Research Article

FATTY ACID COMPOSITION OF OIL EXTRACTED FROM SOYBEAN SEEDS HARVESTED AT DIFFERENT DAYS OF REPRODUCTIVE STAGE

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ABSTRACT

Oil accumulation in soybean is considered as an important trait during seed development which is greatly valued in food, or industrial application. An experiment was done to analyze fatty acid composition of soybean seed harvested at different stages of reproductive period. Six soybean accessions with varied fatty acid composition were sown in two locations- Research field of Kyungpook National University (KNU), and Chungpook National University (CNU), Korea, using four replications for each treatment in both the sites. The pod samples were harvested at an interval of 10 days from the 45th day after flowering, and sampling was continued up to the 75th day until the crop was matured. Fatty acid composition was analyzed by gas chromatography. Significant variation in all five fatty acid compositions was observed among six accessions planted at both locations, except for steric acid, planted at the Research field of CNU. All fatty acid composition was significantly different with respect to the different days (45, 55, 65 and 75), planted in both locations. But, there was no significant interaction found between accessions and days after flowering for both the locations. Among five fatty acids, mean palmitic, steric, and linolenic acid was increased while oleic and linoleic acid was decreased as seed matured for both the locations. These findings provide valuable information in selecting soybean varieties with desirable fatty acid composition to have healthy soybean oil, especially when considered for cooking and industrial purposes.

Key words: Seed development, days after flowering, trait, accessions

INTRODUCTION

Soybean (*Glycine max* L.) is popularly known as a health enhancing food in many Asian countries. Many countries use soybeans in various forms such as soybean sprouts, pastes, soymilk, soybean oil, and tofu as key ingredients in cultural cuisine (Kim et al., 2006). Soybean is used mainly for oil extraction and meal production as it is cheap source of oil and protein (Smith & Circle, 1972), and an important source of vegetable oil for human food (Glaudemans et al., 1998). Soybean oil contains different chemical components. Its quality is primarily dependent on a function of its fatty acid composition. The ratio and amount of saturated and unsaturated fatty acids determine the physical, chemical, and nutritional values of the oil (Gunstone & Norris, 1983). The average fatty acid composition of commercial soybean oil is about 12% palmitic, 4% stearic, 23% oleic, 53% linoleic and 8% linolenic acids (Wilson, 2004), which are predominant fatty acids of soybean oil but ideal fatty acid composition of soybean oil depends on the specific use for which it is intended. So, new soybean genotypes with modified fatty acid composition are constantly being considered for commercial production (Vasilia & Boerma, 2005) to meet the nutritional as well as industrial demand of consumers and industries. A reduction in palmitate (Wilcox et al., 1994) and sterate would enhance the nutritional quality of oil by lowering the total saturated fatty esters (Dutton et al., 1951; Mounts et al., 1988; Smouse, 1979). Intake of diet with saturated acyl components, palmitic and stearic fatty acids may contribute to increase blood serum cholesterol levels (Mensink et al., 1994), thereby increasing the risk of coronary heart disease (Willett, 1994; Uusitalo et al., 1996). Generally, oil with a high content of monounsaturated fatty acid (i.e oleic acid) is less susceptible to oxidative changes during refining, storage and frying which can be heated to higher temperatures without smoking, so that food is cooked faster and absorbs less oil (Miller et al., 1987). Further more, the quality of this oil is retained longer during storage than that of oil with high content of polyunsaturated fatty acids (Robertson & Thomas, 1976). On the other hand, high content of polyunsaturated fatty acids limits the utility of oil, mostly used for cooking and frying, unless it is hydrogenated (Rakow & McGregor, 1973). But, during this hydrogenation chemical treatment, unsaturated fatty acids are converted into saturated fatty acid and also many positional and trans-isomers not normally found in nature are produced. Intake of this artificial fatty acid is related to risk of developing heart diseases (Willet & Ascherio, 1994). These unstable components are also responsible for poor flavor and undesirable oil order, particularly in oils that are heated during the use (Dutton et al., 1951; Mounts et al., 1988; Smouse, 1979).

A crude soybean oil having low saturated fatty acid, high oleic acid and low linolenic acid concentration offers competitive market opportunities in numerous edible and industrial applications. Extensive investigations on complete chemical composition of developing seeds of soybean have been carried out (Sangwan et al., 1986; Yazadi-Samadi et al., 1977; Yao et al., 1983). It is reported that the enzymes that involved in fatty acid biosynthesis in soybean seeds are influenced by both environment and inheritance characteristics (Brim et al., 1968; Hammond

et al., 1972; Singh & Hadley, 1968). Investigation on the comparative changes in fatty acid composition of different soybean genotypes during its maturation is important. So, the objective of this experiment was to analyze fatty acid composition of developing soybean seeds at different stages of reproductive period, and to determine quality of oil content considering cooking food and industrial purposes.

