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Determination of Total Protein and Fatty Acid Composition of Soybean Seed as Affected by Sowing Dates

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The effect of sowing dates and cultivars on total protein, oil and fatty acid composition of soybean seeds grown in southeast Anatolia region was studied. Protein content of soybean varied from 29.26 to 34.94 % and the total oil content of soybean varied from 18.61 to 19.64 %. The protein and oil contents were significantly different among treatments ($p < 0.001$). Sowing dates affected protein, oil, palmitic, stearic, oleic, linoleic and linolenic acids significantly ($p < 0.001$). Palmitic acid varied from 10.64 to 12.92 %, stearic acid varied from 4.01 to 5.17 %, oleic acid varied from 25.27 to 30.09 %, linoleic acid varied from 47.61 to 51.65 % and linolenic acid varied from 5.38 to 7.61 %, respectively. Correlation coefficients between protein and oil content were -0.2170. Sowing dates and sowing dates x cultivars interaction effects were also found to be significant ($p < 0.001$) over the oleic and linoleic acid contents.

Key Words: Soybean, Sowing dates, Protein content, Oil content, Fatty acid composition.

INTRODUCTION

Soybean (*Glycine max* L.) is a leguminous seed crop that is grown widely around the world. Soybean and its processed products have been acclaimed as health foods because of their high protein and essential amino acids contents, omega-3 fatty acids, fat-soluble vitamins, polysaccharides and insoluble fiber[1].

The value of soybeans is based both on protein and oil content. Among cereal and other legume species, soybean seeds contain the highest amount of protein (35-45 %) and a relatively high level of oil (20-25 %)[2]. Soybean oil typically contains 11 % palmitic (16:0), 4 % stearic (18:0), 23 % oleic (18:1), 54 % linoleic (18:2) and 8 % linolenic (18:3) acids, respectively[3,4].

The nutritional value of soybean is determined not only by the quantity but also by the quality of its protein and oil content. One quality factor, which has recently gained much attention, is the fatty acid composition of soybean oil. There is an increasing evidence for a relationship between consumption of saturated fat and elevated serum cholesterol level[5] and that between linolenic acid content and the oxidative stability and loss of flavour in a food system[6].

The actual composition of soybeans depends on many factors, including genotype, growing seasons, geographic location and agronomic practices[7-10]. It is well known that climate has a great influence on the ripeness and chemical composition of vegetable oils[11]. Climate and cultivars both effect the linolenic acid content of soybean oil[12]. The negative correlation between oil and protein contents has been well documented[13,14]. The effect of agronomic factors on the seed protein and oil of soybeans have not been quantified under the growing conditions of southeast Anatolia region and the effect of cultivars and climate on seed composition are also of interest to the soybean industry[15].

The differences in fatty acid content are likely due to the different weather patterns from year to year and from location to location. Studied have indicated that temperature plays a vital role in the synthesis of fatty acids especially unsaturated fatty acids in soybean oil. Studies conducted with common cultivars under extreme temperature conditions have indicated that seeds from soybean plants exposed to high daily temperatures have reduced linoleic and linolenic acids and increased oleic acid content[16-18]. Extreme temperature conditions reduced linolenic acid and elevated stearic acid contents. Higher levels of stearic acid were associated with higher temperatures[19].

The effect of sowing dates on seed composition may be valuable for soybean growers. The objective of this study was to evaluate the influence of different sowing dates on the oil and protein content and fatty acid composition of soybeans.

EXPERIMENTAL

Field experimantation

Soybean cultivars NE3399 and UMUT2002 were grown on five different sowing dates (April 30, May 15, May 30, June 15, June 30) in 2005 and 2006 growing seasons in Diyarbakir province in Turkey. Their seed protein and oil contents as well as the fatty acid composition were evaluated. The experiment was conducted on the trial area of southeast Anatolia Agricultural Research Institute, on the silty-clay soil with pH of 7.65 to 7.80 and a lime content of 8.67 %. It is in the southeast of Turkey with an altitude of 650-700 m above the sea level and with an average daily temperature of 21.7 and 22.3 ºC, respectively. Annual precipitation varies from 170.1 to 257.0 mm distributed in two rain seasons. Meteorological data in Table-1 for the experimental periods were recorded at a site (Turkey Meteorological Department Station in Diyarbakir) located 7 km far the field site. Sowing was performed on irrigated seedbads.

