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ARTICLE

Characterization and Quantification of Odor-Active Compounds in Unsaturated Fatty Acid/Conjugated Linoleic Acid (UFA/CLA)-Enriched Butter and in Conventional Butter during Storage and Induced Oxidation

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Dairy products enriched in unsaturated fatty acids (UFA) and conjugated linoleic acids (CLA) have a higher nutritional value and are suggested to have beneficial health effects. However, such acids are susceptible to oxidation, and off-flavors may be formed during storage. This study was aimed to compare the most important odorants in UFA/CLA-enriched butter to that of conventional butter during storage and induced oxidation. Volatiles were isolated by solvent-assisted flavor evaporation and identified by gas chromatography−olfactometry and mass spectrometry. Aroma extract dilution analysis revealed 18 odorants that were quantified by stable isotope dilution analysis. Another important odorant, 3-methyl-1*H*-indole (mothball-like odor), was quantified by high-performance liquid chromatography. After storage, UFA/CLA-enriched butter showed higher concentrations of pentanal (fatty), heptanal (green), butanoic acid (cheesy), and δ-decalactone (peach-like). Photooxidation of butter samples induced increases in heptanal, (*E*)-2-octenal, and *trans*-4,5-epoxy-(*E*)-2-decenal, especially in conventional butter. The higher vitamin content in UFA/CLA samples may protect this butter from oxidation.

KEYWORDS: Butter; aroma; unsaturated fatty acids; conjugated linoleic acids (CLA); oxidation; stable isotope dilution assay

INTRODUCTION

Consumers are demanding foods that combine a pleasant flavor with improved nutritional value and benefits on human health. Dairy products enriched with unsaturated fatty acids (UFA) and, in particular, conjugated linoleic acids (CLA) offer these advantages, due to a higher content in essential fatty acids and due to potential anticarcinogenic, cholesterol-lowering, and body fat reducing effects (*1*).

Several studies have focused on increasing the amount of UFA/CLA in dairy products by supplementing the ruminant's diet with oils or seeds rich in oleic, linoleic, and linolenic acid (*2*,*3*). For example, Collomb et al. (*3*) showed that a higher CLA content in milk fat is obtained by supplementing the cow's diet with sunflower seeds.

The higher UFA/CLA content may, however, negatively affect the flavor of dairy products, because unsaturated lipids are more susceptible to autoxidation (*4*). The effects of UFA oxidation on the flavor of butter have been first comprehensively described by Badings (*5*), who indicated the formation of several odor-active volatiles during cold storage of butter, such as hexanal (green odor), heptanal (oily), (*E*)-2-nonenal (tallowy), (*E*,*E*)-2,4-heptadienal (metallic, deep-fried), and (*E*,*Z*)-2,6-nonadienal (cucumber-like). These compounds were suggested to be formed by autoxidation of arachidonic, linoleic, and linolenic acid, respectively. Widder et al. (*6*) identified nine odor-active compounds by application of an aroma extract dilution analysis (*7*), including (*Z*)-3-hexenal (green, apple-like), 1-octen-3-one (mushroom-like), (*Z*)-1,5-octadien-3-one (metallic, geranium-like), and (*Z*)- and (*E*)-2-nonenal (fatty, green) as being responsible for off-flavors in butter oil stored for 42 days at room temperature. Off-flavors in butter and generally in dairy products may also be caused by photoinduced lipid oxidation, due to the presence of photosensitizers, such as riboflavin (*8*). For example, light exposure provoked a strong metallic off-flavor in butter oil, due in particular to *trans*-4,5-epoxy-(*E*)-2-decenal, originating from linoleic acid (*9*).

So far, the studies investigating the flavor of UFA/CLA-enriched dairy products versus conventional products have been mostly based on sensory tests (*10*−*12*). No differences in flavor were observed between CLA-enriched milk and butter samples and the respective conventional products (*10*), whereas with respect to cheese, panelists indicated a stronger Cheddar cheese flavor in the CLA-enriched samples after 6 months of ripening (*10*). Jones et al. (*11*) observed no significant flavor differences between CLA-enriched butter and the control. On the other hand, a sensory study on milk showed that CLA-fortified milk

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was less acceptable than milk without CLA addition due to its "grassy/vegetable oil" flavor (*13*).

Our previous work (*14*) employed, in addition to sensory analysis, gas chromatography−mass spectrometry−olfactometry (GC-MS-O) in combination with solid-phase microextraction (SPME) to identify the odor-active compounds in UFA/CLA-enriched butter compared to conventional butter during 8 weeks of storage at 6 °C. Although the sensory analysis found significant differences only in the creamy aroma, which was stronger in UFA/CLA-enriched butter, GC-O was a more sensitive method to detect differences in odor compounds between the two samples. After storage, acids, such as propanoic, butanoic, and 3-methylbutanoic acids, and aldehydes, including pentanal, hexanal, heptanal, and nonanal, had significantly increased in UFA/CLA-enriched butter.

The present study was carried out to complete the characterization of the odor-active compounds of UFA/CLA-enriched butter in comparison to conventional butter, using another aroma extraction technique, that is, solvent-assisted flavor evaporation (SAFE), to identify the potent odorants and to quantify them in fresh and stored samples. Stable isotope dilution assays (SIDA) and mass chromatography were used to quantify the major aroma compounds. In addition, to evaluate the oxidative stability of UFA/CLA-enriched butter compared to conventional butter, important odor-active compounds were identified and quantified after induced oxidation by light exposure as well as in the dark under an oxygen atmosphere.

EXPERIMENTAL PROCEDURES

Chemicals. Diethyl ether, sodium carbonate, anhydrous sodium sulfate, sodium chloride, methanol, potassium phosphate, and anhydrous potassium hydrogenphosphate were obtained from Merck (Darmstadt, Germany). Acetonitrile was supplied from Romil Pure Chemistry (Cambridge, U.K.). Demineralized water was obtained using a Millipore (Schwalbach, Germany) system.

The compounds listed with numbers in **Table 1** were either obtained from commercial sources or synthesized according to published procedures: pentanal (**1**), hexanal (**2**), heptanal (**3**), (*E*)-2-octenal (**4**), (*E*)-2-nonenal (**5**), (*E*,*Z*)-2,6-nonadienal (**6**), decanal (**7**), methional (**8**), δ-octalactone (**9**), δ-decalactone (**10**), δ-dodecalactone (**11**), 1-octen-3-one (**12**), butanoic acid (**13**), and hexanoic acid (**14**) were from Aldrich (Steinheim, Germany). Nonanal (**15**) was from Acros Organics (Geel, Belgium) and (*Z*)-3-hexenal (**16**) from SAFC (Hamburg, Germany). (*Z*)-2-Nonenal (**17**) and *trans*-4,5-epoxy-(*E*)-2-decenal (**18**) were synthesized according to methods in ref *15*, respectively. 2-Methyl-1*H*-indole and 3-methyl-1*H*-indole (skatole) were from Aldrich (Buchs, Switzerland).