MATERIAL AND METHODS

Seed materials

Six soybean accessions, Pungsannamul, Cheongja, Eunha, Hwangkeum, KLG12072 and KLG12228, with altered fatty acid composition were used in this experiment. Among six soybeans, Pungsannamul, Cheongja, Hwangkeum and Eunha had normal fatty acid composition (12% palmitic, 4% stearic, 23% oleic, 53% linoleic and 8% linolenic acids) while KLG12072 and KLG12234 had high oleic acid and low linolenic acid, respectively. The soybean accessions were raised in 2010 at two different locations having different growing environment. The experimental design was a randomized complete block design with four replications. The selected places were experimental farm of KNU and CNU, Korea. The flowers were tagged immediately after their emergence. The pod samples were harvested at intervals of 10 days from the 45th day after flowering, and sampling was continued up to the 75th day when the crop matured. Immediately after harvest, the pods were shelled and the collected seeds were dried. The dried seeds were grounded into fine powder with dry mill. A portion of sample powder (0.5 g) of was used for analysis.

Analysis of Fatty Acid

For oil extraction, each sample (0.5 g) was taken in a test tube and 10 ml of hexane was poured into it. The samples were then placed in a shaking incubator (150 rpm) at 50 ºC for two days. The clear supernatant was transferred into another test tube. Hexane was evaporated by passing air in evaporating unit. The extracted oil (0.15 mL) from each sample was placed in a screw-capped vial, and 5 ml of methylation solution (H_2SO_4 : MeOH : toluene = 1 mL : 20 mL : 10 mL) was added. The sealed vial was heated on a water bath (100 ºC) for 60 min, and allowed to cool at room temperature. Then 5 mL of water was added and shaken. The mixture was separated into two layers, and the upper layer was taken by Pasteur pipette and dried by using anhydrous sodium sulfate for 5 min. Then 1µL of sample was directly injected to the GC using automatic sampler (Agilent 7683B). An Agilent 7890A gas chromatography with a flame ionization detector (FID) and 0.32 mm i.d × 25 m HP-FFAT capillary column was used. The oven temperature was raised from 150 ºC (1 min. holding) to 230 ºC at a constant rate of 2.5 ºC per minute. The injector and detector temperature were kept at 250 ºC and 230 ºC, respectively. The carrier gas was nitrogen at a flow rate of 1 mL per min., and the split ratio at the injector port was 50:1.

Statistical analysis

Analysis of variance (ANOVA) and multiple mean comparisons were performed using the general linear model (GLM) by Statistical Analysis System (SAS 9.1) to identify significant treatment effects and interactions. Differences among mean values were determined using Least Significant Difference at $P \leq 0.05$. Data were analyzed as two factorial completely randomized designs with four replications.

RESULTS AND DISCUSSION

Changes in fatty acid composition of soybean seed oil during its development, planted at two locations (KNU and CNU) are shown in table 1 and 2, respectively. Significant variation in fatty acid composition was observed among six accessions planted at both locations except for steric acid planted in CNU. All fatty acid compositions were significantly different among four different days (45, 55, 65 and 75) planted in both locations. But, no significant interactions between accessions and days after flowering were observed for both locations (Table 1 and 2).

Variation of fatty acid composition of oil of six soybean accessions for different four dates after flowering (for both location KNU and CNU) are shown in Figure 1 and 2, respectively. In this experiment, the increasing or decreasing trend of all five fatty acid composition planted at two locations during seed development period for six accessions was similar. Among five fatty acids, palmitic, steric and linolenic acid were increased while oleic and linoleic acids were decreased as seed matured. But, if we see the individual accessions, KLG12072 and KLG12234 planted in KNU, oleic acid was first increased when days after flowering increased from 45th to 55th day and again decreased up to 75th day. In contrast, linolenic acid decreased from 45th to 55th day and again increased up to 75th day. However, the increasing or decreasing rate was much lower in later stage as compared to initial stage of most of the accessions. Furthermore, the results of increase in oleic and decrease in linolenic acid was consistent with the results of Rubel et al., (1972) and Privett et al., (1973) and contrast to the result reported by Sangwan et al. (1986). The result of Cherry et al. (1984), also supports the inverse pattern of oleic and linolenic acid content. Cherry's

result showed that linolenic acid content in mature seed is inversely proportional to that of oleic acid.

The differences in result regarding changes in fatty acid contents, in addition to varietal differences, observed among the different studies, including this study, may be due to agro-climatic factors, germination condition, germination stage and data analysis method employed. It is known that the enzymes involved in the fatty acid biosynthesis in soybean are influenced by both environmental and inheritance characteristics (Brim et al., 1968; Hammond et al., 1972; Singh & Hadley, 1968).

