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# OXIDATIVE STABILITY AND AROMA OF UFA/CLA (UNSATURATED FATTY ACIDS/CONJUGATED LINOLEIC ACID) ENRICHED BUTTER

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To Tim

and

my parents

*“So eine Arbeit wird eigentlich nie fertig,*

*man muss sie für fertig erklären,*

*wenn man nach Zeit und Umständen das Möglichste getan hat.”*

(Goethe; zur Iphigenie, aus der Italienischen Reise 1787)

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# *Summary*

The enrichment of dairy products with unsaturated fatty acids (UFA) and in particular, with conjugated linoleic acid (CLA) is a possibility to increase their nutritional value and their potential beneficial health effects. On the other hand, the unsaturated lipids are more susceptible to oxidation and could be a source of off-flavours during storage.

The aim of the present investigations was to evaluate the oxidative stability of butter enriched in UFA and CLA in comparison to conventional butter (not enriched), focusing on aroma-active compounds. The aroma profiles of the two kinds of butter were analysed during storage as well as after induced oxidation and the most important odour-active compounds were quantified. The possible origin of odorants from the main isomer of CLA in butter, *cis* 9, *trans* 11, was also investigated in a model study.

The two types of butter were analysed during 8 weeks of storage at 6 °C for their fatty acid composition, vitamins (retinol and α-tocopherol), metal ions (copper and iron), their overall sensory and, in particular, their odour profiles. The UFA/CLA enriched butter and conventional butter had a significantly different fatty acid composition. The enriched butter consisted in particular, of double the amount of total CLA than conventional butter and contained 30 % more of omega 6 fatty acids. Retinol and α-tocopherol were higher in UFA/CLA enriched butter. The iron content was also significantly higher in the enriched butter. The descriptive overall sensory analysis revealed the flavour of the two butter types as very similar during the storage period. Significant differences were found only for the cooked aroma, more intense in the fresh conventional samples, and for the creamy aroma, higher in the stored enriched butter. The UFA/CLA butter showed always a better spreadability.

The olfactometric analyses coupled to solid phase microextraction (SPME) and gas chromatography mass spectrometry (GC/MS/O) showed that the two fresh butter types had similar odour profiles, characterised by milky, soapy and sulphury notes, due to 2-nonanone, nonanal and dimethyl disulphide, respectively. After 6 weeks of storage, the aldehydes increased in both butter types, but especially in UFA/CLA enriched butter. Heptanal (fatty odour), (*E*)-2-octenal (fruity) and (*E,E*)-2,4-decadienal (fried) increased especially in UFA/CLA enriched butter. Aroma extract dilution analysis (AEDA) confirmed these results and in addition, indicated lactones, as δ-decalactone and δ-dodecalactone, with fruity notes, as important odour compounds of UFA/CLA butter. The quantification by stable isotope dilution analysis (SIDA) showed that the content of pentanal, heptanal and δ-decalactone was significantly higher in UFA/CLA enriched butter after storage.

After photo-oxidation and oxidation in the dark in a oxygen atmosphere, the quantification of the odorants in both butter types revealed that heptanal, nonanal, (*E*)- and (*Z*)-2-octenal and *trans*-4,5-epoxy-(*E*)-2-decenal, were higher in the conventional butter. This fact may be explained with the higher α-tocopherol and retinol content of the UFA/CLA enriched samples, protecting this butter type from oxidation.

A model experiment, consisting of a specific CLA ethyl ester and of labelled [$^{13}C_{18}$]ethyl linoleate, submitted to photo-oxidation and oxidation under oxygen atmosphere, was able to explain the formation of specific odorants from the CLA. Hexanal and heptanal, which have been already detected in the enriched butter, were found mainly unlabelled and consequently they stemmed predominantly from EtCLA, under the oxidation conditions applied.

It is concluded that the odorants found in butter may also be formed from CLA, and not only from UFA, during oxidation. Retinol and α-tocopherol may partially inihibit or delay the formation of odorants, due to their antioxidative activity.

# *Zusammenfassung*

Das Anreichern von Milchprodukten mit ungesättigten Fettsäuren (UFA) und im Besonderen mit konjugierten Linolsäuren (CLA) kann den Nährwert und die potentiell gesundheitsfördernde Wirkung erhöhen. Allerdings sind die ungesättigten Fettsäuren anfälliger für Oxidation, und während der Lagerung können daraus Fehlaromen resultieren.

Das Ziel des vorliegenden Forschungsprojektes war es, die oxidative Stabilität von UFA und CLA angereicherter Butter im Vergleich zu konventioneller Butter (nicht angereichert) auf aromaaktive Komponenten hin zu untersuchen. Die Aromaprofile der zwei Buttertypen wurden sowohl während der Kühllagerung, als auch nach induzierter Oxidation bestimmt. Die wichtigsten aromaaktiven Verbindungen wurden quanti-fiziert. Die Bildung von Geruchsstoffen aus der in Milchfett vorherrschenden CLA, dem *cis* 9, *trans* 11-CLA-Isomer, wurde in einer Modellstudie untersucht.

