

## ***trans* Fatty Acid Contents of Crude Soybean Oils Industrially Obtained by Solvent Extraction with Hexane**

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The *trans* fatty acid contents of crude soybean oils, industrially obtained by using continuous solvent (hexane) extraction processes, were determined by capillary gas-liquid chromatography. According to the analysis results, the contents of total *trans* fatty acid were within the ranges of  $0.05 \pm 0.02$ - $0.17 \pm 0.05$  % of total fatty acids. The total C<sub>18:2</sub> *trans* and total C<sub>18:3</sub> *trans* acids were found in all the analyzed samples. The contents of these *trans* fatty acids were  $0.01 \pm 0.00$ - $0.06 \pm 0.02$  and  $0.02 \pm 0.01$ - $0.09 \pm 0.04$  %, respectively. As for the total C<sub>18:1</sub> *trans* acid, its levels were present is less than  $0.02 \pm 0.02$  % of total fatty acids. Also, no total C<sub>18:1</sub> *trans* acid was found in some samples. The *trans* fatty acids were probably formed by heat treatment of soybeans before or during the solvent extraction processes. Although very low values of the *trans* fatty acids were found in the crude soybean oils, the presence of these fatty acids can be caused difficulties to produce refined soybean oils with or without low level of *trans* fatty acid.

**Key Words:** Crude soybean oil, Fatty acid composition, Solvent extraction, *trans* Fatty acid.

### **INTRODUCTION**

Most of the unsaturated fatty acids present in living tissues and foods contain double bonds that have the *cis* configuration. Except for fat from milk and flesh, which contributes to some extent to the daily intake of *trans* isomers in humans, the bulk of such isomers comes from ingestion of partially hydrogenated vegetable oils<sup>1,2</sup>. However, formation of *trans* fatty acids in the vegetable oils during refining processes, particularly in the last refining stage, *i.e.* during deodorization is a well-known fact<sup>3,4</sup>. Furthermore, crude oils may contain very small amounts of *trans* fatty acids. The *trans* fatty acids are probably formed by heat treatment of oilseeds before or during extraction process<sup>5</sup>. Heating the raw material (oilseed or

nuts) may considerably increase the *trans* fatty acid content of the resulting oils<sup>6</sup>. Therefore, vegetable oils are also a little source of *trans* fatty acids, especially *trans* polyunsaturated fatty acids, in human diet. In accordance with nutritionists' proposals, there are strong tendencies in Europe to keep the *trans* fatty acid isomer content of edible oils as low as possible<sup>4</sup>. The isomers that may be formed in vegetable oils during refining are quite different in type and levels from those formed during the hydrogenation process<sup>7</sup>. Whereas in hydrogenated oils and fats the analytical focus is mainly on the monoenoic *cis* and *trans* fatty acid, isomers formed during deodorization are mostly di- and trienoic mono-*trans* fatty acid<sup>8</sup>.

Most crude oils conventionally have been obtained from oilseeds by either mechanical pressing or solvent extraction methods. Many seeds, such as soybeans, are direct extracted and then removing the solvent<sup>9</sup>. Only small amounts of the soybeans are processed by mechanical means<sup>10</sup>. Many of the higher oil content seeds, such as sunflowerseed, are mechanically prepressed prior to solvent extraction to enable solvent extracting to proceed efficiently and trouble-free<sup>11</sup>. There are three major steps in solvent extraction *i.e.*, seed preparation, oil extraction and desolventizing of the oil and meal<sup>10</sup>. Quality properties of crude oils obtained by solvent extraction methods are primarily dependent upon extraction solvents, extraction temperature, pretreatment of oilseeds, *etc.*<sup>12,13</sup>.

Refining has the purpose of not only eliminates impurities, but also minimizes *trans* fatty acid formation<sup>14</sup>. Because, the quality variables for refined oil include the lowest possible content in *trans* fatty acids<sup>15</sup>. Therefore, detailed fatty acid analysis, that separates the fatty acid, not only on the basis of chainlength and double bonds, but also shows the different geometrical and positional isomers, is becoming more important. The data obtained with this type of analysis are used for raw material specifications<sup>8</sup> and can also be utilized to produce refined oils with or without the possible minimum level of *trans* fatty acid.

