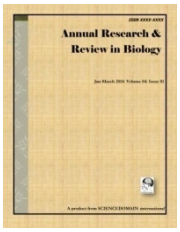

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Exploring Margarine in Anhydrous Milk Fat by Chromatographic Tools

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Authors' contributions

This work was carried out in collaboration between all authors. Author RF designed the study, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Authors JH, SAD, MN and SHP supervised the project *and provided* the analytical tools*. Author SAR performed the statistical analysis. Author MA helped with analysis and revision of the paper. All authors read and approved the final manuscript.*

Original Research Article

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ABSTRACT

Aims: The analysis of 10 anhydrous milk fat (AMF) samples randomly purchased from Tabriz area was carried out to determine the level of adulteration.
Place and Duration of Study: Department of Agriculture (Food Science Labs), Department of Medicine (Central Lab), between April 2010 and October 2011.
Methodology: For adulteration confirmation of the AMF Samples, admixtures of milk fat (MF) with different levels (5, 10 and 15% w/w) of margarine were prepared and analyzed. The authentication was performed employing GC with FID detector and RP-HPLC using fluorescence detector for fatty acid (FA) and tocopherol profiling, respectively.
Results: In market samples, abnormalities in fatty acid profile e.g. significantly high concentration of linoleic acid and low concentration of myristic acid were observed. In addition to high levels of total tocopherol, tocopherols of plant origin like β+γ-tocopherol

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and β+γ-tocotrienol and a compound X with ambiguous identity were also found in tocopherol profile of abnormal samples.
Conclusion: Thus, AMF adulterations arising from admixing with vegetable oils and fats could be successfully detected by applying HPLC for tocopherol profiling.

Keywords: Adulteration; Anhydrous milk fat; Fatty acid; GC, RP-HPLC; Tocopherol.

1. INTRODUCTION

Anhydrous milk fat (called roghan zard in Iran) is a fatty product exclusively made from milk, cream or butter of various animal species by removal of water and nonfat solids [1, 2, and 3]. Anhydrous milk fat is mainly composed of complex mixture of glycerides and a small amount of free fatty acids, phospholipids, sterols and their esters, fat soluble vitamins, carotenoids, carbonyl compounds, hydrocarbons, casein, moisture and traces of elements like copper and iron [4]. A substantial portion of AMF is utilized for cooking and frying of different foods. In deep-frying, it has the privilege of superior flavor and oxidative stability [5].

From a financial point of view, fat is the most important constituent of milk. Substituting more expensive ingredients by inexpensive ingredients (partially) or removal of valuable component of food products (partially) for maximizing profit margin is a common practice which results in food quality deterioration and misbranding, economical loss, legal repercussion and above all health concerns [6].

Due to availability and lower cost, adulteration of MF with vegetable fats and oils has been a usual fraud in countries with few legal controls on food quality or poor monitoring. The assessment of the identity of milk fat (MF) has been made by some classic parameters such as the iodine value (IV) and refractive index (RI), reichert-miessel value (RM), polenske value (PV) and in many cases these are not enough to detect more elaborated frauds because the analytical constants of AMF cover a very wide range, permitting fairly high degree of adulteration while still keeping the constants within normal limits. Those vegetable oils/fats whose analytical constants are close to AMF cannot be detected visually and are preferred for adulteration. Phytosterol acetate test based on the structural differences between phytosterols and animal sterols has also been used [7,8]. Traditional analytical methods of authentication have relied on determination of the amount of marker compound(s) in a suspect material and a subsequent comparison of the value(s) obtained with those established for equivalent material of known origin [6]. Strategies to detect adulterated MF based either on the concentration ranges of individual FA or on the concentration ratios of two or more FA have been also developed [8,9], Although the FA and triacylglycerols (TAG) composition of pure non-milk fats may differ considerably from that of MF, the addition of smaller percentages of foreign fats is difficult to detect due to the variation in MF composition caused by feeding, season or breed [10,11,and12]. Alternatively, the purity of MF can be controlled by GC analysis of TAG based on separation by carbon number. In combination with TAG formulae, the procedure allows the detection and quantification of non-milk fats in bovine milk fat [11,13,14,15]. Being tedious and time-consuming, this approach is less practical for the oil industry and for many research and development programs and limitations have to be considered with technologically processed MF, fat from low-fat milk products such as skim milk or buttermilk, as well as with milk fat from other species than cows [13-16]. Other methods suitable for the detection of adulteration of MF are based on the analysis of minor lipid constituents e.g, diglycerides,

sterols [8,12,17,18,19,20] and steradienes (dehydration products of sterols) [21] and tocopherols [9,20]. The published papers concerning tocol profile of adulterated MFs described the presence of tocopherols, but not the presence of tocotrienols, probably as a consequence of employed procedures. As due to widespread import of palm oil and fat into Iran, it is more probable that AMF can be adulterated with palm oil or fat that is rich in tocotrienols [22,23,24]. Moreover, there is scarce literature regarding the authentication of AMF.

