JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# Enantioselective Analysis of 2- and 3-Hydroxy Fatty Acids in Food Samples

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2-Hydroxy fatty acids (2-OH-FAs) and 3-hydroxy fatty acids (3-OH-FAs) were recently identified at trace levels in dairy products and other food samples (vegetable oils and animal brains). Due to the asymmetric carbon bearing the hydroxy group, they are chiral. This study focused on the enantioselective determination of 2- and 3-OH-FAs in food. For this purpose, extracted saponifiable lipids were converted into methyl esters, and the resulting fatty acid methyl esters (FAMEs) were separated into OH-FAMEs (minor fraction) and non-OH-FAMEs (bulk fraction). OH-FAMEs were then derivatized with (*R*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl chloride [(*R*)-(−)-MTPA-Cl, Mosher's reagent] to produce the corresponding MTPA-*O*-FAMEs. MTPA-*O*-FAME diastereomers were then analyzed by gas chromatography with electron capture negative-ion mass spectrometry (GC/ECNI-MS) in the selected ion monitoring (SIM) mode. In the food samples, both (*S*)- and (*R*)-enantiomers of 2- and 3-OH-FAs were detected, with the (*R*)-enantiomer being enantiopure or predominant with one exception. Especially 2- and 3-OH-16:0 were found to contain relevant proportions of the (*S*)-enantiomer. The differences in enantiomeric composition of 2- and 3-OH-16:0 detected for cheese samples were proposed as markers for authenticity controls.

**KEYWORDS: Hydroxy fatty acids; enantioselective analysis; indirect enantiomer determination; GC/ECNI-MS; foodstuff; cheese; brain**

## INTRODUCTION

Chirality is a specific feature of many natural compounds and is often indispensable for their bioactivity. In the field of fatty acids, naturally occurring unusual 2- and 3- (α- and β-) hydroxy fatty acids (2- and 3-OH-FAs), which are chiral due to the asymmetrical carbon bearing the functional group, are of principal interest.

Although 2- and 3-OH-FAs contribute only minute amounts to the lipids of biological samples, they are nevertheless widespread in nature. The 2-OH-FAs occur in various mammalian tissues as well as in yeast, bacteria, and plants; frequently they are important constituents of sphingolipids (*1*−*7*). For instance, 3-OH-FAs are predominantly found in the lipids of microorganisms. They are representative compounds of lipid A, the lipid portion of the lipopolysaccharides (LPS), which are endotoxins located in the outer membrane of Gram-negative bacteria. In this context, 3-OH-FAs are used to estimate the amounts of endotoxins in clinical and environmental samples (*8*−*10*). Another source of 3-OH-FAs is the Gram-positive bacterium *Lactobacillus plantarum*. The antifungal activity of this lactic acid bacterium was linked to four 3-OH-FAs (*11*). In addition, 3-OH-FAs are formed in the course of β-oxidation of fatty acids in mitochondria and/or peroxisomes. Increased concentrations of free 3-OH-FAs are indicative of disorders in fatty acid oxidation (*12, 13*). Recently, it was shown that food presents a permanent source for the uptake of 2- and 3-OH-FAs in the milligram-per-day range (*14*).

Importantly, the enantioselectivity of OH-FAs affects physiological membrane properties. Incorporating the pure enantiomer instead of the corresponding racemate into biological model membranes stabilized the functional, liquid-crystalline state by reducing the phase transition temperature (*15*). Usually, one enantiomeric form of 2- and 3-OH-FAs predominates in biological samples. For instance, 2-OH-FAs in sphingolipids as well as ester-linked 2-OH-FAs in wool wax and plants were reported to be present enantiopure in the (*R*)-form (*16*−*21*), whereas (*S*)-enantiomers dominated in LPS of bacteria (*19, 22, 23*). Microbial 3-OH-FAs were shown to occur predominantly in (*R*)-configuration (*11, 19, 22*−*24*). On the other hand, 3-OH-FAs resulting from β-oxidation were predicted to share the (*S*)-configuration, but these metabolites were found to be almost racemic (*12*).

