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Determination of storage stability of butter enriched with unsaturated fatty acids/conjugated linoleic acids (UFA/CLA) using instrumental and sensory methods

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ABSTRACT

The oxidative stability of butter enriched with unsaturated fatty acids and conjugated linoleic acids (UFA/CLA butter) was evaluated by chemical, sensory and microbiological analyses during 8 weeks of storage at 6 °C and compared with that of conventional butter. The odour-active compounds were analyzed by gas chromatography–mass spectrometry combined with olfactometry, using solid phase microextraction. Olfactometric analysis showed that both, fresh UFA/CLA butter and fresh conventional butter had similar aroma profiles. After 6–8 weeks of storage, UFA/CLA butter showed stronger fatty (butanoic and 3-methyl butanoic acid), metallic [(*E,E*)-2,4-nonadienal], green [(*E*)-2-hexenol] and creamy (2-pentanone) notes compared with the conventional samples. A sensory panel described the two fresh butter types as having a similar sensory profile, except for a stronger creamy aroma, a less intense cooked milk aroma and a significantly higher spreadability of the UFA/CLA butters. Sensory descriptive analysis showed also that both butter types aged in a very similar way, with an increase in rancid and oxidized notes.

1. Introduction

Recent studies have focused on increasing the amount of unsaturated fatty acids (UFA) and, in particular, of conjugated linoleic acids (CLA) in milk and dairy products (AbuGhazaleh, Schingoethe, Hippen, & Whitlock, 2002; Collomb, Schmid, Sieber, Wechsler, & Ryhänen, 2006; Jones et al., 2005) since they are claimed to have beneficial effects on human health. Milk fat naturally contains UFA in the range of 25–35% depending upon feeding regimen, season, breed and period of lactation. More than 95% of UFA in milk fat is in the form of oleic acid, linoleic acid and α-linolenic acid (21–30%, 2–2.5% and 1–1.3% of total fat, respectively; Collomb, Eyer, & Sieber, 2000).

The concentration of CLA, which is a mixture of different isomers of linoleic acid, can vary within a broad range. Precht and Molkentin (1999) reported average CLA concentrations in milk fat between 0.45 g 100 g^{-1} in winter and 1.20 g 100 g^{-1} in summer. A similar variation in CLA contents was found in butter by Ledoux et al. (2005), who indicated total CLA levels varying from 0.45 g 100 g^{-1} fat in winter to 0.80 g 100 g^{-1} fat in summer. The CLA content of milk fat of pasture-fed cows can be two to five times higher than that of cows given total mixed rations (1.09 versus 0.46 g 100 g^{-1} milk fat: Kelly, Kolver, Bauman, van Amburgh, & Muller, 1998; 2.21 versus 0.39 g 100 g^{-1} milk fat: Dhiman, Arnand, Satter, & Pariza, 1999). Collomb, Bütikofer, Sieber, Jeangros, and Bosset (2002) found especially high CLA values in milk from mountain pasture, varying from 1.90 to 2.80 g 100 g^{-1}. An enrichment of UFA and CLA in milk fat can also be achieved by supplementing the animal diet with rapeseeds, sunflower seeds and linseeds (Collomb, Sieber, & Bütikofer, 2004; Collomb, Sollberger et al., 2004; Ryhänen et al., 2005), or with free oils, such as soybean, linseed and fish oils (AbuGhazaleh et al., 2004).

Collomb, Sieber et al., (2004) and Collomb, Sollberger et al. (2004) showed that the concentrations of oleic (C18:1), linoleic (C18:2) and α-linolenic (C18:3) acid and CLA isomers in milk depend upon the fat source fed to the cows. In particular, when the daily intake of linoleic acid in the cows' diet increased from 281 to 375 g, due to a supplement with sunflower seeds, the total CLA content increased by a factor of two (from 0.87 to 1.79 g 100 g^{-1} fat). A dietary supplementation with sunflower seeds led to the highest content of the *cis*-9, *trans*-11 CLA (c9t11 CLA) isomer, which is considered a very health promoting fatty acid (FA). It represents 75–90% of the total CLA concentration in milk fat (Baumann, Corl, & Peterson, 2003) and was reported to show anticarcinogenic (Ha, Storkson, & Pariza, 1990; Ip, Chin, Scimeca, & Pariza, 1991; Ip et al., 1999; Parodi, 1994), body fat reducing (Pariza, Park, Cook, Albright, & Liu, 1996) and growth-promoting (Chin, Storkson, Albright, Cook, & Pariza, 1994) properties.

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The enrichment of milk fat with UFA and CLA confers higher nutritional value to dairy products. On the other hand, these components are susceptible to auto-oxidation and can negatively affect the flavour and other sensory characteristics of dairy products. In fact, the oxidation of UFA may lead to the formation of secondary oxidation products, such as hydrocarbons, aldehydes and ketones, causing off-flavours and consequently, shorter shelf life of dairy products.

The auto-oxidation of the lipids in dairy products and the resulting off-flavours have been comprehensively studied (Badings, 1970; Swoboda & Peers, 1977; Widder, Sen, & Grosch, 1991). Badings (1970) identified hexanal (green), heptanal (oily), (*E*)-2-nonenal (cucumber-like), (*E,E*)-2,4-heptadienal (metallic), 1-octen-3-one (mushroom-like) as off-flavour compounds in cold stored butter and suggested linoleic, linolenic and arachidonic acids as precursors of these aroma compounds. On the other hand, the auto-oxidation patterns of CLA are still not well known and only few studies indicate the secondary products of CLA auto-oxidation (Eulitz, Yurawecz, & Ku, 1999; Yurawecz, Hood, Mossoba, Roach, & Ku, 1995). *Cis*-9, *trans*-11 CLA methyl ester, exposed to oxygen and ambient light for 8 days, generated volatile compounds, such as heptanal, 2-heptenal and 2-nonenal (Yurawecz, Delmonte, Vogel, & Kramer, 2003).

Several studies on the oxidative stability of milk, cheese and butter enriched in CLA showed no significant differences in flavour and sensory characteristics between CLA enriched and conventional products (Avramis, Wang, McBride, Wright, & Hill, 2003; Lynch et al., 2005; Ryhänen et al., 2005). On the other hand, a study about the sensory characteristics of milk, which was directly fortified with commercially available CLA, containing equal amounts of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA, presented a "grassy/vegetable oil" flavour (Campbell, Drake, & Larick, 2003). Consumer evaluation indicated this CLA enriched milk as less acceptable than milk without CLA addition.

The objective of the present study was to assess the opportunity for producing enriched UFA/CLA butter, which may have a higher nutritional value and, in addition, enhanced physical characteristics, such as reduced hardness and improved spreadability. For this reason, sweet cream butter enriched with UFA/CLA was produced by supplementing the cows' diet with sunflower seeds. The oxidative stability of UFA/CLA butter was compared with that of conventional butter, obtained by feeding cows a conventional diet based on pasture and corn silage.

Instrumental and sensory analyses were applied to UFA/CLA enriched butter and to conventional butter during 8 weeks of storage at 6 °C. Eight weeks of storage corresponds to double the usual shelf life indicated on the label by Swiss butter manufacturers. The odour-active compounds were analyzed by gas chromatography–mass spectrometry coupled with olfactometry (GC/MS/O), and a sensory panel evaluated spreadability, orthonasal odour and flavour. To our knowledge, this is the first study employing GC/O to evaluate odour-active compounds in CLA enriched foods.