TABLE-1
METEOROLOGICAL DATA FOR THE FIELD SITE FOR SOYBEAN GROWING AREA IN 2005 AND 2006

Months	Mean temperature (ºC)		Rainfall (mm)		Mean relative humidity (%)	
	2005	2006	2005	2006	2005	2006
March	8.4	9.2	58.4	26.6	53.0	62.1
April	14.1	14.5	36.5	77.9	52.0	68.9
May	19.6	19.4	26.5	38.4	44.0	53.3
June	25.8	28.5	33.1	0.0	25.0	23.3
July	32.4	31.4	0.0	6.1	11.0	25.0
August	31.8	32.6	0.0	0.0	20.0	16.4
September	25.0	25.0	0.7	3.5	31.0	35.9
October	16.2	17.6	14.9	104.5	40.0	70.9
Mean	21.7	22.3	-	-	34.5	44.5
Total	-	-	170.1	257.0	-	-

Mean = Is the monthly mean for the whole growth period from planting to maturation. Total = Is the cumulative rainfall during seed development.

The experimental design was a completly randomized design of split plot with four replications. Plot size was 2.8 × 6 m. The seeds were sown by the sowing machine at a spacing of 0.05 and 0.70 m within and between the rows, respectively.

Diammonium phosphate (DAP) fertilizer was applied at a rate of 100 kg/ha during the planting. Experimental plots were weeded twice and diseases and pest were controlled by spraying. The seeds were harvested at maturity and air-dried in the laboratory. Irrigation was terminated 15-20 d before the harvest. The length of the growing period varied from 98 to 147 d.

Method of oil and protein extraction: The oil content of seeds was determined by a soxhlet extraction method[20] using *n*-hexane as solvent at 70 ºC for 6 h. Protein content (N × 6.25) of soybean samples were determined according to the Kjeldahl procedure[21] by using a Tecator Kjeltec auto analyzer model 1030.

Preparation of fatty acid methyl esters (FAMEs) and gas chromatography: For every single samples the seeds of a given year were bulked and representative samples were taken for a total fatty acid analyses. Four replicates comprizing healthy looking seeds were analyzed. Total fatty acid content was analyzed by using a method modified by Wu *et al.*[22]. In this method, seed samples were soaked in 2 mL of 2 % sulphuric acid in dry methanol for 16 h at room temperature, followed by 80 min of heating at 90 ºC to convert the fatty acids into methyl derivatives (FAMEs). Methyl-heptadecanoate (17:0-ME) was added as an internal standard. After 2 mL water and 3 mL hexane, were added the FAMEs were extracted for

analyses by gas liquid chromotography. The fatty acid methyl ester composition was analyzed by using a Varian 3400 gas chromotography equipped with a Supelcovax-10 fused slica capillary column (30 m × 0.25 μm film thickness). The column's initial temperature was kept at 160 ºC for 15 min so that an increase in temperature could occur at the rate of 5 ºC min^{-1}. The temperatures of the injector and the dedector (FID) were at 240 and 280 ºC, respectively. The carrier gas was nitrogen with a flow rate of 1-2 mL min^{-1}. Split ratio was adjusted to 30 mL min^{-1} The injected volume of the sample was 1 μL. Fatty acids were identified by retention time relative to that of an authentic standard. The FAMEs were identified by comparing the retention times with those of the standards. Fatty acid content was computed as weight percentage of the total fatty acids by using the GC area counts for various FAMEs.

Statistical analysis: Statistical evaluation was carried out by using JMP package version 5.0.1a. A BUSINESS UNIT OF SAS. (Copyright 1989-2000. SAS Institute Inc.) with general linear model analyses of variance (Anova) with cultivars, sowing dates and years as the main treatment effects. Treatment means were separated by using least significant differences (LSD) at level a probability of 5%. Correlation analysis was performed to explore the relationship among the variables.

RESULTS AND DISCUSSION

Analysis of method and variance

The statistical evaluation of protein and oil as well as the fatty acid composition of soybean seeds are given in Table-2. Sowing dates and cultivar × sowing dates interaction effects were very significant ($p < 0.001$) for the all tasted characteristics. The effect of the cultivars on the protein content, stearic and oleic acids were very significant ($p < 0.001$) and oil content and linolenic acid content was significant ($p < 0.05$) and palmitic and linoleic acids were not significant. The effect of the years on the protein and oil content was also very significant ($p < 0.001$) and palmitic and linoleic acids were significant ($p < 0.05$) and stearic, oleic and linolenic acids were not significant. Year × cultivars × sowing dates interaction effects were very significant for the protein and oil content, but palmitic, stearic, oleic, linoleic and linolenic acids were not significant. Year main effects and cultivar × year × sowing dates interaction influenced the oil and protein content of soybean seeds. Similar findings were reported for soybean[23] and peanut[24].