Compared to the information derived from biological samples, little was known about the enantiomer distribution of 2- and 3-OH-FAs in food. For instance, enantioselective occurrence of 2-OH-FAs was determined in brains (*18*). The lack of a thorough investigation of enantiomeric composition of 2- and 3-OH-FAs in food is possibly based on the high analytical requirements, because only small amounts of 2- and 3-OH-FAs accompanied by complex matrices are expected in foodstuffs.

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10.1021/jf802772a CCC: $40.75 
Published on Web 11/18/2008

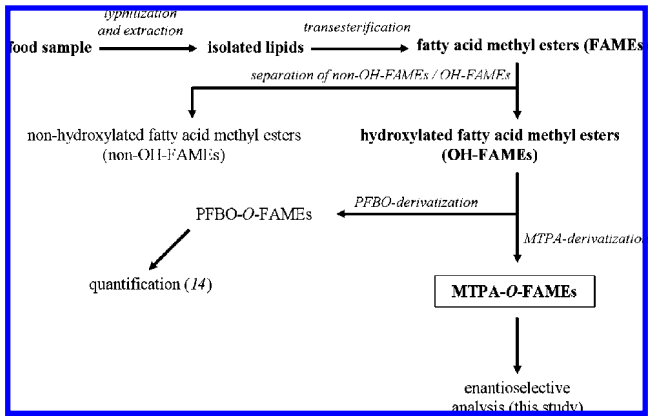


**Figure 1.** Sample preparation scheme for the enantioselective and compound-specific determination of 2- and 3-OH-FAs in food.

The present study was to determine the enantioselectivity of 2- and 3-OH-FAs in food. For this purpose we took advantage of a recently developed method for the quantification of 2- and 3-OH-FAs (*25*). The sample preparation scheme of that study was now combined with a highly sensitive and selective method for the indirect enantioresolution of 2- and 3-OH-FAs (*21*). This method includes the conversion of fatty acids into methyl esters (FAMEs) and the subsequent enantioselective derivatization of OH-FAMEs with Mosher's reagent [(*R*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl chloride ((*R*)-(−)-MTPA-Cl)]. The resulting diastereomeric MTPA-*O*-FAMEs were detected with high sensitivity by gas chromatography/electron capture negative-ion mass spectrometry (GC/ECNI-MS) in the selected ion monitoring (SIM) mode. This method was applied to investigate the enantioselective composition of 2- and 3-OH-FAs in a range of foodstuffs, including milk samples, dairy products, vegetable oils, and brain samples from different animals.

## MATERIALS AND METHODS

**Samples and Chemicals.** The food samples analyzed were bovine milk (Weihenstephan, Freising, Germany), human milk (anonymous donor in Stuttgart, Germany), mare milk (local farm), goat milk (Andecher Creamery, Andechs, Germany), goat cheese (Chevagne Le Berger, Campina, Heilbronn, Germany), goat cream cheese (Zigeli, France), emmental cheese, hand cheese and feta cheese (local market, Stuttgart, Germany), cow feta cheese (Gut and Billig, Aurich, Germany), cow mozzarella (Galbani, Santa Lucia, Italy), buffalo mozzarella (Padania Alimenti, Casalmaggiore, Italy), St. John's wort oil, cedarnut oil, walnut oil, avocado oil (Hees, Stuttgart, Germany), and the brain of pork, bovine, goat, and sheep (from a local farmer).

The 2-OH- and 3-OH-FA standards were purchased from the following commercial sources: 2-hydroxydecanoic acid (2-OH-10:0), 2-hydroxytetradecanoic acid (2-OH-14:0), γ-decalactone, γ-dodecalactone, δ-decalactone, δ-dodecalactone, and δ-tetradecalacton were from Sigma-Aldrich (Steinheim, Germany); 2-hydroxydodecanoic acid (2-OH-12:0), 2-hydroxyhexadecanoic acid (2-OH-16:0), 2-hydroxyoctadecanoic acid (2-OH-18:0), 2-hydroxyeicosanoic acid (2-OH-20:0), 3-hydroxydecanoic acid (3-OH-10:0), 3-hydroxydodecanoic acid (3-OH-12:0), 3-hydroxytetradecanoic acid (3-OH-14:0), 3-hydroxyhexadecanoic acid (3-OH-16:0), and 3-hydroxyoctadecanoic acid (3-OH-18:0) were from Larodan (Malmö, Sweden). The origin and quality of the solvents and chemicals used for sample preparation and derivatization procedure have been presented elsewhere (*21, 25*).

