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Changes in Vitamin E and Essential Fatty Acid Contents and Their Interrelationship in Soybean (*Glycine max* L. Merr.) Seeds During Germination and Storage

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

In this study, vitamin E and linoleic and linolenic acid contents of soybean seeds were estimated with different germination times (24, 48 and 72 hrs) and storage with different packaging materials (aluminum foil, polythene, paper and unpacked) for a period of six months. The highest vitamin E content (12.63 µg/g) was observed in the sample germinated after 48 hrs. The highest amounts of linoleic acid (107.57 mg/g) and linolenic acid (18.27 mg/g) were observed in the ungerminated sample and sample germinated after 72 hrs, respectively. A significant positive correlation (r=0.81) between vitamin E and linolenic acid was observed in the germinated seeds.

Vitamin E content of soybean seeds decreased with time under all four packaging conditions. Linoleic acid content of the seeds stored in aluminum foil and polythene packets increased with time. However linoleic acid content of the seeds stored in paper bags and unpacked seeds decreased significantly with increasing storage time. Linolenic acid content of soybean seeds also decreased with time under all four packaging conditions. A significant positive correlation (r=0.91) between vitamin E and linolenic acid contents and between vitamin E and vitamin E / essential fatty acid ratio (r=0.98) was observed during storage. The rate of loss of vitamin E, linoleic and linolenic acid was comparatively low in seeds packed in aluminum foil and polythene than other packaging types.

INTRODUCTION

Soybean (*Glycine max* L. Merr.) is considered an important world crop owing to its 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 (alpha tocopherol) and essential fatty acids such as linoleic and linolenic acids. 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. Tocopherols are natural antioxidants with heart/vascular, and cancer protective properties (Liu, 2000). Essential fatty acids are necessary for the biosynthesis of compounds

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such as prostaglandins, prostacyclins and thromboxanes in the human body. Several studies have demonstrated health benefits of dietary essential fatty acids. They have anti-inflammatory and antiproliferative effects and facilitate beta oxidation of fatty acid in the liver, and acts as an effective cytotoxic agent against superficial bladder cancer (Goffman and Galletti, 2001).

Vitamins and fatty acid contents change during germination and storage of soybean seeds. Two fold increase in vitamin C content and 1.7 fold increase in riboflavin content and about 23% increase in thiamine was reported in germinated soybean seeds compared to ungerminated sample (Ahmad and Pathak, 2000). In wheat grain the concentration of vitamin E steadily increased with increasing germination time, reaching its peak (10.92 µg /g) after 7 days (Yang *et al.,* 2001).

The vitamin E contents in full fat soy flour decreased with increased storage time at 60^{o}C. However, there was no significant difference in vitamin E contents in samples during storage at -20^{o}C (Lee and Choe, 2002). Vitamin E content degraded quite fast during storage in dhal like black gram and green gram and the rate of degradation increased with storage temperature. At 0^{o}C retention of vitamin E ranged between 62.4% and 86.2% in pre-cooked and dried samples but at 37^{o}C, the retention of vitamin E was reduced and it ranged between 41.4% and 50.0% for the same sample (Arya and Rudramma, 2000).

Vitamin E is a fat-soluble antioxidant, important for the protection of essential fatty acids against oxidative deterioration in both plants and animals. The balance between tocopherol and essential fatty acid contents mainly determine the susceptibility to lipid peroxidation and the storage stability (Goffman and Bohme, 2001). Kamal-Eldin and Andersson (1997) found a positive significant correlation between alpha tocopherol and linoleic acid present in corn oil, but biochemical studies are needed to confirm this relationship.

In this study, changes in vitamin E and essential fatty acid contents with increasing germination time and their interrelationship has been investigated with locally introduced cultivars. In addition, changes in vitamin E and essential fatty acid content with different storage time stored under different packaging types and their interrelationship has also been investigated.

MATERIALS AND METHODS

Sample collection

Soybean seeds of cultivars Pb-1 and PM 13 were obtained from the field crop research center at MahaIlluppallma. The seeds were differentiated according to the size, shape, seed coat colour and hilum colour (DOA, 2000). The seeds were freshly harvested and sun dried for 2 weeks to reduce moisture content to 12% - 13%.

