosynthesis of compounds such as prostaglandins, prostacyclins and thromboxanes. Several studies have demonstrated health benefits of dietary essential fatty acids. It has anti-inflammatory and antiproliferative effects and facilitates fatty acid beta oxidation in the liver and acts as an effective cytotoxic agent against superficial bladder cancer (Goffman and Galletti, 2001). The amount of linoleic and linolinic acid in some Indian soybean cultivars is between 55.8-58.3% and 10.6- 13.1%, respectively (Dhaliwal and Aggarwal, 1999).

Some Indian cultivars like Shivalik (with a mean value of 23.55%) and Punjab No.1 (with a mean value of 20.57%) varied significantly with respect to fat content (Dhaliwal and Aggarwal, 1999). Fat content of 7 different Indian cultivars ranged from 18.8 to 22.4%, cultivar PK-472 and PK-1029 contained the maximum and minimum amount of fat respectively (Krishna *et al.*, 2003). But the detail information about the different fatty acids and vitamin E content of Sri Lankan soybean cultivars are not available.

Vitamin E content of different types of dhal is destroyed during different processing methods like cooking and drying (12% loss), cooking and freeze-thaw and drying (16 % loss) and cooking and flaking and drying (32% loss) (Aria and Rudramma, 2000). Tocopherol concentration gradually decreased (14% loss) in soybean oil prepared from hypocotyls of soybeans roasted in a microwave oven and that the percentages of the losses increased significantly after a 12 min exposure time (Yoshida *et al.*, 1999). However, information on percentage changes in fat and fatty acid and Tocopherol content after different preparation methods is scanty.

Vitamin E is a fat-soluble antioxidant, important for the protection of essential fatty acids against oxidative deterioration in both plants and animals. In general, an intake of 0.6 mg of α-Tocopherol equivalent (1 IU) per gram of essential fatty acid is seen as adequate for human adults, to prevent the development of vitamin E syndrome The balance between Tocopherol and essential fatty acids contents mainly determine the susceptibility to lipid peroxidation and the storage stability (Goffman and Bohme, 2001).

In this study, vitamin E, fat and different fatty acid contents of soybean in different cultivars has been investigated. In addition, changes in vitamin E, fat and different fatty acid contents of soybean during different preparation methods and the relationship between vitamin E content and essential fatty acid contents has also been investigated.

MATERIALS AND METHODS

Sample collection

Soybean seeds of four cultivars collected from the field crop research center, Maha Illuppallma were used in the study. The selected cultivars were Sri Lankan recommended cultivars Pb-1, PM 13 and PM 25 and Bossier. The seeds were differentiated according to the size, shape, seed coat color and helium color (DOA, 2000). The seeds were freshly harvested and sun dried for 2 weeks to reduce moisture content to 12-13%.

Sample preparation

About 100 g of each sample was ground to fine powder by using a blender (model BB 90 E, Waring, U.K). Then the oil was extracted from each sample according to the method described in AOAC (1995). The Tocopherol content of this oil sample was determined by HPLC method (Etenmiller *et al.*, 1998). Gas Chromatographic method (Supelco bulletin 855A, 1994) was used to determine the fatty acid composition in the oil samples.

Determination of Tocopherol

The α-Tocopherol acetate standard solutions were prepared as described by Ye *et al.* (1998). On a molar basis, bioavailability of α-Tocopherol acetate is nearly equivalent to that of α-Tocopherol. The normal phase HPLC system equipped with chromatography manager and Shimadzu SPD-10AV UV detector (Shimadzu Corporation, Colombia, and U.S.A.) was used for the determination of Tocopherol. The column was a Shimpack- GLC-ODS with 6 mm internal diameter and 15 cm length. The wavelength was 290 nm. Column temp was maintained at 55^0C. Methanol was used as the mobile phase. All analytical samples with methanol were passed through the column at a flow rate of 1.5 ml/min. Twenty µl of standard solutions were injected in to the HPLC column by using a micro syringe and the chromatogram of each standard was obtained. Twenty µl prepared samples of different soybean varieties and different preparation methods were also injected into the HPLC column by using the same procedure. Peaks of each sample were identified by comparing their retention time of the chromatogram with that of α-Tocopherol standard.