2. Materials and methods

2.1. Butter samples

Both UFA/CLA enriched butter and conventional butter were produced in May and September 2006. Two groups of Holstein cows (10 for each group), of a similar stage of lactation, were fed different diets to obtain milk with diverse UFA/CLA content. One group grazed on pasture and consumed on average 1.8 kg dry matter (DM) day^{-1} per cow of a sunflower seed mixture. The control group grazed on the same plot and additionally received an average of 5.7 kg DM day^{-1} per cow of corn silage in the stable. The milk was collected separately after 12 days from the two groups (300 L each) during 2 days to produce UFA/CLA enriched butter and conventional butter.

The two butter types were produced under the same conditions. Raw milk was preheated at 45 °C in the pilot plant and then centrifuged. Cream (30 L) containing 35% fat was produced from both types of milk. The cream was pasteurized in a batch heated up to 80 °C. When a temperature of 80 °C was reached, the cream was immediately cooled down with cold water and then stored at 5 °C for 24 h to allow fat crystallization. The cream was churned using an electric 100 L churn (Kasag-Flückiger AG, Langnau, Switzerland) at 40 rpm and at 12 °C. The butter kernels formed after 40 min. Buttermilk was separated, cooled and used to wash the butter. The granules of butter were pressed and worked by hand to remove excess buttermilk. Sweet cream butter (10 kg) was produced, for each kind of milk, wrapped in aluminium foil in 100 g pieces and stored in the dark at 6 °C for 8 weeks. Part of the butter was also stored deep frozen for 2 and 6 weeks at −20 °C and used as a reference.

Microbiological analyses were carried out on fresh butter and after 6 weeks of storage at 6 °C. Moisture, non-fat solids, fat, iron and copper content were determined in the fresh samples. Vitamin A (retinol) and vitamin E (α-tocopherol) were quantified in fresh butter and after 6 weeks of storage at 6 °C; the fatty acid composition was determined in fresh samples and after 8 weeks of storage at 6 °C; the volatile composition analyses were carried out in fresh samples and in butter stored at 6 °C for 1, 2, 4, 6 or 8 weeks. Additionally, frozen samples (−20 °C) were analyzed by GC/MS/O after 2 and 6 weeks. Sensory testing was performed with refrigerated and frozen samples after 1, 2, 4, 6 or 8 weeks of storage.

2.2. Microbiological analyses

Microbiological parameters were determined using standard procedures. Enumeration of aerobic mesophile microorganisms was performed with plate count agar incubated for 72 h at 30 °C. Lipolytic bacteria were counted on Victoria blue butterfat agar incubated for 72 h at 30 °C. Victoria blue butterfat agar consisted of 5 g L^{-1} meat extract (Oxoid, Pratteln, Switzerland), 5 g L^{-1} bacto-peptone (BD, Basel, Switzerland), 12 g L^{-1} agar agar (Oxoid), 5% butterfat and 0.004% Victoria blue B (Fluka, Buchs, Switzerland).

2.3. Determination of moisture, non-fat solids and fat contents

Moisture, non-fat solids and fat contents were determined according to reference procedures (IDF 80/ISO 3727, 2002). The repeatability of the determinations is shown in Table 1 and was calculated from 35 duplicate determinations in accordance with ISO 5725-2 (ISO, 1994).

2.4. Determination of retinol and α-tocopherol

Butter samples (7 g) were mixed with deionized water at 40 °C. Potassium hydroxide (7 g), 50 mL ethanol and a pinch of hydroquinone were added, and the solution was placed in a boiling water bath for saponification over 30 min. The aqueous phase was extracted three times with petrol ether and washed with deionized water. The organic phase was dried with sodium sulphate. The solvent was evaporated using a Rotavapor (Büchi, Flawil, Switzerland) until dry and the extract was re-dissolved in methanol. Aliquots were injected into an HPLC series 1100 system (Agilent Technologies, Santa Clara, CA, USA). Samples were quantified using external standards of retinol and α-tocopherol. The repeatability of retinol and α-tocopherol determinations was calculated by four duplicate analyses of butter samples as shown in Table 1.

Table 1
Chemical composition of UFA/CLA[a] enriched and conventional butter[a]

Chemical composition (in absolute values)	Unit	r[c]	UFA/CLA[b] butter		Conventional butter	
			May	September	May	September
Moisture	g kg^{-1}	3.1	133.7	190.6	120.7	112.8
Fat	g kg^{-1}	2.0	861.1	802.3	873.6	883.1
Non-fat solids	g kg^{-1}	3.5	5.3	7	5.7	4.1
Retinol	mg kg^{-1}	0.6	12	12.5	11	10
α-Tocopherol	mg kg^{-1}	1.1	28	29	24.5	26
Copper	μg kg^{-1}	26	36	17	15	9
Iron	μg kg^{-1}	96	346	209	25	93.5
Chemical composition (values relative to dry mass)		**r[d]**	**UFA/CLA[b] butter**		**Conventional butter**	
			May	September	May	September
Fat	g kg^{-1} dry mass	5.9	994.0	991.0	993.5	995.4
Non-fat solids	g kg^{-1} dry mass	4.1	6.0	8.6	6.5	4.6
Retinol	mg kg^{-1} dry mass	0.8	14.0	15.4	12.5	11.0
α-Tocopherol	mg kg^{-1} dry mass	1.4	32.0	36.0	28.0	29.0
Copper	μg kg^{-1} dry mass	30.3	41.6	21.0	17.0	10.0
Iron	μg kg^{-1} dry mass	112.3	399.4	258.0	28.4	105.4

[a] The results are expressed as absolute values and also reported as relative to dry mass.
[b] Unsaturated fatty acids/conjugated linoleic acid.
[c] Repeatability reported relative to absolute values and calculated according to ISO 5725-2 (ISO, 1994).
[d] Repeatability reported relative to dry mass and calculated by error propagation.

2.5. Determination of copper and iron

For copper determination, 4 g of homogenized butter was placed in an open quartz-glass vessel to allow decomposition of organic matter by wet digestion with 3 mL of nitric acid (purity > 65%, Merck, Darmstadt, Germany). The sample was melted at 45 °C in a water bath and then mixed with 4 mL hexane (Merck). The upper fat phase was removed by aspiration using a vacuum pump and the sample was heated at 75 °C in the water bath to evaporate the rest of hexane. Nitric acid (2 mL) was added and a pressurized mineralization was carried out at 170 °C for 4 h in a closed polytetrafluoroethylene (PTFE) vessel. After cooling, the samples were analyzed by graphite furnace atom absorption spectrometry with Zeeman background correction by Perkin–Elmer AAanalyst 600 (Perkin–Elmer Life and Analytical Sciences, Inc., Waltham, MA, USA) using the following conditions: wavelength 324.8 nm, pre-treatment temperature 1200 °C, atomization at 1900 °C for 5 s. A calibration curve was obtained measuring different dilutions of a Titrisol copper solution (1 g Cu L^{-1}; Merck).

For iron determination, 0.3 g of homogenized butter was placed in a PTFE vessel and mixed with 5 mL of nitric acid. A pressurized mineralization was carried out at 150 °C for 2 h. After cooling, the samples were analyzed by graphite furnace atom absorption spectrometry (Perkin–Elmer AAanalyst 600), using the following conditions: wavelength 348.3 nm, pre-treatment temperature 1400 °C, atomization at 2100 °C for 3 s. A calibration curve was obtained measuring different dilutions of a Titrisol iron solution (1 g Fe L^{-1}; Merck). The repeatability of copper and iron determinations, indicated in Table 1, was calculated by 80 and 9 duplicate analyses of butter samples, respectively.

