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Full Length Research Paper

Effect of heat processing on the profiles of *trans* fatty acids and conjugated linoleic acid in butter oil

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***Sman* or traditional butter oil (TBO) is known to be rich in saturated fatty acids (SFA). Meanwhile, published information about *trans* fatty acids (TFAs) content in TBO remains unexplored. Therefore, a comparison of the fatty acid (FA) composition of traditional butter (TB) and (TBO) with emphasis on geometric and conjugated linoleic acid (CLA) isomers was undertaken. Both TB and TBO accounted for a high level of SFA with significant high content in TBO ($p < 0.05$). Total TFAs in TBO were more than twice the content in TB (8.23% vs. 3.85%, respectively, $p < 0.01$). An increase by 1.81 and 2.9 times was observed for *trans* monounsaturated FAs and *trans* polyunsaturated FAs in TBO compared to TB, respectively. Vaccenic acid (VA), the predominant TFA in both TB and TBO, was increased by 100% in TBO ($p < 0.001$). *Trans*-linoleic acid isomers were 1.84-fold higher in TBO than in TB. The contribution that CLA made to the total FA was increased by 1.48-fold for TBO. In general, it was found that TBO contains high levels of TFAs and CLA. Thus, TBO represents a mixture of FAs with different configurations from natural and technological origins, having potential conflicting effects on human health.**

Key words: *Trans*-fatty acids, conjugated linoleic acid, butter oil.

INTRODUCTION

Trans fatty acids (TFAs) are unsaturated fatty acids containing one or more double bonds in the *trans* configuration. In the *trans* arrangement, the chains are on opposite sides of the double bond and the chain is straight (Micha et al., 2010). In technologically advanced countries, food sources contain a variety of TFAs formed during the processing, hydrogenation and heating of vegetable oils (Scholfield et al., 1967; Beare-Rogers et al., 1979). Many of the same TFA isomers are also present in ruminant milk where they originate from microbial metabolism in the reticulo-rumen (Bauman and Griinari, 2003; Glew et al., 2006). Ruminant-produced *trans* fatty acids (RP-TFA) are made of polyunsaturated fatty acids in the rumen of ruminants such as cow, sheep and goat, and are consequently present in all fats from these animals. For fatty acids (FAs) with 18 carbon atoms, a peak concentration of *trans* double bonds is found in position 9, such as elaidic acid, with a Gaussian distribution of FAs with the *trans* bond in the other positions.

The bacterial desaturation of polyunsaturated fat from grass and vegetables in the rumen also produces *trans* double bonds all over the FAs molecules, but with a distinct preference for the double bond in position 11 of the 18 carbon FAs, as vaccenic acid (Stender et al., 2008). unlike elaidic acid from industrially produced *trans* fatty acids (IP-TFA), vaccenic acid from either IP-TFA or RP-TFA can be converted to rumenic acid, most notably by ruminant animals and also in non ruminant animals as well as in humans. Conjugated linoleic acid (CLA) is a collective term for different positional and geometric isomers of octadecadienoic acid (Guler and Aktumsek,

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2011). The major naturally-occurring isomer of CLA is rumenic acid (18:2 c9,t11) and it is found in plant oils and dairy products (Chin et al., 1992), which may have positive metabolic effects (Stender et al., 2008). CLA are synthesized both in the rumen from dietary linoleic acid (18:2 c9, c12), and in mammary glands from vaccenic acid 18:1 (t11) (Ledoux et al., 2005). Vaccenic acid is produced in the rumen from dietary PUFA such as linoleic and linolenic acids (Griinari and Bauman, 1999). As reported by Ledoux et al. (2000), gas chromatography using polar phases resulted in suitable resolution for linoleic acid geometrical isomers. When using conventional polar (cyanopropyl) gas chromatography (GC) columns, minor CLA isomers in ruminant tissues including t9,c11-, t10,c12- and t11, c13-18:2 are well resolved, but rumenic acid co-elutes with t7,c9-18:2 (Cruz-Hernandez et al., 2004).