Germination of seeds

Soybean seeds of cultivar Pb-1 were selected for this experiment. Initially the seeds were allowed to steep in water overnight in a container. Water was then drained and seeds were allowed to germinate at temperature 26 - 28^{o}C and relative humidity 80% in shallow trays covered with muslin cloth. The soybean seeds were then rinsed with chlorinated water

(20 ppm) at 4 hrs interval to prevent the dryness and microbial contamination (Ahmad and Pathak, 2000). After 24, 48 and 72 hrs of soaking about 100 g samples of germinated seeds were collected separately and dried in a vacuum oven at 70^{o}C to reduce the moisture content to about 12%. Ungerminated seeds of the cultivar Pb-1 were used as the control for this experiment. All four samples were stored in desiccators for further analysis.

Storage conditions

Soybean seeds of variety PM-13 were used for this experiment. This variety was selected for its ability to be stored about six months under ambient temperature and humidity without loosing seed viability. Packaging materials like paper, polyethylene and aluminum (A1) foil were selected to observe the effect of light, moisture and oxygen on vitamin E and essential fatty acids. Two hundred grams of seeds were packed separately in paper (42 GSM), polyethylene (375 µ) and Aluminum Foil Laminate packs and stored for six months at room temperature (22 - 31^{o}C and 73±6 % relative humidity). Another 200 g of seeds were stored for six months without packing at room temperature to serve as the control for this experiment. After three months 100 g of seeds from each pack and the control set were taken out for analysis. After six months the remaining 100 g seeds from each pack were taken for analysis.

Sample preparation for analysis of fatty acids

About 100 g of each sample was ground to fine powder 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 vitamin E (tocopherol) content of this oil sample was determined by High Performance Liquid Chromatography (HPLC) method (Etenmiller *et al.*, 1998). Gas Chromatographic method was used to determine the linoleic and linolenic acid contents in the oil samples (Supelco bulletin 855A, 1994).

Determination of Tocopherol

The α-Tocopherol acetate solutions (Ye *et al.,* 2001) were used as standards since the bio availability of α-Tocopherol acetate is nearly equivalent to that of α-Tocopherol on a molar basis. The normal phase HPLC system equipped with chromatography manager and Shimadzu SPD-10AV, UV detector (Shimadzu Corporation, Colombia, 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 in length. The wavelength was 290 nm. Column temperature was maintained at 55^{o}C. Analytical grade methanol was used as the mobile phase. All analytical samples were run at a flow rate of 1.5 mL/min. A 20 µl sample of standard solution was injected each time for the determination of tocopherol.

Determination of essential fatty acid content

Linoleic and linolenic acid standard solutions were prepared as described in Supelco bulletin 855A (1994). A gas chromatography (Shimadzu GC-14 B) equipped with flame ionization detector (Shimadzu Corporation, Colombia, 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 the column. The column oven

temperature was automatically programmed at initial temperature of 110^{o}C and the final temperature of 220^{o}C. The temperature was increased at a rate of 8^{o}C/min. The flow rate of the carrier gas (Helium) was 20 mL/min. One µl standard solutions of linoleic and linolenic acids were directly injected in to the GC column and the retention time of each standard was noted. One µl of saponified and acidified samples of fatty acids was also injected into the GC coloumn using the same procedure**.**

Statistical analysis

A Complete Randomized Design (CRD) with six replicates per treatment was used as the experimental design to analyze vitamin E and essential fatty acids contents of soybean seeds during germination and storage. The data were analyzed by ANOVA 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% ($\alpha = 0.05$).

RESULTS AND DISCUSSION

The mean vitamin E content of all germinated samples were significantly ($P<0.05$) higher compared to the ungerminated sample. The vitamin E content in soybean was found to increase up to 48 hrs and then decline; a 1.8 fold increase was observed at 48 hrs (Table 1). Such increase in vitamin E content has not been reported earlier for soybean. The increase in vitamin E content during germination may be due to increased lipoxygenase enzyme activity of seeds**.** The vitamin E content of isolated embryonic axes from high vigour soybean seeds was reported to be increased (two fold) after 4 hrs of incubation over a water saturated filter paper in the dark and then declined slightly after 24 hrs of incubation in the same conditions. It was reported that during the initial steps of germination isolated soybean embryonic axes showed a significant increase in lipoxygenase activity and enhanced oxygen production. Vitamin E is one of the best quenchers for oxygen (Simontacchi *et al.,* 1993).