Determination of fatty acid content

Palmitic, stearic, oleic, linoleic and linolinic acid standard solutions were prepared as described in Supelco bulletin 855A (1994). Shimadzu GC-14 B type gas chromatography equipped with flame ionization detector (Shimadzu Corporation, Colombia, and U.S.A.) was used for the determination of fatty acids. Nukol capillary column with 15 m length, 0.53 mm internal diameter and 0.5 µm film thicknesses was used as a column. The column oven temperature was automatically programmed with initial temperature of 110^0C and final temperature of 220^0C. The temperature was increased at a rate of 8^0C/min. Helium was used as a carrier gas at a flow rate of 20 ml/min. One µl standard solutions of palmitic, stearic, oleic, linoleic and linolenic acids were directly injected in to the GC column and the chromatogram of each standard was obtained. The retention time of these standards was noted. One µl of saponified and acidified samples of were also injected into the GC coloumn by using the same procedure**.**

Preparation methods

Soybean seeds of the variety PM-25 were used to study the effect of different preparation methods.

a. Soaking and pressure-cooking

Seeds were soaked in water for 2 h at room temperature. Then excess water was decanted and soaked seeds were pressure cooked at 121^0C and 103.5 kPa steam pressure in an autoclave for 10-15 min.

b. Soaking and boiling

Seeds were soaked in water for 2 h at room temperature. Then excess water was decanted and soaked seeds were boiled at 100-105^{0}C under normal pressure for 30 min. in an open pan.

Seeds prepared according to methods (a) and (b) were dried in a vacuum oven at 70^{0}C separately and the moisture content reduced to about 12%.

c. Baking

Seeds were baked in an oven at 120°C for 5-10 min. Fresh seeds were used as a control.

Statistical analysis

A Complete Randomized Design (CRD) with six replicates was used as the experimental design to analyze fat, vitamin E and different fatty acids of different soybean cultivars. The data of fat, vitamin E and different fatty acids with six replicates were analyzed using Statistical Analysis System (SAS) version 08 modules. Means separation of these data were performed using Least Significant Difference (LSD) procedure at error level of 5% (α = 0.05). Each reported value of fat, vitamin E (Table 1) and different fatty acids (Table 2) are the mean and standard error of 4 samples. Significant difference between two means was indicated by English alphabets.

RESULTS AND DISCUSSION

The fat content of the four Sri lankan Soybean cultivars vary significantly ($P<0.05$) between each other. Among the four Soybean cultivars Pb-1 had the highest fat content followed by Bossier, PM-25 and PM-13. According to Dhaliwal and Aggarwal (1999) Indian soybean cultivars Shivalik and Punjab No.1 had 23.55 and 20.57 % fat, respectively. Fat content of 7 different Indian cultivars ranged from 18.8 to 22.4 %, cultivar PK-472 and PK-1029 contained the maximum and minimum amount of fat, respectively (Krishna *et al.*, 2003). The Sri Lankan soybean cultivars resembled the Indian cultivars in fat content. Even though the Vitamin E content of Soybean varied among cultivars the difference between the cultivars except between Bossier and PM-13 was not significant. Among the four Soybean cultivars Bossier had the highest Vitamin E content followed by Pb-1, PM-25 and PM-13 (Table 1).

Table 1. Fat percentage and vitamin E content of the soybean cultivars.

Variety	Fat (%)*	Vitamin E (µg of α Tocopherol /g of seed)*
Pb - 1	21.10±0.07^a	7.15±0.34^e
PM - 25	18.88±0.16^b	6.23±0.56^e
PM - 13	17.87±0.15^c	5.70±0.20^e
Bossier	20.33±0.09^d	7.41±0.73^f

Note: *Means within a column or within a row followed by the same letter do not differ significantly at 5%.

Dhaliwal and Aggarwal (1999) reported significant differences in fatty acid composition between Indian soybean cultivars Shivalik and Punjab No.1. In their study, cultivar Shivalik showed higher oleic (22.0%) and linoleic (58.3%) acid content compared to cultivar Punjab No.1 (20.1 and 55.8%). Punjab No.1 showed higher palmitic (11.0%) and linolinic (13.1%) acid contents compared to cultivar Shivalik (9.2 and 10.6%). The fatty acid content of different Sri Lankan soybean cultivars also similar to that of Indian cultivars. Cultivar Pb-1 shows the highest linoleic acid content followed by Bossier, PM-25 and PM-13, while cultivar Bossier shows the highest linolenic acid content followed by PM-25, PM-13 and Pb-1 (Table 2). The Indian study also showed that both Indian soybean cultivars are good sources of essential fatty acids. About 68% of the total fatty acids are essential fatty acids. The essential fatty acid percentage of the total fatty acid of different Sri Lankan soybean cultivars ranges from 59-63 %.