2.6. Analysis of fatty acid composition: Short chain fatty acids, unsaturated fatty acids and conjugated linoleic acid isomers

The butter was dissolved in hexane and the glycerides were trans-esterified to the corresponding fatty acid methyl esters by a solution of potassium hydroxide in methanol (Standard 15885, ISO, 1997). The fatty acids were separated and quantified by GC as described by Collomb and Bühler (2000). Isomers of CLA were analyzed and quantified by Ag^+-HPLC according to Collomb, Sieber et al. (2004) and Collomb, Sollberger et al. (2004). The repeatability of the fatty acid determination was below 0.49 g kg^{-1} for all fatty acids except for C4 (0.65 g kg^{-1}), C14 (0.77 g kg^{-1}), C16 (1.89 g kg^{-1}), C18 (0.66 g kg^{-1}) and C18:1c9 (1.34 g kg^{-1}) as determined by 35 duplicate analyses of butter. The repeatability of the CLA isomers was below 0.15 g kg^{-1} as determined by 35 duplicate analyses of butter.

2.7. Extraction and analysis of the odour-active compounds by gas chromatography–mass spectrometry coupled with olfactometry

Samples of butter (9 g) were placed in a 20 mL headspace (HS) vial sealed with a Teflon-lined silicone rubber septum. The HS-solid phase microextraction (SPME) analysis was carried out using a Combi PAL Autosampler (CTC Analytics, Zwingen, Switzerland) and a 2 cm Divinylbenzene/Carboxen/Polydimethylsiloxane fibre (DVB/CAR/PDMS, Supelco, Bellefonte, PA, USA). The volatile compounds of butter were allowed to equilibrate for 45 min at 45 °C, and were then adsorbed on the fibre for 45 min at 45 °C. An Agilent 5890 Series II gas chromatograph (Agilent Technologies), equipped with an HP-5MS column, 30 m ×; 0.25 mm ×; 0.25 μm (Agilent Technologies), was used for the analysis with simultaneous flame ionization detection (FID), mass selective detector (MSD; HP 5971A) and olfactometric detector (Sniffer 9000 system, Brechbühler, Schlieren, Switzerland). The three detectors were mounted in parallel by splitting the flow at the end of the capillary column into three streams.

The MSD operated in the scan mode at 2.9 scans s^{-1} (m/z 29–350) at 70 eV. The GC/O analyses were carried out by two trained sniffers, who described the odours perceived in the effluent at the sniffing port. The oven temperature was programmed at 35 °C for 5 min then increased by 5 °C min^{-1} to 240 °C. Helium was used as the carrier gas at a constant flow of 2.40 mL min^{-1}. The analyses were conducted in duplicate. The repeatability of the method, including extraction, injection, separation and detection, was tested by analyzing the same butter sample in triplicate. The coefficients of variation (CV %) ranged from 2.25% to 8.96% using the same fibre unit.

Identification of the volatile compounds was based on a comparison of the mass spectra with the Wiley 138.L mass spectra library (John Wiley & Sons, Inc., Hoboken, NJ, USA), linear retention indices (LRI) and odour perception with authentic reference compounds. Linear retention indices were calculated by running a C5 to C20 *n*-alkane series under the same working conditions. The LRI were also compared with published data.

2.8. Sensory analysis

2.8.1. Method development

Ten trained panelists of the internal panel of the Agroscope Liebefeld-Posieux (ALP) Research Station participated in the sensory study. They were selected based on their experience in milk product profiling and on their availability to participate in testing sessions over a period of 8 weeks. Training sessions were performed with butter samples from the market to familiarize the judges with the products, to develop a preliminary list of sensory attributes and to establish a testing procedure. In order to obtain a vocabulary including terms that describe possible off-flavours, one part of the market samples were previously artificially oxidized by exposure to fluorescence light (Philips TL40 W/33RS, 2000 lx uniformly at the butter surface) for 6 h at 6 °C.

This preliminary work led to a standardized sensory language for the description of oxidative changes in butter during storage. Eleven attributes, listed in Table 2, were defined and divided into three categories: odour (orthonasal perception), texture (spreadability) and flavour (intended as taste and retronasal odour perception in this study). Each attribute was provided with a quantitative reference for concept alignment. As for the testing procedure, the panelists were instructed to evaluate the odour intensity of the samples first. Then, they were asked to determine the spreadability by spreading 1/3 of 10 g of butter on a filter paper. Finally, they assessed the flavour intensity during sample melting in the mouth. For this last part, an amount of butter the size of a cherry stone was used. After the evaluation, judges were asked to expectorate the sample and to rinse the mouth with a mild black tea. Black tea was chosen for rinsing the mouth between sample evaluation, since previous studies performed at ALP (unpublished work) on palate cleanser for cheese and butter indicated that it could best rinse the palate from the fat.

2.8.2. Sensory sample description during storage

The UFA/CLA enriched butter and the conventional butter samples (both refrigerated and frozen), manufactured in May 2006, were described using the descriptive analysis technique (Stone & Sidel, 2004). During two training sessions, the standardized language, developed in the preliminary phase, was checked for completeness. Moreover, the panel was further familiarized with the testing procedure. For formal testing, samples were presented simultaneously in randomized order in 3-digit coded Petri dishes at a temperature of 14 ± 2 °C. The intensity of attributes was assessed on 10-point unstructured intensity scales anchored on the left with "not" and on the right with "very". A mild black tea (0.6 g tea leaves L^{-1} water, 50–52 °C) and untoasted white toast bread without crust were served to neutralize the palate between samples. The testing sessions were conducted in separate booths under normal light conditions. All the panelists evaluated each sample twice, corresponding to 1, 2, 4, 6 or 8 weeks of storage at 6 °C and at −20 °C.

Table 2
Standardized sensory attributes for the description of oxidative changes in butter during storage

Attribute	Reference	Intensity on a 10-point scale
Odour		
Creamy	Full fat cream (35% fat)pasteurized, 18 ± 2 °C	9
Cooked milk	Full fat milk (3.5% fat), UHT, 18 ± 2 °C	10
Rancid	Butyric acid, 0.5% in H_2O, 18 ± 2 °C	10
Oxidized, metallic	Sweet cream butter exposed to fluorescence light (2000 lx uniformly at the butter surface) for 6 h at 6 °C, served at 14 ± 2 °C	10
Texture		
Spreadability	Sweet cream butter, 14 ± 2 °C	9
Flavour		
Sweet taste	Sweet cream butter, 14 ± 2 °C	6
Sour taste	Lactic acid, 0.2% in H_2O, 18 ± 2 °C	9
Creamy	Full fat cream (35% fat)pasteurized, 18 ± 2 °C	10
Cooked milk	Full fat milk (3.5% fat), UHT, 18 ± 2 °C	10
Rancid (by smelling)	Butyric acid, 0.5% in H_2O18 ± 2 °C	10
Oxidized, metallic	Sweet cream butter exposed to fluorescence light (2000 lx uniformly at the butter surface) for 6 h at 6 °C, served at 14 ± 2 °C	10

2.9. Statistics

Analysis of variance (ANOVA) was carried out for fatty acids and CLA isomers, considering butter type and storage as main effects. Twenty-seven volatile compounds, listed in Table 3, were selected on the basis of GC/MS detection, to carry out an ANOVA using absolute MS responses (arbitrary units) and considering butter type (UFA/CLA enriched and conventional), storage time and period of production (May and September) as factors. All the volatile compounds considered were also perceived by GC/O, except hexane and 2-butanone.