The objective of the present study was to investigate the application of various analytical methods for authentication of AMF.

2. MATERIALS AND METHODS

2.1 Standards and Reagents

Glycerol, NaOH, sulphuric acid, absolute ethanol, sodium thiosulfate, chloroform, hanus solution (analytical grade), acetonitrile (HPLC grade), methanol and BF3 (boron trifluoride) (GC grade) were purchased from Merck (Germany). Pumic stone from Riedel-de Haën (Seelze, Germany), potassium iodide from Applichem (Bio chemical GmbH, Germany) and n-hexane from Scharlau (Spain) were acquired. α-tocopherol standard compound was purchased from Sigma–Aldrich.

2.2 Samples

Commercially available anhydrous milk fat samples were purchased randomly in various retail stores in the Tabriz area in April 2010 and analysis expiration date was in October 2011.

Pure butter from Tabrizlaban (Tabriz, Iran) and margarine from Keshtosanate Shomal Co. with brand name Letka (Amol, Iran) were purchased. Then, MF was extracted from pure butter sample. Three levels (5%, 10% and 15% W/W) of margarine were added to MF.

2.3 Methods

2.3.1 Physicochemical properties

Refractive index, iodine, reichert-meissl and polenske values were analyzed according to standard methods of AOAC [25].

2.3.2 Determination of fatty acid composition

Fat was converted to fatty acid methyl esters according to Savage and McNeil [26]. The ester mixture was analyzed according to Azadmard-Damirchi and Dutta with a slight modification [27]. An YL 6100 GC Young Lin [Anyang, Korea] with flame ionization detector was used. The system had a TR-CN100 Teknokroma capillary column (60 m long, 0.25 mm internal diameter and 0.2 µm film thickness, Barcelona, Spain). Compound identification was carried out using fatty acid methyl esters and reference data [28,29,30]. Helium was the carrier gas and flow was 1ml/min. The oven temperature program was held at 80ºC for 2 mins, increasing by 10ºC/min to 210ºC, and then holding for 25 mins. Split ratio was 100:1 and the detector temperature was 230ºC.

2.3.3 Determination of tocol profile

100 mg of samples dissolved in 10 ml n-hexane were sonicated (Starsonic 35, Bologna, Italy) and centrifuged in 4000 rpm. Then 20 µL aliquot of prepared solutions were injected. Tocopherols were quantified by reversed-phase high-performance liquid chromatography (RP-HPLC). The HPLC system consist of a KNAUERs 1000 HPLC pump, a Eurospher 100-5 C8 KNAUER column (250 × 4.6, 5 µm, KNAUER Inc., Germany) and a KNAUERs fluorescence detector (RF-10XL) (Berlin, Germany). The column was eluted in isocratic mode with a mixture of acetonitrile/water (97:3 V/V) and the flow rate was 1.0 mL/min. Peaks were detected fluoroimetrically (λex: 295nm, λem: 320nm) and using software class for data storing and total control of the system [31]. Compounds were identified on the basis of retention time comparison with standard solution and literature data [32, 33, 34, 35, 36, 37].

* All determinations were done in triplicate.

2.4 Statistical Analysis

The statistical analysis was performed by one-way ANOVA, followed by Duncan's test. The results were expressed as the mean ± SD to show variations in a group. Differences were considered significant when $P < 0.05$.

3. RESULTS AND DISCUSSIONS

3.1 Physicochemical Constants

The addition of margarine to the MF in the ratios of 5, 10 and 15% significantly ($P < 0.05$) affected the reichert-meissl (R-M) and polenske values of the resultant admixtures. However, the presence of margarine in MF up to 15% did not affect iodine value and refractive index significantly. Since all these values were still in normal range of milk fat in all samples, detection of margarine at 5 and 10 and 15% levels in MF by determination of the reichert-Meissl, polenske, iodine values and refractive index was not possible.