The labeled internal standards were synthesized at the Deutsche Forschungsanstalt für Lebensmittelchemie according to the procedures published in the reference given in parentheses: [5,6-$^2H_2$]-hexanal [**2-d** (*16*)], [2,3-$^2H_2$]-(*E*)-2-octenal [**4-d** (*16*)], [2,3-$^2H_2$]-(*E*)-2-nonenal [**5-d** (*17*)], [2,3-$^2H_2$]-(*E*,*Z*)-2,6-nonadienal [**6-d** (*17*)], [5,6-$^2H_2$]-decanal [**7-d** (*18*)], [$^2H_3$]-methional ([$^2H_3$]-3-methylthiopropanal) [**8-d** (*19*)], [8,9-$^2H_2$]-δ-octalactone [**9-d** (*20*)], [10,11-$^2H_2$]-δ-decalactone [**10-d** (*21*)], [3,4-$^2H_2$]-1-octen-3-one [**12-d** (*17*)], [3,4-$^2H_2$]-butanoic acid [**13-d** (*21*)], [5,6-$^2H_2$]-hexanoic acid [**14-d** (*16*)], [5,5,6,6-$^2H_4$]-nonanal [**15-d** (*22*)], [3,4-$^2H_2$]-(*Z*)-3-hexenal [**16-d** (*17*)], [7,7,8,8-$^2H_4$]-*trans*-4,5-epoxy-(*E*)-2-decenal [**18-d** (*17*)]. [12,-13-$^2H_2$]-δ-Dodecalactone was synthesized following a procedure published for [$^2H_2$]-δ-decalactone (*21*).

Butter Samples. Both UFA/CLA-enriched butter and conventional butter were produced at the ALP pilot plant in September 2007. UFA/CLA-enriched butter was obtained from Holstein cows ($n = 5$) fed pasture and sunflower seeds during 2 weeks. The cows had a similar stage of lactation and produced milk with similar contents of UFA/CLA. Control cows ($n = 5$) were fed a conventional diet, composed of pasture and corn silage (*14*). Milk was collected separately from the two groups (150 L each), preheated at 45 °C, and centrifuged to obtain the cream (35% fat). The butter-making process was performed as described previously (*14*). Five kilograms of sweet cream butter was produced from each kind of milk in pieces of 100 g (50 pieces for each type of milk), with the following dimensions: length, 12 cm; width, 6 cm; thickness, 2 cm. Part of the butter, wrapped in aluminum foil, was immediately stored deep frozen at −20 °C and used as a reference. Thirty samples (for each butter type) wrapped in aluminum foil were stored in the dark at 6 ± 1 °C and analyzed fresh (within 1 week) and after 6 weeks of storage.

Table 1. Selected Ions (*m*/*z*) of Analytes and Isotopically Labeled Standards (IST) Used in the Stable Isotope Dilution Assays

no.	odorant	analyte[a] *m*/*z*	IST	IST[a] *m*/*z*	MS response factor
1	pentanal	87	**2-d**	103	0.74
2	hexanal	101	**2-d**	103	1.02
3	heptanal	115	**2-d**	103	1.27
4	(*E*)-2-octenal	127	**4-d**	129	0.88
5	(*E*)-2-nonenal	141	**5-d**	143	0.68
6	(*E*,*Z*)-2,6-nonadienal	139	**6-d**	141	0.80
7	decanal	157	**7-d**	161	0.93
8	methional	105	**8-d**	108	0.50
9	δ -octalactone	143	**9-d**	145	1.06
10	δ-decalactone	171	**10-d**	173	0.68
11	δ-dodecalactone	199	**11-d**	201	0.42
12	1-octen-3-one	127	**12-d**	129	0.55
13	butanoic acid	89	**13-d**	91	0.90
14	hexanoic acid	117	**14-d**	121	0.70
15	nonanal	143	**15-d**	147	0.87
16	(*Z*)-3-hexenal	81	**16-d**	83	0.75
17	(*Z*)-2-nonenal	141	**5-d**	143	0.68
18	*trans*-4,5-epoxy-(*E*)-2-decenal	139	**18-d**	143	1.60

[a] Ion measured in single ion monitoring (SIM), in the chemical ionization (CI) mode.

Oxidation of Butter Samples. Butter samples (six for each type of butter) were placed on a horizontal plate at the distance of 20 cm from a "cool white" fluorescent light (Philips TL40W/33RS, emission spectrum 300−700 nm, 2000 lx) (*23*) at 6 °C for 6 and 12 h, respectively. The samples were turned every 2 h to allow uniform light exposure at the butter surface. In another experiment, the samples (6 for each type of butter) were placed in a desiccator and exposed to a continuous flow of pure oxygen (50 mL/min) for 6 and 12 h, respectively, at 6 °C in the dark.

Standard Analytical Procedures. Moisture, nonfat solids, and fat contents were determined according to methods of ref *24*. Retinol and α-tocopherol were quantified using external standards of retinol and α-tocopherol (*14*). Copper and iron were determined by graphite furnace atomic absorption spectroscopy (*14*). The fatty acids were separated and quantified as described by Collomb and Bühler (*25*). Isomers of CLA were analyzed and quantified by Ag^+-HPLC according to the method of ref *3*. All of the analyses were conducted in triplicate.

Isolation of the Volatiles. Solvent-assisted flavor evaporation (SAFE) (*26*) was used to isolate the volatiles from butter. The sample (100 g) was dissolved in 250 mL of diethyl ether. This solution was added during 1 h (10^{-3} Pa) to the distillation flask (50 °C). The SAFE apparatus was kept at 45 °C. To facilitate GC analysis, the aroma distillate was separated into acidic and neutral/basic fractions. For this purpose, the distillate (about 250 mL) was extracted with sodium carbonate solution (50 mL; 0.5 mol/L; pH 10.0) and washed with 40 mL of a saturated sodium chloride solution. The organic phase, containing the neutral/basic volatiles, was dried over anhydrous sodium sulfate. The pooled aqueous phase was acidified to pH 2 with hydrochloric acid (1 mol/L) and extracted three times with diethyl ether (25 mL each). The extract was then dried over anhydrous sodium sulfate. Both the acidic and neutral/basic fractions were concentrated to 200 μL using a Vigreux column followed by microdistillation. The concentrated extracts were stored at −20 °C prior to GC-MS-O analysis.