Die beiden Buttertpyen wurden während 8-wöchiger Lagerung bei 6 °C auf die Fettsäurezusammensetzung und den Gehalt an Vitaminen (Retinol and α-Tocopherol), und Metallionen (Kupfer und Eisen), sowie chemisch und sensorisch auf ihre Aromaprofile hin untersucht. Die UFA/CLA angereicherte Butter und konventionelle Butter zeigten ein signifikant verschiedenes Muster an ungesättigten Fettsäuren. Die angereicherte Butter enthielt doppelt soviel Gesamt-CLA wie konventionelle Butter und 30% mehr an Omega-6 ungesättigten Fettsäuren. Der Der Gehalt an Retinol and α-Tocopherol sowie an Eisen war in UFA/CLA angereicherter Butter höher als in konventioneller Butter. Nach der deskriptiven sensorischen Analyse war das Aroma beider Buttertypen ähnlich. Signifikante Unterschiede wurden nur für das Attribut „gekochtes Aroma“ gefunden, das in der frischen konventionellen Butter intensiver war,

und für das Attribut "cremig",das in der gelagerten angereicherten Butter ausgeprägter war. Die UFA/CLA Butter zeigte in allen Fällen eine höhere Streichfähigkeit.

Die olfaktometrischen Untersuchungen mit Hilfe der Solid Phase Microextraction (SPME), gekoppelt mit Gaschromatografie-Massenspektrometrie (GC/MS/O), zeigten, dass im frischen, ungelagerten Zustand beide Buttertypen ähnliche Aromaprofile aufwiesen. Ihr Aroma zeichnete sich durch milchige, seifige und schwefelartige Noten aus, verursacht durch 2-Nonanon, Nonanal und Dimethyldisulfid. Nach 6-wöchiger Lagerung nahm der Gehalt an Aldehyden in beiden Buttersorten zu, jedoch stärker in UFA/CLA angereicherter Butter. Heptanal (fettige Note), (*E*)-2-Octenal (fruchtig) und (*E,E*)-2,4-Decadienal (frittiert) stiegen insbesondere in UFA/CLA angereicherter Butter an. Die Aromaextrakt-Verdünnungsanalyse (AEDA) bestätigte diese Ergebnisse and identifizierte zusätzlich Lactone, wie δ-Decalacton und δ-Dodecalacton, mit ihren fruchtigen Aromanoten als wichtige Aromakomponenten in UFA/CLA Butter. Die stabilen Isotopenverdünnungsanalyse (SIDA) mass nach der Lagerung signifikant höhere Konzentrationen für Pentanal, Heptanal und δ-Decalacton in UFA/CLA angereicherter Butter.

Nach Fotooxidation und nach Oxidation unter Lichtausschluss in einer Sauerstoff-Atmosphäre war die Konzentration an Heptanal, Nonanal, (*E*)- und (*Z*)-2-Octenal sowie *trans*-4,5-epoxy-(*E*)-2-Decenal in konventioneller Butter höher als in UFA/CLA angereicherter Butter. Dieser Unterschied kann mit dem höheren Gehalt des oxidationshemmenden α-Tocopherol und Retinol in der UFA/CLA angereicherten Proben erklärt werden.

In einem Modellversuch wurden *cis* 9, *trans* 11-CLA-Ethylester und [$^{13}C_{18}$] isotopenmarkiertes Ethyllinoleat unter derselben Fotooxidation und Oxidation unter Sauerstoff-Atmosphäre unterworfen, um die Bildung von geruchsaktiven Kompo-nenten aus CLA abzuklären. Bei diesem Versuch wurden Hexanal und Heptanal, die bereits in angereicherter Butter als Aromakomponenten identifiziert worden waren, vorwiegend unmarkiert identifiziert. Folglich wurden sie unter den angewandten Versuchsbedingungen hauptsächlich aus dem CLA-Ethylester gebildet.

Damit wurde gezeigt, dass die in der untersuchten Butter gefundenen Aromastoffe während der Oxidation auch aus CLA gebildet werden können, und nicht nur von den ungesättigten Fettsäuren. Retinol und α-Tocopherol scheinen wegen ihrer antioxidativen Wirkung die Bildung von Aromastoffen teilweise zu hemmen oder zu verzögern.