The various physico-chemical measures of 10 cow AMF samples randomly obtained from market are given in Table 1. The average RI for samples ranged from 1.4536-1.4561 which was within the standard limits for MF (1.453-1.457). Increase in degree of unsaturation of fat and length of hydrocarbon chain [38] and autoxidation could cause an increase in RI [39].

The IV ranging from 27.7-44.43 indicates that there were variable differences in IV of AMF samples. Moreover, IV of AMF samples, except for sample no 3 (44.43), were within the limits of the Iranian standard prescribed for AMF [41]. According to Ganguli and Jain AMF is not highly unsaturated, as is evident from its IV of 26 to 38 [5]. Mean value for IV of sheep's AMF reported by Pop was 26.7± 0.5 [3]. Kumar et al. reported the mean IV of cow and buffalo butterfat in the range of 35.16±0.293 and 31.89 ± 0.06, respectively [41]. Dhurvey et al. analyzed IV of cow AMF and found the mean value to be 36.72 ± 0.217 [42]. Obviously, differences in lactation period, feeding, seasonal and geographical variations and processing and storage conditions have impact on iodine value [43,44,45]. Achaya found that IV decreased to varying extents during development of rancidity in cow and buffalo AMFs and the data reported for IV of fresh and rancid AMF by Achaya were in the range of 34.5-39.2 and 22.2- 35.0, respectively [46].

The Reichert-Meissl value of AMF samples were found between 15.9-28.3 and being significantly ($P < 0.05$) low in the samples numbers 3, 6 and 10. In addition, PV of samples ranged from 0.9-2.3 and was significantly ($P < 0.05$) low for 3 samples (2, 3 and 6). Kumar et al. reported the average PV and RM values of cow and buffalo AMF to be 1.60 ± 0.29, 29.45 ± 0.12 and 1.58 ± 0.05, 32.88 ± 0.28, respectively [41]. The Reichert-Meissl number of cow AMF varies from 26 to 29 whereas goat is slightly less. On the other hand, Sheep and buffalo AMF have higher RM numbers of about 32. In general, AMF is required to have a RM number of not less than 28. Polenske value for cow AMF is higher (2 to 3) than buffalos' (1 to 1.5) [5]. Reichert and Polenske values found by Achaya for original cow AMF samples ranged from 23.8-26.2, 1.2-2.1 and for rancid ones the values were 27.9-30.2 and 2.0-3.0, respectively [46].

Table 1. Physicochemical constants of anhydrous milk fat samples acquired from market

AMF Samples	IV	RI	R-M	PV
1	31.0 ± 1.00^{f}	1.4536 ± 0.001^{e}	24.13 ± 0.44^{c}	2.30 ± 0.00^{a}
2	31.8 ± 0.76^{e}	1.4546 ± 0.002^{d}	24.10 ± 0.11^{c}	1.23 ± 0.06^{e}
3	44.4 ± 1.20^{a}	1.4561 ± 0.002^{a}	16.01 ± 0.60^{f}	0.97 ± 0.06^{f}
4	37.3 ± 0.87^{c}	1.4551 ± 0.001^{c}	28.34 ± 0.90^{a}	1.67 ± 0.06^{c}
5	37.3 ± 0.76^{c}	1.4546 ± 0.002^{d}	24.02 ± 0.60^{c}	1.80 ± 0.10^{b}
6	37.4 ± 0.60^{c}	1.4556 ± 0.003^{ab}	17.59 ± 0.79^{e}	1.00 ± 0.00^{f}
7	27.7 ± 0.49^{g}	1.4536 ± 0.002^{e}	25.56 ± 0.32^{b}	2.37 ± 0.11^{a}
8	34.4 ± 0.36^{ed}	1.4546 ± 0.003^{d}	24.27 ± 0.63^{c}	1.50 ± 0.00^{d}
9	35.3 ± 1.04^{d}	1.4556 ± 0.003^{b}	20.28 ± 0.39^{d}	1.63 ± 0.06^{c}
10	39.3 ± 0.70^{b}	1.4561 ± 0.002^{a}	17.53 ± 0.44^{e}	1.60 ± 0.00^{dc}

Values within a column with different letters are significantly different (P < 0.05).