Gas Chromatography−Olfactometry (GC-O). The analysis was performed using a Trace GC (Thermo Fisher Scientific, Dreieich, Germany) equipped with a flame ionization detector (FID) and a sniffing port, heated at 250 °C. The carrier gas was helium with a constant pressure of 140 kPa for a DB-5 column (60 m, 0.32 mm i.d, 0.25 μm film thickness) (J&W Scientific, Folsom, CA) or 75 kPa for the DB-FFAP column (30 m, 0.32 mm i.d., 0.25 μm film thickness) (J&W Scientific). At the end of the column, the flow was split through a Y-type glass splitter (Chrompack,

Frankfurt, Germany) 1:1 to the FID and the sniffing port. The sample was injected by means of the cold on-column technique. The oven temperature was held at 40 °C for 2 min and then raised at 6 °C/min to 240 °C for 5 min. The flavor dilution (FD) factors of the odorants were determined by aroma extract dilution analysis (AEDA) by stepwise diluting an aliquot (50 μL) of the SAFE distillate with diethyl ether (1:1, v/v).

The GC-O analyses and the assessment of the FD factors were performed by two trained panelists in duplicate.

Gas Chromatography−Mass Spectrometry (GC-MS). Analysis was performed by means of an Agilent 5890 series II gas chromatograph (Agilent Technologies, Santa Clara, CA) coupled to a mass selective detector (MSD; Thermo Fisher Scientific). The sample was manually injected cold on-column. The carrier gas was helium with a flow of 1.9 mL/min. The volatiles were separated using either a DB-5 or a DB-FFAP capillary column (both 30 m, 0.25 mm i.d, 0.25 μm film thickness) (J&W Scientific). After 2 min, the oven temperature was raised by 6 °C/min to 240 °C for 5 min. The MSD was operated in the scan mode at 2.9 scans/s (m/z 29−350) at 70 eV. The identification was based on a comparison of the mass spectra with those in the Wiley 138.L database, linear retention indices (LRI), and odor perception of authentic reference compounds. LRI were calculated according to the method of Kovats (*27*). LRI were also compared with published data.

Quantification by Stable Isotope Dilution Assays (SIDA). The labeled internal standards **5-d**, **6-d**, **7-d**, **9-d**, **10-d**, **11-d**, **13-d**, **14-d**, and **15-d** (2.5−25 μg, respectively) dissolved in diethyl ether (250 mL) were added to 100 g of butter, containing the respective analytes in similar concentrations, as determined in preliminary experiments. The same procedure was applied to the oxidized butter samples using **5-d**, **6-d**, **8-d**, **12-d**, **15-d**, **16-d**, **18-d**, and **4-d** (0.5−13 μg, respectively) to quantify the major compounds responsible for the off-odors. The volatiles and the labeled internal standards were isolated by SAFE distillation, as described above.

Quantification was performed by two-dimensional gas chromatography−mass spectrometry (TD-GC-MS) running in the positive chemical ionization mode (CI) as described in ref *28*. Chemical ionization was necessary to enhance the intensities of the molecular ion peaks, which were too weak or not detected in the electron impact mode. The compounds were first separated on a DB-FFAP column (30 m × 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific) and then on a DB-1701 column (30 m × 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific). The oven temperature program for the DB-FFAP was 40 °C for 2 min, then increased by 40 °C/min to 60 °C for 2 min, then raised at 8 °C/min to 180 °C, finally raised at 15 °C/min to 230 °C, and held for 5 min. In the case of DB-1701 the temperature of the oven was 40 °C for 3 min, then raised at 6 °C/min to 240 °C, and held for 5 min. The selected ions of the labeled standards and the odorants (**Table 1**) were analyzed in the single ion monitoring (SIM) mode and their intensities calculated by means of computer programs. Concentrations were calculated and corrected using MS response factors obtained by measuring defined mixtures of respective labeled and unlabeled compounds. Analyses were run in triplicate.

Quantification by SIDA Using Headspace Solid-Phase Microextraction (HS-SPME)-GC-MS. Pentanal, a highly volatile compound, was not recovered by SAFE and, therefore, quantified by SIDA using HS-SPME, which allows the extraction of low-boiling volatile compounds (*29*). Hexanal and heptanal were also quantified by HS-SPME in the same assay using **2-d** as internal standard for all three compounds. Butter (5 g), molten at 40 °C in a water bath, was spiked with **2-d** (6.8 μg in ethanol), homogenized, and equilibrated for 12 h at 8 °C. The HS-SPME analysis was carried out using a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland). The sample was equilibrated at 45 °C for 30 min and then extracted for 45 min at 45 °C using a divinylbenzene/carboxen/polydimethylsiloxane fiber (2 cm; DVB/CAR/PDMS, Supelco, Bellefonte, PA). The analysis was performed using a Trace Ultra GC coupled to a Trace DSQ mass spectrometer (Thermo Fisher Scientific), and separation was performed using the DB-FFAP (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific) column. The oven program was 40 °C for 2 min, then raised at 8 °C/min to 150 °C, and finally raised at 20 °C/min to 240 °C. The mass spectrometer was operated in the positive CI mode using isobutane as the reagent gas at a flow of 2 mL/min. Analyses were run in the SIM mode, considering the ions indicated in **Table 1**. All samples were analyzed in triplicate.

Table 2. Chemical Composition of UFA/CLA-Enriched and Conventional (CONV) Butters

chemical composition	unit	UFA/CLA[a]	CONV[a]
moisture	g/kg	163 ± 11	143 ± 6.4
fat	g/kg	830 ± 11	852 ± 7.4
nonfat solids	g/kg	7 ± 1	5 ± 0.9
retinol	mg/kg	13 ± 0.5	11 ± 0.5
α-tocopherol[b]	mg/kg	36 ± 4	25 ± 0.8
copper	μg/kg	33 ± 14	36 ± 14
iron[b]	μg/kg	382 ± 95	132 ± 54

[a] Mean values (n = 3). [b] $P \leq 0.05$.