Variations between years likely reflect differences in the environmental factors that influence seed composition. Although, year × sowing dates interaction effects were very significant for the protein and oil content ($p < 0.001$) and significant for the stearic acid ($p < 0.01$) and significant for the

TABLE-2
ANALYSIS OF VARIANCE VALUES FOR SOYBEAN SEED COMPOSITION AND AGRONOMIC TRAITS IN 2005 AND 2006

Variables	D.F.	Protein (%)	Oil (%)	Fatty acid composition (%)				
				16:0	18:0	18:1	18:2	18:3
Year	1	‡	‡	*	ns	ns	*	ns
Replication [year]	6	ns	†	ns	ns	ns	ns	ns
Cultivar	1	‡	*	ns	‡	‡	ns	*
Year × cultivar	1	‡	ns	ns	*	ns	ns	ns
Replication × cultivar [year] & random	6	ns	ns	ns	ns	ns	ns	ns
Sowing date	4	‡	‡	‡	‡	‡	‡	‡
Year × sowing date	4	‡	‡	ns	†	*	ns	ns
Cultivar × sowing date	4	‡	‡	‡	‡	‡	‡	‡
Year × cultivar × sowing date	4	‡	†	ns	ns	ns	ns	ns

The fatty acids identified in the samples were palmitik (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), ns = non-significant, *Significant; at $p < 0.05$, †Significant; at $p < 0.01$, ‡Significant; at $p < 0.001$.

oleic acid ($p < 0.05$). Palmitic, linoleic and linolenic acids were not affected year × sowing dates interaction effect.

Protein and oil contents and fatty acid composition: The effect of protein, oil and fatty acid composition averaged over 2005 and 2006 is presented in Table-3. Although, NE3399 cultivar given maximum protein content (31.81 %) and oleic acid content (28.24 %). The UMUT2002 cultivar given maximum oil content (19.06 %), stearic acid content (4.46 %) and linolenic acid content (6.64 %). The lowest protein content (31.00 %) and oleic acid content (27.00 %) were obtained from UMUT2002 and oil content (18.94 %) was obtained from NE3399. Palmitic and linoleic acid contents were not affected both of these cultivars significantly.

TABLE-3
EFFECT OF CULTIVARS ON PROTEIN AND OIL CONTENTS AND FATTY ACID COMPOSITION AVERAGED OVER 2005 AND 2006

Cultivars	Protein (%)	Oil (%)	16:0	18:0	18:1	18:2	18:3
NE3399	31.81a	18.94b	11.47	4.20b	28.24a	49.79	6.50b
UMUT2002	31.00b	19.06a	11.52	4.46a	27.00b	49.93	6.64a
Mean	31.40	19.00	11.49	4.33	27.62	49.86	6.57
LSD (5 %)	1.54	0.09	0.08	0.05	1.13	0.16	0.08
M.S.	13.325‡	0.273*	0.036ns	1.352‡	30.69‡	0.365ns	0.357†

M.S. = mean square, *Significant; at $p < 0.05$, †Significant; at $p < 0.01$, ‡Significant; at $p < 0.001$, ns = non-significant.

The effect of sowing dates on protein and oil contents and fatty acid composition averaged over 2005 and 2006 are shown in Table-4. Palmitic, stearic, oleic, linoleic and linolenic acids are the principal fatty acids that these constitute whole soybean seed fatty acid composition's of 98 %. As indicated in previous publications[25,26] the fatty acid composition of vegetable oils varies depending on seed genealogy, planting date and meteorological factors during the growing season. Protein and oil content varied from 29.79 to 33.55 % and 18.73 to 19.32 %, respectively. The first sowing date *i.e.*, April 30 given highest protein content (33.55 %) and the fifth sowing date *i.e.*, June 30 given the lowest protein content (29.79 %). The highest oil content (19.32 %) was obtained from third sowing date *i.e.*, May 30. Although the highest palmitic acid content (11.90 %) was obtained from the first sowing date *i.e.*, April 30, the lowest palmitic acid content (10.97 %) was obtained from the fifth sowing date of June 30.

