

GRASAS Y ACEITES, 62 (3),
JULIO-SEPTIEMBRE, 284-289, 2011,
ISSN: 0017-3495
DOI: 10.3989/gya.102310

Comparison of geometrical isomerization of unsaturated fatty acids in selected commercially refined oils

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RESUMEN

Comparación de isómeros geométricos de ácidos grasos insaturados en aceites refinados comerciales seleccionados.

Cuatro aceites vegetales refinados comerciales diferentes fueron analizados por cromatografía de gases para determinar el contenido en ácidos grasos *trans*. Los resultados obtenidos mostraron que el contenido total de los FA *trans* de aceites refinados de girasol, maíz, soja y avellana fueron 0.68 ± 0.41 , 0.51 ± 0.24 , 1.27 ± 0.57 , y $0.26 \pm 0.07\%$ de FA totales, respetivamente. Los ácidos grasos totales *trans* comprenden a isómeros de FA C18:1, C18:2 y C18:3. Cinco marcas de aceites de girasol refinado y dos marcas de aceite de avellana contenían cantidades no medibles de ácidos *trans* C18:3 totales. Los ácidos C18:2 *trans* totales fueron los FA *trans* predominantes en el aceite de girasol y maíz refinado, mientras los FA poliinsaturados *trans* fueron encontrados a niveles altos en el aceite de soja refinado. Sin embargo, los ácidos *trans* C18:1 totales fueron los principales FA *trans* en el aceite de avellana refinado. Los aceites vegetales refinados comerciales con un contenido relativamente alto de FA poliinsaturado total contenían cantidades considerable de isómeros poliinsaturados *trans*. Este estudio indica que es necesario optimizar la industria de desodorización, especialmente el tiempo y la temperatura, para cada composición diferente de FA del aceite usado.

PALABRAS CLAVE: Aceite vegetal refinado – Desodorización – Grado de isomerización – Isómeros *trans*.

SUMMARY

Comparison of geometrical isomerization of unsaturated fatty acids in selected commercially refined oils.

Four different commercially refined vegetable oils were analyzed by capillary gas-liquid chromatography for their *trans* fatty acid contents. The results obtained showed that the total *trans* FA contents in refined sunflower, corn, soybean, and hazelnut oils were 0.68 ± 0.41 , 0.51 ± 0.24 , 1.27 ± 0.57 , and $0.26 \pm 0.07\%$ of total FA, respectively. The total *trans* FA comprised isomers of the C18:1, C18:2 and C18:3 FA. Meanwhile, five brands of the refined sunflower oil and two brands of hazelnut oil contained no measurable amounts of total *trans* C18:3 acids. The total *trans* C18:2 acid was the predominant *trans* FA found in the refined sunflower and corn oils, while *trans* polyunsaturated FAs for the refined soybean oils were found at high levels. However, total *trans* C18:1 acid was the major *trans* FA for refined hazelnut oils. The commercially refined vegetable oils with a relatively high

total polyunsaturated FA contained considerable amounts of *trans* polyunsaturated isomers. This study indicates that it is necessary to optimize industrial deodorization, especially the time and temperature, for each different FA composition of oil used.

KEY-WORDS: Degree of isomerization – Deodorization – *trans* Isomer – Refined vegetable oil.

1. INTRODUCTION

The influence of dietary fats/oils on human health and nutrition is related to the quantity of fats/oils consumed, fatty acid (FA) compositions, and the bioactive micronutrients present (Mayamol *et al.*, 2004). Vegetable oils constitute an indispensable part of the human diet and their consumption has been steadily increasing worldwide while that of animal fats has decreased. Vegetable oils intended for normal consumption are refined (Schwarz, 2000). Most consumers use refined oils. On the other hand, consumer interest for minimally processed products has increased. Thus, the use of cold pressed vegetable oils has increased significantly during the last decades (Matthaus and Bruhl, 2003).