Quantification of 3-Methyl-1*H*-indole by HPLC. 3-Methyl-1*H*-indole was determined using a modified method according to ref *30*. Butter (0.5 g) was introduced into a 2 mL centrifuge microtube and heated at 45 °C in a heating block (Dri-Block, Witec AG, Littau, Switzerland). Then, 1 mL of the standard solution ($MeOH/H_2O$ 95:5, v/v), containing 0.05 mg/L 2-methyl-1*H*-indole, was added and vortexed for 30 s. The samples were sonicated (Bandelin Sonorex RK 255H, Schalltec, Mörfelden-Walldorf, Germany) for 5 min at 30 °C, cooled during 20 min in an ice bath, and centrifuged (Biofuge Stratos, Heraeus, Hanau, Germany) at 11000 rpm for 20 min at 4 °C. The supernatant was filtered (Chromafil 0-20/15MS PTFE, Pretech Instruments KB, Sollentuna, Sweden), and 5 μL of the filtrate was injected onto an HPLC column (Zorbax Eclipse XDB-C18 4.6 × 50 mm; 1.8 μm, Agilent). The analysis was carried out at 40 °C using a column oven (Agilent). The mobile phase was potassium phosphate buffer (pH 6.0, 10 mmol/L) and methanol (55:45) at 1.3 mL/min flow. Fluorescence detection was performed using an excitation wavelength of 285 nm and an emission wavelength of 340 nm. Analyses were run in triplicate.

Statistical Analysis. A randomized complete block design, which incorporated the treatments, and three blocks (replicate trials) were used for analysis of the response variables relating to chemical composition, FA composition, and odor compounds of UFA/CLA-enriched butter and conventional butter. Analysis of variance (ANOVA) was performed at $\alpha = 0.05$ using the general linear model (GLM) procedure, where the effect of butter type was estimated for chemical composition and FA composition. ANOVA (GLM) was also performed to estimate the effects of treatments (butter type, storage, photo-oxidation, and oxidation under oxygen atmosphere) and replicates for odor compounds. A split plot design was used to monitor the effects of treatments (butter type, storage, photo-oxidation, and oxidation under oxygen atmosphere) and their interaction on the response variables (odor compounds) measured at 0 and 6 weeks of storage and at 0, 6, and 12 h of photo-oxidation and oxidation under oxygen atmosphere, respectively. All analyses were conducted using Systat for Windows version 12 (Systat Software, Inc., San Jose, CA).

RESULTS AND DISCUSSION

Chemical Composition of UFA/CLA-Enriched Butter and Conventional Butter. The inclusion of sunflower seeds in the cow's diet resulted in marked changes in the chemical composition of UFA/CLA-enriched butter in comparison to that of conventional butter. The fat content of UFA/CLA -enriched butter was lower, but not significantly different from the fat in the conventional butter (**Table 2**). A lower fat content of dairy products was also observed in other studies (*2*, *31*) when the diet of the cows contained a source of UFA. This phenomenon seems to be related to an increase in *trans* fatty acids (FA), such as t10,c12 CLA, which inhibit milk fat synthesis (*32*). However, the mechanisms of this inhibition of fat secretion are yet unclear. The α-tocopherol and iron concentrations were significantly higher in the UFA/CLA-enriched butter (**Table 2**), also confirming previous data (*14*). The higher contents of these components are probably related to the cow's diet supplemented with sunflower seeds being rich in α-tocopherol and iron (*33*).

As expected, compared to conventional butter, the UFA/CLA-enriched butter had significantly higher concentrations of monounsaturated fatty acids (MUFA), polyunsaturated FA (PUFA),

Table 3. Fatty Acid Composition of UFA/CLA-Enriched and Conventional (CONV) Butters

fatty acid[a]	g/100 g of fat	
	UFA/CLA[b]	CONV[b]
saturated**	49 ± 0.70	62 ± 1.76
butanoic acid C4:0	2.7 ± 0.11	3.0 ± 0.19
hexanoic acid C6:0**	1.4 ± 0.02	2.0 ± 0.07
octanoic acid C8:0***	0.7 ± 0.02	1.2 ± 0.02
decanoic acid C10:0**	1.6 ± 0.07	3.0 ± 0.21
dodecanoic acid C12:0**	1.8 ± 0.06	3.0 ± 0.09
tetradecanoic acid C14:0**	7.6 ± 0.56	10 ± 0.03
palmitic acid C16:0**	20 ± 0.36	27 ± 1.23
stearic acid C18:0*	9.5 ± 0.61	8.0 ± 0.09
monounsaturated***	35 ± 0.57	25 ± 0.09
oleic acid (C18:1 c9)**	23 ± 0.70	17 ± 0.01
polyunsaturated**	6.0 ± 0.23	4.5 ± 0.50
linoleic acid (C18:2 c9c12)*	1.4 ± 0.14	1.0 ± 0.14
α-linolenic acid (C18:3 c9c12c15)	0.7 ± 0.07	0.6 ± 0.03
sum CLA***	2.0 ± 0.02	1.2 ± 0.26
C18:2 c9t11***	1.9 ± 0.14	1 ± 0.02
C18:2 t7c9**	0.080 ± 0.004	0.040 ± 0.003
C18:2 t11c13	0.040 ± 0.007	0.030 ± 0.006
C18:2 t8c10	0.030 ± 0.004	0.025 ± 0.004
C18:2 t10c12	0.010 ± 0.003	0.005 ± 0.002
sum omega-3[c]	1.2 ± 0.17	1.3 ± 0.22
sum omega-6*[d]	3.5 ± 0.19	2.0 ± 0.16

[a] *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. [b] Mean values ($n = 3$). [c] C18:2 t11c15 + C18:2 c9c15, C18:3 c9c12c15, C20:3 (n-3), C20:5 (n-3), C22:5 (n-3) and C22:6 (n-3). [d] C18:1 t12, C18:1 c12, C18:2 t9t12, C18:2 c9t12 + (c,c-MID + t8c13), C18:3 c6c9c12, C20:2 cc (n-6), C20:3 (n-6) and C20:4 (n-6).

including CLA and omega-6, and significantly lower saturated fatty acid levels (**Table 3**). Butanoic acid, α-linolenic acid, and omega-3 FA were not significantly different in the two butter types.

The UFA/CLA-enriched butter was almost twice as rich in CLA as the conventional butter (2 vs 1.2 g/100 g of fat, respectively). These results confirmed the observation of Collomb et al. (*3*), who found that total CLA increased by a factor of 2 when the daily intake of linoleic acid increased from 280 to 375 g. **Table 3** also indicates the major CLA isomers, such as C18:2 c9t11 representing 90% of the total CLA in milk fat, C18:2 t7c9, and C18:2 t11c13.