3.2 Fatty Acid Composition

The result of GC analysis of MF and admixtures of MF with margarine showed that by increasing the margarine level in MF, caproic, caprilic, capric, lauric, myristic and stearic acids decreased and palmetic, oleic and linoleic acids increased.

The major fatty acids of 10 AMF samples determined by GC presented in Table 2. The total saturated fatty acid (SFA) of the AMF ranged from 53.71 to 63.19%. According to Mariod et al. the proportion of total SFA of AMF samples was very high (ranged 63.38 – 68.81%), which were higher than SFA of Indian cow AMF (60%) [47]. Mean values of total saturated fatty acids in AMF of indigenous and creamery method preparation irrespective of the species (cow and buffalo) and diets (green fodder and concentrate) observed by Tyagi et al. was 64.67±0.94 and 64.54±0.85, respectively [48]. The major fatty acids of AMF samples were myristic, palmitic, stearic and oleic acids. These results were in agreement with previous studies [2,3,47,49,50,51]. Sample number 3 contained lowest value of caproic, caprylic and capric acid, which only caproic acid content was out of limit required by Iranian standard for AMF. Lauric acid was present at 1.18–4.58% and its content was lower in samples numbers 3, 6, 9, and 10 in comparison with result presented by Hussein et al. [52]. Myristic acid (C14:0) ranged from 5.99–12.91% which was significantly low in samples numbers 3, 6, 9 and 10.

The dominant FA among the unsaturated group was oleic acid and its range varied from 14.02-30.17%. AMF number 3 contained exceptionally high level of oleic acid. Adulteration of MF with common oils expected to increase the linoleic acid content.

Standard MF should contain 1.0–2.4% of linoleic acid and 0.25–1.1% of linolenic acid [8,9]. The linoleic acid was quite high in samples numbers 3, 4, 6, 9 and 10 and out of range of Iranian standard for AMF and Hussein et al. [40,53].

Although the species is major factor contributing to variability in lipid composition of MF, other factors such as feed, seasonal and geographic variation and processing can contribute to further variability [12,20]. Moreover, adulteration of MF using low percentage of vegetable oils with similar composition to MF (e.g. Palm oil) makes standard FA analysis difficult [53].

For increasing the sensitivity of analysis of FAs, the concentration ratios of two FAs were also investigated. Results showed that C18:2/C8:0 ratios were significantly high for samples numbers 3, 6, 9 and 10, while the lowest value determined for C14:0/C18:2 ratios was also observed in the same samples. However, these formulas can only be used to give a rough expression of the percentage of adulteration in mixed MF from large number of cows and various plant oils and fats.

3.3 Tocopherol Profile

Tocopherol is present in MF as well as in vegetable oils but is considerably higher in the latter. Thus, tocopherols and tocotrienols (often called ‘‘tocols’’) could be used as markers for the detection of adulterations in MF. HPLC is currently a widely used technique for determination of tocopherols in foodstuffs. Although several detectors have been used in analysis of these compounds, the more common include the ultra-violet and the fluorescence detectors. With method and instruments used in this study, the ultra-violet detector and normal phase HPLC did not give comparable results. RP-HPLC does not allow the complete resolution of β and γ isomers, and consequently, in RP-HPLC these two vitamers are quantified together [54]. Tocopherol profile of genuine MF and margarine is given in Fig. 1. It is clear that addition of margarine to MF will cause an increase in tocopherol content of resultant mixture.

The tocopherol and tocotrienol composition of 10 AMF samples are given in Table 3 and related chromatograms presented in Fig. 2. The average total tocol content for samples number 2 (123.77 mg kg^{-1}), 3 (325.60 mg kg^{-1}), 6 (509.09 mg kg^{-1}), 9 (323.56 mg kg^{-1}) and 10 (480.39 mg kg^{-1}) were significantly ($P < 0.05$) higher and high above the typical range for MF. The only tocopherol detected for AMF samples numbers 1, 4, 5, 7, 8 was α-tocopherol with amount approaching or slightly exceeding the upper limit for MF [9,55,56 and 57]. Judd et al. reported a figure of 76 mg kg^{-1} for α-tocopherol and 38 mg kg^{-1} for γ-tocopherol in butter [58]. It was not possible to compare our results about tocopherol content in AMF because of lacking data in literature. In sample number 2, β+γ-tocopherol together with α-tocopherol and in samples numbers 6 and 10 all tocol variety except δ-tocopherol were also found. In addition to α-tocopherol, sample number 3 contained β+γ-tocotrienol, δ-tocotrienol and compound X. Tocols observed in sample number 9 were β+γ-tocopherol, β+γ-tocotrienol and compound X.

