

trans-Fatty Acid Isomers in Two Sesame (Sesamum indicum L.) Seed Byproducts under Processing

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The present study has been inspired by the growing need for rigorously controlling the nutritional quality and safety of food products. The impact of application in the food industry on fatty acids composition, trans-fatty acids (TFAs), and conjugated linoleic acid (CLA) profiles were investigated in a highly consumed candy byproduct of sesame seed (chamia) in comparison to fresh sesame seed oil (SSO) and heated SSO under simulated frying experiments. The effect of treatment on SSO was studied by determining the TFA and CLA changes. Results showed significant differences between the two byproducts in TFA and CLA amounts. Total TFAs were found to be significantly higher in chamia than fresh SSO (1.31 versus 0.066%, respectively; p < 0.05) and even higher than all heated SSO from 2 to 10 h at 180 °C (1.31 versus 0.33%, respectively; p < 0.05). A significant linear relationship was found between trans-monounsaturated fatty acid (MUFA), trans-polyunsaturated fatty acid (PUFA), and total TFA and the time of processing, with a correlation coefficient (R^2) greater than 0.9 for TFA and PUFA, with a higher correlation assigned to PUFA (r = 0.988; p < 0.9880.001), followed by TFA (r = 0.959; p < 0.01) and MUFA (r = 0.844; p < 0.05). Principal component analysis of the fatty acid (FA) profiles showed discrimination between chamia and both fresh and heated SSO. A high stability of SSO against isomerization reactions as compared to their chamia sample counterpart has been noted. These findings suggest that the food industry engenders relatively higher changes in fatty acid configurations than the frying process.

KEYWORDS: Sesame; chamia; trans-fats; conjugated linoleic acid; processing

INTRODUCTION

Sesame seed is the seed of Sesamum indicum L. (family Pedaliaceae) believed to be indigenous to tropical Africa and cultivated in India, China, and Nigeria (1) Sesame serves as a nutritious food for humans and is used widely in bakery and confectionery products (2). As an oleaginous plant, sesame seed (SS) contains about 50–60% oil, 8% protein, 3.2% crude fiber, 18% carbohydrate, 5.7% ash and is very rich in minerals, such as calcium, phosphorus, and vitamin E(3). Sesame oil has a pleasant flavor and regarded as a superior vegetable oil. It ranges second with regard to nutritional value after olive oil (4). Sesame oil is widely used in cooking and as an ingredient of confectionery for making margarine (5). Sesame seed provides highly stable oil and nutritious protein and meals, used in sweetmeats and confectionery foods, and has a variety of medicinal properties (6). In addition to their edible oil, sesame seeds were largely used in the food industry for manufacturing chamia (CHM). CHM is a candy product obtained exclusively by the mixture of at least 50% "tahina" and a maximum content of sugar of 45%. "Tahina" is a paste obtained by crushing peeled sesame seeds.

The presence of *trans*-fatty acids (TFAs) in food is believed to have negative health effects (7). The term "*trans*-fatty acids"

covers a wide range of fatty acids (FAs) with large variations in structure and property. TFAs in food have three major sources, partial hydrogenation of fats, high-temperature processing of edible oils, and the natural occurrence of TFAs in ruminant meat and dairy products. Foods that are high in TFAs or saturated FAs are associated with an increased risk of cardiovascular disease and diabetes (8). An emerging consensus underscores the importance of oxidative events in vascular disease, including excess production of reactive oxygen/nitrogen species (5). A possible association has been shown between the intake of TFAs and the risk of coronary heart disease. They are proven to produce adverse effects on blood lipids, including an increasing low-density lipoprotein (LDL-cholestrol) concentration and a decreasing high-density lipoprotein (HDL-cholestrol) concentration (9). Dietary trans-fats not only include mono- and polyunsaturated FAs but also conjugated linoleic acids (CLAs) (10). Research has mainly been focused on sesame lignans, which are present in small amounts in sesame oil, and, particularly, on their efficacy in inhibiting lipid oxidation (11), as well as their possible synergistic action with tocopherols (12). On the contrary, TFA content in sesame byproducts has not received much attention in the literature. To our knowledge, *trans* isomerization of sesame seed oil (SSO) under industrial processing remains unexplored. To date, the published information on TFAs in food products in Tunisia remains scarce and of questionable accuracy. The Scientific Panel on Dietetic Products, Nutrition, and Allergies (NDA)

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of the European Food Safety Authority (EFSA) found that the intake of TFAs varies between countries, with lowest intakes found in Mediterranean countries.