# *Abbreviations*

AEDA: Aroma Extract Dilution Analysis

CharmAnalysis$^{TM}$: Combined Hedonic Aroma Response Measurement

CLA: Conjugated Linoleic Acids

DMS: Dimethyl Sulphide

DMTS: Dimethyl Trisulphide

FD: Flavour Dilution Factor

FID: Flame Ionisation detector

GC x GC: Two Dimensional Gas Chromatography

GC-MS: Gas Chromatography Mass Spectrometry

GC-O: Gas Chromatography Olfactometry

MUFA: Monounsaturated Fatty Acids

OAV: Odour Activity Value

SAFE: Solvent Assisted Flavour Evaporation

SIDA: Stable Isotope Dilution Assay

SNIF: Surface Nasal Impact Frequency

SPME: Solid Phase Microextraction

TOF-MS: Time-of-flight Mass Spectrometry

UFA: Unsaturated Fatty Acids

# 1. General Introduction

The fact that food items may exhibit health benefits beyond their nutritional value has been recognised since a long time. Many traditional recommendations on food selection have included this view. In more recent years the interest in food with specific health benefits has greatly increased and stimulated the development of respective products for the food market. At the same time large efforts are made to substantiate health claims by validated experimental methods.

Dairy products have become to play an important role in this context. Within dairy products, those enriched with unsaturated fatty acids (UFA) and particularly conjugated linoleic acids (CLA) present a promising example. CLA are a group of positional and geometric isomers of linoleic acid which occur naturally in milk and meat from ruminants as a result of rumen biohydrogenation and endogeneous conversion from vaccenic acid. Potential anti-carcinogenic, anti-atherogenic and body fat reducing effects are attributed to CLA. Accordingly, a considerable number of studies were initiated to increase the concentration of UFA/CLA in dairy products. This may be achieved by direct fortification of milk with synthetic CLA, by feeding cows on mountain pastures which enhances the content of UFA/CLA in milk fat, or by supplementing the animal diet with fish oil or vegetable oil and oleaginious seeds, all rich in oleic, linoleic and linoleic which in turn are transferred to milk and partly converted to CLA.

It is foreseeable that UFA/CLA enriched products are more susceptible to lipid oxidation than conventional products so that the storage stability and in consequence the flavour quality may be impaired more rapidly. So far, only few sensory

investigations have been carried out on the flavour of UFA/CLA enriched dairy products. The identification of the most important odour-active compounds in these products by the gas chromatography olfactometry (GC/O) technique has not yet been carried out. Besides, some of the results available to date are controversial. While there are reports that no difference exists in flavour between the enriched and the conventional products other studies found that "oily/vegetable" like notes are characteristic for enriched but not for conventional products.

In the present dissertation the aroma compounds of UFA/CLA enriched butter in comparison to conventional butter and their stability and changes during cold storage and induced lipid oxidation were investigated. UFA/CLA enriched butter was produced from milk that was obtained from cows, which were fed on pasture with sunflower seeds as supplement rich in linoleic acid.

After a literature review in Chapter 2, which focuses on odour-active compounds analysed by GC/O, a comprehensive study on the storage stability of UFA/CLA enriched butter by instrumental and sensory methods is presented in Chapter 3. As a next step the most important odorants of fresh and stored UFA/CLA enriched butter and conventional butter were identified by aroma extraction dilution analysis (AEDA) coupled to gas chromatography, mass spectrometry and olfactometry (GC/MS/O) and quantified by stable isotope dilution analysis (SIDA). In addition, both types of butter were subjected to photo-oxidation and oxidation in the dark in an oxygen atmosphere and the influence of these treatments on odour compounds was investigated. These experiments are presented in Chapter 4. The third study, presented in Chapter 5, attempts to explain the odour formation in UFA/CLA enriched butter by introducing a butter model. Different model systems, containing linoleic acid and CLA in ester form, in the same proportion present in real UFA/CLA butter, were subjected to oxidation and again analysed by GC/MS/O. Isotopically labelled ethyl linoleate was

used to trace the origin. Finally, Chapter 6 draws the main conclusions of the dissertation and discusses the perspectives of future research.

# 2. Literature Review*

The present review shows that more than 230 volatile compounds have been identified in butter. However, only a small number of them can be considered as key odorants of butter aroma. Gas chromatography olfactometry was used to determine the character impact odorants of different kind of butter. Sweet cream butter is characterised by lactones with fruity and creamy notes and by sulphur compounds, having corn-like and garlic odours. The key odour compounds of sour cream butter are diacetyl (buttery-like), butanoic acid (cheesy) and δ-decalactone (peach), mainly due to lactic acid bacteria fermentation. The aroma of butter oil is characterised by aldehydes, such as (*E*)- and (*Z*)-2-nonenal and (*E*,*E*)-2,4-decadienal, conferring green and oily notes. Olfactometric studies of heated butter showed the formation of new aroma compounds during heating, such as 3-methylbutanoic acid (cheesy), methional (potato-like) and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (caramel-like). High temperature treatment of butter can also induce off-flavour development. Off-odours in butter can originate from auto-oxidative and as well as from lipolytic reactions, microbial contamination and animal feeding.