The decrease in levels of palmitic and oleic acid with seed maturity are in agreement with other workers and the pattern of change is also expected as the short-chain saturated fatty acids are believed to be the intermediates in biosynthesis of higher unsaturated fatty acids (Kannangara et al., 1973). Total unsaturated fatty acid (free fatty acid) composition in all the accessions decreased as soybean became more matured as reported by others (Urbanski et al., 1980 and Yao et al., 1983). The result is expected since the free fatty acids are being utilized for oil synthesis during seed maturation (Yao et al., 1983).

Table 1. Changes in fatty acid composition (relative %) in soybean during seed development, grown in Research farm of KNU, Korea.

Accession	Days after flowering	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
Pungsannamul	45	13.4	4.9	26.3	47.0	8.6
	55	10.1	4.1	28.4	50.4	7.2
	65	10.3	3.8	27.4	51.0	7.7
	75	9.5	3.8	27.2	51.8	7.8
Cheongja	45	10.6	4.2	22.7	52.7	10.0
	55	10.0	3.8	24.6	53.1	8.8
	65	9.3	3.6	23.9	54.6	8.8
	75	8.9	3.6	23.6	55.4	8.7
Eunha	45	10.8	4.3	19.9	56.0	9.1
	55	10.0	3.8	21.9	56.5	8.0
	65	9.5	3.6	22.0	57.8	7.3
	75	9.1	3.3	22.6	57.8	7.4
Hwangkeum	45	10.2	4.1	25.3	51.3	9.7
	55	9.5	3.8	27.3	51.7	7.9
	65	9.5	3.7	26.6	52.6	7.8
	75	9.1	3.4	27.2	52.9	7.7
KLG12072	45	9.6	5.1	48.1	29.3	8.0
	55	9.1	4.3	50.3	28.7	7.8
	65	8.7	4.2	48.8	31.1	7.4
	75	8.6	3.9	48.9	31.5	7.2
KLG12234	45	4.2	3.9	26.5	60.1	5.5
	55	3.9	3.8	30.0	58.1	4.3
	65	3.7	3.5	27.5	61.3	4.1
	75	3.3	3.3	28.0	61.6	3.9
	LSD (0.05%)	1.8	0.9	6.9	7.0	1.1
Accession		**	*	**	**	**
Days after flowering		**	**	ns	ns	**
Accession × Days after flowering		ns	ns	ns	ns	ns

* = Significant ($p<0.05$), ** = Significant ($p < 0.01$) and ns = not significant at $p=0.05$
LSD (0.05%) is for all values of days after flowering of each column

Table 2. Changes in fatty acid composition (relative %) in soybean cultivars during seed development grown in Research farm of CNU, Korea.

Accession	Days after flowering	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
Pungsannamul	45	11.7	3.8	24.6	51.6	8.5
	55	10.6	3.5	25.9	52.4	7.8
	65	10.2	3.6	26.6	52.7	7.1
	75	9.7	3.4	27.1	53.4	6.7
Cheongja	45	11.7	4.5	21.6	52.9	9.5
	55	10.9	3.9	22.4	54.4	8.6
	65	10.4	3.7	23.3	54.8	7.9
	75	10.0	3.4	24.2	54.8	7.7
Eunha	45	12.0	3.7	21.5	54.6	8.4
	55	11.2	3.3	22.2	55.4	8.1
	65	10.3	3.1	23.0	56.3	7.7
	75	9.8	3.0	23.8	56.4	7.3
Hwangkeum	45	10.4	3.5	25.4	51.4	9.4
	55	10.0	3.3	26.8	51.9	8.2
	65	9.5	3.1	27.4	52.4	7.8
	75	9.3	3.1	28.1	52.8	7.0
KLG12072	45	9.8	4.1	47.3	30.5	8.4
	55	9.4	3.9	47.9	31.3	7.7
	65	9.0	3.6	48.6	31.6	7.3
	75	8.9	3.3	49.1	32.2	6.8
KLG12234	45	4.6	4.1	27.8	58.8	4.9
	55	4.3	3.8	28.7	59.5	4.3
	65	4.0	3.5	29.0	59.7	4.0
	75	3.7	3.2	29.2	60.3	3.9
	LSD (0.05%)	1.2	0.9	8.5	7.5	2.0
Accession		**	ns	**	**	**
Days after flowering		**	*	ns	ns	**
Accession × Days after flowering		ns	ns	ns	ns	ns

* = Significant ($p<0.05$), ** = Significant ($p < 0.01$) and ns = not significant at $p=0.05$
LSD (0.05%) is for all values of each day after flowering of each column