In the present study, we are interested in comparing the FA composition, TFA isomers, and CLA profiles of a highly consumed sesame seed byproduct (CHM) manufactured by industrial processing to those in SSO at fresh state and under different cycles of heating at frying conditions.

MATERIALS AND METHODS

Reagents and Standards. Reagents and solvents were of analytical or high-performance liquid chromatography (HPLC) grade, supplied by Sigma-Aldrich (Buchs, Switzerland). In the present study, a mixture of standards containing the following methyl esters of lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), *trans*-9-elaidic acid (18:1n-9), oleic acid (18:1n-9), *trans*-11-vaccenic acid (18:1n-7), linolelaidic acid (all *trans*-18:2n-6; *t9*,*t12*, *t9*,*c12*, *c9*,*t12*, and *c9*,*c12*), linoleic acid (18:2n-6), linolenic acid (18:3n-3), arachidic acid (20:0), behenic acid (22:0), erucic acid (22:1n-9), arachidonic acid (20:4n-6), and lignoceric acid (24:0) were obtained from E. Merck (Darmstadt, Germany) and Supelco (Bellefonte, PA).

Samples. Sesame seeds for CHM manufacturing and oil extraction have the same origin and were supplied by a local manufacturer. Chamia samples were industrially manufactured (Ariana, Tunisia).

Lipid Extraction. The seed material, finely ground in a mill, lipid fraction was extracted by the Soxhlet method with hexane for 8 h at the boiling point of the solvent (68–70 °C). The lipid extracts were collected in a flask and subsequently treated with sodium sulfate to remove traces of water. After filtration, the extract was then taken to dryness on a rotary evaporator at 40 °C. The extracted lipids were stored under nitrogen at -20 °C for further analyses.

Oxidation Experiment of Crude Oils. Samples of crude oils (SSO) were placed in a series of Pyrex flasks having a volume of 200 mL each. The oxidation reaction was accelerated in an oven set at 180 °C for 2, 4, 6, 8, and 10 h. Aliquots of the oil samples were removed at predetermined time intervals to assess their FA profiles, and oil samples were withdrawn for triplicate analyses.

Analysis of Fatty Acid Methyl Esters (FAMEs). The process of converting free or esterified FAs into methyl esters is referred to a transesterification, with a methanolic solution of potassium. FAME analysis was carried out according to the European Union Commission modified Regulation EEC 2568/91 (13) on a Hewlett-Packard gas chromatograph (Hewlett-Packard, Palo Alto, CA), fitted with a flame ionization detector and a split-splitless injector, set at 270 °C. The carrier gas was nitrogen (1 mL/min), and elution was performed with a fused silica Agilent DB23 capillary column (60 m length, 0.32 mm inner diameter, and 0.25 µm film thickness). Conditions were as follows: injector temperature, 270 °C; flame ionization detector, 280 °C; injector split ratio, 1:50; the initial column temperature, 130 °C; step 1, 6.5 °C/ min to 170 °C; step 2, 2.8 °C/min to 215 °C and maintained for 12 min; step 3, 40 °C/min to 230 °C and maintained for 20 min. FAMEs were identified by comparing their relative and absolute retention times to those of authentic cis-fatty acid (CFA) and TFA standards. The FA composition was reported as a relative percentage of the total peak area using a HP Chemstation integrator.

Oxidative Susceptibility (OS). The oil OS was estimated according to Cert et al. (*14*) by means of the following equation:

 $OS = monounsaturated fatty acids + (45 \times linoleic acid)$

$+(100 \times \text{linolenic acid})$

Statistical Analysis. The results were reported as mean values of three replicates. Data were compared on the basis of the standard deviation of the mean values. Significant differences among samples studied were determined by an analysis of variance, which applied Duncan's test with a 95% significant level (p < 0.05). The proportions of individual TFA and CLA were used as input values in principal component analysis (PCA) to determine if the FAME signatures of sesame byproducts varied after food industry application. PCA was conducted using XLSTAT 2010, version 3.06. The factor loading scores for the individual TFA and CLA were used

to assess the relative importance of each one in the calculation of the principal component axes.