* This chapter is an adapted version of the review article published as: Mallia, S., Escher, F., Schlichtherle-Cerny, H. (2008). Aroma-active compounds of butter – a review. European Food Research and Technology, 226, 315-325.

## 2.1 General

Butter is a traditional food which is widely consumed all over the world, directly or as an ingredient in processed foods such as pastries and convenience dishes. Its nutritional value, due to a high content of fats, vitamins and minerals, and its unique and pleasant flavour make butter particularly appreciated by the consumers.The nature of this flavour has since long intrigued chemists and flavourists who studied the aroma compounds of butter extensively and tried to reproduce an "artificial" butter aroma (Winter et al., 1963; Urbach et al., 1972). Various review articles of butter aroma are also available (Forss, 1971; Badings and Neeter, 1980; Nursten, 1997).

Different methods have been used for the isolation of the volatile compounds of butter, mainly consisting in steam distillation and high-vacuum distillation techniques (Forss et al., 1967) and static and dynamic headspace methods, such as solid phase microextraction (SPME) (Shooter et al., 1999), static headspace analysis (Peterson and Reineccius, 2003a, b), simultaneous purging-solvent extraction (Adahchour, 1999) and using a purge and trap system (Povolo and Contarini, 2003). Gas chromatography coupled to mass spectrometry (GC/MS) is the separation technique usually applied for the identification and quantification of volatile compounds in butter and generally in foods (Maarse and Belz, 1982).

The aroma composition of butter depends on animal feeding (Azzara and Campbell, 1992), season of production (Day et al., 1964), manufacturing process (Schieberle and Grosch, 1987) and storage conditions (Widder et al., 1991; Christensen and Hølmer, 1996).

Depending on the manufacturing process, three main types of butter exist, each having a specific flavour: sour cream butter, obtained from cream inoculated with starter cultures; sweet cream butter, derived from unfermented cream; acidified cream butter,

produced with sweet cream, that is acidulated in a subsequent step with lactic acid and a flavour concentrate.

Removing the aqueous phase from butter by decantation or evaporation yields butter oil, an important product. The aroma of butter oil was also widely studied over the years (Forss et al., 1967; Widder et al., 1991). In an early study on the volatile composition of sweet cream butter, Siek and Lindsay (1968) identified over 100 compounds in the steam distillates from butter-fat, including alkanals, alkanones, alcohols, esters, hydrocarbons and aromatic compounds. Stark and co-workers (Stark et al., 1973; Stark et al., 1976a, b) did several studies on a top quality Australian butter oil, obtained from sweet cream. They identified alkanones, alkanoic acids, δ-lactones, phenolic compounds, dimethyl sulfone, indole and 3-methylindole (skatole) and used flavour threshold studies to detect which compounds contribute to the aroma of butter oil. Their conclusion was that decanoic acid, lauric acid, δ-octalactone, δ-decalactone, indole, and skatole are important volatile compounds for the butter oil aroma, whereas the phenolic compounds are of only borderline significance.

The flavour of sour cream butter is composed of aroma compounds which also occur in sweet cream butter and additionally those from starter cultures (Badings and Neeter, 1980). The reproduction of the flavour of sour cream butter was attempted by Lindsay and co-workers (1967) and later by Badings (1973), who found 2,3-butanedione (diacetyl), acetic acid and lactic acid as the most important aroma compounds, stemming from the metabolism of the lactic acid bacteria. δ-Dodecalactone, δ-decalactone, γ-decalactone, hydrogen sulphide and dimethyl sulphide, derived from sweet cream butter, also contribute to the flavour of the cultured butter (Badings, 1973). Another study on sour cream butter aroma (Mick et al., 1982) confirmed the identification of lactones as the main volatile components of this butter type. In addition 2-methylketones and alcohols were found as important contributors. Additionally Shooter and co-workers (1999) accomplished a selective

study on volatile sulphur compounds in butter, showing an increase of methanethiol and dimethyldisulfide concentration in spring butter, due to the pasture composition, and a significant decrease of these compounds during storage.

An extensive study to identify the aroma compounds present in the water fraction of butter (Adahchour et al., 1999) found 23 compounds, such as 1-methoxy-2-propanol, 3-hydroxy-2-butanone, 1-ethoxy-2-propanol, 2,3-butanediol, butanoic acid and benzoic acid. It was observed that the heat treatment of butter at 170°C for 5 min induces rapid formation of 2,5-dimethyl-4-hydroxy-3-(2H)-furanone and 3-hydroxy-2-methyl-pyran-4-one (maltol). The same research group recently improved the identification of flavour compounds in butter (Adahchour et al., 2005), using two-dimensional gas chromatography (GC x GC) coupled to flame ionisation (FID) and time-of-flight mass spectrometric (TOF/MS) detection. This led to the detection of aldehydes, 2-enals, ketones, alcohols, fatty acids and lactones which were not identified in the first study. Furan derivatives and heterocyclic compounds such as pyrroles and pyridines were exclusively found in the heat-treated samples.