The objective of refining oils is to remove unacceptable materials with the least possible effect on desirable components and the least possible loss in the quantity of the oil (Jawad *et al.*, 1983). On the other hand, chemical or physical refining subjects the oil to high temperatures, alkali and metal processing equipment that can alter their chemical composition (Karaali, 1985). The typical oil refining process includes degumming, chemical or physical refining, bleaching and deodorization (Erickson, 1995). Each refining step has a specific function for removing certain minor components, which can act as prooxidants or antioxidants (Jung *et al.*, 1989). In the refining process, vegetable oils are commonly heated to between 60 and 100°C before deodorization. During the deodorization stage, the temperature is increased to 180-270°C (Martin *et al.*, 2007). The deodorization stage is a principal step in the vegetable oil refining process. Free fatty acids, aldehydes, unsaturated hydrocarbons and ketones, which cause undesirable odors and flavors in the oil,

are eliminated during this stage (Zehnder, 1995). However, the high temperature used in this stage may induce the geometrical isomerization of unsaturated FA, such as C18:2 and C18:3 (Tasan and Demirci, 2003). During this stage, other factors which also affect the formation of *trans* FA include heating time, pressure and stripping steam dosage (Wolff, 1993; Bruggen *et al.*, 1998; Martin *et al.*, 2008). The rate of conversion also depends on the level of unsaturation of the FA molecule (Bruggen *et al.*, 1998). On the other hand, *trans* FA isomers that may be formed in vegetable oils during refining are quite different in type and level from those formed during the hydrogenation process (Ackman *et al.*, 1974). Whereas in hydrogenated oils/fats the analytical focus is mainly on the monoenoic *cis* and *trans* FA, isomers formed during deodorization are mostly dienoic and trienoic mono-*trans* FA (Duchateau *et al.*, 1996).

Knowledge about the *trans* FA content of fats and oils is important not only for the manufacturer as an aid in processing optimization but also for health authorities due to the fact that the potential health hazards of *trans* FA intake are well known (Ferrari *et al.*, 1996). Although refined vegetable oils make almost the lowest contribution to the total *trans* FA intake in the diet, they contain *trans* polyunsaturated FA formed in small amounts during refining, mainly during the deodorization stage. In addition, because of their high consumption, i.e., in the Mediterranean countries, evaluation of the *trans* FA contents in refined vegetable oils becomes important. In accordance with nutritionists' proposals, there is a strong tendency in the vegetable oil industry to keep the *trans* FA contents of vegetable oils as low as possible (Kemeny *et al.*, 2001). On the other hand, the US Food and Drug Administration (FDA, 2003) has issued the rule requiring manufacturers to include *trans* FA or *trans* fat on the nutrition facts label. Therefore, there has been much effort on the part of the vegetable oil industry to meet the labeling requirement. From these perspectives, the aim of the present work was to gather an overview of the FA compositions with special emphasis on the *trans* FA of commercially refined oils of different botanical origins. Such data can also present details on the variability in *trans* FA content within a group of refined vegetable oil products. In addition, the *trans* FA contents of the analyzed oils were compared and discussed with respect to their formation.

2. MATERIALS AND METHODS

2.1. Standards and reagents

FA methyl ester (FAME) standards (99% purity) were provided by Nu-Check-Prep Inc. (Elysian, MN). All chemicals were of analytical or chromatographic grade.

2.2. Sample selection

The most commonly marketed brands of the refined vegetable oils in Turkey were purchased from several local supermarkets and immediately analyzed. The samples were selected from the most popular commercially refined vegetable oils, namely refined sunflowerseed oil, refined corn oil, refined soybean oil and refined hazelnut oil. These oils were produced by industrial manufacturers which are major producers of the refined vegetable oils in all regions of Turkey. Three samples from each brand were taken over a 9-month period. The samples of refined sunflower, corn, soybean and hazelnut oils were evaluated in 30, 18, 15 and 9 brand groups, respectively. The samples were stored at 4°C until analyzed. Data were given as arithmetic means \pm standard deviation (SD) for each group of the refined vegetable oils unless otherwise indicated.