Table 2. Fatty acid contents of different Soybean cultivars.

Cultivar	Fatty acids (mg/g of seed)*				
	C16: 0	**C18: 0**	**C18: 1**	**C18: 2**	**C18: 3**
Pb-1	25.15±0.39^{a}	7.55±0.01^{d}	57.37±0.24^{f}	107.57±0.75^{j}	13.95±0.19^{m}
PM-13	22.65±0.66^{b}	7.27±0.22^{d}	38.90±0.71^{g}	91.17±0.48^{k}	17.37±0.68^{n}
PM-25	22.40±0.29^{b}	7.07±0.12^{d}	42.12±0.44^{h}	99.50±1.42^{l}	17.60±0.41^{n}
Bossier	31.00±0.48^{c}	8.77±0.13^{e}	44.50±0.27^{i}	101.55±0.41^{l}	17.92±0.51^{n}

Note: C16: 0 - Palmitic acid; C18: 0 - Stearic acid, C18: 1 - Oleic acid; C18: 2 - Linoleic acid; C18: 3 - Linolenic acid. * Means within a column or within a row followed by the same letter do not differ significantly at 5%.

Soybean cultivars of the current study differed significantly ($p<0.05$) in fat content, but not in vitamin E content. The fat content varied from 17.9-21.1 % and vitamin E content ranged from 5.7-7.4 µg/g of seed. These cultivars also differ significantly ($p<0.05$) in essential fatty acids like linoleic acid and linolinic acid content. The linoleic acid content of these cultivars ranged from 91.2-107.6 mg/g of seed and linolenic acid content from 13.9-17.9 mg/g of seed.

Table 3 shows that there is a significant correlation between essential fatty acids like linoleic and linolenic acids content and Vitmin E content of different soybean cultivars. Linoleic and linolenic acid contents also showed a significant correlation between them. A significant correlation was observed between vitamin E/essential fatty acid ratio and vitamin E and linolenic acid content. Kamal-Eldin and Andersson (1997) also found a positive correlation between alpha Tocopherol and linoleic acid present in corn oil, but biochemical studies are needed to confirm this relationship.

Uncooked PM-25 cultivar seeds were used as control. According to Table 4, comparing with the control, the mean fat content in boiled and baked samples were reduced by 3.5 and 3.0%, respectively and they differed significantly ($P<0.05$). All cooked samples had slightly lower fat content compared to the control. The Vitamin E content of all cooked samples differed significantly ($P<0.05$) from the uncooked sample (Table 4). Vitamin E is

liable to be destroyed during heating (Gregory, 1996). Baking operations completely destroyed Vitamin E. The extent of loss is less in boiled and pressure-cooked samples. About 76% of the vitamin E was lost during pressure-cooking while about 95% of vitamin E was lost during boiling. The extent of vitamin E loss is high in dry heating method (baking) compared to wet heating methods (boiling and pressure cooking).

Table 3. Correlations among vitamin E, linoleic acid, linolenic acid and vitamin E/EFA ratio in 4 soybean cultivars.

	Linoleic acid	Linolenic acid	Vit. E/EFA ratio
Vitamin E	0.48^{*}	0.52^{*}	0.92^{*}
Linoleic acid		0.30^{*}	0.22^{ns}
Linolenic acid			0.31^{*}

Note: * Significant at 5% level; ns - not significant.

Table 4. Fat and Vitamin E content of soybean cultivar PM - 25 under different preparation methods.

Preparation methods	Fat (%)	Vitamin E (µg of α Tocopherol /g of seed)
Control	18.88 ± 0.16^{a}	6.23 ± 0.56^{d}
Boiled sample	18.22 ± 0.08^{b}	0.30 ± 0.01^{e}
Pressure cooked sample	$18.60\pm0.07^{a,c}$	1.46 ± 0.01^{f}
Baked sample	$18.31\pm0.07^{b,c}$	00.00^{g}

Note: Means within a column or within a row followed by the same letter do not differ significantly at 5%.