It is well known that the types of fat have a more important role in determining risk of coronary heart diseases (CHD) than the total amount of fat in the diet (Hu et al., 2001; He et al*.,* 2007). The results from observational studies concerning the intake of TFA and CHD demonstrate that the intake of RP-TFA has not been associated with or has been negatively associated with the risk of CHD (Weggemans et al., 2004). Other studies have proven that *trans* fats from partially hydrogenated oils are more harmful than those naturally-occurring oils (Anderson et al., 1961; Gerberding, 2009). Since artificial TFA are not only produced by processing the vegetable oils, it is important to discriminate between natural RP-TFA and those induced by processing of milk byproducts. Milk fat is a natural product with incomparable organoleptic properties, which make it an important ingredient in a wide variety of food applications (Munro and Illingworth, 1986). Milk fat is probably the most complex of all edible fats. More than 400 different FA have been detected in milk lipids so far, from C2 to C28, including even and odd-numbered, saturated, monounsaturated, and polyunsaturated, *cis* and *trans*, linear and branched, and various keto- and hydroxyl- fatty acids (Deman and Deman, 1983; Sommerfeld, 1983; Collomb and Bűlher, 2000).

A variety of fermented milk products are prepared in Tunisia and other African and Asiatic countries by rural women who usually use their traditional knowledge of fermentation in the bio-preservation of milk for storage and future consumption. Two products result from the spontaneous fermentation of milk in the South of Tunisia: the first is rich in proteins and is locally called ‘‘Leben’’, while the second is rich in fat and is locally called ‘‘traditional butter’’ (Samet-Bali et al., 2009). Traditional butter oil (TBO) is a highly consumed food commodity in various parts of the world that has been used for long time in culinary preparations and recently it is being used as an ingredient in bakery products. There are no standard procedures for the preparation of traditional butter oil and in Tunisia there are no industrially produced butter oil under controlled conditions of temperature and pressure. However, all the butter oil locally available are produced by the pastoral population employing the traditional method of manually churning the naturally fermented milk and heating the butter to obtain traditional butter oil (Sawaya et al., 1984). Tunisian traditional butter oil called “sman” is obtained by heating traditional butter and separating fat (butter oil) from milk serum (Samet-Bali et al., 2009). A major portion of butter oil is used for culinary cooking (Özkanli and Kaya, 2005).

In the framework of nutritional quality control of milk byproducts, the physicochemical and microbial characteristics and storage stability of TBO were previously studied by Samet-Bali et al. (2009). However, in both butter and butter oil, TFAs either of natural or technological origins have not been studied until now. The aim of this study was to compare between the FA composition of traditional butter and traditional butter oil with emphasis on TFAs and conjugated linoleic acid isomers.

MATERIALS AND METHODS

Reagents and standards

The reagents and solvents used were of analytical or high performance liquid chromatography (HPLC) grade, supplied by Sigma-Aldrich (Buchs, Switzerland). The fatty acid methyl esters (FAMEs) were identified by comparing their retention times with those of *cis* and *trans* fatty acid standards: butanoic acid (4:0), dodecanoic acid (12:0), tetradecanoic acid (14:0), hexadecanoic acid (16:0), octadecanoic acid (18:0), *trans*-9-octadecenoic acid (18:1n-9), *cis*-9-octadecenoic acid (18:1n-9), *trans*-11-octadecenoic acid (18:1n-7), *trans*-octadecadienoic acid isomers: *trans*-18:2n-6 (t9,t12; t9,c12; c9,t12), *cis*-9,*cis*-12-pctadecadienoic acid (18:2n-6), *cis*-9,*trans*-11-pctadecadienoic acid (18:2 c9,t11 CLA), *trans*-10,*cis*-12-octadecadienoic acid (18:2 t10,c12 CLA), *cis*-6,*cis*-9,*cis*-12-octadecatrienoic acid (18:3n-3), eicosanoic acid (20:0), docosanoic acid (22:0), cis-13-docosenoic acid (22:1n-9), 5,8,11,14-eicosa-tetraenoic acid (20:4n-6), tetracosanoic acid (24:0) were obtained from Merck (Darmstadt, Germany) and Supelco (Bellefonte, PA, USA).