2.3. Preparation of FAMES

FAMES were prepared according to the American Oil Chemists Society (AOCS, 1992) official method Ce 2-66. The FAMES were obtained from the lipid fractions after alkaline hydrolysis, followed by methylation in methanol with 12.5% boron trifluoride catalyst. The final concentration of the FAMES was approximately 7 mg/mL in heptane.

2.4. Capillary gas-liquid chromatography (GLC) analyses of FAMES

Analyses of the FAMES by capillary GLC were carried out on a Hewlett-Packard 6890 chromatograph (Hewlett-Packard, Wilmington, DE) equipped with a Chrompack Autosampler-M911 (Chrompack, Middelburg, the Netherlands) for split-type injection and a flame-ionization detector. A fused-silica capillary column (Chrompack) was used for the FAME analysis: CP-Sil 88, 100 m \times 0.25 mm i.d., 0.20 μ m film thickness. 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 temperature, 200°C; detector temperature, 250°C; carrier gas, helium at a flow rate of 1 mL/min; split ratio 1:100; and volume of injected sample 1 μ L. The peaks were tentatively identified by comparing the retention times and area percentages with those of authentic standards of FAMES obtained from Nu-Chek-Prep Inc. (Elysian, MN) and on the basis of literature data for *trans* FA (Wolff, 1992; Duchateau *et al.*, 1996). The final results were expressed as relative percentage of individual FA.

2.5. Statistical data analysis

Differences in the mean values of each FA, total *trans* polyunsaturated FA, and total *trans* FA contents were tested by analysis of variance using an SPSS software program (SPSS Inc., Chicago, IL), and the

significance of differences among the mean values was determined using the Duncan's multiple range tests.

3. RESULTS AND DISCUSSION

A comparison of *trans* FA contents (mean \pm SD, % of total FA) from the commercially refined sunflower, corn, soybean, and hazelnut oils are presented in Table 1. The degrees of isomerization of the C18:1, C18:2 and C18:3 FA are also given in Table 1.

The C16:0 and C18:0 acids were the main saturated FA for all refined vegetable oils analyzed. The C16:0 acid contents obtained for refined corn and soybean oils were significantly higher ($P < 0.01$) than those of the others. For the C18:0 acid, the refined soybean oils exhibited a significantly higher mean value ($P < 0.01$) than the others did. The main monounsaturated FA, the total C18:1 acid, ranged from 29.13 ± 0.95 and $80.90 \pm 0.99\%$ in all groups of the refined vegetable oils and the mean value obtained for refined hazelnut oil was significantly higher ($P < 0.01$) than those obtained for the others. Among the polyunsaturated FA, the total C18:2 acid levels ranged from 10.01 ± 0.70 and $59.29 \pm 3.03\%$ in all groups of the refined vegetable oils, with significantly higher mean values ($P < 0.01$) observed for refined sunflower and corn oils in comparison with the others. As for the total C18:3 acids, their low levels (approximately 0.10%) for refined sunflower and hazelnut oils were statistically similar ($P > 0.01$). In contrast, the mean values of the total C18:3 acid in the refined corn oils were $0.78 \pm 0.10\%$, whereas those of refined soybean oils were $7.10 \pm 0.53\%$. The sum of six minor FA (C14:0, C16:1, C20:0, C20:1, C22:0, C24:0), and unidentified FA were below 2% of total FA. All obtained FA profiles were within the ranges found in the literature.