**Sample Preparation and Derivatization.** The preparation of food samples and the MTPA derivatization were carried out as summarized in **Figure 1**. An aliquot of sample solutions prepared for the quantification of 2- and 3-OH-FAs (*14*) was used for their simultaneous enantioselective identification. The food samples except vegetable oils were lyophilized, and lipids were obtained by means of an accelerated solvent extractor (ASE 200, Dionex, Idstein, Germany) using the azeotropic mixture of ethyl acetate/cyclohexane (54:46, v/v) as the solvent (*26*). Subsamples of the extracted lipid phases were transesterified according to the $BF_3$ method C-VI 11d of the German Society for Fat Science (*27*). After separation of non-OH-FAMEs, which dominated in the samples, the OH-FAMEs were derivatized with Mosher's reagent (*21*). After conversion, the corresponding MTPA-*O*-FAMEs (**Figure 2**) were subjected to enantioselective analyses by gas chromatography with electron capture negative-ion mass spectrometry.

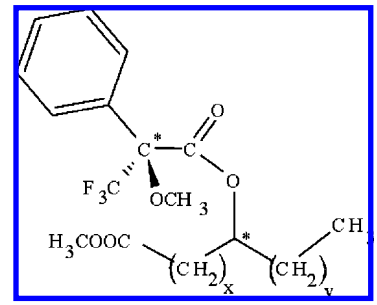


**Figure 2.** General structure of (*R*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) derivatives of 2-OH-FA ($x = 0$ and $y = 7-17$) and 3-OH-FA ($x = 1$ and $y = 6-14$) methyl esters.

**Gas Chromatography with Electron Capture Negative-Ion Mass Spectrometry (GC/ECNI-MS).** A CP-3800 gas chromatograph coupled to a 1200 mass spectrometer (Varian, Darmstadt, Germany) was used with parameters described in detail by Jenske and Vetter (*21*). GC analyses were performed with a Factor Four VF-5 ms column (30 m, 0.25 mm i.d., 0.25 μm $d_f$; Varian). The oven temperature program started at 60 °C (hold time, 1.5 min) and was then raised at 40 °C/min to 180 °C (hold time, 2 min), at 2 °C/min to 220 °C (hold time, 35 min), and, finally, at 10 °C/min to 300 °C (hold time, 5.5 min). The total run time was 75 min. Mass spectra of both 2- and 3-MTPA-*O*-FAMEs exhibit $[M - 31]^-$ as characteristic fragment ion, which was used as an analysis ion that specifies the molecular weight of each 2- and 3-OH-FA (*21*). In addition, the $^{13}C$ isotopic peak was used for verification. The respective ions were determined in the selected ion monitoring (SIM) mode using the following six time windows: *m/z* 387/388 (2- and 3-MTPA-*O*-10:0-ME) was measured from 5 to 22 min, *m/z* 415/416 (2- and 3-MTPA-*O*-12:0-ME) from 22 to 29 min, *m/z* 443/444 (2- and 3-MTPA-*O*-14:0-ME) from 29 to 40 min, *m/z* 471/472 (2- and 3-MTPA-*O*-16:0-ME) from 40 to 55 min, *m/z* 499/500 (2-MTPA-*O*-18:0-ME) from 55 to 67 min, and *m/z* 527/528 (2-MTPA-*O*-20:0-ME) from 67 to 75 min (end of the analysis).

## RESULTS AND DISCUSSION

**Quality of the Method.** Low concentrations of 2- and 3-OH-FAs in foodstuffs such as milk, dairy products, and brains (*14*) required a selective and sensitive method of determination. For this purpose, we merged two previous methods based on (i) the selective enrichment of OH-FAs by separation of nonhydroxylated FAs (*25*) and (ii) GC/ECNI-MS-SIM determination of OH-FA diastereomers obtained after derivatization with Mosher's reagent (*21*) (**Figure 1**). All samples were previously analyzed for OH-FA quantitites (*14*).