The average fatty acid composition of commercial soybean oil is 12% palmitic, 4% stearic, 23% oleic, 53% linoleic and 8% linolenic acids (Wilson, 2004). There was a large variation in fatty acid composition within six soybean accessions for each day after flowering planted in two locations. Pungsannamul Cheongja, Eunha and Hwangkeum were selected for normal fatty acid composition also showed normal fatty acid composition in each day after flowering. Similarly, KLG12072 and KLG12234 showed high oleic acid and low linolenic acid, respectively. The highest palmitic and stearic acid content i.e 13.4% and 5.1%, respectively were measured in Pungsannmul and KLG12072 soybean at 45th days after flowering while the lowest palmitic and stearic acid content (3.3%) was measured in KLG12234 soybean at 75th days after flowering (matured stage). In case of oleic acid and linolenic acid, all stages of KLG12072 and KLG12234 showed higher and lower composition of oleic and linolenic acid, respectively as compared to each stages of other accessions.

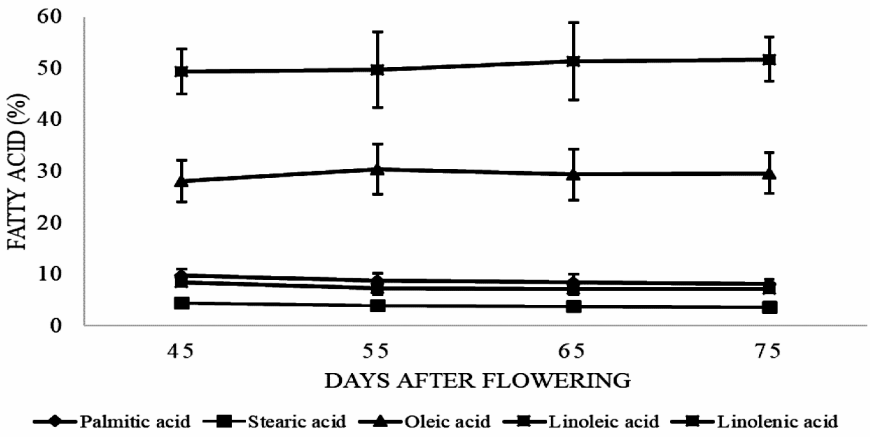

Figure 1. Variation of fatty acid composition in seeds of six soybean accessions for different four dates after flowering, grown in Research farm of KNU, Korea.

Note: Pungsannamul, Cheongja, Hwangkeum and Eunha-normal fatty acid concentration;KLG12072- high oleic acid concentration, and KLG12234- low linolenic acid concentration.
Data for each genotype are the mean ± standard error from four replicate s.

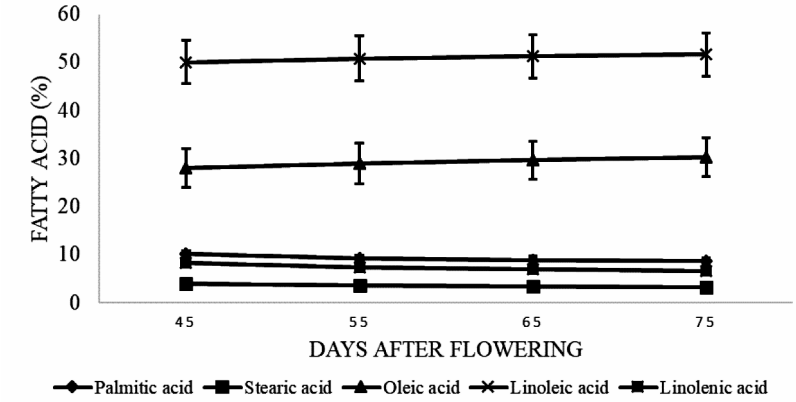

Figure 2. Variation of fatty acid composition in seeds of six soybean accessions for different four dates after flowering, grown in Research farm of CNU, Korea.

Note: Pungsannamul, Cheongja, Hwangkeum and Eunha-normal fatty acid concentration; KLG12072- high oleic acid concentration, and KLG12234-low linolenic acid concentration.
Data for each genotype are the mean ± standard error from four replicates.

CONCLUSION

The content of palmitic, stearic and linolenic acid decreased, but oleic and linoleic acid increased while the maturity days increased from 45th to 75th day. This pattern of fatty acid composition of soybean oil from developing seeds indicate that oil of mature seeds is superior to that of immature ones as it contents low saturated, high oleic and low linolenic acid which is desirable composition for edible and industrial soybean oil. This information on genetic variability in fatty acid composition of developing soybean seed will be useful in future efforts to the quantitative and qualitative improvement in oil content.

ACKNOWLEDGEMENTS

This work was carried out with the support of 'Lab fund' provided by Plant Genetics Lab, School of Applied Biosciences, Kyungpook National University, Republic of Korea.

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