Three-way analysis of variance was performed with the sensory data on the main factors of butter type, storage time and storage conditions (refrigerated and frozen) with interactions by attribute. Differences among 1-week-old samples were assessed with three-way analysis of variance on factors sample, judge and replications with interactions. Significant differences between the samples were established using Fisher's least significant difference (LSD) at the 5% level.

A regression analysis (GLM, General Linear Model) was performed using selected GC/MS/O data and sensory data. For the GC/MS/O data the same compounds were taken as for the ANOVA analysis (Table 3). For the sensory data, the mean values of odour and flavour intensities for the attributes creamy, cooked milk, rancid and oxidized-metallic were considered. Systat for Windows version 11 (Systat Software, Inc., San Jose, CA, USA) was used for the statistical analyses.

3. Results and discussion

3.1. Microbial parameters

The results of the microbiological analyses are shown in Table 4. These parameters indicated a good microbiological quality of the fresh manufactured samples. After 6 weeks of storage at 6 °C, mesophilic microrganisms and lipolytic bacteria were significantly higher in UFA/CLA butter than in conventional samples. This could be due to the slightly higher moisture content of UFA/CLA butter, as indicated in Table 1. Nevertheless, these samples conformed to the microbiological quality parameters defined by the Swiss Federal Hygiene Regulation (2004).

3.2. Chemical composition

The chemical compositions of fresh UFA/CLA enriched butter and conventional butter are shown in Table 1. The fat content of UFA/CLA butter was lower than in conventional butter, especially in butter produced in September.

A decrease of the fat content in milk and dairy products was also observed by other authors when sources of UFAs, especially linoleic acid, were supplemented with the diet of dairy cows (AbuGhazaleh et al., 2004; Chilliard, Ferlay, & Doreau, 2001). Banks, Clapperton, and Girdler (1990) associated the fat decrease in milk enriched with UFA with an increase in *trans*-C18:1 FA. The amount of *trans*-FA present in the mammary gland influences the amount and type of

Table 3
Significant effects in the ANOVA on the volatile composition of UFA/CLA[a] enriched and conventional butter

Compound[b]	LRI[c]	Significance of effects in the ANOVA[d]		
		Butter type	Storage[e]	Month of production
Fatty acids				
Propanoic acid	678	***	***	***
Acetic acid	723	*	***	***
Butanoic acid	791	***	***	***
3-Methyl butanoic acid	863	***	***	***
Hexanoic acid	986	***	***	***
Alcohols				
Ethanol	470	***	***	***
1-Butanol	667	NS	NS	NS
2-Methyl-1-butanol	754	NS	***	***
1-Hexanol	870	**	***	**
Aldehydes				
3-Methylbutanal	652	**	***	**
Pentanal	700	***	***	**
Hexanal	803	NS	*	NS
Heptanal	907	***	***	**
Nonanal	1114	*	***	***
Ester				
Hexanoic acid methyl ester	927	NS	***	***
Hydrocarbons				
2-Methylpentane	570	NS	***	***
Hexane	610	NS	***	***
Toluene	772	***	***	**
(*E*)-2-Octene	806	***	***	***
Ketones				
2-Propanone	522	***	***	***
2,3-Butanedione	596	**	***	**
2-Butanone	607	***	***	***
2-Pentanone	687	***	***	***
2-Heptanone	892	NS	***	***
2-Nonanone	1090	***	***	NS
2-Undecanone	1295	***	***	***
Lactone				
δ-Hexalactone	1095	***	***	*

[a] Unsaturated fatty acids/conjugated linoleic acid.
[b] Compound selected by larger peak area found by gas chromatography/mass spectrometry.
[c] LRI, linear retention index using the DB-5MS column.
[d] NS, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.
[e] 1–8 weeks of storage at 6 °C.

FA produced. However, the fat decrease mechanism by *trans*-C18:1 is not yet known (AbuGhazaleh et al., 2003).

α-Tocopherol and iron contents were significantly higher in UFA/CLA butter. The results showed that retinol and copper were also higher in UFA/CLA samples but the difference was not significant. The higher content of these components in the enriched butters is probably related to the cows' feed supplemented with sunflower seeds, which contain carotenoids, vitamin E, copper and iron (Sauvant, Perez, & Tran, 2004; Souci, Fachmann, & Kraut, 2000). All these parameters remained constant during storage, except for α-tocopherol which was significantly lower, in both butter types, after 6 weeks of storage.

Table 4
Microbiological parameters of UFA/CLA[a] enriched and conventional butter (fresh and after 6 weeks of storage at 6 °C)

	UFA/CLA[a] enriched butter		Conventional butter	
	Fresh	6 Weeks	Fresh	6 Weeks
Mesophile microorganisms (cfu g^{-1})	260	6000	280	530
Lipolytic bacteria (cfu g^{-1})	<10	650	< 10	<10

[a] Unsaturated fatty acids/conjugated linoleic acid.

3.3. Fatty acid composition of butter

The UFA/CLA enriched butter and conventional butter had a significantly different fatty acid (FA) composition. Table 5 shows the FA composition of fresh butter and in butter after 8 weeks of storage at 6 °C. Compared with conventional butter, the UFA/CLA enriched butter had significantly higher concentrations of monounsaturated FAs (MUFAs) (+30%), polyunsaturated FAs (PUFAs) (+33%), including CLA (+60%), C18:1 *trans*-FA (+44%), C18:2 *trans* FA without CLA (+31%) and omega-6 FA (+31%). Storage of butter significantly reduced C18:1t9 and C18:1t12, the other FA were not influenced by the storage of butter.

The concentrations of the CLA isomers in UFA/CLA enriched butter and in conventional butter are shown in Table 6. The CLA isomers c9t11 and t7c9 were significantly higher in UFA/CLA enriched butter. The concentration of the most important CLA (c9t11) amounted to about 88% of the total CLAs. The CLAs were very stable during the storage period. These results are in agreement with previous studies on oxidative stability of CLA enriched dairy products during storage (Campbell et al., 2003; Lynch et al., 2005; Ryhänen et al., 2005).

3.4. Odour-active compounds in unsaturated fatty acid/conjugated linoleic acid enriched and in conventional butter

In total 68 odour-active compounds were identified in the various butter types. The number of perceived compounds increased during storage, particularly in UFA/CLA enriched butter, indicating the development of secondary products from lipid oxidation.

Table 7 summarizes the odour-active compounds found in UFA/CLA enriched and conventional butter during 8 weeks of storage at 6 °C. The odour profiles of fresh butter and butter after 1 and 2 weeks of storage were practically identical and, for this reason, they are both qualified as "fresh butter" in Table 7. The fresh UFA/CLA butter and the fresh conventional butter had a very similar odour profile, characterized by creamy (2,3-butanedione), milky (2-nonanone), fatty (2-methyl-1-butanol), soapy (2-heptanone and nonanal) and sulphur (dimethyl disulphide) notes. The compounds 2,3-butanedione, 2-methyl-1-butanol and dimethyl disulphide were perceived with higher intensity only in the fresh butter. The amount of 2,3-butanedione probably decreases by reduction to acetoin and further to 2-butanone and 2-butanol (Mallia, Fernández-García, & Bosset, 2005). Dimethyl disulphide also disappeared during storage. Similar findings were observed by Shooter, Jayatissa, and Renner (1999). Enriched and conventional butters kept for 2 and 6 weeks at −20 °C were also analyzed by GC/O and compared with the samples stored 2 and 6 weeks at 6 °C. Samples kept at −20 °C showed an odour profile identical to the one of fresh butter.