Whereas the (*S*)-enantiomer was eluted first in either case, MTPA derivatives (**Figure 2**) of 2-OH-FAs were better resolved than 3-OH homologues (*21*). Compared to our previous study, improvement of the oven temperature (see Materials and Methods) led to chiral resolutions ($R_S$) of 1.0 or higher for all compounds studied (*21*). However, the second-eluting 2-MTPA-O-FAME diastereomer was not fully resolved from the first-eluting 3-MTPA-O-FAME diastereomer. Yet, the enantioselective determination of all target compounds could be determined without interference in one GC run (**Table 1**). Individual MTPA derivatives of lactones (4- and 5-OH-FAMEs, see Materials and

**Table 1.** Gas Chromatographic Data[a] of 2- and 3-Hydroxy Fatty Acid Methyl Esters as Diastereomeric *O*-Terminated MTPA Derivatives

| | $t_R$ (*S*)[b] (min) | $t_R$ (*R*)[c] (min) | $R_S$[d] |
|---|---|---|---|
| **2-OH-** | | | |
| 10:0 | 18.26 | 18.91 | 6.00 |
| 12:0 | 24.52 | 25.24 | 5.72 |
| 14:0 | 32.25 | 33.34 | 5.47 |
| 16:0 | 45.65 | 47.56 | 5.10 |
| 18:0 | 64.93 | 65.60 | 4.34 |
| 20:0 | 69.00 | 69.25 | 2.78 |
| **3-OH-** | | | |
| 10:0 | 19.11 | 19.31 | 1.55 |
| 12:0 | 25.38 | 25.57 | 1.54 |
| 14:0 | 33.51 | 33.80 | 1.39 |
| 16:0 | 47.85 | 48.31 | 1.20 |
| 18:0 | 65.73 | 65.86 | 1.00 |

[a] Capillary column, VF-5 ms (30 m, 0.25 mm i.d., 0.25 $\mu$m $d_f$); GC oven temperature program, 60 °C (hold time, 1.5 min), which then was raised at 40 °C/min to 180 °C (hold time, 2 min), at 2 °C/min to 220 °C (hold time, 35 min), and, finally, at 10 °C/min to 300 °C (hold time, 5.5 min). [b] Retention time of the respective (*S*)-enantiomer. [c] Retention time of the respective (*R*)-enantiomer. [d] Chiral resolution.

**Table 2.** Enantioselective Identification of 2-OH-FAs in Food Samples

| | EF[a] (%) | | | | |
|---|---|---|---|---|---|
| ME/MTPA derivative | 2-OH-10:0 | 2-OH-12:0 | 2-OH-14:0 | 2-OH-16:0 | 2-OH-18:0 |
| **milk samples** | | | | | |
| bovine | nd[b] | nd | 100 | 50 | nd |
| human | 100 | 100 | nd | 50 | nd |
| mare | 76 | 68 | 67 | 67 | nd |
| goat | 67 | 67 | nd | 50 | nd |
| **cheese samples** | | | | | |
| emmental | nd | nd | nd | 50 | nd |
| goat | 68 | nd | nd | 50 | nd |
| goat cream | nd | nd | nd | 50 | nd |
| hand | 84 | nd | nd | 83 | nd |
| cow mozzarella | nd | nd | nd | 50 | nd |
| buffalo mozzarella | nd | nd | nd | 50 | nd |
| cow feta | nd | nd | nd | 50 | nd |
| feta | nd | nd | nd | 83 | nd |
| **brain samples** | | | | | |
| pork | nd | nd | nd | 62 | 62 |
| bovine[c] | nd | nd | nd | 71 | 72 |
| goat[c] | nd | nd | nd | 68 | 71 |
| sheep[c] | nd | nd | nd | 68 | 71 |
| **vegetable oils** | | | | | |
| St. John's wort | 56 | nd | nd | 100 | nd |
| avocado | nd | nd | nd | 78 | nd |
| cedarnut | nd | nd | nd | 100 | nd |
| walnut | nd | nd | nd | 77 | nd |

[a] Enantiomer fraction of (*R*)-enantiomer. [b] Not detectable (limits of detection: 2-OH-10:0, 0.07 mg/100 g; 2-OH-12:0, 0.10 mg/100 g; 2-OH-14:0, 0.20 mg/100 g; 2-OH-16:0, 0.25 mg/100 g; 2-OH-18:0, 0.40 mg/100 g; 2-OH-20:0, 0.42 mg/100 g); 2-OH-20:0 was not detected in any sample. [c] BSE specified risk material, must be destroyed during slaughter in the European Union.