Traditional butter and traditional butter oil preparation

Milk fresh samples were obtained from a local farm in the South area of Tunisia. Each sample of pooled milk (8 to 10 L) was taken daily from the same herd of ewes for eight weeks (March and April 2010). Butter and butter oil samples were prepared by traditional method. Raw milk was left at room temperature until coagulation for at least 20 h. After fermentation of the milk, the obtained yoghourt product is called ‘‘rayeb’’. Following vigorous shaking (churning) for at least 1 h, the fat-rich fraction called “traditional butter” is separated from an aqueous fraction (protein, lactose and mineral). The obtained butter is salted (~5% w/w). After heating the salted butter for 30 min with intermitted agitation until all water has boiled off, the upper phase was manually removed with a spatula and the remained-dehydrated phase is called traditional butter oil (Figure 1). This natural process avoids preservation of butter oil without refrigeration.

Lipid extraction

The total lipids of the samples were isolated and purified based on the method of Folch (1957). Briefly, the samples (1 g) were weighed

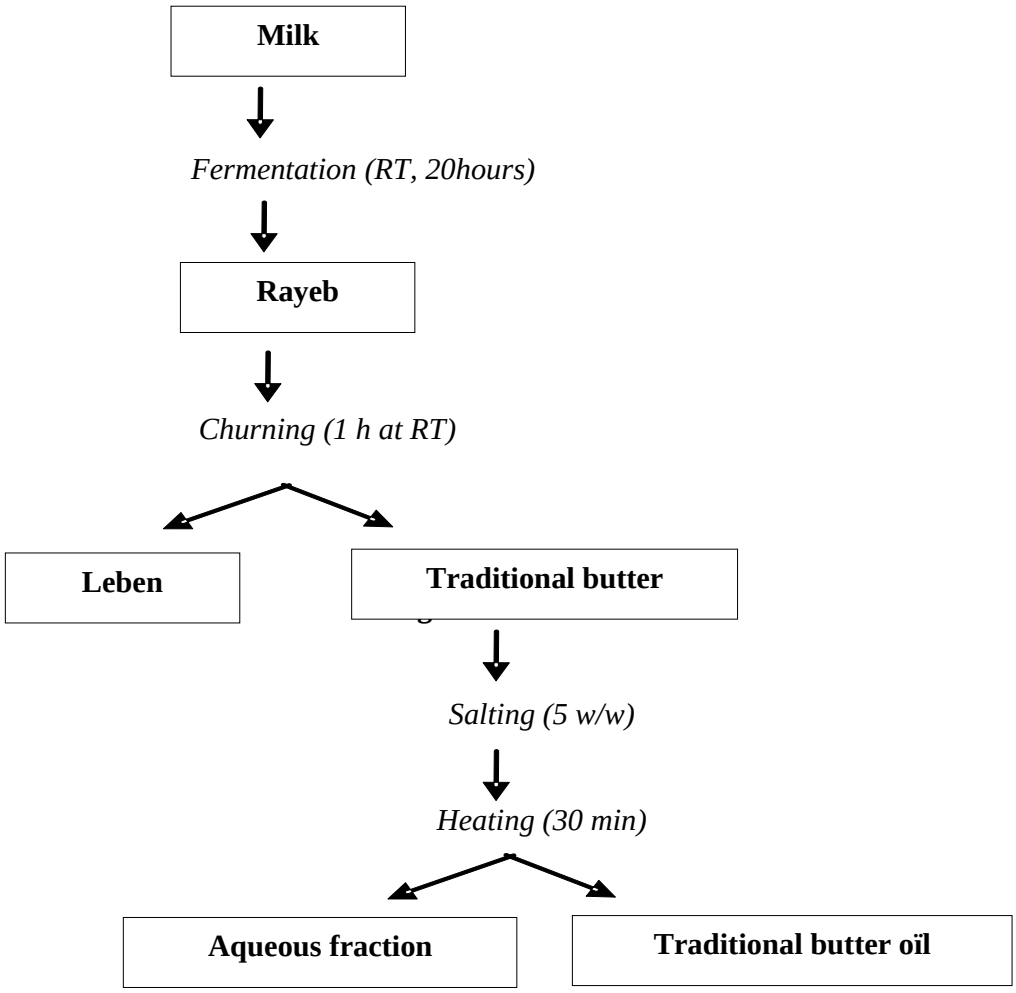

Figure 1. Schematic diagram of traditional method for Tunisian butter oil preparation.

in a 50 ml extraction tube prior to lipid extraction using chloroform/methanol (2:1, v/v) at a final dilution of 20 fold. Following filtration and washing of the extract with KCl (0.88%, w/v) in distilled water (2 ml) and centrifugation, the supernatant was discarded and the organic phase was separated and evaporated to dryness under a gentle stream of nitrogen.

Fatty acid methyl esters (FAMEs) analysis

The process of converting fatty acids into fatty acids methyl esters (FAMEs) is referred to a *trans*-esterification with a methanolic solution of potassium. FAMEs analysis was carried out according to EEC 2568/91 on a Hewlett-Packard gas chromatograph model 5890 Series II instrument (Hewlett-Packard, Ca Palo Alto, Calif.), 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 J&W DB-23 capillary column (60 m length, 0.32 mm i.d., and 0.25 µM film thickness). Experimental conditions were as follows: injector