Our previous study (*14*) indicated similar values for the FA composition in UFA/CLA-enriched butter and conventional butter and also showed that these FA were not influenced by the storage of butter at 6 °C for 8 weeks.

Aroma Extract Dilution Analysis (AEDA). Among the 58 odorants detected by GC-O in the butter extracts (data not shown), 20 odorants in fresh samples and 22 in stored butter showed FD factors of >4 (**Table 4**). The fresh UFA/CLA-enriched butter showed the highest FD factors for 2-nonanone (milky odor, FD = 64), nonanal (soapy, FD = 64), 3-methylbutyl acetate (orange-like, FD = 32), methyl 2-methylbutanoate (fruity, FD = 32), δ-decalactone, (peach-like, FD = 32), hexanal (green, FD = 32), and (*E*,*Z*)-2,6-nonadienal (cucumber-like, FD = 32). The fresh conventional butter had the highest FD factors for the same compounds, but often by a factor of 2 lower compared to those of fresh UFA/CLA-enriched butter. Only 2-phenylethyl acetate, with a fruity odor, showed a higher FD factor in fresh conventional butter than in the fresh UFA/CLA-enriched samples (FD = 64 versus 16). Previous investigations had also reported 2-nonanone, hexanal, nonanal, (*E*,*Z*)-2,6-nonadienal, and δ-decalactone as important aroma compounds in fresh sweet cream butter (*21*, *34*, *35*).

Diacetyl, with a typical butter odor, was not found in UFA/CLA-enriched butter nor in conventional butter. This odor compound results from the activity of lactic acid bacteria, which are added to the cream during butter production. In the present study only sweet cream butter was analyzed, produced without a fermentation process, which may explain the absence of diacetyl.

With the exception of 2-phenylethyl acetate, the same odorants were perceived at the sniffing port in the two butter types after 6 weeks of storage. In particular, δ-dodecalactone and δ-decalactone, both perceived as peach-like, (*E*,*Z*)-2,6-nonadienal (cucumber-like), and heptanal (soapy) showed the highest FD factors in the stored UFA/CLA-enriched butter. In addition, hexanoic acid (animal-like), decanal (green), and *trans*-4,5-epoxy-(*E*)-2-decenal (metallic), which were not perceived in the fresh butter, became important odorants in the stored samples. On the other hand, the FD factor of methyl 2-methylbutanoate was reduced from 32 to 8 after storage in both butter types.

Most of these odor-active compounds were already identified in our previous study using HS-SPME (*14*). However, several odor compounds, such as 3-methylbutyl acetate, methyl 2-methylbutanoate, octanal, (*E*,*Z*)-2,6-nonadienal, decanal, 3-methyl-1*H*-indole, γ- and δ-octalactone, and δ-dodecalactone, had previously not been found. This could be due to the different extraction type used, SPME being more effective for the extraction of low-boiling volatile compounds, whereas SAFE yields more high-boiling odor compounds. Besides, in the two studies butter samples produced in different years (2006 and 2007) were investigated, showing a possibly slightly different aroma profile probably caused by seasonal variations.

Quantitative Analysis. AEDA is considered to be a screening method to locate odor-active compounds among the bulk of odorless volatiles, and the results are not corrected for, for example, losses during the workup procedures. Therefore, 13 odorants with FD factors of ≥64, considered to be important in the fresh and stored samples, were quantified by means of SIDA (**Table 5**). In addition, the high-boiling 3-methyl-1*H*-indole (mothball-like odor), which was not well detected by GC-MS, was quantified by HPLC. The results showed that δ-decalactone had the highest concentration in both fresh and stored samples, but especially in UFA/CLA-enriched butter. Pentanal, butanoic acid, and heptanal were significantly higher in UFA/CLA-enriched butter, in both fresh and stored samples. In contrast, δ-octalactone and δ-dodecalactone had significantly higher concentrations in fresh and stored conventional butters. Interestingly, the concentration of hexanal, considered to be an oxidation marker (*36*), did not significantly change during cold storage. The concentration of 3-methyl-1*H*-indole was not significantly different in the two butter types and remained constant during storage.

To estimate the sensory contribution of the odorants to the overall odor of the butter samples, odor activity values (OAVs) were calculated by dividing the concentration by the nasal odor threshold concentration determined in oil (*37*) (**Table 6**). The OAV calculated for pentanal in fresh UFA/CLA-enriched butter and in fresh conventional butter was < 1, meaning that this compound is not important for the overall odor of fresh butter.

δ-Decalactone, δ-dodecalactone, and butanoic acid had the highest OAVs in both fresh UFA/CLA-enriched and fresh conventional butter, with δ-decalactone being slightly more important in the UFA/CLA samples (OAV = 7 vs OAV = 5 in conventional butter) and δ-dodecalactone in the conventional samples (OAV = 12 vs OAV = 11 in UFA/CLA-enriched butter). The highest OAV was calculated for δ-decalactone in UFA/CLA-enriched butter after 6 weeks of storage (OAV = 18). Then, δ-decalactone was the most prominent odorant in the stored UFA/CLA-enriched butter. Butanoic acid, heptanal, and pentanal had higher OAVs in stored UFA/CLA-enriched butter than in the conventional one. The stored conventional butter showed

Table 4. Most Odor-Active Compounds (FD Factor ≥ 4) in Fresh and 6 Weeks Stored (6 °C) UFA/CLA-Enriched and Conventional (CONV) Butters

				FD factor[a]			
		RI		fresh		stored	
odorant[b]	odor quality[c]	DB-5	FFAP	UFA/CLA	CONV	UFA/CLA	CONV
3-methylbutyl acetate	orange-like	nd[d]	1116	32	16	16	16
pentanal	fat, green	738	973	8	8	64	16
methyl 2-methylbutanoate	fruity	776	1016	32	32	8	8
hexanal	green	810	1082	32	16	64	64
butanoic acid	cheesy	856	1620	4	4	64	64
heptanal	soapy	910	1170	8	8	128	32
ethyl hexanoate	orange-like	1000	1223	16	8	16	8
octanal	almond, fat	1007	1277	8	4	8	4
hexanoic acid	animal-like	1022	1800	nd	nd	64	32
2-nonanone	milk	1101	1402	64	64	64	64
2-acetyl-2-thiazoline	popcorn	1108	1751	8	4	8	4
nonanal	soapy, citrus	1109	1385	64	64	64	32
(*Z*)-2-nonenal	hay	1148	1510	4	4	64	32
(*E*,*Z*)-2,6-nonadienal	cucumber-like	1154	1567	32	16	128	64
(*E*)-2-nonenal	grass	1163	1529	8	4	64	32
decanal	green	1250	1486	nd	nd	64	32
2-phenylethyl acetate	fruity	1250	1810	16	64	nd	nd
γ-octalactone	sweet	1260	1878	8	4	8	8
δ-octalactone	fruity	1290	1923	8	8	64	64
trans-4,5-epoxy-2-(*E*)-decenal	fat, metallic	1380	2017	nd	nd	16	8
3-methyl-1*H*-indole	mothball	1399	2500	16	16	32	16
δ-decalactone	peach-like	1469	2201	32	16	128	64
δ-dodecalactone	peach-like	1509	2420	8	8	256	128