Table 2. Fatty acid composition of anhydrous milk fat samples collected from market

Fatty acids	Anhydrous milk fat samples									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	10
C6	2.46±0.04^{b}	2.32±0.03^{c}	1.06±0.03^{g}	2.17±0.03^{d}	2.97±0.03^{a}	2.17±0.02^{d}	1.91±0.02^{e}	2.20±0.02^{d}	1.58±0.03^{f}	1.88±0.02^{e}
C8	1.42±0.03^{e}	1.57±0.02^{c}	0.47±0.03^{j}	1.48±0.02^{d}	1.89±0.03^{a}	1.27±0.02^{g}	1.28±0.01^{f}	1.68±0.02^{b}	0.88±0.02^{i}	1.09±0.01^{h}
C10	2.80±0.03^{d}	3.37±0.02^{c}	0.85±0.02^{g}	3.33±0.03^{c}	3.98±0.02^{a}	2.59±0.02^{d}	2.80±0.02^{d}	3.59±0.00^{b}	1.88±0.03^{t}	2.13±0.01^{e}
C12	3.30±0.02^{e}	4.06±0.02^{b}	1.20±0.02^{i}	3.85±0.03^{c}	4.57±0.01^{a}	2.94±0.04^{f}	3.35±0.03^{e}	3.77± 0.01^{d}	2.21±0.02^{h}	2.63±0.03^{g}
C14	11.47±0.03^{c}	12.28±0.01^{b}	6.03±0.04^{l}	11.10±0.02^{d}	12.89±0.02^{a}	9.33±0.03^{t}	10.79±0.01^{e}	11.45±0.03^{c}	8.68±0.02^{g}	7.56±0.02^{h}
C16	29.67±0.02^{t}	28.50±0.03^{h}	35.46±0.03^{a}	25.49±0.02^{i}	30.13±0.03^{e}	33.40±0.01^{c}	29.4±0.02^{g}	30.31±0.04^{e}	34.45±0.03^{b}	31.12±0.02^{d}
C16:1	2.32±0.03^{b}	2.35±0.03^{b}	1.71±0.02^{e}	1.59±0.02^{f}	2.57±0.01^{a}	1.91±0.02^{d}	1.98±0.02^{c}	2.54±0.025^{a}	1.98±0.02^{c}	1.50±0.01^{g}
C18	9.21±0.03^{d}	7.67±0.03^{f}	10.41±0.02^{c}	11.41±0.03^{a}	6.76±0.03^{i}	6.90±0.02^{h}	10.90±0.01^{b}	8.50±0.02^{e}	7.71±0.02^{f}	7.30±0.03^{g}
C18:1 trans	1.55±0.01^{e}	3.05±0.03^{b}	nd^{h}	3.12±0.02^{a}	1.14±0.02^{g}	1.29±0.01^{t}	nd ^{h}	1.75±0.04^{d}	nd ^{h}	2.83±0.04^{c}
C18:1	19.77±0.02^{g}	16.46±0.02^{i}	30.20±0.03^{a}	19.04±0.02^{h}	14.05±0.03^{j}	21.30±0.02^{f}	23.13±0.04^{d}	22.37±0.02^{e}	24.80±0.05^{b}	24.43±0.02^{c}
C18:2	2.09±0.01^{g}	2.03±0.02^{h}	3.88±0.03^{c}	3.65±0.01^{e}	1.27±0.02^{j}	4.63±0.03^{b}	2.38±0.02^{t}	1.97±0.02^{i}	3.82±0.03^{d}	4.68±0.02^{a}
MUFA	23.13±0.06^{t}	21.88±007^{g}	31.91±0.05^{a}	23.75±0.07^{et}	17.78±0.05^{h}	24.06±0. 07^{e}	25.11±0.01^{d}	26.67±0.02^{c}	26.78±0.03^{c}	28.76±0.00^{b}
SFA	60.32±0.03^{c}	59.76±0.20^{c}	55.48±0.01^{t}	56.82±0.05^{e}	63.21±0.06 ^{a}	58.73±0.2^{d}	60.46±0.07^{c}	61.50±0.03^{b}	57.45±0.05^{e}	53.74±0.02^{g}
C18:2/C8	1.47±0.03^{ef}	1.29±0.01^{f}	8.35±0.7^{a}	2.46±0.03^{d}	0.67±0.00^{g}	3.60±0.05^{c}	1.85±0.01^{e}	1.19±0.05^{f}	4.47±0.16^{b}	4.26±0.05^{b}
C14/C18:2	5.48±0.08^{d}	6.04±0.05^{b}	1.55±0.00^{i}	3.04±0.00^{f}	10.13±0.18^{a}	2.01±0.05^{h}	4.51±0.06^{e}	5.81±0.01^{c}	2.27±0.01^{g}	1.61±0.00^{i}