temperature of 270°C, flame ionization detector at 280°C, nitrogen carrier gas at 1 ml/min, injector split ratio of 1:50, the initial column temperature was 130°C; step 1: 6.5°C/min to 170°C; step 2: 2.8°C/min to 215°C, maintained for 12 min; step 3: 40°C/min to 230°C and maintained for 20 min. FAMEs were identified by comparing their relative retention times with those of authentic *cis* and *trans* fatty acid standards.. The FA composition was reported as a relative percentage of the total peak area using a HP Chemstation integrator.

Statistical analysis

All analyses were carried out in triplicate. The results are reported as mean values of three replicates ± standard deviation. Student's test was performed to discriminate among means values of individual *cis* and *trans* fatty acids of the studied samples using the SPSS program, release 11.0 for Windows (SPSS, Chicago, IL, USA). The data provided by gas chromatography (GC) analysis were subjected to principal component analysis (PCA) using the XLSTAT pro 7.5.2 (Addinsoft, France).

RESULTS AND DISCUSSION

Saturated and *cis*-unsaturated fatty acids

All FAs present in the lipid fraction of traditional butter (TB) were also detected in traditional butter oil (TBO).

However, there were several remarkable differences. Table 1 gives a list of saturated fatty acids (SFA) in TB and TBO from 4 to 22 carbons with their trivial names and shorthand designations. The major FA was palmitic acid (C16:0), followed by myristic acid (C14:0). These results are in agreement with other studies (Samet-Bali et al., 2009). Both TB and TBO contained a high level of SFA (71.41 vs. 67.44%, respectively) with significant high content of this fraction in TB ($p < 0.05$). Özkanli and Kaya (2005) had found a lower SFA level (59.13%) in butter produced from ewes' milk (69.10%). Moreover, Sağdiç et al. (2004) reported that the percentages of SFA were 73.88 and 69.10% in butter made from goats' and ewes' milk respectively. The majors FAs in TBO samples were C14:0, C16:0, C18:0 and C18:1. These results are in agreement with the results of previous studies (Al-Khalifah and Al-Kahtani, 1993; Iskander et al., 1985; Özkanli and Kaya, 2005; Sawaya et al., 1984).