The effect of storage on the volatile fraction of butter was evaluated by Christensen and Hølmer (1996) and by Povolo and Contarini (2003). The first authors studied the oxidative rancidity in butter during 14 weeks and chose hexanal as an indicator for lipid oxidation. Povolo and Contarini, using a purge and trap technique as well as SPME, identified 48 aroma compounds in butter, belonging to the chemical classes of ketones, aldehydes, alcohols, esters, acids, sulphur compounds, hydrocarbons and terpenes.

More than 230 volatiles have been identified as natural constituents of butter (Maarse and Visscher, 1996), but only a small number of those is recognized as key odorants of butter flavour. The study of the odour-active compounds, which actually contribute to the aroma of a specific food, is possible using instrumental methods in combination with sensory techniques. The application of gas chromatography-olfactometry

(GC/O), using the human nose as a detector, provides both odour descriptors and odour activity measurement.

The human nose can be more sensitive than an instrumental detector, having in fact a detection limit down to 10-19 moles for certain odorants (Reineccius, 1994). During olfactometric analysis, a trained panelist describes the odour quality and indicates the duration of the odour perception (Acree et al., 1976).

Several GC/O methods are available to estimate the importance of a particular aroma compound in food: Aroma Extract Dilution Analysis (AEDA) (Schmid and Grosch, 1986) based on serial dilutions until an odour is no longer perceivable; CharmAnalysis™ (Combined Hedonic Aroma Response Measurement) (Acree et al., 1984), also based on serial dilutions of the aroma extract; Osme (from the Greek word meaning odour) (McDaniel et al., 1990; Da Silva et al., 1994), consisting of the analysis of a single concentration of the extract, to establish quality, duration and odour intensity; Surface Nasal Impact Frequency (SNIF) (Pollien et al., 1997), based on the detection of odour frequencies by a panel of trained sniffers. Although olfactometric analysis may be dependent on subjective factors related to the psychophysical conditions of the panelist, it can result in reproducible measurements, if the panelists are trained with reference chemicals and agreed on the odour attributes.

## 2.2 Odour-active compounds in butter

The character impact odour compounds of butter primarily originate from the cream used to make it. Up to now, however, only few publications exist on the aroma of cream. Haverkamp and co-workers (1964) found (Z)-4-heptenal to be important for the cream flavour. Pionnier and Hugelshofer (Pionnier and Hugelshofer, 2006) analysed cream from different processes (pasteurisation, sterilisation, UHT) and having different fat levels by GC/O. They identified 35 key odorants, such as diacetyl (buttery), 2-pentanone (caramel-like, cream-like), 2-heptanone (dairy-like), 3-

hydroxy-2-butanone (buttery), dimethyl trisulfide (cabbage-like), 2-nonanone (hot milk-like), acetic acid (acidic), furfural (caramel-like), butanoic acid (cheese-like). The aroma of cream is mainly due to the contribution from the aqueous phase of milk and from the fat globule membrane (Badings and Neeter, 1980), while butter aroma is primary derived from the volatile compounds present in the fat fraction.

### 2.2.1 Sweet cream butter

The key aroma compounds of sweet cream butter (SwCB) were studied by AEDA by Budin and co-workers (Budin and Reineccius, 2001) and by Peterson and Reineccius (2003a). In the first study, lactones, ketones and aldehydes were found to have high aroma dilution factors: δ-decalactone (512), δ-dodecalactone (256), (*Z*)-6-dodecen-γ-lactone (128), 1-hexen-3-one (256), 1-octen-3-one (128), (*E*)-2-nonenal (64), (*E,E*)-2,4-decadienal (64), *trans*-4,5-epoxy-(*E*)-2-decenal (64) and (*Z*)-2-nonenal (32). Interestingly skatole was also found as key odorant of SwCB, showing an aroma dilution factor of 128.