RESULTS AND DISCUSSION

FA Profile. Results on FA composition of the two sesame seed byproducts, CHM and fresh SSO, showed a noticeable difference in their profiles. Typical chromatograms of studied samples were illustrated in Figure 1. In this chromatogram, there were significant peaks in oleic acid (C18:1n-9) and linoleic acid (18:2n-6) present in both CHM and fresh SSO. There were noticeable difference in the numbers of peaks and FA isomer types between CHM and fresh SSO (panels A and B of Figure 1). As shown in Table 1, the major FAs in unused SSO and CHM were oleic acid (39.36 and 36.42%), linoleic acid (41.86 and 39.1%) and palmitic acid (14.39 and 19.4%), respectively. The differences in the amount of various FAs were given as a ratio of unsaturated/ saturated FAs (UFA/SFA). UFA/SFA in SSO was higher than in CHM (5.67 versus 3.89, respectively). As a result of the industrial application, monounsaturated fatty acid (MUFA) losses occurred and caused a relative increase of SFA in CHM, which is in accordance with the idea that unsaturated fats are more easily oxidized than saturated fats (15, 16). Oleic acid (C18:1n-9) and linoleic acid (C18:2n-6) were the major MUFA and polyunsaturated fatty acid (PUFA) in both SSO and CHM, respectively. The high oxidative susceptibility of CHM (OS = 1974.43) compared to SSO (OS = 1843.41) can be an excellent index of the oxidation state of the first (Table 1). The presence of a high content of PUFAs increases the susceptibility of oil to oxidation (17). UFA/ SFA is considered to be a major factor affecting oil oxidation.

TFA and CLA. Under high temperature, isomerization reactions of UFAs occurred.

Zambonin et al. (18) reported that *cis/trans* isomerization of carbon double bonds is promptly carried out by a free radical attack. **Scheme 1** shows the reaction mechanism involving reversible addition of a radical X[•] to a double bond to form a radical adduct. The reconstitution of the double bond is obtained by β -elimination of X[•], and the result is in favor of *trans* geometry, the most thermodynamically stable configuration. In this mechanism, X[•] acts as a catalyst for *cis/trans* isomerization and double-bond shifts are not allowed. The efficiency of the attacking radical.

As shown in Table 1, significant differences among FA isomers in the two byproducts were observed. The major *trans* isomer obtained in fresh SSO was trans-hexadecenoic acid C16:1n-7 (trans), which is statistically different when compared to the principal trans isomer found in CHM, which is the cis, trans-linoleic acid C18:2n-6 (c9,t12). As shown by the chromatogram (Figure 1), the peaks belonging to trans-linoleic acid C18:2n-6 (c9,t12) were observed in CHM and fresh SSO with significant difference in importance. The chromatogram of Figure 1A showed two peaks corresponding to CLA detected in CHM samples, but any traces are detected in fresh SSO. As seen from Figure 2, there were statistically significant differences between the groups in terms of TFA isomers and CLA contents (p < 0.05). The different TFA fractions were presented in Table 1. In opposition to CHM, elaidic acid (trans-C18:1n-9) was not detected in fresh SSO. Similar observations on TFAs in refined oils have been reported by Bansal et al. (19) and Greyt et al. (20). In relation to the trans-18:2 isomers, the higher relative content of these isomers in CHM than in SSO (1.215 versus 0.018%, respectively) seemed to indicate that they are more generated by industrial applications (Table 1). CHM had higher amounts of both SFA (21.74 ± 0.33 and $14.39 \pm$ 0.40) and TFA $(1.31 \pm 0.19 \text{ and } 0.066 \pm 0.01)$ than fresh SSO, respectively. In the prospective cohort studies that compared the

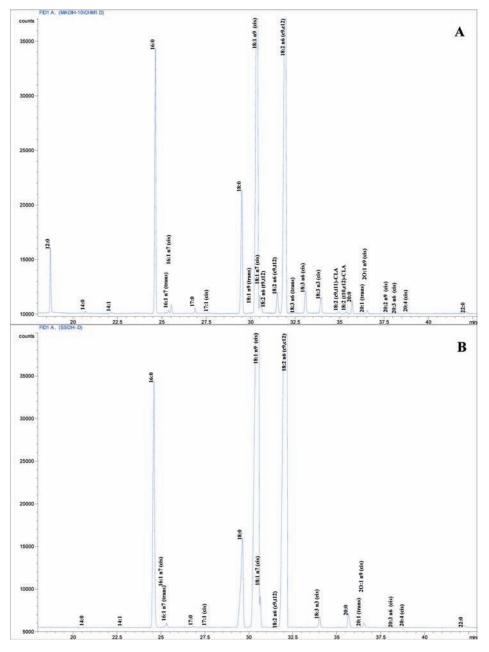


Figure 1. Chromatograms of TFA and CFA isomers in (A) CHM and (B) fresh SSO.

effects of TFA and SFA, the effects of TFA were stronger than those of a mixture of SFA. A number of these studies allow for a comparison of food sources, and these results could explain the positive association with the risk of cardiovascular diseases and the intake of TFA from industrial food sources (21).