In this study linoleic acid content in soybean seeds declined significantly within 24 hrs of germination and remained at the same level thereafter. Dhaliwal and Aggarwal (1999) reported that the linoleic acid content expressed as percentage of free fatty acids first increased in oils extracted from 36 hrs germinated soybean (60%) with compared to control (56.1%). However, decrease in linoleic acid was noticed in oils, extracted from 48 hrs germinated soybeans (57%).

In this present study linolenic acid content was found to increase significantly after 48 hrs of germination after a decline at 24 hrs. Soybean sample germinated after 72 hrs showed the highest linolenic acid content. According to Dhaliwal and Aggarwal (1999) in the control, linolenic acid content expressed as percentage of free fatty acids was 12.5%, in 36 hrs germinated seeds it was10.0% and in 48 hrs germinated seeds it was increased to 13%.

Table 1. Vitamin E, Linoleic and Linolenic acid content of soybean seeds of cultivar Pb-1 during germination.

Germination time	Vitamin E (µg of tocopherol/g of seed)	Linoleic acid (mg/g of seed)	Linolenic acid (mg/g of seed)
Control	7.15±0.34 [a]	107.57±0.75[d]	13.95±0.19[f]
24 hrs	7.08±0.42[a]	103.95±0.78[e]	13.22±0.14[g]
48 hrs	12.63±0.54[b]	103.72±0.87[e]	18.15±0.22[h]
72 hrs	10.18±0.31[c]	103.65±1.03[e]	18.27±0.17[h]

Note: Means within a coloumn followed by the same letter do not differ significantly at P = 0.05.

A significant positive correlation was observed between vitamin E and linolenic acid content and vitamin E/essential fatty acid ratio of soybean cultivar Pb-1 with different germination time (Table 2). But linoleic acid showed a negative correlation with vitamin E, linolenic acid contents and vitamin E/essential fatty acid ratio. Vitamin E has been identified as a nonenzymatic protector against peroxidation of lipids. In plants, vitamin E is able to protect chemical damage invivo. The increase observed in the content of vitamin E at the early stages of germination could be due to an increase in active oxygen production as imbibition progressed (Simontacchi *et al.,* 1993).

Table 2. Correlations among vitamin E, linoleic acid, linolenic acid and vitamin E/essential fatty acid (E/EFA) ratio of soybean seeds of cultivar Pb-1 with different germination time.

	Linoleic acid	Linolenic acid	Vit. E/EFA ratio
Vitamin E	-0.31[ns]	0.81[*]	0.99[*]
Linoleic acid		-0.40[ns]	-0.35[ns]
Linolenic acid			0.80[*]

Note: *Significant at P = 0.05; ns-not significant.

Vitamin E content of soybean seeds decreased with time under all four packaging conditions (Figure 1). Unpacked seeds showed the highest decrease in vitamin E content after three months and six months storage followed by seeds packed in paper. The rate of decrease of vitamin E is lower in seeds packed in Al foil and polythene. Seeds packed in aluminum foil showed highest vitamin E content after three months storage among all four packaging conditions while those packed in polythene showed the highest vitamin E content after six month storage. The vitamin E content is significantly ($P<0.05$) decreased in samples stored for three months compared to fresh sample in all four packaging conditions. The vitamin E content also decreased significantly ($P<0.05$) in samples stored for six months compared to three months stored and fresh sample. The decrease in vitamin E content may be due to oxidation of vitamin by oxygen and light. In a previous study, Lee and Choe (2002) reported that full fat soy flour samples stored at -20^{o}C under dark did not show any significant difference in tocopherol contents. However, contents of tocopherol in the above sample decreased with storage time when stored at 60^{o}C and light.