SFA are classified according to their chain length into short (C4 to C6), medium (C8 to C12), and long (C16 to C22) chain SFA. Medium-chain fatty acids (MCFAs) are markedly different from long-chain fatty acids (LCFAs) with regard to physical properties, digestion/absorption, biodegradation, and body fat accumulation (Takeuchi et al., 2008). Medium-chain triacylglycerol (MCT) are rapidly digested, absorbed and suppresses body-fat accumulation. MCT are completely hydrolyzed to FA and glycerol by pancreatic lipase, and rapidly absorbed. However, MCT has disadvantages: low smoking point and foaming in deep frying (Takeuchi et al., 2008). LCFAs in esterified form increase the rigidity of membranes. As shown in Table 1, the FA composition of TB presents a relatively high content of short-chain fatty acids (SCFAs), C4:0 to C6:0 (2.58%) compared to that of TBO samples ($p < 0.001$). Considerable amounts of MCFAs are detected in TBO (13.23%) that were significantly ($p < 0.001$) lower than those in TB (17.55%). However, TB and TBO present significant ($p < 0.001$) different amounts of LCFAs (51.12 *vs.* 52.42% respectively) (Table 1). Hence, TBO preparation engenders a significant decrease of SCFAs and MCFAs. According to these results, the decrease of total SFA was due to SCFAs and MCFAs decline and not to LCFAs variation.

Considering the total unsaturated fatty acid (UFAs) contents (%), as displayed in Table 2, the total amounts of UFAs in TB and TBO were found to be 23.88 and 22.76%, respectively ($p < 0.05$). In TBO, the monounsaturated fatty acid (MUFA) levels were significantly lower than in TB samples ($p < 0.01$). Total MUFA contents in TB and TBO were determined as 22.96 and 22.17%, respectively. The predominant MUFA in TB and TBO was oleic acid with significant difference (16.53 vs. 14.61%, respectively; $p < 0.01$). For polyunsaturated fatty acids (PUFA), there were relatively low levels of the two essential FA, linoleic acid and α-linolenic acid (Table 2). Linoleic acid 18:2 (c9, c12) showed a steady increase in TB. Variation in the FA composition between TB and TBO could be explained by several reactions as: enzymatic interconversion, oxidation, isomerization, elongation, and desaturation during the storage and the processing of butter for preparing butter oil. This is in agreement with Glew et al. (2006) who indicated that endogenous milk enzymes and microbial processes could have inter-converted FA by oxidation, desaturation and elongation or hydrogenation reactions.

Geometric and positional fatty acids isomers

Trans fatty acid isomers

Figure 2 illustrates the proportions of TFA fractions in TB and TBO. The total TFAs in TBO represent more than twice that of TB (8.23 *vs*. 3.85%, respectively $p < 0.01$). Statistical analysis using student's test showed significant increase of *trans* MUFA ($p < 0.001$) as well as of *trans* PUFA contents ($p < 0.01$). As shown by the partial chromatograms (Figure 3), the peaks belonging to *trans*-18:1 and *trans*- 18:2 were observed in both TB and TBO, with significant difference. Compared to TB samples, there was an increase by 1.81- and 2.9-fold of *trans* MUFAs and *trans* PUFAs levels, respectively for TBO (Table 3). As illustrated in the chromatograms in Figure 3 (panel A and B), *trans*-18:1 n-7 (vaccenic acid) was the major *trans* isomer in TB and TBO, with more important peak for TBO samples. This isomer represents 1.86 and 3.54 % of the total FAs in TB and TBO, respectively (Table 3).