SSOs were free of CLAs, whereas CHM contains about 0.25% of total FAs, represented by both 18:2 (*cis-9,trans-*11) and 18:2 (*trans-*10,*cis-*12). Therefore, thermal isomerization is not exclusively geometrical isomerization, leading to isomers with the double bonds in the same position as in the original FA, but the food industry also causes isomerization reactions of linoleic acid. As reported earlier, CLAs are a specific group of different positional and geometrical isomers derived from linoleic acid that can also be formed by heating, refining, or partial hydrogenation of oils (22, 23). Usually, the native tocopherols that are normally present in the oils are sufficient to protect the oils against oxidative deterioration during storage at ambient temperature (24). The stability of oils during storage or upon heating to the frying temperature is an important measure for ensuring good oil

performance at elevated temperatures (24). During the frying of oil, a large number of reactions take place (hydrolysis, oxidation, isomerization, polymerization, and cyclization), which give rise to a lot of desirable and non-desirable compounds (25, 26). For this reason, heating at frying conditions was investigated on SSO to compare the *trans* isomers and CLA profiles between the two sesame seed byproducts, CHM and the heated SSO.

Chemometric Analysis. PCA of sesame seed byproduct FA profiles (*cis, trans*, and CLA isomers) was conducted to determine the effects of processing on FA distribution. In the PCA of the FA profiles of the studied seed byproducts, the first-component axis accounted for 62.02% of the variance and the second-component axis accounted for 22.71% of the variance (**Figure 3**). This explained 84.73% of the total variance. The PCA separated the samples into three distinct clusters. The first cluster contained the CHM. The second cluster contained the fresh SSO. The third cluster contained the heated SSO for 10 h. Whereas the first cluster (CHM) was positively associated with PC1, the second and third clusters were negatively associated with PC1 (**Figure 3**).

Table 1. FA Composition (%) and Oxidative Susceptibility of CHM Compared to Fresh SSO^a

to Fresh SSO ^a	shorter form	СНМ	fresh SSO
FA (%)	shorter torm	CHIM	liesh 550
lauric acid	C12:0	2.34 ± 0.02	nd
tetradecanoic acid	C14:0	0.09 ± 0.02	0.01 ± 0.00
palmitic acid	C16:0	11.18 ± 0.02	7.97 ± 0.11
margaric acid	C17:0	0.32 ± 0.11	0.06 ± 0.01
stearic acid	C18:0	7.06 ± 0.15	5.6 ± 0.04
eicosanoic acid	C20:0	0.58 ± 0.02	0.68 ± 0.23
behenic acid	C22:0	0.17 ± 0.01	0.03 ± 0.00
saturated	\sum SFA	21.74 ± 0.33	14.39 ± 0.40
cis-tetradecenoic acid	C14:1	0.18 ± 0.17	0.01 ± 0.00
cis-hexadecenoic acid	C16:1n-7 (<i>cis</i>)	0.14 ± 0.04	0.11 ± 0.01
cis-heptadecenoic acid	C17:1	0.04 ± 0.01	0.07 ± 0.02
oleic acid	C18:1n-9 (<i>cis</i>)	34.99 ± 0.17	41.86 ± 0.79
vaccenic acid	C18:1n-7	0.90 ± 0.10	0.68 ± 0.01
cis-eicosamonoenoic acid	C20:1n-9	0.16 ± 0.01	0.17 ± 0.02
monounsaturated	∑ <i>cis</i> -MUFA	36.42 ± 0.5	41.9 ± 0.87
cis, cis-linoleic acid	C18:2n-6 (c9,c12)	$\textbf{38.01} \pm \textbf{0.13}$	39.36 ± 0.76
<i>cis,cis,cis-γ</i> -linolenic acid	C18:3n-6 (cis)	1.09 ± 0.24	nd
$cis, cis, cis, cis-\alpha$ -linolenic acid	C18:3n-3 (cis)	0.91 ± 0.00	0.3 ± 0.01
eicosadienoic	C20:2n-9	0.09 ± 0.02	0.01 ± 0.00
dihomo- γ -linolenic acid	C20:3n-6	0.01 ± 0.02	0.09 ± 0.02
cis-arachidonic acid	C20:4	0.07 ± 0.02	0.05 ± 0.00
polyunsaturated	∑ <i>cis</i> -PUFA	39.1 ± 0.42	39.81 ± 0.8
	UFA/SFA ratio	3.89	5.67
trans-hexadecenoic acid	C16:1n-7 (trans)	0.042 ± 0.01	0.03 ± 0.00
elaidic acid	C18:1n-9 (trans)	0.042 ± 0.02	nd
trans-eicosanoic acid	C20:1 (trans)	0.01 ± 0.00	0.01 ± 0.00
trans-monounsaturated	∑ <i>trans</i> -MUFA	0.09 ± 0.03	0.05 ± 0.01
	trans-/cis-MUFA ratio	0.25	0.11
	trans-/cis-PUFA ratio	3.01	0.04
trans, trans-linoleic acid	C18:2n-6 (<i>t</i> 9, <i>t</i> 12)	0.08 ± 0.01	nd
trans, cis-linoleic acid	C18:2n-6 (19,c12)	nd	nd
cis, trans-linoleic acid	C18:2n-6 (c9,t12)	1.04 ± 0.21	0.02 ± 0.00
trans-linolenic acid	C18:3n-3 (trans)	0.09 ± 0.06	nd
trans-polyunsaturated	∑ <i>trans</i> -PUFA	1.21 ± 0.17	0.02 ± 0.00
rumenic acid	C18:2 (<i>c</i> 9, <i>t</i> 11)-CLA	0.13 ± 0.03	nd
alternate CLA isomer	C18:2 (<i>t</i> 10, <i>c</i> 12)-CLA	0.12 ± 0.00	nd
conjugated linoleic acid	\sum CLA	0.25 ± 0.00	nd
	∑TFA	1.31 ± 0.19	0.07 ± 0.01
oxidative susceptibility	OS	1974.43	1843.41