Values within a row with different letters are significantly different (P < 0.05).
Nd= not detected

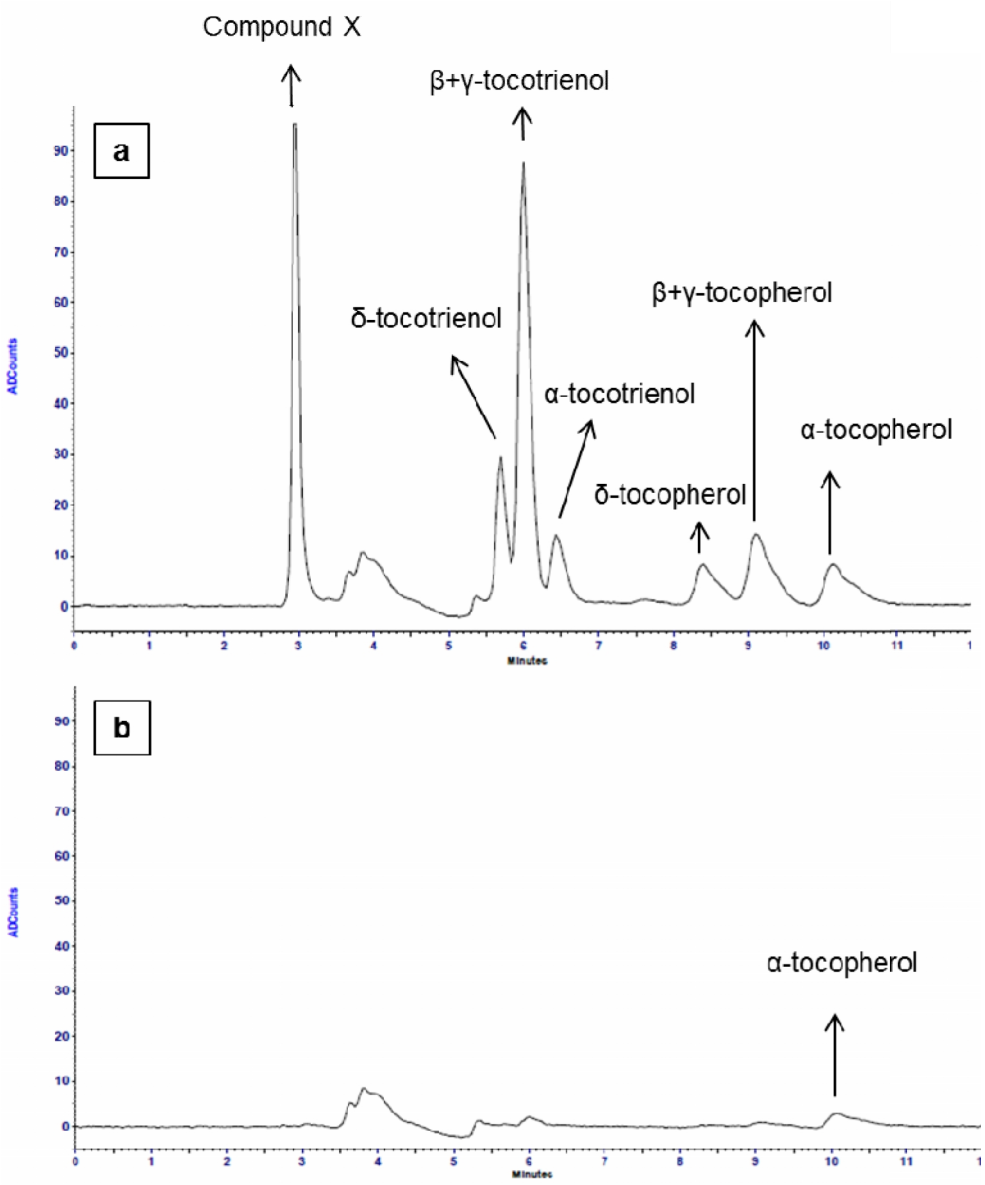

Fig. 1. Tocopherol profiles of margarine (a) and pure MF (b)