After 6–8 weeks of storage, UFA/CLA enriched butter was characterized by cheesy and fatty notes, mainly due to butanoic and 3-methyl butanoic acid, by fruity and green odours, probably originating from ethanol, (*E*)-2-hexenol, heptanal and nonanal, and by creamy and milky notes, due to 2-pentanone, 2,3-pentanedione, 2-heptanone, 2-nonanone and 2-undecanone. Esters, like butanoic acid methyl ester and acetic acid 2-phenylethyl ester with fruity notes, were found only after 6 weeks of storage and mostly in UFA/CLA samples. A metallic smell, attributed to (*E,E*)-2,4-nonadienal and *trans*-4,5-epoxy-2-decenal, was intensively perceived in the UFA/CLA enriched butter. (*E,E*)-2,4-Nonadienal was already described as "fatty" in butter oil stored for 42 days in the dark and at room temperature (Widder, 1994). *Trans*-4,5-epoxy-2-decenal was found as an important odour-active compound in puff-pastries prepared with butter (Gassenmeier & Schieberle, 1994). Both aldehydes arise from the auto-oxidation of linoleic acid (Widder, 1994). Interestingly, two unknown compounds (RI = 918 and 1591),

Table 5
Fatty acid composition (g 100 g^{-1} fat) of fresh and 8 weeks stored UFA/CLA[a] enriched and conventional butter

Fatty acid	Fresh		8 Weeks of storage		Significance of the effects in the ANOVA[b]	
	UFA/CLA	Conventional	UFA/CLA	Conventional	Butter type	Storage
C4:0	2.86	3.28	2.80	3.30	NS	NS
C6:0	1.43	2.10	1.42	2.10	**	NS
C8:0	0.72	1.20	0.70	1.21	*	NS
C10:0	1.50	2.77	1.47	2.76	**	NS
C12:0	1.72	3.13	1.70	3.12	**	NS
C14:0	6.80	9.96	6.80	9.98	**	NS
C15:0	0.83	0.90	0.84	0.87	NS	NS
C16:0	19.48	25.25	19.50	25.43	**	NS
C16:1 t	0.27	0.15	0.27	0.14	*	NS
C16:1 c[c]	1.26	1.28	1.26	1.30	NS	NS
C18:0	10.37	8.13	10.42	8.20	**	NS
C18:1 t6–8	0.35	0.12	0.30	0.14	NS	NS
C18:1 t9	0.66	0.32	0.59	0.24	**	**
C18:1 t10–11	4.50	2.53	4.56	2.63	**	NS
C18:1 t12	0.42	0.22	0.40	0.20	*	**
C18:1 t13–14 + c6–8	1.15	0.80	1.16	0.80	**	NS
C18:1 c9	24.02	16.98	23.90	16.97	**	NS
C18:1 c11	0.95	0.76	0.95	0.77	*	NS
C18:1 c12	0.60	0.34	0.60	0.35	*	NS
C18:1 t16 + c14	0.57	0.33	0.50	0.36	NS	NS
C18:2 c9t13 + (t8c12)	0.37	0.20	0.36	0.20	*	NS
C18:2 c9t12+(c,c-MID + t8c13)	0.39	0.27	0.36	0.26	NS	NS
C18:2 t11c15 + t9c12	0.30	0.24	0.26	0.24	NS	NS
C18:2 c9c12	1.60	1.20	1.55	1.20	*	NS
C18:3 c9c12c15	0.45	0.50	0.46	0.50	NS	NS
Sum saturated FA[d]	48.55	59.50	48.54	59.74	**	NS
Sum C18:1[e]	33.43	22.52	33.12	22.57	*	NS
Sum C18:2[f]	4.92	2.96	4.70	2.92	*	NS
Sum unsaturated FA[g]	41.50	28.94	40.97	28.90	*	NS
Sum monounsaturated[h]	35.82	25.13	35.50	25.16	*	NS
Sum polyunsaturated[i]	5.67	3.80	5.47	3.73	*	NS
Sum C18:1 t[j]	7.74	4.33	7.57	4.39	*	NS
Total CLA (GC)[k]	1.98	0.82	1.98	0.82	*	NS
Sum C18:2t without CLA t[l]	1.26	0.87	1.14	0.85	*	NS
Trans total without CLA t[m]	9.31	5.40	9.02	5.40	**	NS
Sum omega-3[n]	0.96	0.98	0.92	0.94	NS	NS
Sum omega-6[o]	3.23	2.23	3.07	2.20	*	NS

[a] Unsaturated fatty acids/conjugated linoleic acid.
[b] NS, not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
[c] t, *trans*; c, *cis;* MID, methylene interrupted diene.
[d] C4 bis C10, C12, C12 iso, C12 aiso, C13 iso, C14, C14 iso, C14 aiso, C15, C15 iso, C16, C16 iso, C16 aiso, C17, C17 iso, C17 aiso, C18, C19, C20 and C22.
[e] C18:1 -t4, -t5, -t6–8, -t9, -t10–11, -t12, -t13–14 + -c6–8, -c9, -c11, -c12, -c13, -16 + c14.
[f] C18:2 – ttNMID (non methylene interrupted diene), -t9,t12, -c9,t13 + -t8,c12, -c9,t12 + -c,c-MID + -t8,c13, -t11,c15 + -t9,c12, -c9,c12, -c9,c15, -c9,t11 + -t8,c10 + -t7,c9, -t11,c13 + -c9,c11, -t9,11.
[g] C10:1, C14:1 ct, C16:1 ct, C17:1 t, ∑C18:1, ∑ C18:2, C20:1 t, C18:3c6,c9,c12, C20:1c5, C20:1c9, C20:1c11, C18:3c9,c12,c15, C18:2c9,t11 + t8,c10 + t7,c9, C18:2 t11c13 + c9,c11, C18:2t9,t11, C20:2c,c (n-6), C20:3 (n-6), C20:3 (n-3), C20:4 (n-6), C20:5 (EPA) (n-3), C22:5 (DPA) (n-3), C22:6 (DHA) (n-3).
[h] C10:1, C14:1 ct, C16:1 ct, C17:1 ct, ∑C18:1, C20:1 t, C20:1c5, C20:1c9, C20:1c11.
[i] C18:2, C18:3 c6c9c12, C18:3 c9c12c15, C20:2 c,c (n-6), C20:3 (n-3), C20:4 (n-6), C20:5 (EPA) (n-3), C22:5 (DPA) (n-3), C22:6 (DHA) (n-3).
[j] C18:1 t4, C18:1 t5, C18:1 t6-8, C18:1 t9, C18:1 t10–11, C18:1 t12, C18:1 t13–14 + c6–8.
[k] ∑(C18:2 c9t11 + t8c10 + t7c9) + (C18:2 t11c13+c9c11), C18:2 t9t11.
[l] C18:2 – ttNMID, -t9,t12, -c9,t13 + -t8,c12, -c9,t12 + -c,c-MID + -t8,c13, -t11,c15 + -t9,c12.
[m] C14:1 t, C16:1 t, C17:1 t, C20:1 t, C18:1 trans + C18:2 trans (without CLA trans).
[n] C18:2 t11c15 + C18:2c9c15, C18:3 c9c12c15, C20:3n-3, C20:5, C22:5 and C22:6.
[o] C18:1 t12, C18:1 c12, C18:2 t9t12, C18:2 c9t12 + (c,c-MID + t8c13), C18:2c9c12, C18:3 c6c9c12, C20:2 cc, C20:3n-6 and C20:4n-6.

with chemical and fatty odours, were detected by the sniffers only in the UFA/CLA enriched butter.