^and, not detected; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; CLA, conjugated linoleic acid.

Scheme 1. Reaction Mechanism for the *cis/trans* Isomerization of UFAs Catalyzed by X* Radicals (18)

$$X^{\cdot} + R^{1} R^{2} \longrightarrow R^{2} R^{2} R^{2} R^{2} R^{2}$$

This indicated that clear differentiation exists between sesame seed byproducts. Processing in the food industry increases the relative proportion of total TFA isomers when compared to fresh SSO and heated SSO at the frying temperature. This was also illustrated by the fact that SFA (C12:0, C14:0, C16:0, C18:0, C20:0, and C22:0), elaidic acid C18:1n-9 (*trans*), *trans*-PUFA [C18:2n-6 (*t*9,*t*12) and C18:2n-6 (*c*9,*t*12)], total TFA, and CLA [C18:2 (*c*9,*t*11)-CLA and C18:2 (*t*10,*c*12)-CLA] are more represented to the right of PC1. PC1 was heavily weighted by TFA isomers. Therefore, we can conclude that manufacturing in the food industry affects the *trans*-fat configurations and their distribution more than by culinary applications under frying conditions.

Correlations. Results showed that examined food samples differed substantially in the content of TFA. After 10 h of heating at 180 °C of SSO, the *trans*-MUFA isomers significantly increased

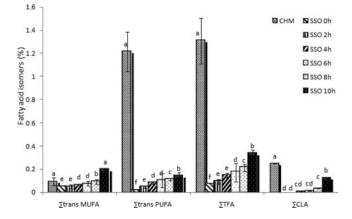


Figure 2. TFA and CLA amounts in CHM and fresh and heated SSO. Different letters indicate significantly different values at ($p \le 0.05$) according to Duncan's test.

4 times compared to their counterparts of fresh SSO (0.195 versus 0.048%, respectively). Moreno et al. (27) reported that intensive heating of oils caused an increase in TFA. Higher amounts of elaidic acid C18:1n-9 in heated oil samples as compared to control oil samples and CHM could be explained by the release of these TFAs by the frying process, which is in accordance with results reported by Yaacoub et al. (28), proving that heating and frying processes induced production of elaidic acid, which was absent in the fresh oil samples. An increase in TFA and CLA content was observed in heated oils, and consequently, significant positive correlations were noted. Figure 4 shows a linear relationship between trans-MUFA, trans-PUFA, and total TFA and the time of heating, with a correlation coefficient (R^2) greater than 0.9 in PUFA and TFA (r = 0.959; p < 0.05), with a higher significant correlation assigned to PUFA (r = 0.988; p < 0.001), followed by TFA (r = 0.959; p < 0.01) and MUFA (r = 0.844; p < 0.05).