Methods) were prepared to verify that they did not interfere with the target compounds.

The enantiomeric composition was expressed as the percentage proportion of the (*R*)-enantiomers (*28*), which is related to the enantiomer fraction [EF; (*R*/*R*+*S*)]. This turned out to be more suitable for describing enantiomer signatures than enantiomer ratios (*R*/*S*) (*29*).

**Enantiomeric Composition of 2-OH-FAs in Food Samples.** In the different food samples, 2-OH-FAs were identified predominantly with higher proportions of the second-eluting (*R*)-enantiomers (**Table 2**). However, the EF could not be determined for all 2-OH-FAs quantified before (*14*). MTPA derivatives (three fluorine substituents supporting electron capture)

**Table 3.** Enantioselective Identification of 3-OH-FAs in Food Samples

| | EF[a] (%) | | | |
|---|---|---|---|---|
| ME/MTPA derivative | 3-OH-10:0 | 3-OH-12:0 | 3-OH-14:0 | 3-OH-16:0 |
| **milk samples** | | | | |
| bovine | 100 | 100 | 100 | 50 |
| human | 100 | 100 | 100 | 50 |
| mare | 100 | 100 | 100 | 55 |
| goat | 100 | 100 | 100 | 76 |
| **cheese samples** | | | | |
| emmental | 100 | 100 | 100 | 50 |
| goat | 100 | 85 | 100 | 75 |
| goat cream | 100 | 77 | 100 | 56 |
| hand | 100 | 100 | 100 | 50 |
| cow mozzarella | 100 | 100 | 100 | 50 |
| buffalo mozzarella | 100 | 100 | 100 | 34 |
| cow feta | 100 | 100 | 100 | 55 |
| feta | 100 | 100 | 100 | 75 |
| **brain samples** | | | | |
| pork | nd[b] | nd | nd | 50 |
| bovine[c] | nd | nd | nd | 50 |
| goat[c] | nd | nd | nd | 50 |
| sheep[c] | nd | nd | nd | 50 |
| **vegetable oils** | | | | |
| St. John's wort | 58 | 100 | 100 | 50 |
| avocado | 100 | 100 | nd | nd |
| walnut | nd | nd | 100 | nd |

[a] Enantiomer fraction of (*R*)-enantiomer. [b] Not detectable (limits of detection: 3-OH-10:0, 0.02 mg/100 g; 3-OH-12:0, 0.03 mg/100 g; 3-OH-14:0, 0.04 mg/100 g; 3-OH-16:0, 0.07 mg/100 g; 3-OH-18:0, 0.09 mg/100 g); 3-OH-18:0 was not detected in any sample. [c] BSE specified risk material, must be destroyed during slaughter in the European Union.

gave lower responses and thus higher detection limits compared to PFBO derivatives (five fluorine substituents). Irrespective from that, 2-OH-10:0, 2-OH-12:0, and 2-OH-14:0 in bovine and human milk as well as 2-OH-16:0 in St. John's wort oil and cedarnut oil were found to be enantiopure (*R*)-enantiomers in either case [EF (%) = 100]. In contrast, most of the milk and cheese samples contained both enantiomers of 2-OH-16:0, frequently in an almost racemic composition [i.e., EF (%) = 50]. No difference was found between the EF of 2-OH-16:0 (racemic composition) in goat milk, goat cheese, and goat cream cheese and that in bovine milk and sweet milk cheeses made from it (**Table 2**). In the curdled milk cheese (hand cheese) the EF of (*R*)-2-OH-16:0 was 83%. In addition, feta cheese made from sheep milk also showed an EF of 83%, whereas cow feta cheese possessed equal amounts of both enantiomers [EF (%) = 50]. Future research should be directed to the verification of this result in higher sample numbers because the EF of 2-OH-16:0 may be used as a marker in authenticity control. Unfortunately, only very few additional data could be obtained for other 2-OH-FA homologues. Generally, the (*R*)-enantiomer was enantioenriched and amounted to 56−100% (**Table 2**). Enantiopurity is a rather common feature in nature, and the presence of both enantiomers of 2-OH-FAs in milk and diary products may be due to different sources for (*S*)- and (*R*)-enantiomers. For instance, 2-OH-FAs are known to contribute to the fatty acid pattern in sphingolipids of milk fat (*30*).