The objective of this study was to evaluate the *trans* fatty acid contents of crude soybean oils obtained by solvent extraction with hexane under industrial conditions, by using capillary gas liquid chromatography.

## EXPERIMENTAL

**Crude soybean oil samples:** Crude soybean oils, produced by using continuous solvent extraction (hexane) processes under usual industrial conditions, were used for analysis. Hexane-extracted crude soybean oils were obtained directly from 15 different oil companies which are major producers of the vegetable oils in Turkey. Samples were taken three times over a 9 months period. Each company was coded with a number (1, 2, 3, ...). Dark glass containers were purged with nitrogen gas after filling to prevent oxida-

tion and stored at 4 °C until analyzed. Fatty acid methyl esters (FAMES) standards (99 % purity) were purchased from Nu-Chek-Prep Inc. (Elysian, MN).

**Preparation of fatty acid methyl esters:** fatty acid methyl esters (FAMES) were prepared according to American Oil Chemists' Society Official Method Ce 2-66<sup>16</sup>. The FAMES were obtained from the soybean oils after alkaline hydrolysis, followed by methylating in methanol with 12.5 % BF<sub>3</sub> (boron trifluoride) catalyst. The final concentration of the FAMES was approximately 7 mg/mL in heptane.

**Capillary gas liquid chromatography analysis:** Analyses of the FAMES by capillary GLC were carried out on a Hewlett-Packard 6890 chromatograph, equipped with a flame ionization detector (FID) on a split injector. A fused-silica capillary column (Chrompack, Middleburg, The Netherlands) was used for the FAMES analysis; CP<sup>TM</sup>-Sil 88, 50 m × 0.25 mm i.d., 0.2 µm film. GLC operating conditions were: a temperature program of 130 °C for 5 min increasing at a rate of 2 °C/min to 177 °C; injector and detector temperatures, 200 and 250 °C, respectively. The carrier gas was helium at a flow rate of 1 mL/min; the split ratio was 1:50; the volume of injected sample was 1 µL. The peaks were identified by comparing the retention times with those of authentic standards of FAMES obtained from Nu-Chek-Prep Inc. (Elysian, MN). The final results were expressed as relative percentage of individual fatty acids.

**Statistical analysis:** The analytical data concerning the total C<sub>18:1</sub> *trans*, total C<sub>18:2</sub> *trans*, total C<sub>18:3</sub> *trans*, total *trans* fatty acid collected with three replications of each sample were subjected to analysis of variance using MSTAT software program. The Duncan multiple range test was applied to the results using the same program<sup>17</sup>.

## RESULTS AND DISCUSSION

Fatty acid compositions and *trans* fatty acid contents of the crude soybean oil samples are presented in Table-1. Three samples of each oil company were taken and each sample was analyzed in triplicate. The results are given as arithmetic means ( $n = 3 \times 3$ ) for each oil company. The palmitic acid (C<sub>16:0</sub>; 9.13-11.84 %), stearic acid (C<sub>18:0</sub>; 3.84-4.33 %), oleic acid (C<sub>18:1</sub> *cis*; 23.65-27.12 %), linoleic acid (C<sub>18:2</sub> *cis*; 49.46-54.27 %) and linolenic acid (C<sub>18:3</sub> *cis*; 5.69-7.27 %) were the principal fatty acids for all the analyzed samples. The sum of myristic (C<sub>14:0</sub>), palmitoleic (C<sub>16:1</sub>), arachidic (C<sub>20:0</sub>), gadoleic (C<sub>20:1</sub>), behenic (C<sub>22:0</sub>), lignoceric (C<sub>24:0</sub>) acids were below 1.7 %. The results were within the common natural ranges.