[a] FD factor determined by two panelists. The FD factors between the two panelists differed by a factor of not more than 2. [b] The compounds were identified by comparison with the reference substance on the basis of the following criteria: retention index (RI) on two columns of different polarities, mass spectra obtained by MS (EI), and odor quality perceived at the sniffing port. [c] Odor quality perceived at the sniffing port. [d] nd, not detected.

Table 5. Concentrations of Odorants Quantified in Fresh and Stored UFA/CLA-Enriched and Conventional (CONV) Butters

	concentration[a] (μg/kg)						
	fresh		stored		between subjects effects[b]	within subjects effects[b]	
odorant[c]	UFA/CLA	CONV	UFA/CLA	CONV	butter type	storage	butter type × storage
pentanal	235 ± 30	64 ± 6	661 ± 8	289 ± 21	***	***	***
hexanal	10 ± 1	11 ± 1	10 ± 0.6	12 ± 0.6	ns	ns	ns
butanoic acid	1606 ± 8	1568 ± 7	1885 ± 9	1820 ± 8	***	***	**
heptanal	963 ± 30	364 ± 35	1703 ± 80	1140 ± 62	***	***	ns
hexanoic acid	802 ± 3	806 ± 4	1240 ± 5	1237 ± 5	ns	***	*
nonanal	68 ± 5	59 ± 1	72 ± 1	73 ± 2	ns	**	*
(*Z*)-2-nonenal	0.07 ± 0.02	0.10 ± 0.05	0.36 ± 0.04	0.32 ± 0.04	ns	***	ns
(*E*,*Z*)-2,6-nonadienal	17 ± 4	17 ± 3	17 ± 4	20 ± 2	ns	ns	ns
(*E*)-2-nonenal	6.8 ± 0.2	6.6 ± 0.5	6.8 ± 0.4	6.2 ± 0.7	ns	ns	ns
decanal	24 ± 2	12 ± 3	16 ± 4	14 ± 2	*	*	**
δ-octalactone	114 ± 6	137 ± 2	303 ± 3	487 ± 3	***	***	***
3-methyl-1*H*-indole	108 ± 3	111 ± 4	104 ± 3	109 ± 4	ns	ns	ns
δ-decalactone	2858 ± 12	2061 ± 10	7245 ± 18	5293 ± 16	***	***	***
δ-dodecalactone	1314 ± 6	1464 ± 5	1491 ± 7	1536 ± 6	***	***	***

[a] Mean values ($n = 3$). [b] Effects of ANOVA: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ns, not significant. [c] The compounds were quantified by a stable isotope dilution assay (SIDA), except 3-methyl-1*H*-indole.

higher OAVs for (*E*,*Z*)-2,6-nonadienal, δ-octalactone, and δ-dodecalactone.

Studies on Light- and Oxygen-Induced Changes in Odorants. UFA/CLA-enriched and conventional butters were exposed to fluorescent light for 6 and 12 h, respectively. Selected odorants formed during exposure were compared to those present in the butter before illumination. Hexanal, which remained constant during 6 weeks of cold storage, considerably increased in both butter types and especially in UFA/CLA-enriched butter after 6 h of light exposure (**Table 7**). Photo-oxidation provoked a stronger metallic off-flavor in the conventional samples, probably due to a higher amount of *trans*-4,5-epoxy-(*E*)-2-decenal compared to UFA/CLA-enriched butter. Surprisingly, heptanal, which showed a higher concentration in UFA/CLA-enriched butter during cold storage, in this case revealed significantly higher values in conventional butter during illumination. Light exposure also induced a significant increase in nonanal and (*E*,*Z*)-2,6-nonadienal in conventional butter.

The lower concentrations of aldehydes in light-exposed UFA/CLA-enriched butter might be explained by its higher contents of α-tocopherol and retinol, which may act as antioxidants. A potential antioxidant activity of CLA could also protect UFA/CLA-enriched butter from lipid oxidation. However, the role of CLA as antioxidant is disputed by contradictory studies (*38*).

Light exposure induced the formation of methional (potato-like), which was not detected in butter during cold storage. This compound originates from photodecomposition of methionine,

and it is mainly responsible, together with other sulfur-containing volatiles, for the light-activated flavor in dairy products (*8*).

A calculation of the OAVs on the basis of the quantitative data (**Table 8**) revealed the highest values for hexanal in UFA/CLA-enriched butter and *trans*-4,5-epoxy-(*E*)-2-decenal in conventional butter. In particular, after light exposure the OAVs of *trans*-4,5-epoxy-(*E*)-2-decenal and (*E*,*Z*)-2,6-nonadienal in conventional butter were more than 2.5-fold higher than their respective OAVs in the enriched butter. The OAVs of pentanal and methional were higher in the UFA/CLA-enriched samples.

Samples exposed to an oxygen atmosphere for 6 and 12 h, respectively, developed fatty and metallic odor notes, due to an increase in aldehydes, such as pentanal, hexanal, heptanal, nonanal, (*E*)-2-octenal, and *trans*-4,5-epoxy-(*E*)-2-decenal (**Table 9**). The conventional butter showed significantly higher concentrations of heptanal, (*E*)-2-octenal (fatty), and (*Z*)-2-octenal (nutty) than the UFA/CLA-enriched samples. 1-Octen-3-one, with a mushroom-like odor, also significantly increased in conventional butter exposed to the oxygen atmosphere and exceeded the enriched butter by a factor of 1.6 after 6 h and by a factor of 3.4 after 12 h of exposure to oxygen. A calculation of the OAVs showed that, in particular, the values for heptanal in conventional butter were twice as high compared to those of UFA/CLA-enriched butter (**Table 10**). Pentanal was a more prominent odorant in UFA/CLA-enriched butter after 12 h of induced oxidation under oxygen atmosphere.

