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Changes in concentration of tocopherols and fatty acids during germination and maturation of soybean(*Glycine max*)

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Abstract: The concentrations of tocopherols and compositions of fatty acids during germination and maturation of soybean seeds were determined by HPLC and GC. In germination stages, when the length of seedling axis was about 10 cm, the contents of total tocopherols and lipids were the highest. At the early phase of pod filling on field condition, the concentration of δ-tocopherol in soybean seeds was the highest, but, in further pod filling, the content of δ-tocopherol decreased continuously, and α- and γ-tocopherol increased. Accumulation of oil during pod filling seems to be determined at the beginning. There seem positive correlations between several tocopherol homologues and fatty acids(Received March 5, 1993; accepted April 13, 1993).

The soybean (*Glycine max*) is different from the other oil seeds in high content of oil and protein. Also, a high proportion of polyunsaturated lipids and lack of cholesterol are additional nutrition characteristics of soybean.[6] Especially, because soybean oil contains high content of tocopherols which are natural antioxidants related to aging of human, soybean is good source of vitamin E (tocopherol) in human diet.[8,10,16]

The vitamin E group consists of α-, β-, γ-, and δ-tocopherol and four corresponding unsaturated derivatives, α-, β-, γ-, and δ-tocotrienol in nature to a different extent methylated.

All tocopherols and tocotrienols are potent antioxidants, which easily oxidize, and act as an antioxidant in adipose tissues. Being antioxidants by their chemical structure, tocopherols in nature might function as interruption of the reaction chain in the first steps of fatty acid oxidation.[4] In agreement with this theory, some oil seeds with high concentration of tocopherols have high concentration of polyunsaturated fatty acids. In fact, most of soybean seeds have a good ratio of tocopherols to polyunsaturates.[12]

However, there are few informations related to tocopherols and fatty acids during growth of soybean in Korea.

The study investigated contents of tocopherol homologues and compositions of fatty acids during germination and maturation of soybean seed. And the relationships between tocopherols and unsaturated fatty acids in the aspect of the role of antioxidation were considered.

Materials and Methods

Materials

Soybean (*Glycine max*) seeds, which had been produced in Chungnam R.D.A., were germinated in the laboratory for sprouts at room temperature. The bean sprouts were collected at each stage of germination as shown in Table 1 and stored at −4℃ until analyzed. Each of the sample was ground after being dried in the ventilation drying oven at 50℃ for 12 hours. The experiments were performed in the dark and light condition of a 60-watt incandes-

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cent lamp, respectively.

For experiments on maturation, soybean seeds were sown and allowed to grow under ordinary farm conditions until the end of the experiments. Sample seeds were collected at the different stages of pod maturation as shown in Table 2 and stored at −4℃ for analysis. Some Physico-chemical properties of field soil prior to seeding were analyzed by R.D.A. standard method[14] (Table 3).

Reagents

The tocopherol standards and solvents for HPLC analysis were obtained from E. Merck Co., and standard mixture of fatty acid methyl esters and 14% boron trifluoride-methanol were purchased from Sigma Chemical Company. The extraction solvents were analytical reagent grade.

Analysis of tocopherols

2.5g of dried sample added 90 ml of 75% ethanol and 0.25g of ascorbic acid was purged with nitrogen and then extracted at 75℃ water bath with a reflux system for 2 hours. Tocopherol fraction was separated three times with 30 ml of n-hexane in separatory funnel. The combined hexane fraction was washed twice with 30 ml of distilled water and evaporated in a rotary vacuum evaporator at 40℃ under nitrogen stream. The hexane concentrates were dissolved in 2 ml of methanol and filtered through a Millipore 0.45 μm membrane for the analysis of tocopherols by HPLC.

Analysis of tocopherol homologues was performed with a Waters Associates Model 510 HPLC, U.S.A., equipped with a Waters 486 Tunable Absorbance UV detector. The separation and analysis of tocopherols were carried out on a C_{18} reverse phase μ-Bondapak stainless steel column, 10 μm particle size, 30 cm×3.9 mm I.D. with methanol/H_2O (95 : 5, v/v) as mobile phase at 1.5 ml/minute. The wavelength of UV detector was 280 nm. Each unknown components were identified by authentic standards.

Analysis of fatty acids

0.5g of dried sample was shaken with 40 ml of ethyl ether and 10 mg of tridecanoic acid ($C_{13:0}$) as an internal standard for 5 hours at 30℃. After filtration of the sample, the filtrate was evaporated in a rotary vacuum evaporator at 40℃. The concentrates of lipids were saponified and methylated by the method of Metcalfe *et al.*[13] To protect oxidation of fatty acids, all of the system were displaced with nitrogen.