The ANOVA carried out on 27 volatile compounds, of which 25 were odour-active, showed significant effects concerning butter type, storage time and period of production (Table 3). The effect most influencing the results was the storage period (1–8 weeks at 6 °C) regarding 26 volatile compounds. After 6 weeks of storage, propanoic, butanoic and 3-methyl butanoic acids, ethanol, pentanal, hexanal, heptanal, nonanal, and hexanoic acid methyl ester significantly increased in UFA/CLA butter, as well as hexane, 2-propanone, 2-butanone and 2-heptanone. Only 2-octene was significantly higher in conventional butter after 6 weeks of storage.

The period of production (May and September) also affected the volatile composition of butter. Both 1-hexanol (soapy) and 2-methyl-pentane (chemical) were found only in butter produced in May, whereas (*E*)-2-octene (mushroom-like) and 2-butanone were found in butter manufactured in September. These differences may be due to the seasonal variation, resulting in a different chemical butter composition and flavour formation.

Twenty odour compounds were found at significantly higher concentrations in UFA/CLA butter; among these were butanoic and hexanoic acids, 2-pentanone, 2-nonanone, 2-undecanone, pentanal, heptanal, toluene and (*E*)-2-octene. The more elevated intensities of aldehydes, ketones and hydrocarbons in UFA/CLA enriched butter indicated a higher oxidation rate in these samples. Fig. 1 shows heptanal as an example for the increase in signal intensity of the volatile aldehydes in UFA/CLA enriched butter during storage, in comparison with conventional samples. Hexanal, a reported indicator of lipid oxidation in food (Christensen & Hølmer, 1996), was surprisingly not significantly higher in UFA/CLA

Table 6
PUFA[a] contents and CLA[b] isomers (g 100 g^{-1} fat) of fresh and 8 weeks stored UFA/CLA[c] enriched and conventional butter

18:2 CLA	Fresh		8 Weeks of storage		Significance of the effects in the ANOVA[d]	
	UFA/CLA	Conventional	UFA/CLA	Conventional	Butter type	Storage
C18:2 t12 t14	0.014	0.013	0.014	0.013	NS	NS
C18:2 t11 t13	0.025	0.024	0.024	0.024	NS	NS
C18:2 t10 t12	0.013	0.003	0.013	0.003	NS	NS
C18:2 t9 t11	0.015	0.010	0.015	0.010	NS	NS
C18:2 t8 t10	0.004	0.003	0.004	0.004	NS	NS
C18:2 t7 t9	0.007	0.004	0.005	0.004	NS	NS
C18:2 t6 t8	0.001	0.001	0.001	0.001	NS	NS
C18:2 c/t 12, 14	0.003	0.004	0.004	0.004	NS	NS
C18:2 t11 c13[e]	0.040	0.020	0.040	0.020	NS	NS
C18:2 c11 t13	0.003	0.002	0.002	0.002	NS	NS
C18:2 t10 c12	0.010	0.005	0.010	0.004	NS	NS
C18:2 c9 t11[e]	1.800	0.730	1.808	0.733	**	NS
C18:2 t8 c10	0.035	0.020	0.038	0.014	NS	NS
C18:2 t7 c9[e]	0.080	0.040	0.081	0.040	**	NS
∑C18:2 c9t11,t8c10,t7c9	1.915	0.790	1.927	0.787	**	NS
Total CLA	2.050	0.880	2.060	0.876	**	NS

[a] Polyunsaturated fatty acids.
[b] Conjugated linoleic acid.
[c] Unsaturated fatty acids/conjugated linoleic acid.
[d] NS, not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
[e] The most important CLA isomers in butter.

Table 7
Odour-active compounds of UFA/CLA[a] enriched butter and conventional butter during 8 weeks of storage at 6 °C, as detected by GC/MS/O[b]

Compound	LRI[c]	Odour descriptor	Identification[d]	Fresh/1, 2 weeks[e]		4 Weeks		6 Weeks		8 Weeks	
				UFA/CLA	CONV[f]	UFA/CLA	CONV[f]	UFA/CLA	CONV[f]	UFA/CLA	CONV[f]
Acids											
Propanoic acid	673	Fatty	MS, PI, R					++		++	
Butanoic acid	807	Cheesy, rancid	MS, PI, R					+++	+	+++	++
2-Methyl butanoic acid	826	Rancid, yeast	MS, PI, R	+	+	+	+	++	++		
3-Methyl butanoic acid	865	Fatty	MS, PI, R					++	++	+++	+++
Hexanoic acid	1003	Soapy, fat	MS, PI, R	+	+	++	+	++	++	++	++
Octanoic acid	1284	Cream, whey	MS, PI, R					++	++	++	++
Alcohols											
Ethanol	440	Fruity/alcohol	MS, PI, R	++	+	++	+	+++	++	+++	++
2-Methyl-3-buten-2-ol	626	Solvent/green	PI, R	+	+	+	+	++	++	++	++
1-Butanol	667	Chemical	MS, PI, R					+++	+	+++	++
2-Methyl-1-butanol	750	Fatty	MS, PI, R	++	++	+	+	+	+	+	+
1-Pentanol	765	Roasted	MS, PI, R			+	+	+	+	++	++
2,3-Butanediol	810	Fruity	MS, PI, R			+	+	+	+	+	+
(*E*)-2-Hexenol	852	Fruity/cheesy	MS, PI, R	+	+	++	+	+++	++	+++	++
(Z)-3-Hexenol	856	Orange	MS, PI, R					++	++	++	++
1-Hexanol	870	Soapy	MS, PI			+		+	+	++	++
3-Heptanol	886	Flower	MS, PI, R				+	+	++	+	++
2-Pentanol	950	Green, mould	MS, PI, R	+	+			+	+	++	++
2-Heptanol	975	Green, fatty	MS, PI, R	+	+	+				+	+
1-Octen-3-ol	991	Mushroom	PI, R	+	+	+	+	+	+	++	++
1-Octanol	1076	Mushroom	MS, PI, R	+	+	+		+++	+	+++	++
Aldehydes											
2-Methylbutanal	643	Creamy	MS, PI, R			+	+	+	+	+++	+++
3-Methylbutanal	652	Fruity/malty	MS, PI, R	+	++	+	++	+	++	+	++
2-Methylpropanal	656	Chemical, fat	MS, PI, R					+	+	+	+
Pentanal	699	Fatty/perfume	MS, PI, R			++	+	++	++	++	++
Hexanal	801	Green, metallic	MS, PI, R	+	+	++	+	+++	++	+++	++
(*E*)-2-Hexenal	840	Soapy, fat, green	MS, PI, R	+	+	+	+	++	++	+++	+++
Heptanal	909	Green/fatty	MS, PI, R	+	+	++	++	+++	++	+++	++
Benzaldehyde	960	Roasted, almond	MS, PI, R			+	+	+	+	+	+
(*E*)-2-Octenal	1058	Flower	MS, PI, R					+++	++	+++	++
Nonanal	1121	Soapy, milk	MS, PI, R	++	+	+++	++	+++	++	+++	++
(*E*)-2-Nonenal	1170	Green	PI, R	+	+	+	+	++	++	++	++
(*Z*)-2-Nonenal	1183	Fruit	PI	+	+	+	+	++	++	++	++
(*E,Z*)-2,4-Nonadienal	1196	Butter	PI, R			+	+	++	++	+++	+++
(*E,E*)-2,4-Nonadienal	1219	Metallic, soapy	PI, R		+	+	+	+++	++	+++	++
(*E,E*)-2,4-Decadienal	1319	Coffee	PI, R							++	++
(*E*)-2-Undecenal	1350	Animal, green	MS, PI, R		+	+	+	++	+	++	+
trans-4,5-Epoxy-2-decenal T	1380	Fatty, metallic	MS, PI	+	+	+	+	+++	+	+++	+
Tridecanal	1504	Oily	MS, PI, R					+++	+	+++	+