Small amounts of the *trans* fatty acids were also detected by capillary gas-liquid chromatography analysis in the crude soybean oils. Generally, the total *trans* fatty acids in the samples examined comprised isomers of

TABLE-1  
FATTY ACID COMPOSITIONS AND TRANS FATTY ACID CONTENTS OF CRUDE SOYBEAN OILS\*

Fatty acid†	Sample code														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
14:0	0.06	0.07	0.09	0.08	0.07	0.06	0.10	0.08	0.07	0.07	0.08	0.06	0.09	0.07	0.10
16:0	10.14	11.25	11.33	9.92	10.52	9.54	10.76	9.13	11.59	10.78	9.89	9.35	11.20	11.84	10.87
16:1 <i>cis</i>	0.14	0.13	0.15	0.10	0.11	0.14	0.11	0.13	0.12	0.14	0.13	0.10	0.11	0.12	0.14
18:0	4.25	4.16	4.28	4.33	4.19	4.21	4.12	4.18	4.05	3.84	4.02	4.18	4.30	3.95	4.21
18:1 <i>cis</i>	23.65	25.41	26.01	24.35	25.92	27.12	25.74	24.35	24.48	25.42	24.40	26.27	27.08	25.65	26.29
Σ 18:1 <i>trans</i>	0.01	0.01	–‡	0.02	–	0.01	0.01	0.01	–	–	0.02	0.02	0.01	0.01	0.01
18:2 <i>cis</i>	53.27	51.30	50.89	52.53	51.46	51.70	50.39	54.27	51.90	52.32	53.66	51.54	49.46	49.79	50.13
Σ 18:2 <i>trans</i>	0.03	0.02	0.02	0.06	0.01	0.04	0.03	0.03	0.02	0.02	0.05	0.06	0.03	0.04	0.04
18:3 <i>cis</i>	6.98	6.24	5.75	7.22	6.38	5.69	7.27	6.35	6.34	5.92	6.30	6.93	6.25	7.08	6.69
Σ 18:3 <i>trans</i>	0.08	0.04	0.06	0.05	0.02	0.08	0.06	0.08	0.03	0.06	0.04	0.09	0.05	0.06	0.08
20:0	0.40	0.40	0.41	0.40	0.38	0.41	0.40	0.39	0.42	0.42	0.41	0.41	0.40	0.42	0.42
20:1 <i>cis</i>	0.28	0.26	0.30	0.31	0.28	0.28	0.31	0.27	0.26	0.30	0.31	0.28	0.31	0.30	0.30
22:0	0.48	0.50	0.51	0.42	0.46	0.49	0.48	0.51	0.52	0.48	0.49	0.50	0.48	0.46	0.51
24:0	0.23	0.21	0.20	0.21	0.20	0.23	0.22	0.22	0.20	0.23	0.20	0.21	0.23	0.21	0.21

\*Values are average of three samples of each oil company analyzed individually in triplicate and are expressed as a weight percentage of total FAMES.

†Fatty acids are designated by number of carbon atoms; number of double bonds.

‡Not detected or trace amounts, <0.01%.