The fatty acids in each sample were analyzed with a Varian Model 3700 GC, equipped with flame ionization detector (FID). A fused silica capillary column (Supelco Inc.), 30 m×0.32 mm I.D. coated SP-2340, was used to separate the fatty acids. The column oven temperature was 180℃, and injector

Table 1. Stages of the germinating seeds

Growth stages	Description	Length of axis, cm
G-0	Original seed	0
G-1	Early white axis	~1
G-2	Seedling axis(5 cm)	5
G-3	Seedling axis(10 cm)	10
G-4	Early lateral root	15
G-5	Early first leaf	20

Table 2. Stages of pod filling of the seeds

Growth stages	Days after seeding	Date	Description
M-1	105	Sep. 1	Early seed formation
M-2	120	Sep. 15	Middle pod filling
M-3	135	Oct. 1	Green pod filling
M-4	150	Oct. 15	Brown pod filling (ripening)

Table 3. Physico-chemical properties of soil prior to seeding

pH	OM	T-N	Ava. P_2O_5	Exchangeable cation (me/100g, dw)			
(1 : 5)	(%)	(%)	(ppm)	Ca	Mg	K	Na
6.6	0.88	0.04	254	2.7	1.4	0.25	0.07

Table 4. Tocopherol compositions of soybean seeds germinated in the dark and light condition

Growth stages	Dark condition				Light condition			
	Tocopherols(%)			Total tocopherol	Tocopherols(%)			Total tocopherol
	α	γ	δ	(mg/100g, dw)	α	γ	δ	(mg/100g, dw)
G-0	9.5	59.9	30.6	40.4	9.5	59.9	30.6	40.4
G-1	9.9	62.0	28.1	30.9	9.6	66.6	23.7	27.8
G-2	11.3	69.4	19.3	39.8	13.0	66.1	20.9	33.6
G-3	14.3	60.4	25.3	63.0	18.7	56.6	24.7	44.8
G-4	15.1	59.3	25.6	45.5	22.1	56.3	21.6	38.2
G-5	12.6	61.2	26.2	34.6	28.7	50.8	20.5	30.5

and detector temperatures were 240℃. Nitrogen was used as carrier gas at a flow rate of 1 ml per minute with a split ratio of 1 : 100. The response factor of fatty acid methyl esters on the same analytical condition of GLC was determined with a standard mixture of fatty acid methyl esters. Each fatty acid was identified by matching the retention time of the unknown peak with that of the standard compound.

Table 5 Tocopherol compositions of soybean seeds during maturation under field conditions

Growth stages	Tocopherols(%)			Total tocopherol (mg/100g, dw)
	α	γ	δ	
M-1	2.9	36.9	60.2	29.0
M-2	2.7	49.8	47.8	28.8
M-3	2.7	65.3	32.0	28.2
M-4	11.5	66.6	21.9	34.0

Results and Discussion

Changes in the concentration of tocopherol homologues during seed germination

The changes of composition of tocopherol homologues during germination of soybean seeds in the dark and light are shown in Table 4.

Ungerminated soybean seeds (G-0 stage) predominantly contained γ-tocopherol (24.2 mg/100, dw, ca. 60% of total), and δ- and α- tocopherol followed. At the G-1 stages, there was a temporary decrease of total tocopherols. In further stages of the dark condition, the concentration of total tocopherols gradually increased till G-3 stage, having 8.9 (α-tocopherol), 38.1 (γ-tocopherol) and 15.9 mg (δ-tocopherol) per 100g of dry weight. And, during the next stages, they all continously decreased and at G-5 stage the values were lower than those of original seeds. However, the tocopherol contents of soybean seeds germinated in artificial light differ from that of the dark. Although the change of non-α-tocopherol was similar to that of the dark, only the ratio of α-tocopherol to the total increased gradually till the last stages. The result suggested that there was a close relation between α-tocopherol and chlorophylls because the chlorophyll occurred in the light conditions only. In higher plants, in fact, α-tocopherol is accumulated in the lamellae and osmiophilic globules of the chloroplast in green tissue and the leucoplasts, chromoplasts, and etioplasts of nonphotosynthetic tissue.[17] In chloroplast, α-tocopherol could protect vulnerable lipids from photooxidation by chlorophyll.