The results clearly showed that photo-oxidation and oxidation in the dark under an oxygen atmosphere induced the generation of a different set of odorants in UFA/CLA-enriched butter and in conventional butter. In particular, heptanal, (*E*,*Z*)-2,6-nonadienal, (*E*)-2-octenal, and *trans*-4,5-epoxy-(*E*)-2-decenal increased more in conventional butter when subjected to oxidation. These findings suggest that the higher retinol and α-tocopherol concentrations of UFA/CLA-enriched butter in combination with a potential antioxidative activity of CLA may be protective, even despite the higher prooxidant iron content in this kind of butter.

The formation of odor compounds in butter during storage or oxidation in the presence of oxygen involves the conversion of unsaturated fatty acids to hydroperoxides, which decompose into odor-active secondary oxidation products. Arachidonic, linoleic, and linolenic acid are considered to be the major precursors of odor compounds in butter (*5*). However, CLA could also be considered a source for odorants. Yurawecz et al. (*39*) suggested that the autoxidation of unsaturated fatty acids via a free radical mechanism is not probable to occur in CLA, because a higher

Table 6. Odor Activity Values (OAV) for Eight Odorants Quantified in Fresh and Stored Butter Samples

		OAV[a]			
		fresh		stored	
odorant	odor threshold (μg/L)	UFA/CLA[b]	CONV[c]	UFA/CLA	CONV
pentanal	240	<1	<1	3	1
butanoic acid	135	12	12	14	13
heptanal	250	4	1	7	5
(*E*,*Z*)-2,6-nonadienal	3.8	4	4	4	5
δ-octalactone	120	<1	1	3	4
3-methyl-1*H*-indole	15.6	7	7	7	7
δ-decalactone	400	7	5	18	13
δ-dodecalactone	120	11	12	12	13

[a] Odor activity values were calculated by dividing the concentration by the odor threshold in oil (*37*). [b] Unsaturated fatty acid/conjugated linoleic acid enriched butter. [c] Conventional butter.

Table 8. Odor Thresholds and Odor Activity Values (OAV) of the Most Important Odorants of UFA/CLA-Enriched and Conventional (CONV) Butters Exposed to Fluorescent Light

		OAV[a]			
		6 h of light		12 h of light	
odorant	odor threshold (μg/kg in sunflower oil)	UFA/CLA	CONV	UFA/CLA	CONV
pentanal	240	7	3	11	4
hexanal	210	26	24	25	23
heptanal	250	7	8	4	6
(*E*,*Z*)-2,6-nonadienal	3.8	3	10	7	8
trans-4,5-epoxy-(*E*)-2-decenal	1.3	21	69	25	69
methional	0.2	2	1	2	1

[a] Odor activity values were calculated by dividing the concentration by the odor threshold (*37*).

Table 7. Changes in the Concentration of Odorants in UFA/CLA-Enriched and Conventional (CONV) Butters Induced by Light Exposure

	concentration[a] (μg/kg)								
	0 h		6 h		12 h		between subjects effects[b]	within subjects effects[b]	
odorant[c]	UFA/CLA	CONV	UFA/CLA	CONV	UFA/CLA	CONV	butter type	time exposure linear contrast	butter type × time exposure quadratic contrast
pentanal	235 ± 30	64 ± 6	1699 ± 18	791 ± 14	2590 ± 20	826 ± 15	***	***	***
hexanal	10 ± 1	11 ± 1	5494 ± 16	5032 ± 18	5185 ± 14	4822 ± 13	***	***	***
heptanal	963 ± 30	364 ± 35	1681 ± 14	1905 ± 13	1059 ± 14	1513 ± 12	**	***	***
nonanal	68 ± 5	59 ± 1	360 ± 8	405 ± 9	278 ± 8	379 ± 9	***	***	***
(*E*)-2-nonenal	6.8 ± 0.2	6.6 ± 0.5	21 ± 2	23 ± 2	28 ± 2	29 ± 3	ns	***	*
(*E*,*Z*)-2,6-nonadienal	17 ± 4	17 ± 3	13 ± 2	38 ± 3	25 ± 3	30 ± 3	**	**	*
trans-4,5-epoxy-(*E*)-2-decenal	<0.05	<0.05	27 ± 3	90 ± 8	33 ± 2	90 ± 3	***	ns	ns
3-methyl-1*H*-indole	108 ± 3	111 ± 4	93 ± 3	84 ± 4	nd[d]	nd	ns	***	**
methional	<0.05	<0.05	0.43 ± 0.08	0.30 ± 0.04	0.33 ± 0.03	0.26 ± 0.05	ns	*	ns
1-octen-3-one	0.06 ± 0.005	0.05 ± 0.005	0.31 ± 0.02	0.36 ± 0.04	0.39 ± 0.03	0.40 ± 0.03	ns	***	***
(*Z*)-3-hexenal	0.85 ± 0.9	0.73 ± 0.03	0.76 ± 0.03	0.66 ± 0.03	0.64 ± 0.03	0.58 ± 0.04	*	**	ns
(*E*)-2-octenal	7.5 ± 1	2.4 ± 0.2	88 ± 4	93 ± 5	114 ± 2	101 ± 6	***	***	***
(*Z*)-2-octenal	nd	nd	85 ± 5	91 ± 4	135 ± 11	108 ± 5	ns	***	***

[a] Mean values ($n = 3$). [b] Effects of ANOVA: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ns, not significant. [c] The compounds were quantified by a stable isotope dilution assay (SIDA), except 3-methyl-1*H*-indole. [d] nd , not detected.