(*continued on next page*)

Table 7 (*continued*)

Compound	LRI[c]	Odour descriptor	Identification[d]	Fresh/1, 2 weeks[e]		4 Weeks		6 Weeks		8 Weeks	
				UFA/CLA	CONV[f]	UFA/CLA	CONV[f]	UFA/CLA	CONV[f]	UFA/CLA	CONV[f]
Esters											
Butanoic acid methyl ester	723	Fruity	MS, PI, R					++	+	++	+
Hexanoic acid methyl ester	924	Fruity	MS, PI, R					+++	+	+++	+
Acetic acid 2-phenylethyl ester	1257	Flower	MS, PI, R	+				++	+	++	+
Furanones											
Ethyl furanone	1400	Coffee	MS, PI, R	+	+			++	+	++	+
Hydrocarbons											
2-Methyl-pentane	559	Chemical/sweet	MS, PI, R	+	+	+	+	++	+	++	+
Toluene	773	Chemical	MS, PI, R	++	+	++	+	+++	++	+++	++
(*E*)-2-Octene	806	Mushroom	MS, PI, R	++	+	++	+	++	+	++	+
Ketones											
2-Propanone	503	Solvent	MS, PI, R	+	+	++	++	+++	++	+++	++
2,3-Butanedione	596	Creamy	MS, PI, R	++	++	+	+	+	+	+	+
2-Pentanone	687	Creamy	MS, PI, R					+++	+	+++	++
2,3-Pentanedione	709	Chocolate, soapy	MS, PI, R				+	+++	++	+++	++
3-Hydroxy-2-butanone	719	Buttery, creamy	MS, PI, R					+	+	+	+
2-Heptanone	892	Soapy, fatty	MS, PI, R	++	+	++	+	+++	++	+++	++
1-Octen-3-one	986	Mushroom, earthy	PI, R	++	++	++	++	+++	++	+++	++
2-Nonanone	1095	Milk	MS, PI, R	++	++	++	++	+++	++	+++	++
2-Undecanone	1295	Green, nutty	MS, PI, R	++	+	++	+	+++	++	+++	++
Lactones											
δ-Hexalactone	1105	Fruity, fatty	MS, PI, R	+	+	+	+	+++	+++	+++	+++
γ-Nonalactone	1366	Fruity	MS, PI, R	+	+	++	+	+++	++	+++	++
γ-Decalactone	1464	Fruit	MS, PI, R		+	+	+	++	++	++	++
δ-Decalactone	1522	Flower	MS, PI, R	+	+	+	+	++	++	+++	+++
δ-Undecalactone	1610	Biscuit, flower	MS, PI, R	+	+	+	+	++	++	++	++
Sulphur compounds											
Dimethyl disulfide	742	Sulphur/animal	PI, R	++	++						
Dimethyl sulfone	927	Fatty, sulphur	MS, R		++	++	++	++	++		
Dimethyl trisulfide	976	Garlic	PI, R	+	++	+	++				
Terpenes											
α-Pinene	935	Fresh	MS, PI, R	+	+	++	+	++	+	++	+
Limonene	1024	Citrus, green	MS, PI, R	+	+	++	+	++	++	++	++
Unknown compounds											
Unknown	819	Oxidized fat	–			+	+	+++	+++	+++	+++
Unknown	918	Chemical, fatty	–	+	+		+	+++		+++	
Unknown	981	Burnt	–					++	++	++	++
Unknown	1228	Sulphur	–			+	+	+	+	+	+
Unknown	1332	Fatty, coffee	–	+	+			++	+++	++	++
Unknown	1440	Fatty, fruit	–					++	++	++	++
Unknown	1591	Fat	–		+			+++		+++	

[a] Unsaturated fatty acids/conjugated linoleic acid.
[b] Gas chromatography/mass spectrometry/olfactometry.
[c] Linear retention index using a DB-5MS column.
[d] Identification, MS, mass spectra comparison using Wiley library; PI, comparison with published LRI; R, comparison with LRI and odour of authentic standards injected; tentatively identified (T).
[e] +Weak, ++medium, +++strong odour intensity perceived by two trained panelists.
[f] Conventional butter.

butter compared with conventional butter. The compound 3-methylbutanal was the only one significantly higher in the conventional butter, perhaps already present in milk.

The increase of aldehydes, ketones and hydrocarbons after refrigerated storage is also reported by other authors. Christensen and Hølmer (1996) indicated an increase of these compounds after 14 weeks of storage at 4 °C in cultured butter containing 1–2% sodium chloride. A recent study (Lozano, Miracle, Krause, Drake, & Cadwallader, 2007) showed an increase of ethyl acetate, hexanal, 2-heptanone, butanoic acid and lactones after 6 months of storage at 4 °C in salted butter. The increase of aroma compounds observed after the varied periods of storage might be due to the diverse types of butter analyzed in these studies: sweet cream, cultured, salted or unsalted butter.

3.5. Sensory analysis

The fresh (1-week old) UFA/CLA enriched butter showed a higher spreadability and a less intense cooked milk aroma and creamy odour than the fresh conventional butter (Fig. 2). No significant differences were found regarding the odour of the samples, with the attributes of creamy and cooked milk odour showing medium intensities. The attributes of rancid odour/aroma and oxidized odour/aroma were of very low intensity in the fresh samples. The UFA/CLA enriched butter was significantly more easily spreadable than the conventional one. This is in line with the findings from Ryhänen et al. (2005). In their study, differences in spreadability between CLA enriched butter and control butter were observed and were explained by a softer

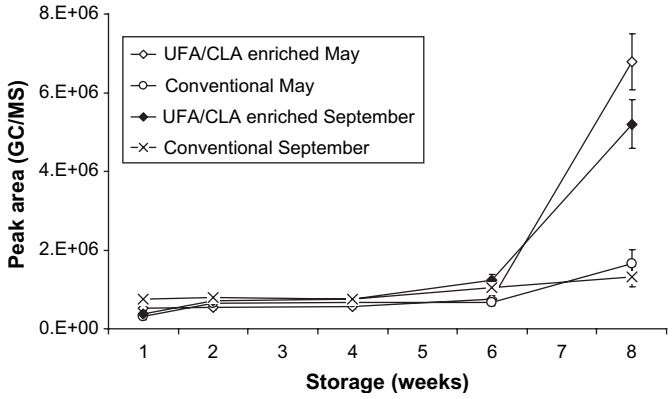

Fig. 1. Heptanal content development during storage at 6 °C in unsaturated fatty acid/conjugated linoleic acid (UFA/CLA) enriched and conventional butter, produced in May and September.

texture of the CLA enriched butter, due to the unsaturated fatty acids present.

During the 8 weeks of storage, the UFA/CLA enriched butter and the conventional one aged in a very similar way. In fact, the results from ANOVA showed that most of the significant effects were related to aging and not to storage conditions or butter type. Storage time had a significant impact on the rancid and oxidized odour and on all flavour parameters. Rancid odour/aroma and oxidized odour/aroma significantly increased during storage in both UFA/CLA and conventional butter. The rise of the oxidized/rancid notes is in agreement with the GC/O findings. Compared with results from Krause, Miracle, Sanders, Dean, and Drake (2008) and Lozano, Miracle, Krause, Drake, and Cadwallader (2007), who observed the development of a "refrigerator/stale" flavour in butter only after 6 months of storage at 4–5 °C, in the present study the development of off-flavours was observed after a shorter period of time. Differences may be explained by the different butter types, manufacturing, sample sizes (bulk or sticks) and packaging.