As illustrated in Table 1, there was a remarkable variation in the amounts of the total *trans* FA among the different refined vegetable oils. The mean values of total *trans* FA varied from 0.26 ± 0.07 to $1.27 \pm 0.57\%$ of total FA for all refined vegetable oils ($P < 0.01$). The total *trans* FA comprised isomers of the C18:1, C18:2 and C18:3 acids. Meanwhile, five brands of refined sunflower oil and two brands of hazelnut oil contained no measurable amounts of the total *trans* C18:3 acid. All samples studied contained total *trans* C18:1 acid and total *trans* C18:2 acid. The refined soybean oils had higher total *trans* FA content than the others. In addition, similar results were observed for the total *trans* polyunsaturated FA in all groups of refined vegetable oils, which varied from 0.09 ± 0.01 to $1.21 \pm 0.55\%$ of total FA ($P < 0.01$). A lower mean value was obtained for refined hazelnut oil. Thus, the highest difference between the total *trans* FA and the total *trans* polyunsaturated FA contents was found in this type of oil. Wolff (1992) and Kellens (1997) reported that the *trans* FA contents

for refined soybean oils were 0.16-2.99 and 0.9-3.5%, respectively. Ferrari *et al.* (1996) reported that the total *trans* FA contents of refined corn, soybean and rapeseed oils were 1.5, 4.6, and 2.4%, respectively. De Greyt *et al.* (1996) expressed that the total *trans* FA contents of the commercial sunflower, peanut, corn and soybean oils ranged from 0.1 to 4.6%. Medina-Juarez *et al.* (2000) found that *trans* FA contents in refined vegetable oils varied from 0.90 to 2.93%. Tasan and Demirci (2003) revealed that the concentration of total *trans* FA in physically refined sunflower oil ($2.56 \pm 0.25\%$) was higher than in chemically refined sunflower oil ($0.76 \pm 0.27\%$). Van Hoed *et al.* (2006) demonstrated that the high temperature of the deodorization process led to the formation of *trans* isomers, resulting in 0.8% of *trans* FA in fully refined rice bran oil. García *et al.* (2006) revealed that the content of total *trans* FA in refined safflower oil with high oleic acid was 0.34% of the total FA. Martin *et al.* (2008) reported that the mean *trans* FA levels ranged from 0.8 and 2.6% for refined soybean oils.

The total *trans* C18:1 monounsaturated FA was the predominant *trans* FA found in refined hazelnut oil, with a mean value of $0.17 \pm 0.05\%$. A higher mean value of total *trans* C18:1 acid was obtained for refined hazelnut oil than those obtained for the other oils, with mean values lower than 0.1%. For *trans* polyunsaturated FA, the total *trans* C18:2 acid was the predominant *trans* FA found in the refined sunflower and corn oils, while *trans* polyunsaturated FA for the refined soybean oil was found at high levels. Refined hazelnut oil exhibited a considerably lower mean value for total *trans* C18:2 acid than the other refined oils. Meanwhile, the mean values of the total *trans* C18:1 and total *trans* C18:2 acids in refined corn oil were very similar. The low mean values of total *trans* C18:3 acid were detected in the refined vegetable oils, which varied from 0.01 ± 0.01 to $0.07 \pm 0.03\%$, except for soybean oil, whose mean value was $0.71 \pm 0.28\%$. The differences among the refined vegetable oils for both the total *trans* C18:1 acid and the total *trans* C18:2 acid, except for refined hazelnut oil, were not statistically significant ($P > 0.01$). As for total *trans* C18:3 acid, no significant differences ($P > 0.01$) existed among total *trans* C18:3 acid of the refined vegetable oils, except for refined soybean oil. The distribution of the C18:1, C18:2, and C18:3 acids in the starting vegetable oils is quite different. Consequently, the amounts of detectable *trans* FA formed during refining are different (Bruggen *et al.*, 1998). Also, fluctuations in the conditions of industrial processing such as temperature, duration, vacuum, humidity, stripping steam dosage and different sample origin can cause variations in *trans* polyunsaturated FA concentrations (Tasan and Demirci, 2003; Martin *et al.*, 2008).