As reported by Glew et al. (2006) and Micha and Mozaffarian (2008), the predominant TFA in ruminant milk and dairy products is vaccenic acid (VA). Compared to TB, VA was increased by 100% for TBO ($p < 0.001$). These results are in agreement with those of Glew et al. (2006) who reported that butter oil contained a large amount of VA than butter. The high level of VA in butter oil in comparison with butter can be explained by the fact that this isomer was probably produced during the processing at high temperature during the preparation. For PUFAs isomers, only the linoleic acid is concerned by geometric and positional isomerization. As shown in Figure 3, three individual isomers of *trans* linoleic acid were eluted before linoleic acid [18*:2* n-6 (c9, c12)] in the following order: 18:2 (t9, t12), 18:2 (t9, c12) and 18:2 (c9, t12). The level of total *trans* linoleic acid isomers was 1.84-fold higher in butter oil than in butter (Table 3). This is in accordance with our previous study on the effect of processing on *trans* isomerization reactions of linoleic acid by either domestic or industrial treatment of sesame seed by-products (Dhibi et al., 2010). Sağdiç et al. (2004) have reported that *trans* unsaturated fatty acid (UFA) contents (%) as 18:2 n-6 (t9, t12) of the ewe's butter were about 0.26 % ± 0.01, which seemed to be very low when compared to our results for TB and TBO. *Trans*-6-9-12 linolenic acid (C18:3) isomer content was non-detectable

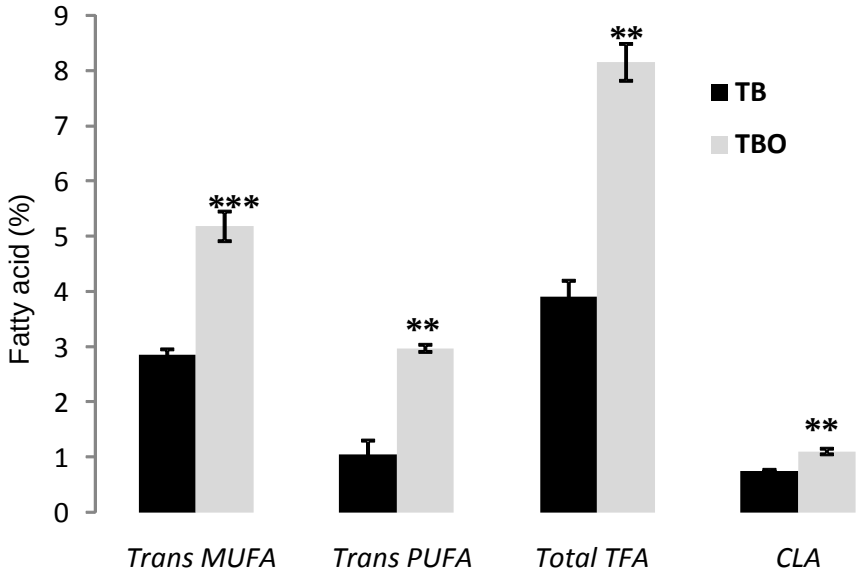

Figure 2. *Trans* fatty acid (TFA) and conjugated linoleic acid (CLA) isomers in Tunisian traditional butter and traditional butter oil. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