Tocopherol content for cow and buffalo AMF reported by Kumar et al. was 31.55 ± 1.109 and 27.95 ± 1.02 mg kg^{-1}, respectively [36]. According to Schwartz et al. butter fat contained 20 mg kg^{-1} α-tocopherol and 1.5 mg kg^{-1} α-tocotrienol [59]. In the MF only naturally occurring tocopherols are α- and γ-tocopherol, however, the proportion of the γ homologue in total tocopherol content can be about 4% [9,60]. According to Sundram et al., AMF (ghee) contained α-tocopherol (32.7 mg kg^{-1}), δ- tocopherol (33.8 mg kg^{-1}) and total tocol of 66.5 mg kg^{-1} [61]. In other study the alpha and gamma tocopherol content in milks from different species collected in summer time in South-eastern Sicily (Italy) analysed and the

predominant isomer detected was α–tocopherol and differences species-specific have been found with a higher content of γ- isomer in milk from goat and buffalo compared to other varieties [62].

Observations suggest that samples numbers 2,3,6,9 and 10 were adulterated by adding vegetable fat. Most oils contain only tocopherols, while palm, palm kernel and rice bran oil contain significant amounts of tocotrienols [61,63,64].

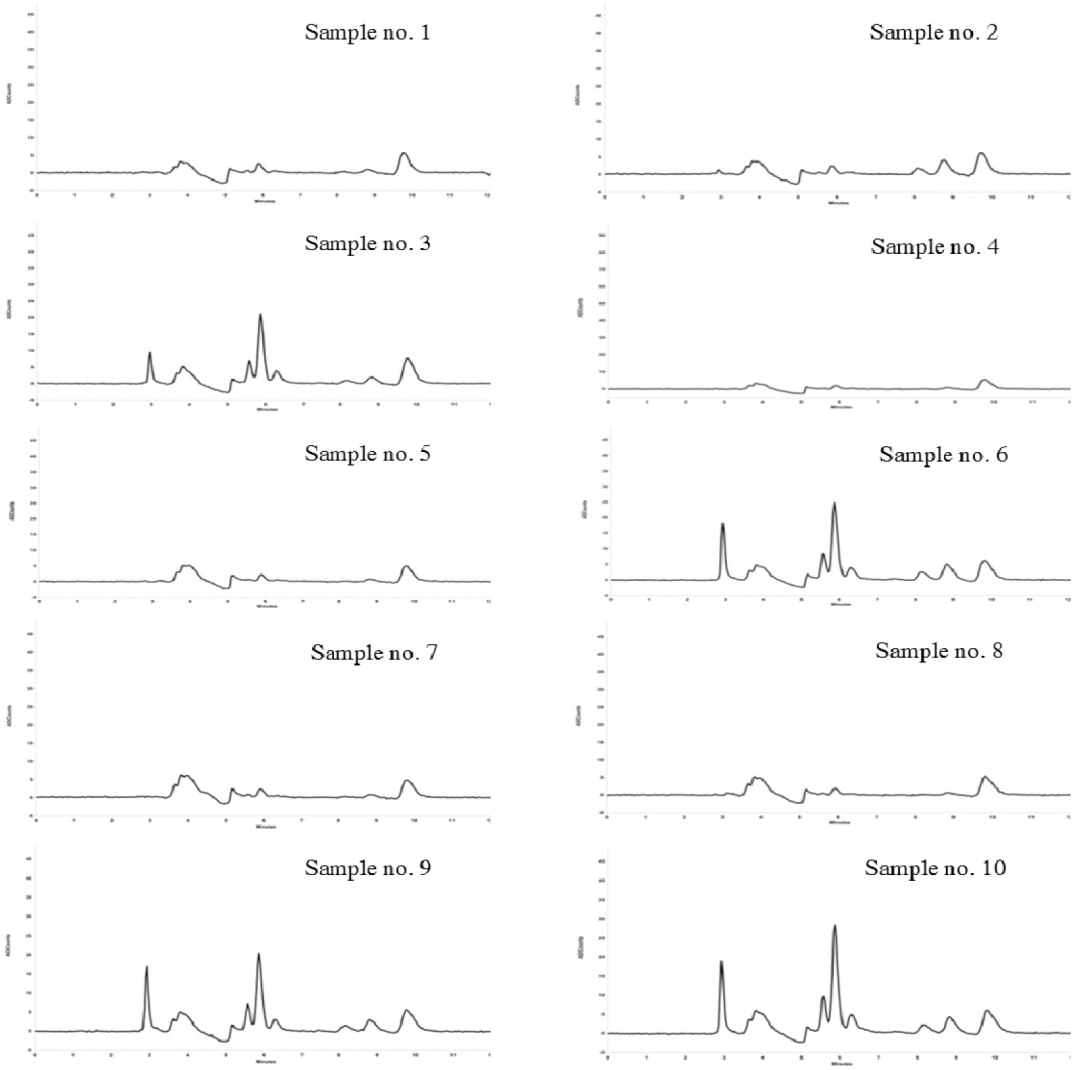

Fig. 2. RP-HPLC chromatograms related to tocopherol profiles of 10 anhydrous milk fat samples

Table 3. Tocopherol profiles of 10 anhydrous milk fat samples determined by RP-HPLC (mg/kg)

AMF Samples	α-tocopherol	β+γ-tocopherol	δ-tocopherol	β+γ-tocotrienol	δ-tocotrienol	Compound x	Total tocol
1	65.43 ± 4.9^{e}	nd^{e}	nd^{c}	nd^{d}	nd^{d}	nd^{e}	65.43 ± 4.9 ^{c}
2	82.47 ± 1.3^{c}	41.30 ± 3.2^{b}	nd^{c}	nd^{d}	nd^{d}	nd^{e}	123.77 ± 2.2 ^{c}