Peterson and Reineccius identified by headspace analysis δ-decalactone, 1-hexen-3-one, 1-octen-3-one, (*E*)- and (*Z*)-2-nonenal and skatole as potent odorants of SwCB, which is in agreement with the study of Budin and co-workers. Additional aroma compounds were hydrogen sulphide, acetaldehyde, dimethyl sulphide, diacetyl, hexanal, 2-methylbutanal, 3-methylbutanal, butanoic acid, dimethyl trisulphide, hexanoic acid, δ-hexalactone, nonanal, δ-octalactone and γ-dodecalactone. Quantification of key odorants was performed by purge and trap-GC-MS using standard addition. The identified volatile compounds were added, according to their concentrations, to a model system to reconstitute the aroma of SwCB. Nineteen panellists rated the similarity of the aroma of the butter model vs. the fresh butter obtained directly from the manufacturing plant, which was used as reference. The sensory analysis indicated that the butter model was significantly different from the reference, but it was ranked the same in similarity as an unsalted commercial butter.

According to Peterson and Reineccius (2003a), SwCB was characterised by δ-octalactone, δ-hexalactone and γ-dodecalactone. In particular, δ-hexalactone and γ-dodecalactone had creamy and peach-like odours, respectively, but were identified in SwCB only by Peterson and Reineccius. The authors found, additionally, dimethyl sulphide (DMS) and dimethyl trisulphide (DMTS) as key odorants, which have already been detected by GC/O in milk and treated milks (Christensen and Reineccius,1992; Bosset et al., 1994), however not in butter. Day and co-workers (1964) identified DMS in butter already earlier and considered it a desirable component that smoothes the strong flavour of diacetyl.

In a study carried out by Schieberle and co-workers (Schieberle et al., 1993), the overall odour impression of SwCB was evaluated by a trained sensory panel and compared with the odorants of different types of sour cream butter (SoCB). The results showed that the diacetyl concentration is lower without a fermentation process as in SwCB, resulting in an overall mild and sweet odour impression. Odour activity values (OAVs = concentrations of the odorants divided by their odour thresholds) were calculated: diacetyl (<2), δ-decalactone (32), butanoic acid (19), (*Z*)-6-dodecen-γ-lactone (<1), hexanoic acid (<1). Table 2.1 summarises the concentrations, odour thresholds and odour activity values of odour-active compounds found in SwCB by the different authors.

### 2.2.2 Sour cream butter

Schieberle and co-workers (1993) studied different kinds of butter: sour cream butter (SoCB), Irish SoCB, German farm SoCB and cultured butter and compared them with SwCB. An AEDA of Irish SoCB, showing the most intense odour during a preliminary sensory analysis, revealed 18 odour-active compounds: diacetyl, 1-penten-3-one, hexanal, 1-octen-3-one, (*Z,Z*)-3,6-nonadienal, (*E*)- and (*Z*)-2-nonenal, (*E,E*)-2,4-nonadienal, (*E,E*)-2,4-decadienal, γ-octalactone, *trans*-4,5-epoxy-(*E*)-2-decenal, skatole, δ-decalactone, (*Z*)-6-dodecen-γ-lactone, acetic acid, butanoic acid,

hexanoic acid and an unknown compound with fatty-nutty odour. δ-Decalactone, (*Z*)-6-dodecen-γ-lactone and diacetyl, which had the highest dilution factors (FD) of 4096, 512 and 256, respectively, were quantified by stable isotope dilution assay (SIDA). Butanoic as well as hexanoic acid, which were major compounds in the acidic fraction of the volatiles, were quantified using unlabeled standards. Table 2.1 lists the most important odour-active compounds of SoCB. Sunflower oil was spiked with diacetyl, δ-decalactone and butanoic acid at the same concentrations occurring in cultured butter that was chosen as standard for the most typical butter odour. The results indicated that the sunflower oil containing the three odorants exhibited an aroma note which in quality and intensity was very similar to the odour of the cultured butter.

The higher amounts of diacetyl, δ-decalactone (*Z*)-6-dodecen-γ-lactone, butanoic and hexanoic acids in SoCB, might result from the lactic acid bacteria, which are added to the cream during production.

Volatile compounds of traditional SoCB, such as Ghee (Wadodkar et al., 2002) and in particular of Smen, a fermented butter produced in Morocco and in other Arab countries, were studied by GC/O (Triqui and Guth, 2001). The results of an AEDA indicates butanoic and hexanoic acid as potent odorants. The primary mechanism of aroma development in this product is lipolysis.

### 2.2.3 Butter oil

Widder and co-workers (Widder et al., 1991; Widder, 1994a, b) investigated the key odorants of butter oil (BO), using vacuum distillation for the isolation and GC/MS combined with olfactometry for the identification of the volatile compounds. Sixteen potent odorants were identified by AEDA: diacetyl, acetic and butyric acid, 1-hexen-3-one, (*Z*)-3-hexenal, 1-octen-3-one, (*Z*)-1,5-octadien-3-one, guaiacol, (*Z*)- and (*E*)-2-nonenal, (*E,E*)-2,4-decadienal, skatole, 4-hydroxy-3-methoxybenzaldehyde (vanillin), (*Z*)-6-dodecen-γ-lactone, δ-octalactone and δ-decalactone. Vanillin has been reported by the authors for the first time in BO. The most important aroma compounds with the

highest flavour dilution (FD) factors were 1-octen-3-one (FD=128), (*Z*)-3-hexenal (64), (*Z*)-2-nonenal (64), (*E*)-2-nonenal (32) and (*E,E*)-2,4-decadienal (32) (Widder et al., 1991).