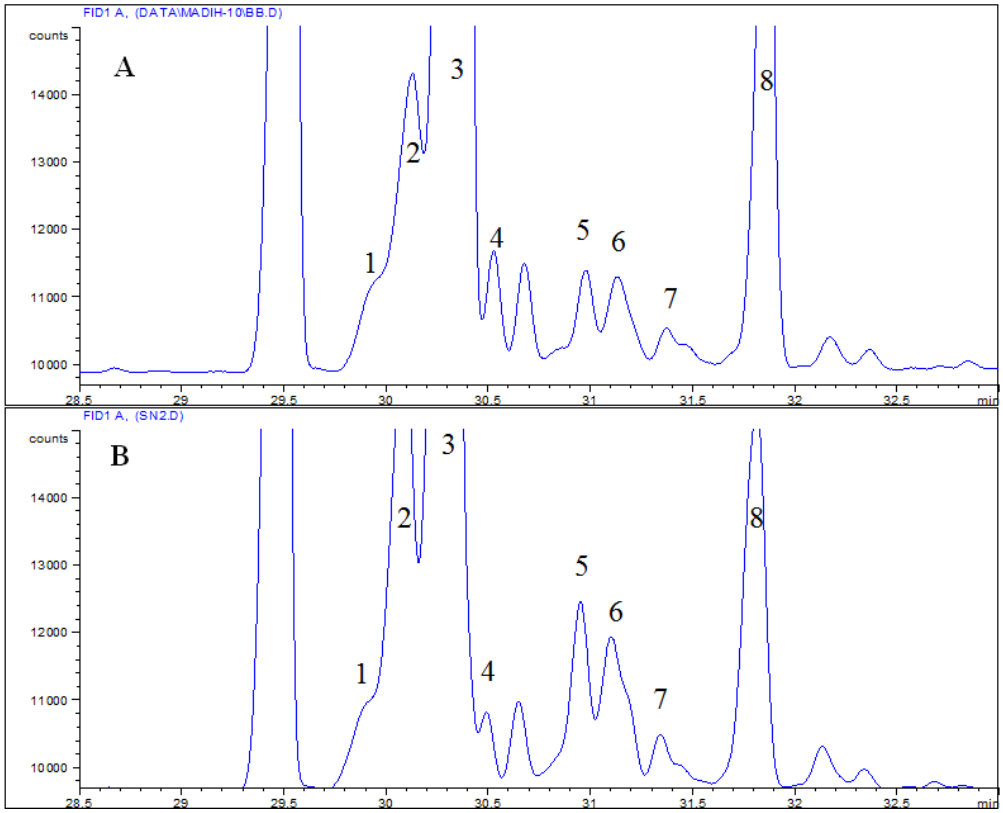

Figure 3. Partial chromatograms of 18:1 and 18:2 *cis* and *trans* isomers in TB (panel A) and TBO (panel B). **1**, *Trans*-18:1 n-9; **2,** t*rans*-18 :1 n-7; **3,** c*is*-18:1 n-9; **4,** cis-18 :1 n-7; **5,** 18:2 (t9, t12); **6,** 18:2 (t9, c12); **7,** 18:2 (c9, t12); **8,** 18:2 (c9, c12).

in both TB and TBO.

Certainly, TBO contains process-induced TFAs with considerable amounts exceeding the naturally-occurred TFAs that are derived from TB, except for elaidic acid

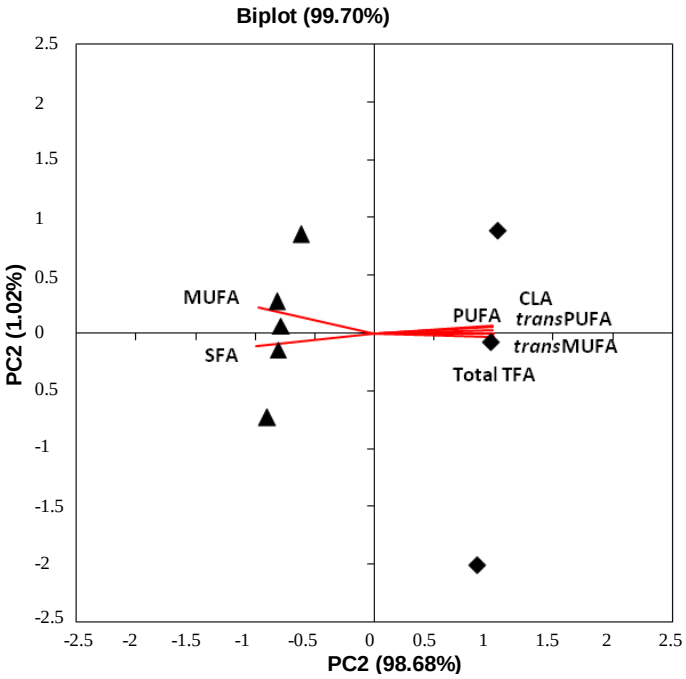

Figure 4. Biplot of samples and variable loads obtained by principal components analysis (PCA) of the fatty acid (FA) fractions of traditional butter (TB) and traditional butter oil (TBO). FA isomers to the right in the plot indicate those that are more represented in TBO, while FA isomers to the left in the plot indicate those that are present in TB. The variance explained by each principal component axis is shown in parentheses (▲: TB; ♦: TBO).