TABLE-2  
DEGREES OF ISOMERIZATION FOR OLEIC, LINOLEIC AND LINOLENIC ACIDS IN CRUDE SOYBEAN OILS

Sample code	$\Sigma$ 18:1 <i>trans</i>	DI <sub>o</sub>	$\Sigma$ 18:2 <i>trans</i>	DI <sub>L</sub>	$\Sigma$ 18:3 <i>trans</i>	DI <sub>Ln</sub>	$\Sigma$ <i>trans</i> fatty acid	DI <sub>T</sub>
1	0.01 ± 0.00 <sup>b</sup>	0.04	0.03 ± 0.01 <sup>bc</sup>	0.06	0.08 ± 0.03 <sup>c</sup>	1.13	0.12 ± 0.02 <sup>cd</sup>	0.14
2	0.01 ± 0.01 <sup>b</sup>	0.04	0.02 ± 0.01 <sup>ab</sup>	0.04	0.04 ± 0.03 <sup>bc</sup>	0.64	0.07 ± 0.04 <sup>abc</sup>	0.08
3	0.00 ± 0.00 <sup>a</sup>	-	0.02 ± 0.02 <sup>ab</sup>	0.04	0.06 ± 0.02 <sup>d</sup>	1.03	0.08 ± 0.03 <sup>abc</sup>	0.10
4	0.02 ± 0.01 <sup>c</sup>	0.08	0.06 ± 0.02 <sup>c</sup>	0.11	0.05 ± 0.02 <sup>cd</sup>	0.69	0.13 ± 0.02 <sup>cd</sup>	0.15
5	0.00 ± 0.00 <sup>a</sup>	-	0.01 ± 0.00 <sup>a</sup>	0.02	0.02 ± 0.01 <sup>a</sup>	0.31	0.03 ± 0.03 <sup>d</sup>	0.04
6	0.01 ± 0.00 <sup>b</sup>	0.04	0.04 ± 0.03 <sup>cd</sup>	0.08	0.08 ± 0.04 <sup>e</sup>	1.39	0.13 ± 0.04 <sup>cd</sup>	0.15
7	0.01 ± 0.01 <sup>b</sup>	0.04	0.03 ± 0.02 <sup>bc</sup>	0.06	0.06 ± 0.02 <sup>d</sup>	0.82	0.10 ± 0.02 <sup>abc</sup>	0.12
8	0.01 ± 0.00 <sup>b</sup>	0.04	0.03 ± 0.01 <sup>bc</sup>	0.06	0.08 ± 0.02 <sup>e</sup>	1.24	0.12 ± 0.01 <sup>cd</sup>	0.14
9	0.00 ± 0.00 <sup>a</sup>	-	0.02 ± 0.02 <sup>ab</sup>	0.04	0.03 ± 0.00 <sup>ab</sup>	0.47	0.05 ± 0.02 <sup>ab</sup>	0.06
10	0.00 ± 0.00 <sup>a</sup>	-	0.02 ± 0.02 <sup>ab</sup>	0.04	0.06 ± 0.02 <sup>d</sup>	1.00	0.08 ± 0.03 <sup>abc</sup>	0.10
11	0.02 ± 0.02 <sup>c</sup>	0.08	0.05 ± 0.02 <sup>de</sup>	0.09	0.04 ± 0.00 <sup>bc</sup>	0.63	0.11 ± 0.04 <sup>bcd</sup>	0.13
12	0.02 ± 0.02 <sup>c</sup>	0.08	0.06 ± 0.02 <sup>e</sup>	0.12	0.09 ± 0.04 <sup>e</sup>	1.28	0.17 ± 0.05 <sup>d</sup>	0.20
13	0.01 ± 0.00 <sup>b</sup>	0.04	0.03 ± 0.00 <sup>bc</sup>	0.06	0.05 ± 0.02 <sup>cd</sup>	0.79	0.09 ± 0.01 <sup>abc</sup>	0.11
14	0.01 ± 0.01 <sup>b</sup>	0.04	0.04 ± 0.02 <sup>cd</sup>	0.08	0.06 ± 0.01 <sup>d</sup>	0.84	0.11 ± 0.06 <sup>bcd</sup>	0.13
15	0.01 ± 0.02 <sup>b</sup>	0.04	0.04 ± 0.01 <sup>cd</sup>	0.08	0.08 ± 0.03 <sup>e</sup>	1.18	0.13 ± 0.04 <sup>cd</sup>	0.16

Values represent means of triplicated ± standard deviation (SD).

Values in the columns with different letters (a-e) are significantly different ( $p < 0.01$ ).

DI<sub>o</sub>, degree of isomerization for the indicated fatty acid, ratio of  $\Sigma$  18:1 *trans* on  $\Sigma$  18:1 times 100.

DI<sub>L</sub>, degree of isomerization for the indicated fatty acid, ratio of  $\Sigma$  18:2 *trans* on  $\Sigma$  18:2 times 100.

DI<sub>Ln</sub>, degree of isomerization for the indicated fatty acid, ratio of  $\Sigma$  18:3 *trans* on  $\Sigma$  18:3 times 100.