Table 9. Concentrations of Selected Odorants in UFA/CLA-Enriched and Conventional (CONV) Butters Exposed to Oxygen

	concentration[a] (μg/kg)						between subjects effects[b]	within subjects effects[b]	
	0 h		6 h		12 h				
odorant[c]	UFA/CLA	CONV	UFA/CLA	CONV	UFA/CLA	CONV	butter type	oxidation time linear contrast	butter type × oxidation time quadratic contrast
pentanal	235 ± 30	64 ± 6	368 ± 6	166 ± 5	673 ± 10	372 ± 4	***	***	***
hexanal	10 ± 1	11 ± 1	1663 ± 5	1815 ± 6	1577 ± 6	1567 ± 5	***	***	***
heptanal	963 ± 30	364 ± 35	1360 ± 6	2511 ± 8	1000 ± 5	1896 ± 7	***	***	***
nonanal	68 ± 5	59 ± 1	557 ± 7	504 ± 5	712 ± 8	462 ± 6	***	***	***
(*E*,*Z*)-2,6-nonadienal	17 ± 4	17 ± 3	16 ± 3	15 ± 3	19 ± 4	20 ± 3	ns	ns	ns
(*E*)-2-nonenal	6.8 ± 0.2	6.6 ± 0.5	17 ± 2	16 ± 2	17 ± 2	18 ± 3	ns	**	**
trans-(*E*)-4,5-epoxy-(*E*)-2-decenal	<0.05	<0.05	0.46 ± 0.04	0.26 ± 0.04	0.33 ± 0.05	0.35 ± 0.05	*	ns	**
1-octen-3-one	0.06 ± 0.005	0.05 ± 0.005	0.20 ± 0.02	0.36 ± 0.05	0.31 ± 0.03	1.05 ± 0.09	***	***	***
(*Z*)-3-hexenal	0.85 ± 0.9	0.73 ± 0.03	0.79 ± 0.07	0.43 ± 0.03	0.59 ± 0.04	0.66 ± 0.05	*	**	***
(*E*)-2-octenal	7.5 ± 1	2.4 ± 0.2	75 ± 4	92 ± 5	72 ± 5	88 ± 3	*	***	***
(*Z*)-2-octenal	nd[d]	nd	69 ± 3	112 ± 3	69 ± 2	80 ± 2	***	***	***

[a] Mean values (n = 3). [b] Effects of ANOVA: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ns, not significant. [c] The compounds were quantified by a stable isotope dilution assay (SIDA), except 3-methyl-1*H*-indole. [d] nd, not detected.

Table 10. Odor Thresholds and Odor Activity Values (OAV) of the Most Important Odorants of UFA/CLA-Enriched and Conventional (CONV) Butters Exposed to Oxygen

		OAV[a]			
		oxygen 6 h		oxygen 12 h	
odorant	odor threshold (μg/kg in sunflower oil)	UFA/CLA	CONV	UFA/CLA	CONV
pentanal	240	1.5	<1	3	1.5
hexanal	210	8	9	7.5	7.5
heptanal	250	5.5	10	4	8
(*E*,*Z*)-2,6-nonadienal	3.8	4	4	5	5

[a] Odor activity values were calculated by dividing the concentration by the odor threshold (*37*).

activation energy is required for conjugated double bonds. In the present study, the low temperature, that is, 6 °C, used in the oxidation experiments probably reduces or even inhibits the formation of the hydroperoxides from CLA. The formation of hydroperoxides from CLA was reported to be negligible even at 30 °C by Luna et al. (*40*). Thus, in the present study, the formation of volatile compounds from the oxidation of CLA can probably be attributed to a cycloaddition of oxygen, as proposed by Yurawecz et al. (*39*). They observed also that different CLA isomers formed different odorants during oxidation. *cis*9,*trans*11-CLA, kept in glass vials exposed to oxygen and ambient light for 8 days, generated heptanal and 2-nonenal among others. On the other hand, t10,c12-CLA developed mainly hexanal, methyl nonanoate, and decadienal under the same conditions.

Cold storage seemed to affect particularly the levels of pentanal and heptanal in UFA/CLA-enriched butter, with an increase in fatty and green notes. Pentanal and heptanal have been reported to be formed in oxidized oil and triacylglycerols, containing equal amounts of *c*9,*t*11-CLA and *t*10,*c*12-CLA isomers (*41*). These two compounds, which were found to be significantly more abundant in UFA/CLA-enriched butter during 6 weeks of storage, could thus also originate from CLA and not only from other UFA, such as linoleic and linolenic acid, as previously reported (*4*).

Overall, the results obtained in this study showed that autoxidation, induced photo-oxidation, or induced oxidation in the dark under oxygen atmosphere had different effects on odorant formation in butter. Different chemical pathways and kinetics seem to be involved, depending on the lipid composition, especially related to the presence of long-chain unsaturated fatty acids, and oxidation conditions (temperature, oxygen pressure, intensity of light, time of exposure, presence of antioxidants or pro-oxidants). Previous studies also showed the formation of different odor-active compounds when different oxidation processes were involved. Butter oil kept at room temperature for 42 days developed strong green/cardboard notes, due to hexanal, (*Z*)-3-hexenal, and (*E*)-2-nonenal, and mushroom odor correlated to the presence of 1-octen-3-one and (*Z*)-1,5-octadiene-3-one (*6*). On the other hand, butter oil exposed to fluorescent light for 48 h developed fried, tallowy, and metallic off-notes, due to high concentrations of (*E*,*E*)-2,4-decadienal, (*E*)-2-nonenal, and *trans*-4,5-epoxy-(*E*)-2-decenal, respectively (*9*).

Induced accelerated photo-oxidation or induced oxidation in the dark under an oxygen atmosphere of butter samples offered the opportunity to rapidly evaluate the formation of potential off-flavors that could develop during long storage of butter. However, there might be differences in the reaction pathways and kinetics involved between accelerated oxidation and oxidation during storage. The results of the accelerated oxidation may probably not be applied in all aspects to the stored samples, as was shown by the higher heptanal concentration in the stored UFA/CLA-enriched butter in contrast to the lower heptanal concentration after 12 h of exposure to oxygen in the enriched butter.

In conclusion, metallic and oxidized odor notes were not found in UFA/CLA-enriched butter after 6 weeks of storage at 6 °C. As described recently (*14*) a sensory expert panel did not find significant flavor differences, which might be related to oxidation processes between the two butter types during storage. Induced photo-oxidation and oxidation in the dark with oxygen revealed differences and significantly stronger off-notes in conventional butter compared to UFA/CLA-enriched butter, due to *trans*-4,5-epoxy-(*E*)-2-decenal, heptanal, and (*E*,*Z*)-2,6-nonadienal. The UFA/CLA-enriched butter seemed to be quite stable, taking into account the higher contents of UFA compared to the conventional butter. UFA/CLA-enriched butter has presumably been protected from oxidation by its high content of α-tocopherol and perhaps also by the potential antioxidant properties of CLA.

A production of UFA/CLA-enriched butter can be envisaged without strong off-flavor formation for a certain limited period of storage. However, further studies on the storage stability of UFA/CLA-enriched dairy products would be advisable to evaluate

possible flavor changes during longer periods of storage and at different temperatures. Sensory tests for consumer acceptance would also be helpful.

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