TABLE-4
EFFECT OF SOWING DATES ON PROTEIN AND OIL CONTENTS AND FATTY ACID COMPOSITION AVERAGED OVER 2005 AND 2006

Sowing dates	Protein (%)	Oil (%)	16:0	18:0	18:1	18:2	18:3
April 30	33.55a	19.32a	11.90a	4.05e	27.68d	49.85b	6.16d
May 15	32.53b	18.89b	11.88a	4.15d	27.99c	49.61c	6.33c
May 30	30.86c	18.73b	11.07c	4.60a	28.32a	49.45c	6.50b
June 15	30.29d	19.19a	11.57b	4.49b	28.15b	48.98d	6.56b
June 30	29.79e	18.88b	10.97c	4.37c	25.96e	51.42a	7.31a
Mean	21.40	19.00	11.47	4.33	27.62	49.86	6.57
LSD (5%)	0.35	0.20	0.78	0.05	0.13	0.16	0.08
M.S.	39.995‡	0.920‡	3.427‡	0.867‡	14.714‡	13.788‡	3.142‡

M.S. = mean square, *Significant; at $p < 0.05$, †Significant; at $p < 0.01$, ‡Significant; at $p < 0.001$.

However, the highest stearic and oleic acid contents (4.60 and 28.32%) were obtained from third sowing date *i.e.*, May 30, the highest linoleic and linolenic acid contents (51.42 and 7.31 %) was obtained from fifth sowing date *i.e.*, June 30 and the lowest stearic acid content (4.05 %) was obtained from the first sowing date *i.e.*, April 30 and the lowest oleic acid content (25.96%) was obtained from the fifth sowing date *i.e.*, June 30 and the fourth sowing date June *i.e.*, 15 was given the lowest linoleic acid content (48.98 %) and the initial sowing date *i.e.*, April 30 was given the lowest linolenic acid content (6.10 %).

The interaction effect of cultivars and sowing dates average values in 2005 and 2006 are presented in Tables 5 and 6. Protein content varied from 29.26 to 34.94 %. The highest protein content (34.94 %) was obtained from NE3399 at the initial sowing date *i.e.*, April 30 and the lowest protein content (29.26 %) was obtained from UMUT2002 at the fourth sowing date *i.e.*, June 30.

TABLE-5
EFFECT OF SOWING DATES AND CULTIVARS ON PROTEIN AND OIL CONTENTS AND PALMITIC AND STEARIC ACIDS COMPOSITION AVERAGED OVER 2005 AND 2006

Sowing dates	Protein (%)		Oil (%)		16:0		18:0	
	NE	UM	NE	UM	NE	UM	NE	UM
April 30	34.94a	32.15c	19.64a	18.99c	12.37b	11.62c	4.25d	3.84g
May 15	30.88d	34.18b	18.63d	19.15bc	10.83e	12.92a	4.56c	3.74g
May 30	30.88d	29.69ef	18.61d	18.86cd	11.29d	10.85e	4.03ef	5.17a
June 15	32.46c	29.26f	18.96c	19.41ab	11.58c	11.56c	4.16de	4.83b
June 30	29.90e	29.69ef	18.88cd	18.89cd	11.30d	10.64e	4.01f	4.73b
Mean	31.81	30.99	18.94	19.06	11.47	11.51	4.20	4.46
LSD (5%)	0.49		0.29		0.23		0.13	
M.S.	27.046‡		0.899‡		5.517‡		2.791‡	

NE = NE3399; UM = UMUT2002; M.S. = mean square, *Significant; at $p < 0.05$, †Significant; at $p < 0.01$, ‡Significant; at $p < 0.001$.

TABLE-6
EFFECT OF SOWING DATES AND CULTIVARS ON OLEIC, LINOLEIC AND LINOLENIC ACIDS COMPOSITION AVERAGED OVER 2005 AND 2006

Sowing dates	18:1		18:2		18:3	
	NE	UM	NE	UM	NE	UM
April 30	30.09a	25.27g	48.25g	51.44a	5.38h	6.94c
May 15	29.67b	26.31e	49.45ef	49.78d	5.86f	6.80d
May 30	29.40c	27.24d	49.29f	49.62de	6.28e	6.71d
June 15	26.46e	29.83b	50.34c	47.61h	7.40b	5.72g
June 30	25.56f	26.35e	51.65a	51.20b	7.61a	7.02c
Mean	28.23	27.00	49.79	49.93	6.50	6.63
LSD (5%)	0.18		0.24		0.11	
M.S.	43.586‡		17.932‡		6.586‡	

NE = NE3399; UM = UMUT2002; M.S. = mean square, *Significant; at $p < 0.05$, † Significant; at $p < 0.01$, ‡Significant; at $p < 0.001$.