DI<sub>T</sub>, degree of isomerization for the indicated fatty acid, ratio of  $\Sigma$  *trans* fatty acid on the sum of  $\Sigma$  18:1,  $\Sigma$  18:2,  $\Sigma$  18:3 times 100.

oleic (C<sub>18:1</sub>), linoleic (C<sub>18:2</sub>) and linolenic (C<sub>18:3</sub>) acids. In addition, the degrees of isomerization for oleic (C<sub>18:1</sub>), linoleic (C<sub>18:2</sub>) and linolenic (C<sub>18:3</sub>) acids are presented in Table-2.

The contents of total *trans* fatty acid in all the analyzed samples were within the ranges of 0.05 ± 0.02-0.17 ± 0.05 % of total fatty acids, corresponding to the degrees of isomerization equal to 0.04-0.20 %. All the samples under study contained the total C<sub>18:2</sub> *trans* and total C<sub>18:3</sub> *trans* acids. The amounts of total C<sub>18:3</sub> *trans* acid were generally higher than the total C<sub>18:2</sub> *trans* acid in the samples. The total C<sub>18:2</sub> *trans* and total C<sub>18:3</sub> *trans* acids were 0.01 ± 0.00-0.06 ± 0.02 and 0.02 ± 0.01-0.09 ± 0.04 %, respectively. Their degrees of isomerization varied from 0.02 to 0.12 and from 0.31 to 1.39 %, respectively. The ratio between the degrees of isomerization calculated for C<sub>18:3</sub> and C<sub>18:2</sub> acids (DI<sub>Ln</sub>/DI<sub>L</sub>; values not included in Table-2) indicates that the probability of C<sub>18:3</sub> acid isomerization is higher than that of C<sub>18:2</sub> acid. No total C<sub>18:1</sub> *trans* acid was detected in some samples, while the total C<sub>18:1</sub> *trans* acid was present in less than 0.02 ± 0.02 % of total fatty acids, representing DI<sub>o</sub> of 0.04-0.08 %. As illustrated in Table-2, there was a significant variation (p < 0.01) in the amounts of total *trans* fatty acid and individual *trans* fatty acids between the different crude soybean oils. This could indicate that the operation conditions used during the solvent extraction steps varied among the different oil companies. From the results obtained it can be assumed that hexane-extracted soybean oils with higher amounts of total *trans* fatty acid than 0.1 % were exposed to improper conditions during the solvent extraction processing. Similarly, Matthaus and Bruhl<sup>18</sup> reported that an evidence for an improper extraction processing of crude oils, especially cold-pressed oils may be the presence of *trans* fatty acids.

According to Schwarz<sup>3</sup>, raw vegetable oils include a negligible amount of *trans* fatty acid, ranging between 0.1 and 0.3 % of the total fatty acid content. Similar observations were made by several research studies. Ferrari *et al.*<sup>19</sup> reported that crude corn, soybean and rapeseed oils contained small amounts of total *trans* fatty acid (0.1 %). Among the three crude oils, the total *trans* fatty acids of crude soybean oil comprised isomers of C<sub>18:1</sub> (< 0.1 %), C<sub>18:2</sub> (0.1 %) and C<sub>18:3</sub> (< 0.1 %). The obtained values for *trans* isomers of C<sub>18:1</sub> and C<sub>18:3</sub> are in accordance with present results. Bruhl<sup>6</sup> revealed that the C<sub>18:1</sub> *trans* and C<sub>18:2</sub> + C<sub>18:3</sub> *trans* acid contents of soybean oils in % labelled as cold pressed were 0.07 and 0.03-0.08 %, respectively. Matthaus and Bruhl<sup>18</sup> reported that most of the cold pressed rapeseed oils contained amounts of *trans* fatty acids between 0.1 and 0.2 %, with an average of 0.18 %. Tasan and Demirci<sup>5</sup> showed that crude sunflower oils (mixed equal amounts of pressed and hexane-extracted oils) contained no measurable amounts of C<sub>18:3</sub> *trans* acid and only small amounts of C<sub>18:2</sub> *trans* (0.04-0.05 %)