Palmitic acid content of the baked sample was not significantly different from control sample. But it was significantly different from all other cooked sample. Palmitic acid content of the pressure-cooked sample was not significantly different from boiled sample and it was slightly higher than boiled sample. But it was significantly different from all other samples. Palmitic acid content of the boiled sample was also significantly different from all other samples except pressure-cooked sample (Table 5).

Stearic acid content of the baked sample also significantly differed from all other samples. Stearic acid content of the boiled sample and pressure-cooked sample were not significantly different from each other and with control sample. But they were significantly different from baked samples. Baked sample showed highest oleic acid content compared to other samples and it was significantly ($P<0.05$) higher than other samples. Oleic acid content of the boiled sample and pressure-cooked sample were significantly different from each other and also from other samples. The linoleic and linolenic acid contents of cooked samples were significantly lower compared to the control sample irrespectively of the preparation method. This observation indicates that heating of soybeans leads to loss of essential fatty acids. Linoleic acid content of the boiled and baked samples were not

significantly differed from each other ($P<0.05$). Linolenic acid content of the pressure-cooked sample did not significantly differ from boiled and baked samples. Linolenic acid content of the boiled and baked samples was significantly different from other samples except pressure-cooked sample.

A significant positive correlation was observed between linoleic and linolenic acids content and Vitmin E content of soybean cultivar PM-25 under different preparation methods. Linoleic and linolenic acid contents also showed a significant correlation between them. A significant correlation was observed between vitamin E/essential fatty acid ratio and linoleic acid content. But vitamin E and linolenic acid contents did not showed significant correlation with vitamin E/essential fatty acid ratio (Table 6).

Table 5. Fatty acid content of soybean cultivar PM-25 processed under different preparation methods.

Preparation methods	Fatty acids (mg/g of seed)*				
	C16 : 0	C18 : 0	C18 : 1	C18 :2	C18 :3
Control	22.40±0.29^{a}	7.07±0.12^{d}	42.12±0.44^{g}	99.50±1.42^{k}	17.60±0.41^{o}
P.Cooked Sample	25.62±0.29^{b}	6.72±0.11^{d,e}	49.00±0.23^{h}	92.50±0.62^{l}	11.85±0.17^{p}
Boiled Sample	24.60±0.22^{b}	7.00±0.01^{d,e}	55.62±0.45^{i}	83.95±0.59^{m}	11.72±0.17^{p}
Baked sample	21.25±0.25^{a,c}	4.52±0.32^{f}	59.27±0.19^{j}	86.12±0.41^{n}	10.92±0.11^{q}

Note: C16: 0 - Palmitic acid; C18: 0 - Stearic acid; C18: 1 - Oleic acid; C18: 2 - Linoleic acid; C18: 3 - Linolenic acid. *Means within a coloumn followed by the same letter do not differ significantly

Table 6. Correlations among vitamin E, linoleic acid, linolenic acid and vitamin E/EFA ratio of cultivar PM- 25 under different preparation methods.

	Linoleic acid	Linolenic acid	Vit. E/EFA ratio
Vitamin E	0.82*	0.93*	0.15^{ns}
Linoleic acid		0.73*	0.37*
Linolenic acid			0.05^{ns}

Note: * Significant at 5% level; ns - not significant.

CONCLUSIONS

This study shows that among the four Soybean cultivars, Bossier had the highest Vitamin E content and cultivar Pb-1 had the highest fat content. The vitamin E content appeared to parallel the fat content in the cultivars. The essential fatty acid contents (linoleic and linolenic acid) of different Sri Lankan soybean cultivars ranged from 59-63%. Oleic, palmitic and stearic acids are the other 3 important fatty acids found in soybean cultivars. During pressure cooking, boiling and baking the Vitamin E loss was around 76, 95 and

100%, respectively. During heating loss of essential fatty acids of soybeans was considerable. The loss of linoleic and linolenic acid in pressure cooking, baking and boiling was 9,15 and 15%, respectively. A positive correlation was observed between vitamin E and linoleic and linolenic fatty acids content under different preparation methods. This indicates that both vitamin E and the essential fatty acids destroyed due to heating.

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