Whereas Karlsson and Pascher (*17*) as well as Tatsumi et al. (*18*) detected only (*R*)-enantiomers of 2-OH-FAs in sphingolipids of brain and yeast, (*S*)-2-OH-FAs were identified in some LPSs of bacteria (*22, 23*). However, both (*S*)- and (*R*)-enantiomers were also found to coexist in environmental samples (*19*). These results suggest that different enzymes or at least enzymes with different enantioselectivities must be involved in the formation of 2-OH-FAs in biota. Whereas the nature of these enzymes could not be determined, it is the merit of the

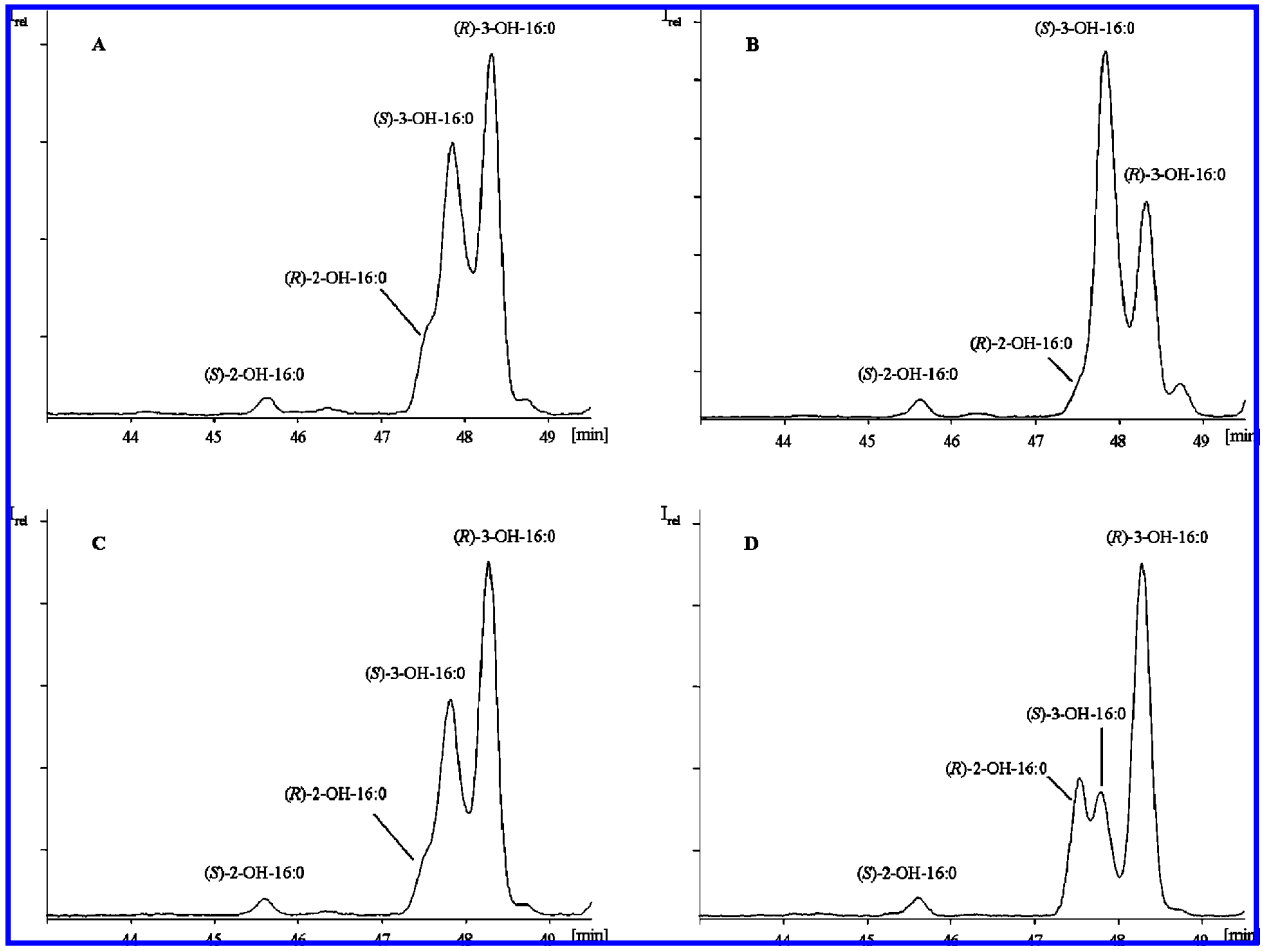


**Figure 3.** Partial GC/ECNI-MS-SIM chromatogram of (*R*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) derivatives of 2- and 3-OH-16:0 methyl esters in (**A**) cow mozzarella, (**B**) buffalo mozzarella, (**C**) cow feta cheese, and (**D**) feta cheese (for enantiomer fractions, see **Tables 2** and **3**).

enantioselective analysis to identify such potential differences in their formation.

Because of the previous reports of 2-OH-FAs in animal brains (*4, 5, 7*), we also analyzed this tissue from different species. Due to the bovine spongiform encephalopathy (BSE) crisis, brain tissues from cow, goat, and sheep were classified as specified risk materials by the European Union (EU) in 2000. Thus, these previous food items must be separated during slaughtering and destroyed so that they do not reach consumers. Nevertheless, the identification of BSE risk material in food is a very important task in risk assessment studies. In this context, bovine brain-originating 2-OH-FAs have been suggested as marker compounds for quality controls of meat products (*4, 5, 7*). In addition, brains of different animals in all kinds of preparation serve as food delicacies in numerous countries all over the world such as the United States, India, Indonesia, and also in the EU.

Unexpectedly, both enantiomers of 2-OH-16:0 and 2-OH-18:0 were detected in brain samples with an EF of the (*R*)-enantiomer of 62−72% (**Table 2**). No considerable differences were determined in brain samples from different species (**Table 2**). Noticeable as well, the EF values of 2-OH-16:0 and 2-OH-18:0 were virtually identical in all brain samples. These results were surprising because other studies reported the exclusive presence of the (*R*)-enantiomers in bovine brain and calf brain (*17, 18*). The same was found in samples from