Taking into account all the available data (Table 1), the degrees of isomerization of the C18:1, C18:2 and C18:3 acids generally showed a wide variation within each group of refined

Table 1
**Trans FA contents and degree of isomerization
 in the commercially refined vegetable oils.**

Fatty acids ¹	Sunflower oils (n = 30) ⁶	Hazelnut oils (n = 9)	Corn oils (n = 18)	Soybean oils (n = 15)
16:0	5.83 ± 0.20 ^a	5.62 ± 0.62 ^a	10.74 ± 0.31 ^b	10.11 ± 0.58 ^b
18:0	3.62 ± 0.23 ^c	2.47 ± 0.20 ^b	1.96 ± 0.16 ^a	4.13 ± 0.18 ^d
Σ 18:1 <i>trans</i> ²	0.08 ± 0.04 ^a	0.17 ± 0.05 ^b	0.08 ± 0.03 ^a	0.06 ± 0.02 ^a
18:1 <i>cis</i>	29.27 ± 3.23 ^a	80.73 ± 1.02 ^b	29.05 ± 0.93 ^a	26.37 ± 1.02 ^a
Σ 18:1	29.35 ± 3.22 ^a	80.90 ± 0.99 ^b	29.13 ± 0.95 ^a	26.43 ± 1.03 ^a
Σ 18:2 <i>trans</i> ³	0.59 ± 0.39 ^b	0.08 ± 0.02 ^a	0.36 ± 0.22 ^b	0.50 ± 0.29 ^b
18:2 <i>cis</i>	58.70 ± 2.84 ^c	9.93 ± 0.69 ^a	55.71 ± 1.27 ^c	49.80 ± 1.70 ^b
Σ 18:2	59.29 ± 3.03 ^c	10.01 ± 0.70 ^a	56.07 ± 1.21 ^c	50.30 ± 1.62 ^b
Σ 18:3 <i>trans</i> ⁴	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.07 ± 0.03 ^a	0.71 ± 0.28 ^b
18:3 <i>cis</i>	0.09 ± 0.03 ^a	0.08 ± 0.01 ^a	0.78 ± 0.10 ^b	6.39 ± 0.37 ^c
Σ 18:3	0.10 ± 0.04 ^a	0.09 ± 0.02 ^a	0.85 ± 0.11 ^b	7.10 ± 0.53 ^c
Others ⁵	1.81 ± 0.11 ^c	0.91 ± 0.06 ^a	1.25 ± 0.11 ^b	1.93 ± 0.08 ^c
Σ <i>trans</i> PUFA	0.60 ± 0.38 ^{bc}	0.09 ± 0.03 ^a	0.43 ± 0.23 ^b	1.21 ± 0.55 ^c
Σ <i>trans</i> FA	0.68 ± 0.41 ^{ab}	0.26 ± 0.07 ^a	0.51 ± 0.24 ^a	1.27 ± 0.57 ^b
DI 18:1	0.27 (0.06-0.59) ⁷	0.21 (0.15-0.23)	0.28 (0.17-0.43)	0.23 (0.12-0.34)
DI 18:2	1.00 (0.16-1.99)	0.80 (0.67-0.97)	0.64 (0.19-1.28)	0.99 (0.41-1.94)
DI 18:3	10.00 (0-30.0)	11.11 (0-18.18)	8.24 (4.69-13.25)	10.00 (4.67-13.18)
Trans/ <i>cis</i>	0.78 (0.15-1.58)	0.29 (0.22-0.36)	0.59 (0.21-1.03)	1.41 (0.65-2.53)