Changes in the concentration of tocopherol homologues during maturation

As shown in Table 5, at early phase of pod filling, the ratio of concentration of δ-tocopherol to the total was the highest. In further pod filling, however, the δ-tocopherol was rapidly decreased, whereas α- and γ-tocopherol increased gradually. The δ-tocopherol seems to be a preliminary compound which is converted to other forms of tocopherol having higher biological activity in final ripening stage. And, biosynthesis of the γ-tocopherol seems to be associated with the final stages of soybean seed

Table 6. Fatty acid compositions of soybean seeds germinated in the dark

Growth stages	Oil content (g/100g, dw)	Fatty acid Composition(%)					Saturated fatty acid (%)	Unsat'd fatty acid (%)
		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$		
G-0	15.0	9.2	2.3	17.0	65.0	6.5	11.5	88.5
G-1	15.9	9.3	2.4	16.6	64.6	7.1	11.7	88.3
G-2	18.1	8.6	2.4	16.0	66.2	6.8	11.0	89.0
G-3	18.7	9.3	2.6	16.9	64.6	6.6	11.9	88.1
G-4	17.8	9.8	2.5	14.7	65.7	7.3	12.3	87.7
G-5	16.6	9.6	2.6	15.6	64.1	8.1	12.2	87.8

Table 7. Fatty acid compositions of soybean seeds germinated in the light

Growth stages	Oil content (g/100g, dw)	Fatty acid Composition(%)					Saturated fatty acid (%)	Unsat'd fatty acid (%)
		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$		
G-0	15.0	9.2	2.3	17.0	65.0	6.5	11.5	88.5
G-1	16.0	9.5	2.4	16.5	64.5	7.1	11.9	88.1
G-2	17.6	9.6	2.4	16.2	64.8	7.0	12.0	88.0
G-3	18.5	9.7	2.5	16.0	64.5	7.3	12.2	87.8
G-4	18.1	10.3	2.5	16.4	63.7	7.1	12.8	87.2
G-5	18.2	9.3	2.6	16.4	63.6	8.1	11.9	88.1

ripening.

Also, when tocopherol compositions in Table 4 and 5 are calculated in terms of the concentrations, the concentrations of tocopherol homologues of original soybean seeds used in this research were 3.8 mg (as α-tocopherol), 24.2 mg (as γ-tocopherol), and 12.4 mg (as δ-tocopherol) per 100g of dry weight (Table 4), whereas the concentrations of tocopherol homologues in soybean seeds harvested on the field were lower than original ones as 22.6 mg of γ-tocopherol and 7.5 mg of δ-tocopherol per 100g of dry weight, with the exception of the α-tocopherol (Table 5). The results suggest that the concentration of tocopherols in the plant is changed by the growing condition. In fact, the tocopherol content in plants is influenced by plant genetics, weather conditions during the growing and harvest seasons, the state of maturity at harvest, and handling after harvesting.[3-5]

Changes in the concentration of fatty acids and oils during germination

The data of fatty acids and oil of soybean seeds germinated in the dark and light were shown in Table 6 and 7.

The changing pattern of fatty acids during germination of soybean seeds was very similar between dark and light-grown seedlings, as well as the ratio of saturated to unsaturated lipids. Major fatty acids in both dark and light-grown seedlings were oleic and linoleic acid, and the amount of unsaturated fatty acids was very predominace as above 85% of total.

During germination in the dark and light, oil content of soybean seeds slowly increased till G-3 stage, while, in further growing stages, oil contents of soybean seed constantly kept at G-4 and G-5 stages in the light and decreased in the dark.

There was an increasing tendency gradually in stearic and linolenic acid during germination of soybean seeds in the dark and light, while oleic and linoleic acid decreased irregularly. Shin[15] reported an increase of stearic and a decrease of linoleic, and, in contrast, Brown *et al.*[1] observed a continuous increase of linoleic acid in the cotyledon of soybeans germinated in the dark. Holman[7] reported a gradually decrease in the linoleic and linolenic acid content of whole soybean seedlings during 6

Table 8. Fatty acid compositions of soybean seeds during maturation of pod under field conditions

Growth stages	Oil content (g/100g, dw)	Fatty acid composition (%)					Saturated fatty acid (%)	Unsat'd fatty acid (%)
		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$		
M-1	8.8	13.6	2.7	27.5	46.5	9.7	16.3	83.7
M-2	17.5	11.9	2.1	23.6	55.7	6.7	14.0	86.0
M-3	17.0	13.1	2.4	21.6	55.6	7.3	15.5	84.5
M-4	15.6	11.4	2.5	19.9	59.0	7.2	13.9	86.1

Table 9. Simple correlation coefficients between tocopherol and fatty acid compositions during germination of soybean seeds in the dark and light conditions