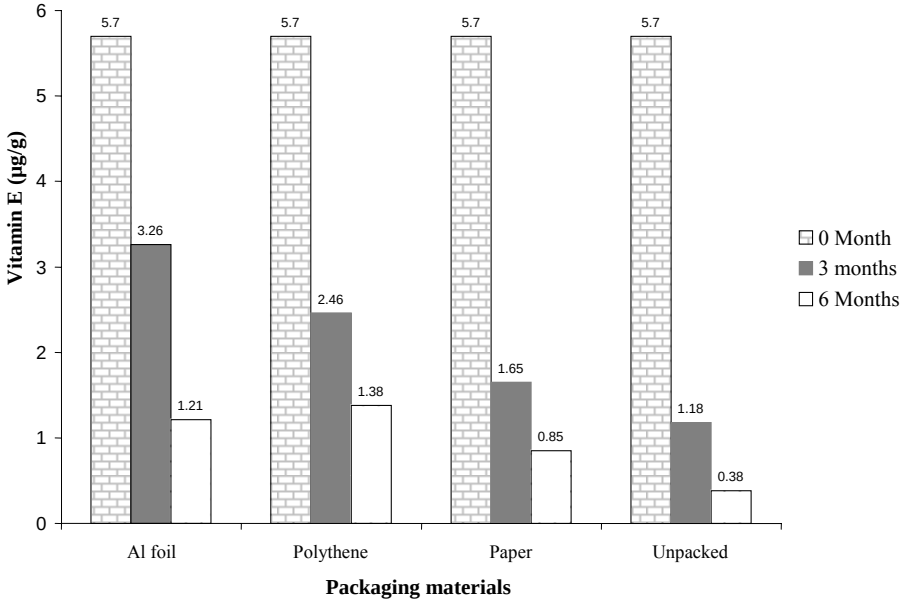

Figure 1. Vitamin E content of variety PM-13 seeds stored for different duration packed with different packaging materials.

Linoleic acid content of the soybean seeds stored in Al foil packets for three months is significantly higher than the freshly harvested seeds but it is not significantly differ from seeds stored for 6 months (Figure 2). Linoleic acid content of the soybean seeds stored in polythene packets also showed similar results like seeds packed in Al foil. Linoleic acid content of seeds packed in paper bags and unpacked seeds decreased significantly with increasing storage time; seeds stored for six months showed the lowest linoleic acid content. Stability of linoleic acid of the cultivar PM-13 soybean seeds was high when packed in Al foil and polythene even after six months storage. Packing of seeds with paper resulted in a decline in linoleic acid much similar to that of unpacked seeds.

In a previous study with soybean seeds of variety PM-13 stored for nine months under ambient conditions and controlled atmospheric conditions an increase in total free fatty acid content was observed. The increase was 4 fold in ambient storage conditions and 2 fold in controlled storage conditions (constant at $20^{o}C \pm 1^{o}C$ and 50±1% RH). However no reports were available for detailed study on each of the major fatty acids in soybean (Arulnandy and Senanayake, 1989).

Linolenic acid contents of all soybean seed samples decreased significantly with increasing storage time irrespective of the packaging type; seeds stored for 6 months showed the lowest linolenic acid content in all four storage conditions compared to other storage periods (Figure 3). Stability of linolenic acid of the cultivar PM-13 soybean seeds was better when packed in Al foil up to 3 months. Decline in linolenic acid was more or less similar in unpacked, polythene or paper packed samples. Linolenic acid content of the seeds packed in Al foil for 3 months was significantly higher than that of unpacked seeds or those packed in polythene and paper.

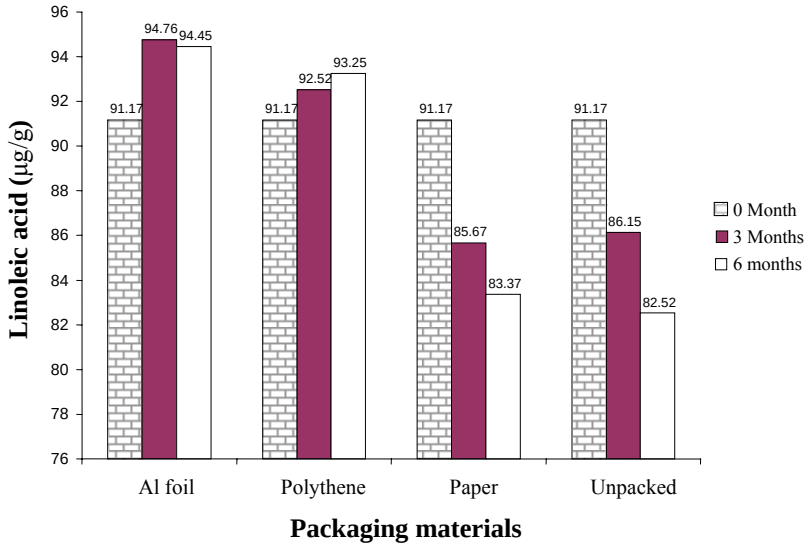

Figure 2. Linoleic acid content of variety PM-13 seeds stored for different duration packed with different packaging materials.