Data are the mean ± standard deviation (SD), and are expressed as a weight percentage of total FA methyl esters. The means in each row with different letters (a-d) are significantly different ($P < 0.01$, Duncan's multiple range test). ¹FA are designated by number of carbon atoms: number of double bonds. FA, fatty acid; PUFA, polyunsaturated FA. ²Constituted of 18:1 $\Delta 9$ *trans*. ³Sum of only $\Delta 9$ *cis*, $\Delta 12$ *trans* and $\Delta 9$ *trans*, $\Delta 12$ *cis* isomers for the C18:2. ⁴Sum of *trans* isomers were calculated as one group for the C18:3. ⁵Other fatty acids include 14:0, 16:1, 20:0, 20:1, 22:0, 24:0, and unidentified fatty acids. ⁶n = number of brands within group analyzed. ⁷Range (in parentheses). DI 18:1, degree of isomerization for the indicated fatty acid ratio of Σ 18:1 *trans* on Σ 18:1 times 100. DI 18:2, degree of isomerization for the indicated fatty acid ratio of Σ 18:2 *trans* on Σ 18:2 times 100. DI 18:3, degree of isomerization for the indicated fatty acid ratio of Σ 18:3 *trans* on Σ 18:3 times 100.

vegetable oils. In particular, refined sunflower oils showed a rather heterogeneous distribution of degree of isomerization for these unsaturated FA. This indicates that the operation conditions used during the deodorization stage could vary greatly from one producer to another. The degree of isomerization of unsaturated FA is an important indicator of the intensity of the thermal treatment applied to refined vegetable oil in the deodorization stage. Generally, isomerization is reported to begin at between 180 and 200°C and to increase with increasing temperature and prolonged heating time (De Greyt *et al.*, 1996). A deodorization temperature of 220-230°C seems to be the critical point above which C18:3 acid isomerization substantially increases. The critical temperature is higher for C18:2 acid, over 240°C (Henon *et al.*, 1999). Kellens (1997) reported that chemical refining should be done at 230-235°C and physical refining at 235-240°C to obtain deodorized oils with low levels of *trans* FA (< 1%). Kemeny *et al.* (2001) found an

increase in the relative percentage of isomerized C18:2 and C18:3 acids with an increase in either the deodorization time or the temperature. On the other hand, in this study, the mean values of the degree of isomerization of C18:3 acid were higher than those obtained for the C18:2 acid, while these values for C18:1 acid were found at very low levels for the groups of refined vegetable oils, as expected. De Greyt *et al.* (1996) reported that more C18:3 acid *trans* isomers are formed in soybean oil in spite of the fact that the initial level of C18:2 acid is higher. This phenomenon is described in the literature (Wolff 1992) and explained by the fact that the probability for a C18:3 molecule to be isomerized during heat treatment is about 10 to 12 times high than for a C18:2 acid molecule. Bruggen *et al.* (1998) reported that the lowest isomerization rate is observed for the monoenoic FA. For the formation of geometrical isomers of unsaturated FA to be avoided during deodorization, the temperature in the deodorizer should be

relatively low and the heating time sufficiently short to minimize isomerization (Wolff, 1994). Refining has the purpose of not only eliminating impurities, but also minimizing *trans* FA formation (Kellens, 1997). Because the quality variables for refined vegetable oils include the lowest possible content in *trans* fatty acids, this is more and more important as a contemporary quality criterion (Leon-Camacho *et al.*, 1999; Henon *et al.*, 1999).

4. CONCLUSIONS

The results demonstrate an important disparity in the *trans* FA contents among the studied commercially refined vegetable oils and within each group of oils due to their different unsaturated FA levels and variable industrial refining methods employed. Because of their high levels of monounsaturated FA, refined hazelnut oils contained limited amounts of total *trans* polyunsaturated FA and also total *trans* FA. However, other commercially refined vegetable oils with a relatively high total polyunsaturated FA contained considerable amounts of these FA. The obtained results revealed that it is important to be aware of the nutritional quality of refined vegetable oils. As a corollary, deodorization conditions during industrial refining processes should be carefully evaluated in order to prevent the formation of *trans* FA or to keep their content at a low level in the vegetable oils that are rich in polyunsaturated FA, particularly soybean oils.

ACKNOWLEDGEMENTS

This work was supported by the Trakya University Scientific Research Projects Fund (Project No. TUBAP-484).

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Recibido: 5/10/10
Aceptado: 10/12/10