and C<sub>18:1</sub> *trans* acids (0.01 %). Similarly, Ortega-Garcia *et al.*<sup>20</sup> found that crude high oleic safflower oils contained very small amounts of individual *trans* unsaturated fatty acids (< 0.1 %). Cmolik *et al.*<sup>21</sup> revealed that *trans* isomers of C<sub>18:1</sub>, C<sub>18:2</sub> and C<sub>18:3</sub> acids were detected in crude rapeseed oils produced by prepressing in screw presses, followed by solvent extraction with hexane, 0.01-0.06, 0.01-0.04 and 0.04-0.06 %, respectively. Wagner<sup>22</sup> also reported that total *trans* fatty acid contents in cold pressed oils was the maximum 0.1 %. On the other hand, Van Hoed *et al.*<sup>23</sup> revealed that *trans* fatty acids were not detected in the crude rice bran oil, produced by using solvent extraction (hexane) process. Perretti *et al.*<sup>24</sup> announced that no C<sub>18:1</sub> *trans* acid was found in the sunflower seed oils mechanically extracted and conventional solvent extracted. But, only latter contained C<sub>18:2</sub> *trans* acid at 0.4-0.5 %.

Some of the potential abuses to both beans and oil that can directly affect crude soybean oil quality is shown as improperly bean and crude oil storage (time/temperature), poor conditioning beans for extraction, solvent-stripping oil (overheating) and oil from stripper (overheating)<sup>25</sup>. All of these potentially contribute to increased levels of impurities and, in some cases, *trans* fatty acids are also probably formed in the crude soybean oils. When oil is excessive heated during processing, *cis* double bonds naturally occurring in vegetable oils can change to *trans* double bonds<sup>22</sup>. Thus, *trans* fatty acids detected in the vegetable oil are an indication for heating of the vegetable oil<sup>6</sup>. Tasan and Demirci<sup>5</sup> also reported that the factor affecting the formation of *trans* fatty acids in the crude sunflower oils are most likely excessive heating at the extraction processes. According to Matthaus and Bruhl<sup>18</sup>, *trans* isomers were formed by heating of the seeds during the drying process. Even though the raw material is carefully pressed, previous hot drying or even roasting of seeds, especially nuts, will increase the *trans* fatty acid content in the products<sup>6</sup>. On the other hand, different authors such as Cmolik *et al.*<sup>21</sup> and Engeseth *et al.*<sup>26</sup>, expressed that the presence of *trans* fatty acids in crude oils can also due to the lipxygenase activity in seeds before seed processing.

The results presented in this paper demonstrate that there was the effect of the solvent (hexane) extraction processes on the formation of *trans* fatty acids in crude soybean oils. Nevertheless, very low amounts of the *trans* fatty acids were found in these oils. On the other hand, the important parameter for an assessment of the quality of crude oils is the content of *trans* fatty acid. Because, the quality variables for the refined oil include the lowest possible content in *trans* fatty acids. As is known, deodorization is the main step of oil refining that contributes to increases in the content of *trans* fatty acids. The formation of geometrical isomers of fatty acid is especially time and temperature dependent<sup>5</sup>. The quantity of originating



*trans* fatty acids in refined oil depend also on the initial fatty acid composition of crude oil used in refining process<sup>4</sup>. As a corollary, the small quantities of *trans* fatty acid in crude oils, formed during improper extraction processing, may also make contributions to some extent to these fatty acid contents of refined oils. The low-*trans* level for the refined oils was explained by the fact that these oils were cold pressed<sup>27</sup>.

The knowledge of the *trans* fatty acid content in crude oils can play an significant role for the manufacturers as an aid in refining process optimization. The manufacturers must take into account that in European countries the quality parameters for refined vegetable oils include low levels of *trans* fatty acid (< 1 %). The presence of *trans* fatty acids in the crude soybean oils can be caused difficulties to produce refined soybean oils without or with low level of *trans* fatty acid. Therefore, processing conditions should be carefully evaluated in order to prevent the formation of *trans* fatty acids in the crude soybean oils during solvent extraction with hexane processes.

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