The cooked milk aroma significantly decreased over time until 6 weeks of storage. A decrease of cooked aroma during storage was also observed by Lozano et al. (2007), who attributed this fact to a decrease of sulphur compounds during storage. Their observations agree with the GC/MS/O findings of the present study, which indicated a decrease of dimethyl disulphide and dimethyl trisulphide after 2 and 4 weeks of storage, respectively. However, the cooked milk aroma intensities increased after 8 weeks and its intensity in the UFA/CLA butter was similar compared with the beginning of the test. This result may be explained by the presence of distinct rancid and oxidized notes, which could have influenced the evaluation of the cooked milk parameter.

Sweetness also significantly decreased during storage. The changes in sourness are difficult to interpret because this was lower in the first 2 weeks, increasing in the following weeks and decreasing again towards the end of the testing period. Fig. 3 shows the sensory profile of UFA/CLA and conventional samples after 8 weeks of storage at 6 °C.

The storage conditions (refrigeration or freezing) only significantly influenced the rancid odour, which was significantly more intense in the refrigerated samples than in the frozen ones. In a storage study performed on commercial salted sweet cream butter, Lozano et al. (2007) reported a more pronounced increase of a "refrigerator/stale" flavour in the refrigerated samples than in frozen ones. In the present study, significant differences were observed for the rancid odour only, and not for the rancid flavour. However, the periods of storage also differ considerably between the study of Lozano et al. (2007), Krause et al. (2008) and the present one. Finally, the butter type was found to significantly impact spreadability and creamy aroma. Spreadability and creamy aroma were always higher in the UFA/CLA enriched butter than in the conventional one.

3.6. Statistical correlation between analytical and sensory analysis

No direct correlation was found between GC/MS/O data and sensory analyses. The technique of GC/MS/O evaluates the odorants individually after GC separation, whereas during sensory analysis

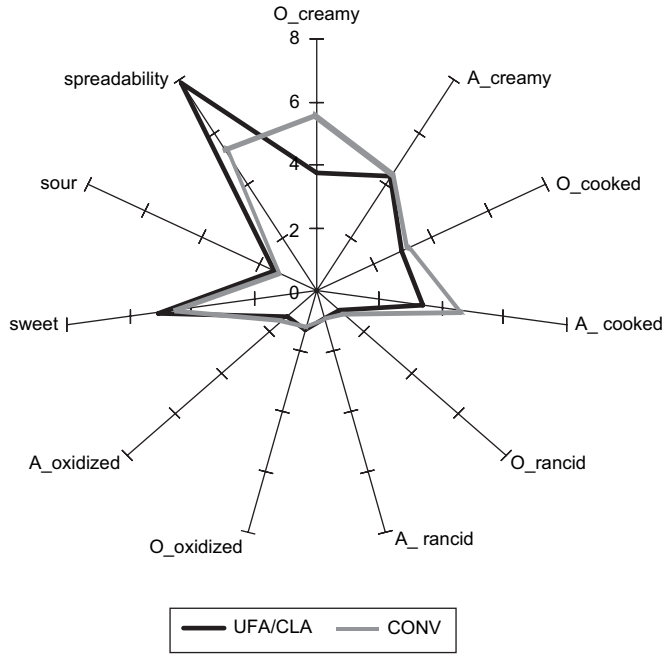

Fig. 2. Odour (O), aroma (A) and texture attributes of the unsaturated fatty acid/conjugated linoleic acid (UFA/CLA) enriched butter and conventional butter after 1 week of storage at 6 °C.

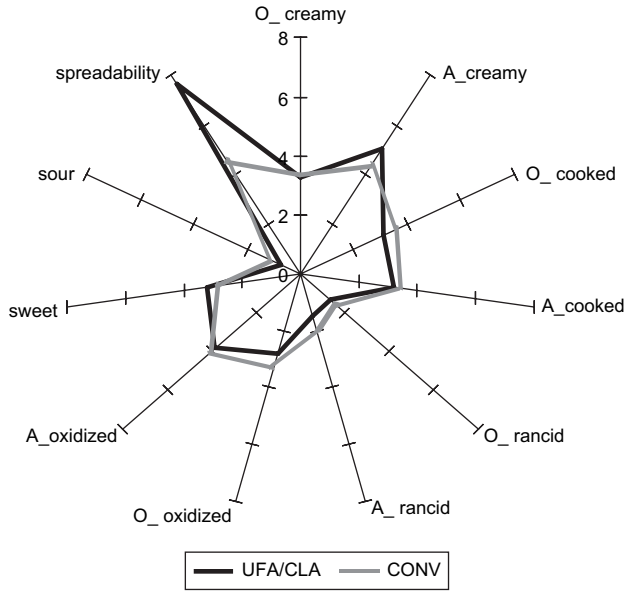

Fig. 3. Odour (O), aroma (A) and texture attributes of the unsaturated fatty acid/conjugated linoleic acid (UFA/CLA) enriched butter and conventional butter after 8 weeks of storage at 6 °C.

the odour-active compounds affect and interact with each other as well as with the matrix. In the literature, there are examples of the differences between odours of foods perceived by GC/O and by sensory analysis. For example, methional, showing a boiled potato-like odour, was found as an important odorant in French fries by aroma extraction dilution analysis (AEDA), whereas a sensory panel rated methional as not affecting its flavour by omission tests (Wagner & Grosch, 1998).

A highly significant correlation ($P < 0.001$), however, was found between the "oxidized odour" sensory attribute and the peak intensity of hexane ($r^2 = 0.80$). Hexane was not perceived by GC/O, probably due to its high odour threshold (Amoore & Hautala, 1983). However, hexane could be used as an easily measurable marker of lipid oxidation, which confirms the findings of Christensen and Hølmer (1996).

4. Conclusions

Among the diverse analyses carried out on UFA/CLA enriched and conventional butter, GC/MS/O was found to be a suitable and sensitive method to detect the differences of aroma profiles between the two samples. In fact, this technique allows the detection and identification of even traces of odour-active compounds. After 6 weeks of storage, the UFA/CLA enriched butter showed more intense cheesy, rancid, chemical, mushroom-like, green and metallic notes than conventional butter. Fatty acids, alcohols, aldehydes and ketones were mainly responsible for the development of these odours.

The sensory evaluation described the fresh UFA/CLA butter as more easily spreadable and with a more intense creamy and a weaker cooked milk aroma. The panel could not find differences between the two butter types during storage, except for the spreadability and the creamy aroma (always higher in UFA/CLA butter) and described the two kinds of butter as aging in the same way. No significant differences were detected with regard to the attributes related to oxidative processes, i.e., rancid and oxidized odour/aroma. These notes increased in both butter types during storage and were perceived, in particular, from 6 weeks.

The differences between GC/O and the sensory analyses can be explained by the different test conditions. During GC/MS/O the odorants are perceived separately from each other without mutual interaction. In contrast, during sensory analysis the odours are perceived as a mixture at almost the same time and as a result of odour–odour and odour–matrix interactions, such as masking effects.

On the basis of our chemical and sensory findings, the shelf life of UFA/CLA enriched butter at 5–6 °C was comparable to that of conventional sweet cream butter. For further characterisation of the odour-active compounds of UFA/CLA enriched butter, the use of more than a single aroma extraction technique is necessary. To provide more precise data on the differences between the UFA/CLA enriched butter and conventional butter, the quantification of important odour-active compounds will be necessary.

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