Despite the generation of *trans* isomers of MUFA, PUFA, and CLA in heat-treated SSO, they usually remain significantly less that those found in CHM (p < 0.05), except for *trans*-MUFA after 10 h of heating (**Figure 2**). The oil fraction (SSO) showed a remarkable stability to oxidation. This could be attributed to endogenous antioxidants (sesamol, sesamolin, and sesamin) together with tocopherols. Sesame oil is known to be significantly resistant to oxidative rancidity, although it contains nearly 85% UFAs (29).

There are several explanations of the high amount of TFA content in CHM compared to SSO. The isomerization rate could be a result of the manufacturing conditions of CHM, which needs many phases, such as dehulling, humidification, drying at 80 °C for 15 min with spinning, and roasting for 1 h at 120 °C, as previously described by Elleuch et al. (*30*). As reported by Yaacoub et al. (*28*), sesame seeds are particularly rich in both lipids and proteins, making them very susceptible to oxidation and Maillard reactions during thermal treatment. Consequently, the loss of nutritional value, changes in the organoleptic quality, and accumulation of reaction products (oxidized lipids, TFA, and carboxymethyllysine) occurred. Lipid oxidation is a principal chemical change of foods that depends upon the level of oxygen, degree of unsaturation of fatty acids, energy (heat/light), and metals (*31*).

In conclusion, differently processed sesame seeds engender isomerization reactions with significantly different levels among the CHM and SSO samples. Significant amounts of TFAs may be accumulated by consuming oleaginous plant byproducts under food industry applications, such as CHM, adding to other principal origins (shortenings, hydrogenated oils, fried foods, dairy

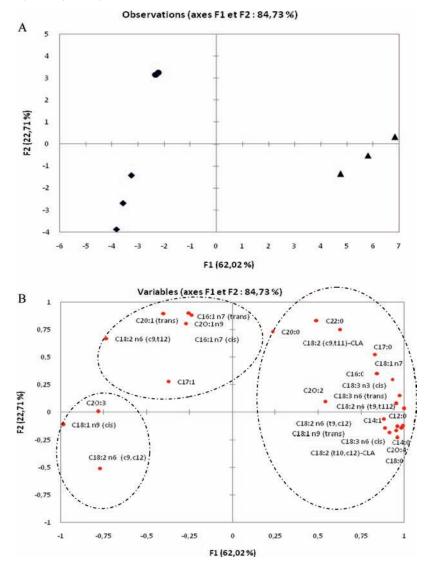


Figure 3. Effect of the processing application on the FA pattern of the sesame seed byproducts. (A) Ordination plots of the PCA based on the FA profile. (B) Loadings of the individual FAs from the PCA of the FA data. FA isomers to the right in the plot indicate those that are more represented in CHM. FA isomers to the left in the plot indicate those that are more represented in heated SSO for 10 h. The variance explained by each principal component axis is shown in parentheses (\blacktriangle , CHM; \blacklozenge , fresh SSO; \blacklozenge , heated SSO for 10 h).

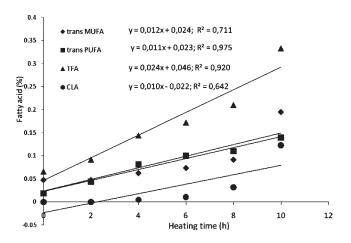


Figure 4. Correlations between the *trans*-MUFA, *trans*-PUFA, and total TFA isomers and CLA and the time of heating of SSO at 180 $^{\circ}$ C for 10 h.

products, etc.). Negative health implications associated with consuming *trans*- and saturated fats may be reversed by altering the intake of these heart unhealthy fats and replacing them with

polyunsaturated fats. Present results suggested the feasibility of using SSO as a frying oil instead of other highly polyunsaturated vegetable oils. Despite the promising effects of industrial applications, it remains a significant challenge for industrial adoption of methods of quality control with high accuracy, such as analysis of TFA content, in baked foods and, especially, the oleaginous seed byproducts. Industrial operations need to be precisely controlled and optimized to produce a good quality product that has the lowest TFA content. Finally, recommendations are needed to regulate TFAs by declaring them on the nutrition label in food supply with more efficient accuracy.

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