In the same study, the odour-active compounds of fresh BO were compared with those in BO after 42 days storage at room temperature. The FD factors of the carbonyl compounds formed by lipid peroxidation increased. This topic will be discussed later concerning oxidative off-flavours formed during storage.

Table 2.1 summarises the major odour-active compounds found in BO and describes their odour quality. Concentrations, nasal and retronasal odour thresholds in oil and OAVs of the odour compounds are listed, when available. The odour threshold data vary from author to author (Guth and Grosch, 1990a,b; Preininger and Grosch, 1994; Reiners and Grosch, 1998), especially for (*E*) and (*Z*)-2-nonenal, 2-methylbutanal, hexanal and nonanal. The retronasal odour threshold of (*E*)-2-nonenal, for example, varies from 0.066 (Guth, 1991) to 66 mg/kg oil (Widder, 1994a, b). The chemical structures of selected odour-active compounds of butter and BO are represented in Figure 2.1.

**Table 2.1:** Odour-active compounds in Sweet Cream Butter (SwCB), Sour Cream Butter (SoCB) and Butter Oil (BO) as determined by gas chromatography-olfactometry

| No[a] | Compound | Odour quality | Concentration (µg/kg butter) | | | Odour threshold (mg/kg oil)[b] | | OAV[c] |
|---|---|---|---|---|---|---|---|---|
| | | | SwCB[d] | SoCB[e] | BO[f,g] | nasal | retronasal | |
| | Acids | | | | | | | |
| 1 | Butanoic acid | Buttery, sweaty, cheesy | 192 | 4480 | nq | 0.135[e] | | 19[q];33[e] |
| | Hexanoic acid | Pungent, musty, cheesy | 732 | 1840 | nq | 5.4[e] | | <1[e,q] |
| | Aldehydes | | | | | | | |
| 2 | 2-Methylbutanal | Chocolate, fruit | 4.9 | - | - | 0.0022[h]; 0.140[i] | 0.0082[h]; 0.023[i,j] | |
| | 3-Methylbutanal | Chocolate | 11.9 | - | - | 0.0054[k]; 0.013[i] | 0.0108[k] | |
| 3 | Hexanal | Green, fatty | 29 | nq | nq | 0.120[i]; 0.300[l] | 0.073[m]; 0.190[n] | |
| 4 | Nonanal | Waxy, fatty, floral | 43 | - | - | 1[i]; 13.5[o] | 0.26[o] | |
| 5 | (*E*)-2-Nonenal | Green, fatty, tallowy | 10 | nq | 6.75 | 0.9[l] | 0.066[m]; 66[f]; 45[f] (BO) | <1[f] |
| | (*Z*)-2-Nonenal | Green, fatty | nq | nq | 0.2 | 0.0045[l] | 0.0006[m]; 0.6[l]; 3[f] (BO) | <1[f] |
| | (*Z*)-4-Heptenal | Creamy, biscuit-like | nq | nq | 0.3 | 0.002[o] | 0.001[o]; 0.75[l]; 3.5[f] (BO) | <1[f] |
| | Ketones | | | | | | | |
| 6 | 2,3-Butanedione | Buttery, creamy | 6.6 | 620 | nq | 0.0045[e]; 0.010[k] | 0.01[k]; 0.055[n] | 2[q]; 138[e] |
| | 1-Hexen-3-one | Vegetable-like, metallic | 0.004 | - | nq | | | |
| 7 | 1-Octen-3-one | Mushroom-like | 0.58 | nq | 1.1 | 0.010[l] | 0.3[l]; 3[f] (BO) | <1[f] |

## Table 2.1 continued

| No[a] | Compound | Odour quality | Concentration (µg/kg butter) | | | Odour threshold (mg/kg oil)[b] | | OAV[c] |
|---|---|---|---|---|---|---|---|---|
| | | | SwCB[d] | SoCB[e] | BO[f,g] | nasal | retronasal | |
| | Lactones | | | | | | | |
| | δ-Hexalactone | Creamy, chocolate | 47.9 | - | - | | | |
| | δ-Octalactone | Coconut-like, peach | 72.8 | - | nq | | | |
| 8 | δ-Decalactone | Coconut-like, peach | 1193 | 5000 | nq | 0.4[k]; 0.12[c] | 1.4[n]; 1.6[k] | 32[q]; 42[c] |
| 9 | γ-Dodecalactone | Peach | 441 | - | - | | | |
| | (Z)-6-Dodeceno- γ-lactone | Peach | - | 260 | nq | 0.25[e] | | 1[q]; 1[c] |
| | Sulphur containing compounds | | | | | | | |
| 10 | Dimethyl sulphide | Corn-like, pumpkin | 20 | - | - | 0.0012[p] | 0.0023[p] | |
| 11 | Dimethyl trisulphide | Garlic, sulphury | 17.4 | - | - | 0.0025[p] | 0.0042[p] | |
| | Nitrogen containing compound | | | | | | | |
| 12 | 3-methyl-1H-indole (Skatole) | Mothball, fecal | 12.6 | nq | nq | 0.0156[k] | 0.05[k] | |