and VA (Table 3). This raises the question about the consumption of this by-product. Chardigny et al. (2008) reported that TFAs from industrially produced and from natural sources have different effects on CHD risk factors in women. The two main industrially produced *trans*-isomers, 18:1t-and 18:2t fatty acids have well documented adverse effects on health (Katan et al., 1995; Mozaffarian et al., 2006). Brouwer et al. (2010) suggested that all such FAs with a double bond in the *trans* configuration raise LDL and decrease HDL cholesterol. The authors reinforce the widespread advice to reduce intake of ruminant fats, because these are also the major source of SFA in affluent diets. Foods that are rich in TFAs or SFAs are associated with an increased risk of cardiovascular disease and diabetes (Mozaffarian et al., 2006). Other studies considered that the lack of evidence for a harmful effect of ruminant produced-TFA intake, and the fact that these FA cannot be removed from ruminant fat and have been part of human food in millennia, places the burden of proving that these fats are more harmful than saturated fats (Stender et al., 2008).

Conjugated linoleic acid (CLA)

Kay et al. (2004) reported that the enzymatic insertion of a *cis*-9 double bond into VA results in synthesis of rumenic acid. Approximately, 19% of dietary VA apparently is converted to rumenic acid in human tissues (Turpeinen et al., 2002). As illustrated in Figure 3, rumenic acid, the unique conjugated dienoic isomer of linoleic acid in TB appears to have been significantly increased during the preparation of TBO ($p < 0.01$). The contribution that CLA made to the total FA was increased 1.5-fold by the butter oil preparation process (Table 3). As previously reported by Dhibi et al. (2010), processing under high temperature induces positional isomerization and increases the CLA amount in heated sesame seed oil. Hence, we can confirm the occurrence of process-induced CLA under heat treatment (Table 3). As known, CLA presents biological, physiological and nutritional properties, which are very interesting for consumer health (Parodi, 1997; Molkentin, 1999). Glew et al. (2006) reported that rumenic acid in butter oil may have anti-inflammatory, antioxidant or anti-cancer effects in those individuals whose diet contains substantial quantities of this dairy product. However, it remains unclear if the process-induced CLA causes adverse effect on health or not.

Chemometric analysis

To confirm the statistical difference between TB and TBO FA distribution, a discriminative analysis was conducted. As can be seen in Figure 4 for the principal component

analysis (PCA) of the present data, principal component 1 (PC1) explained 98.68% of the overall variance. However, PC2 accounted for only 1.02% of the variance; this explained 99.7% of the total variance. It is clear that TBO samples are located at the positive side of PC1, while TB samples are located at the negative side of PC1. PCA provides a clear discrimination between the TB and TBO samples base on SFA, MUFA, PUFA, *trans* MUFA, *trans* PUFA and CLA. PC1 was heavily weighted by geometric and positional FA isomers. Therefore, these results prove that the traditional method for TBO preparation engenders a new distribution of FA fractions with an increase of geometric and positional isomers.

Conclusion

The present results on FA isomers in traditional butter oil and traditional butter offer an important contribution to the literature about changes in FA composition induced by preparation process. Both TB and TBO are characterized by the relatively low level of monounsaturated and polyunsaturated FAs and by the high levels of saturated fatty acid (SFA). TBO lipids are not only rich in SFA since it is derived from ruminant, but also contain considerable levels of TFAs and CLA isomers. The major TFA isomers in each of these FAs classes are vaccenic acid and rumenic acid for TB and TBO. The main finding of this study was that TBO contains large amounts of TFAs and CLA isomers of both natural and processing origins. Nevertheless, it could present biological, physiological and nutritional properties due to the important CLA and VA amounts. Meanwhile, this assumption might be substantiated by scientific data from experimental studies.

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