plants in which only (*R*)-enantiomers had been detected (*2, 16*). The EF of the 2-OH-FAs in brain was probably altered during food processing. The (*R*)-2-OH-16:0 also dominated in vegetable oils, whereas it was found to be enantiopure in St. John's wort oil and cedarnut oil. However, in walnut oil and avocado oil it was accompanied with (*S*)-2-OH-16:0. The (*S*)-enantiomers identified in brains and vegetable oils possibly resulted from bacterial sources.

**Enantiomeric Composition of 3-OH-FAs in Food Samples.** The EF of four even-chained 3-OH-FAs could be detected in all milk samples and dairy products (*14*). As was observed for 2-OH-FAs, (*R*)-3-OH-FAs dominated in all but one food sample (**Table 3**). With few exceptions, enantiopure (*R*)-3-OH-FAs with chain lengths from $C_{10}$ to $C_{14}$ were detected in the samples. Whereas (*S*)-3-OH-10:0 was (only) determined in St. John's wort oil, small proportions of (*S*)-3-OH-12:0 (15−23% of total 3-OH-12:0) were found in cheeses made from goat milk (**Table 3**). Thus, the EF in the goat cheeses deviated from the one determined in goat milk, where only (*R*)-3-OH-12:0 was determined.

Again, noticeable variations in the EF were detected only for 3-OH-16:0. Both 3-OH-16:0 enantiomers were detected in all samples. In the brain samples, St. John's wort oil, and some milk and cheese samples, (*S*)- and (*R*)-enantiomers of 3-OH-16:0 were on one level (i.e., racemic). Goat milk contained a proportion of 76% of (*R*)-3-OH-16:0. The same enantiomer distribution of 3-OH-16:0 was also detected in goat cheese, whereas goat cream cheese contained a lower proportion of (*R*)-3-OH-16:0 (i.e., only 56%). In bovine milk, 3-OH-16:0 was racemic [EF (%) = 50], and this result was also determined for curdled milk cheese (hand cheese), a sweet milk cheese made from raw bovine milk (emmental cheese), and for pasteurized bovine milk (cow mozzarella). The only exception was cow

feta cheese, which contained 55% of 3-OH-16:0 in the form of the (*R*)-enantiomer. Feta cheese produced from sheep milk showed an enantiomer proportion of 3-OH-16:0 similar to goat cheese (**Table 3**). Particularly noticeable was the proportion of only 34% of (*R*)-3-OH-16:0 in buffalo mozzarella. This was the only sample with an excess of the (*S*)-enantiomer of any 2- or 3-OH-FAs in the food samples (**Tables 2** and **3**). By contrast, cow mozzarella contained racemic 3-OH-16:0.