3	98.53 ± 4.5^{a}	nd ^{e}	nd^{c}	185.40 ± 2.6 ^{b}	2.50 ± 0.4^{c}	39.17 ± 3.5^{d}	325.60 ± 6.5^{ab}
4	72.50 ± 0.1^{d}	nd^{e}	nd^{c}	nd^{d}	nd^{d}	nd^{e}	72.50 ± 0.1^{c}
5	58.67 ± 3.7^{f}	nd^{e}	nd^{c}	nd^{d}	nd^{d}	nd^{e}	58.67 ± 3.7^{c}
6	91.20 ± 2.2^{b}	65.33 ± 2.0^{a}	3.60 ± 0.8^{b}	236.93 ± 0.8^{a}	16.70 ± 0.4^{b}	95.33 ± 2.2 ^{a}	509.09 ± 1.7^{a}
7	53.40 ± 0.7 ^{f}	nd^{e}	nd^{c}	nd^{d}	nd^{d}	nd^{e}	53.40 ± 0.7^{c}
8	71.53 ± 1.5^{de}	nd^{e}	nd^{c}	nd^{d}	nd ^{d}	nd^{e}	71.53 ± 0.7^{c}
9	72.93 ± 4.7^{d}	16.26 ± 3.5^{c}	nd^{c}	171.87 ± 3.0^{b}	nd^{d}	62.50 ± 3.8^{c}	323.56 ± 2.1^{b}
10	82.30 ± 5.7^{c}	39.03 ± 3.5^{b}	14.33 ± 2.0^{a}	236.93 ± 3.9^{a}	23.93 ± 4.3^{a}	83.87 ± 7.0 ^{b}	480.39 ± 1.4^{a}

Values within a column with different letters are significantly different (P < 0.05).
Nd= not detected

4. CONCLUSION

Although the wide variation in edible oils from different origins is a limiting factor in the interpretation of data with regard to adulteration of MF, the evaluation of the qualitative and quantitative tocopherol profile by means of HPLC with fluorescence detector is a suitable tool to assess the authenticity of AMF based on the results of present study. For increasing the sensitivity of FA analysis, in addition to linoleic acid content as a marker of MF adulteration, using the concentration ratios of specific FAs (e.g. C18:2/C8:0, C14:0/C18:2) rather than their concentrations as criteria for the naturalness of MFs seem to be more practical.

COMPETING INTERESTS

Authors declare that no competing interests exist with other people or organizations that could inappropriately influence our work.

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