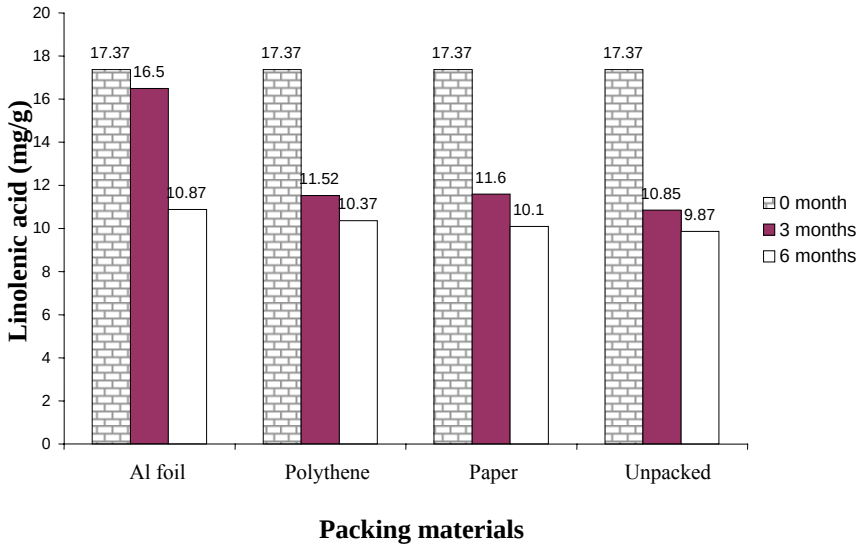

Figure 3. Linolenic acid content of variety PM-13 seeds stored for different duration packed with different packaging materials.

Table 3 Correlations among vitamin E, linoleic acid, linolenic acid and vitamin E/essential fatty acid (E/EFA) ratio of soybean seeds of cultivar PM-13 during storage with different packaging materials.

	Linoleic acid	Linolenic acid	Vit. E/EFA ratio
Vitamin E	0.40*	0.91*	0.98*
Linoleic acid		0.44*	0.36*
Linolenicacid			0.90*

Note: *Significant at p = 0.05.

A significant positive correlation was observed between vitamin E, linoleic and linolenic acid content and vitamin E/essential fatty acid ratio of soybean cultivar PM-13 during storage with different packaging materials (Table 3). Linoleic and linolenic acid contents show a significant positive correlation and also a significant correlation with vitamin E/essential fatty acid ratio. Kamal-Eldin and Andersson (1997) found a positive significant correlation between alpha tocopherol and linoleic acid present in corn oil, but biochemical studies are needed to confirm this relationship.

CONCLUSIONS

This study shows that the vitamin E content in soybean increases up to 48 hrs during germination and then decline; a 1.8 fold increase was observed by 48 hrs. The 1.8 fold increase in vitamin E content in soybean observed in this study is thus a new finding. The linoleic acid content of the germinated soybean seeds decreased with germination time but linolenic acid content increased with germination time. The linoleic acid content of the germinated seeds ranged from 103.65 - 107.57 mg/g of seed and linolenic acid from 13.22 - 18.27 mg/g of seed. A significant positive correlation between vitamin E and linolenic acid (r=0.81) was observed in the germinated seeds.

Vitamin E and Linolenic acid content of soybean seeds decreased with time under all four packaging conditions. Linoleic acid content of the seeds stored in aluminum foil and polythene packets increased with time. However, linoleic acid content of the seeds stored in paper bags and unpacked seeds decreased significantly with increasing storage time. A significant positive correlation (r=0.91) between vitamin E and linolenic acid contents and between vitamin E and vitamin E/essential fatty acid ratio (r=0.98) was observed during storage. The rate of loss of vitamin E, linoleic and linolenic acid was comparatively low in seeds packed in aluminum foil and polythene than other packaging types.

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