Growth condition	Tocopherols	Fatty acids				
		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Dark	α-Tocopherol	0.525	0.787	−0.596	0.077	0.267
	γ-Tocopherol	−0.858	−0.245	0.016	0.554	−0.136
	δ-Tocopherol	0.530	−0.237	0.347	−0.623	−0.028
Light	α-Tocopherol	0.266	0.944	−0.396	−0.891	0.848
	γ-Tocopherol	−0.066	−0.732	0.077	0.706	−0.630
	δ-Tocopherol	−0.431	−0.723	0.677	0.658	−0.694

Table 10. Simple correlation coefficients between tocopherol and fatty acid compositions during pod filling of soybean seeds in the field conditions

Tocopherols	Fatty acids				
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
α-Tocopherol	−0.706	0.218	−0.647	0.578	−0.240
γ-Tocopherol	−0.541	−0.253	−0.975	0.889	−0.721
δ-Tocopherol	0.634	0.155	0.981	−0.891	0.663

days germination in the dark. The results suggest that fatty acids of soybean seedlings are randomly used for their energy metabolism during germination.

Changes in the concentration of fatty acids and oils during maturation

Table 8 shows the composition of fatty acids and content of oil during pod filling of soybean seed.

In the first phase of seed formation (M-1 stage), the composition of fatty acids was different from that of germination stages as shown in Table 6 and 7, in that only the ratio of linoleic acid is lower than that of germination stages and remaining ones are higher. Also, the ratio of unsaturated fatty acids is lower than that of germination stages. But, in further pod filling, the concentration of linoleic acid in contrast to remaining ones increased markedly, although, at the final stage, its content did not reach the level of seedlings.

The oil content in the M-1 stage was as low as 8.8% but its content at the M-2 stage rapidly increased up to the level of 17.5%. The result indicated that the accumulation of oil during maturation of soybean seeds achieved in the early phase.

Correlation of tocopherols and fatty acids in the soybean seed

The simple correlation coefficients between compositions of tocopherols and fatty acids during germination of soybean seeds in the dark and light, and pod filling were shown in Table 9 and 10.

During germination of soybean seeds in the dark and light, the correlation coefficients between α-tocopherol and stearic acid were 0.787 ($P<0.1$) and 0.944($P<0.01$), and those between γ-tocopherol and linoleic acid were 0.554 and 0.706 although there were no significances. Also, the correlation coefficients between γ-tocopherol and linoleic acid during pod filling was 0.889 and that between δ-tocopherol and oleic acid was 0.981($P<0.02$). That is, there seem positive correlations between α-tocopherol and stearic acid in germination, γ-tocopherol and linoleic acid in germination and maturation, and δ-tocopherol and oleic acid in maturation, respectively.

While, current evidence indicates that γ-tocopherol is a better antioxidant than α-tocopherol. In vitro, γ-tocopherol had greater antioxidant activity than α-tocopherol since the latter was more easily oxidized by air. Lea[11] has shown, using a series of accelerated oxidation tests, that α-tocopherol is the least effective in vitro antioxidant among the tocopherol homologues. Cillard and Cillard[2] reported than α-tocopherol exhibited prooxidant property, whereas γ-tocopherol was antioxidant in aqueous media. Also, Khafisov *et al.*[9] concluded that γ-tocopherol was a better antioxidant that α-tocopherol in cotton seed oil. Therefore, the predominance of γ-tocopherol in soybean seeds having abundant linoleic acid seems to be significance in relation to its nutritional value and the protection of relatively large quantitatives of stored lipid for long periods.

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대두의 발아 및 성숙과정중 토코페롤과 지방산의 변화

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초록 : 대두의 발아 및 종실의 성숙과정중 토코페롤 및 지방산 함량의 변화를 알아보기 위하여 발아과정은 실험실에서 명조건과 암조건으로 구분하여 수행하였고, 종실의 성숙과정 시험은 포장에서 실시하였으며, 토코페롤과 지방산의 함량은 HPLC와 GC를 사용하여 분석하였다. 발아과정중 각 토코페롤 및 지방질 함량은 배축부의 길이가 약 10 cm로 생육시 가장 높았으며, 발아과정 후기에서는 명조건하의 α-토코페롤을 제외하고 모두 감소하였다. 종실의 초기 생성과정중 δ-토코페롤은 가장 높은 함량을 보였으나 성숙과정의 진행과 동시에 점차 감소되었고, 역으로 γ- 및 α-토코페롤은 종실의 성숙과정중 꾸준히 증가하였다. 성숙과정중 종실의 유지함량은 초기단계에서 현저하게 증가하였고, 지방산조성의 변화에서는 점진적인 oleic acid의 감소와 더불어 linoleic acid의 증가가 관찰되었다. 토코페롤 동족체들과 지방산들 사이의 상관성을 검토한 결과, 일부 처리구간에서 정의 상관관계가 인정되었다.