The enantiomeric composition of 3-OH-FAs determined in this study was rather unexpected because bacteria were expected to be the key sources for 3-OH-FAs, which in turn were thus far known to be exclusively (*R*)-configured (*11, 22*−*24*). The enantiopure identification of (*R*)-3-OH-10:0, (*R*)-3-OH-12:0, and (*R*)-3-OH-14:0 is in agreement with bacteria as the source. However, (*S*)-3-OH-16:0 was detected in all food samples. Notably, Gradowska and Larsson (*19*) also identified both (*R*)- and (*S*)-3-OH-FAs in dust samples without being able to explain the origin of the (*S*)-3-OH-FAs. Until now, the existence of (*S*)-enantiomers was found only in conjunction with the $\beta$-oxidation of fatty acids. Further studies are needed to evaluate the possible origin of (*S*)-enantiomers in food samples. As already found for 2-OH-FAs, the results from this study indicate that different enzymes or at least different enantioselectivities of enzymes are involved in the formation of 3-OH-FAs in different food sources.

**Potential for Using Enantiomer Fractions (EF) of 2- and 3-OH-FAs in Authenticity Control.** Recently, different amounts and fingerprints of 3-OH-FAs were reported in buffalo mozzarella and cow mozzarella as well as feta cheese and cow feta cheese (*14*). Concentrations of individual 3-OH-FAs were slightly higher in cow feta cheese than in traditional feta. In mozzarella from bovine milk, the concentration of 3-OH-FAs was ~4-fold higher than in buffalo mozzarella. The 3-OH-10:0 was even about 40-fold more concentrated in cow mozzarella. Consequently, specific 3-OH-FAs were proposed as tracers for authenticity controls (*14*). The enantiomeric compositions of 2- and 3-OH-FAs may probably serve as further markers for the authenticity of buffalo mozzarella or feta cheese.

Mozzarella is an Italian cheese made from either buffalo or bovine milk. The original mozzarella is made from buffalo milk in southern Italian regions. In 1996, this "mozzarella di bufala campana" received a protected designation of origin (PDO) status from the European Union (registry no. 1107/96). In the samples analyzed, the EF values of 3-OH-16:0 were different for buffalo mozzarella and cow mozzarella (**Figure 3A**,**B**). For verification, it should be tested if other authentic buffalo mozzarella samples also contain an excess of (*S*)-3-OH-16:0 and if samples made from bovine milk generally do not. In this case, the EF of 3-OH-16:0 could be used as a tracer for the authenticity of both buffalo milk and mozzarella.

On the other hand, feta initially was a brined curd cheese traditionally made in Greece from sheep or goat milk. In recent decades, this sheep cheese has enjoyed increasing popularity in many European countries. As a result, brined cheeses from other European regions were sold as "feta", although they were mostly made from bovine milk, which is cheaper. On this account the EU specified (registry no. 1829/2002) that the term "feta" is reserved for cheese matured in brine that is made exclusively from sheep (70% or more) or goat milk in Greece. The PDO legislation valid within the EU for "feta" came into force in 2002. To control the authenticity of feta cheese, again the EF of OH-FAs might be used as a tracer (**Figure 3C**,**D**). A higher excess of (*R*)-3-OH-16:0 may verify the presence of sheep milk. Furthermore, in cow feta cheese 2-OH-16:0 was composed of equal ratios of (*S*)- and (*R*)-enantiomers, whereas the original feta cheese possessed an excess of (*R*)-2-OH-16:0 (**Table 2**). However, further investigations including the analysis of a larger sample size have to be carried out to verify this proposal of authenticity control. The method used in this study is suggested for this purpose because it allows for the sensitive and selective enantioselective analysis of 2- and 3-OH-FAs in food.

## LITERATURE CITED


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**Received for review September 8, 2008. Revised manuscript received November 2, 2008. Accepted November 2, 2008.**

JF802772A