The oil content varied 18.61 to 19.64 % and the highest oil content (19.64 %) was obtained from NE3399 at the first sowing date *i.e.*, April 30 and the lowest oil content (18.61 %) was obtained from NE3399 at the third sowing date *i.e.*, May 30. The oleic acid content varied from 25.27 to 30.09 %. The highest oleic acid content (30.09 %) was obtained from NE3399 at the initial sowing date *i.e.*, April 30 and the lowest oleic acid content (25.27 %) was obtained from UMUT2002 at the first sowing date *i.e.*, April 30 too.

The palmitic, stearic, linoleic and linolenic acid contents varied from 10.64 to 12.92 %, 3.74 to 5.17 %, 47.61 to 51.65 % and 5.38 to 7.81 %, respectively. Although, the highest palmitic acid content (12.92 %) was

obtained UMUT2002 at the second sowing date *i.e.*, May 15. The highest stearic acid content (5.17 %) was obtained UMUT2002 at the third sowing date May 30. The highest linoleic content (51.65 %) was obtained NE3399 at the fifth sowing date June *i.e.*, 30 and the lowest linoleic acid content (47.61%) was obtained from UMUT2002 at the fourth sowing date *i.e.*, June 15. The highest linolenic acid content (7.61%) was obtained from NE3399 at the fifth sowing date *i.e.*, June 30 and the lowest linolenic acid content (5.38 %) was obtained from NE3399 at the initial sowing date *i.e.*, April 30.

Correlation analysis of protein and oil content and fatty acid composition: Correlation analysis was performed to explore the trend of associations between protein and oil contents and individual fatty acids and also between the fatty acids in soybean seeds. The data presented that protein content had a significantly negative correlation with the oil content, stearic, linoleic and linolenic acids in contrast had a significantly positive correlation with palmitic and oleic acids (Table-7). These findings are in agreement with the negative correlation between oil and protein content[20,21]. Analysis was done by using combined data from both years revealed that the oil content also had significantly positive correlation with palmitic, stearic and oleic acids but, had an inverse relationship with protein content, linoleic and linolenic acids. The results are compatible with those reported earlier[27,28]. In the other hand, palmitic acid had a positive correlation with oleic acid but showed negative correlation with stearic, linoleic and linolenic acids. Stearic acid contents were found to associate positively with oil content and oleic acid but had a reverse relationship with protein, palmitic, linoleic and linolenic acids. Oleic acid showed positive correlation with protein and oil content, palmitic and stearic acids but showed negative correlation with linolenic acids had an inverse effect with all the traits. Linolenic acid had negatively correlated with both oleic and stearic acids. This findings have been previously reported for sesame[29] and some other oilseed crops like rapeseed, sunflower, peanut and soybean.

TABLE-7
CORRELATION COEFFICIENTS FOR PROTEIN AND OIL CONTENTS AND SOME FATTY ACID COMPOSITION DATA OF 2005 AND 2006

	Protein (%)	Oil (%)	16:0	18:0	18:1	18:2	18:3
Protein (%)	1.0000	-0.2170*	0.5363	-0.4473	0.0423	-0.1205	-0.1458
Oil (%)	-0.2170	1.0000	0.2605	0.1003	0.0651	-0.0819	-0.0842
16:0	0.5363‡	0.2605*	1.0000	-0.5665	0.0036	-0.2553	-0.1680
18:0	-0.4473‡	0.1003	-0.5665‡	1.0000	0.3293	-0.3324	-0.2556
18:1	0.0423	0.0651	0.0036	0.3293†	1.0000	-0.8646	-0.8959
18:2	-0.1205	-0.0819	-0.2553*	-0.3324†	-0.8646‡	1.0000	0.8553
18:3	-0.1458	-0.0842	-0.1680	-0.2556*	-0.8959‡	0.8553‡	1.0000

*Significant; at $p < 0.05$, †Significant; at $p < 0.01$, ‡Significant; at $p < 0.001$.

Conclusions

The findings reported here will be beneficial for the future work towards improving the oil yield and quality of soybean for the growers. In the other hand, cultivars and sowing dates appeared to have an effect on the seed composition of soybeans grown in the southeast Anatolia region of Turkey. In addition, these results show that is important to carry out further investigations to find out the effects of the different environmental and agronomical factors on soybean seed chemical composition in different locations.

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