nq = compound detected but not quantified; [a]Numbers refer to Figure 2.1; [b]Thresholds in vegetable oil, except for BO = odour threshold determined in Butter Oil; [c]Odour activity value (ratio of concentration to odour threshold) for SoCB and BO; [d]Literature data (concentration determined by standard addition method) according to Peterson and Reineccius (2003a); [e]Literature data refer to Irish sour cream butter (concentration determined by SIDA), according to Schieberle et al. (1993); [f]Literature data refer to fresh butter oil (concentration determined by SIDA) according to Widder (1994a); [g]Literature data according to Widder et al. (1991); [h]Odour threshold according to Reiners and Grosch (1998); [i]Odour threshold according to Guadagni et al. (1972); [l]Odour threshold according to Wagner and Grosch (1998); [m]Odour threshold according to Preininger and Grosch (1994); [n]Odour threshold according to Guth and Grosch (1990a, b); [o]Odour threshold according to Guth (1991); [p]Odour threshold according to Maarse and Visscher (1996); [q]Odour threshold according to Belitz and Grosch (1992); [r]Odour threshold according to Kubícková and Grosch (1998)

## 2.3 Odour-active compounds in heated butter

Butter generates potent odorants during heating (Grosch, 1987). Although the volatile composition of heated butter is well known and more than 170 compounds have been identified (Maarse and Visscher, 1996), only few studies have identified and quantified the odour-active compounds responsible for its aroma. Budin and co-workers (2001) studied odorants in heated SwCB using AEDA. The volatile fraction of butter, heated to 105-110 °C for 15 min, was isolated by high vacuum distillation. The odorants with the highest aroma dilution factors were 1-hexen-3-one, 1-octen-3-one, (*E*)-2-nonenal, (*Z*)-2-nonenal, (*E,E*)-2,4-decadienal, *trans*-4,5-epoxy-(*E*)-2-decenal, 2,5-dimethyl-4-hydroxy-3-(2H)-furanone, methional, δ-octalactone, δ-decalactone, δ-dodecalactone and skatole. The quantification of 10 odour-active compounds was performed by SIDA and the OAVs were also calculated. The data are shown in Table 2.2. The key aroma compounds of heated butter were compared with those of fresh butter: δ-decalactone, skatole, 1-octen-3-one, (*E*)- and (*Z*)-2-nonenal, (*E,E*)-2,4-decadienal and *trans*-4,5-epoxy-(*E*)-2-decenal had higher aroma dilution values in heated butter.

Schieberle and co-workers (1993) determined the sensory threshold of δ-decalactone as 120 µg/kg sunflower oil. It is present in heated butter approximately 50 times above its threshold, which suggests that it is the most important odorant in heated butter (Budin et al., 2001). Due to their OAVs, 1-octen-3-one, methional, 2,5-dimethyl-4-hydroxy-3-(2H)-furanone and *trans*-4,5-epoxy-(*E*)-2-decenal were found to contribute to heated butter aroma. These results are in general qualitative agreement with those from Dickerson (1996), who found 1-hexen-3-one, 1-octen-3-one, methional, δ-octalactone, δ-decalactone, 2,5-dimethyl-4-hydroxy-3-(2H)-furanone, butanoic acid and skatole important for the aroma of heated butter. The quantitative results of Budin, obtained by isotope quantitation, are different from those of Dickerson, who found higher concentrations for 1-hexen-3-one, 2,5-dimethyl-4-hydroxy-3-(2H)-

furanone and δ-octalactone using a sensory panel. Peterson and Reineccius (2003b) studied the key aroma compounds of heated butter, using static headspace analysis, and confirmed methional, (*E*)-2-nonenal, 1-hexen-3-one, 1-octen-3-one, δ-octalactone, δ-decalactone, 2,5-dimethyl-4-hydroxy-3-(2H)-furanone and skatole as potent odorants. In addition, they found hydrogen sulphide, methanethiol, acetaldehyde, diacetyl, 2-heptanone, dimethyl trisulphide, nonanal, butanoic acid, 3-methylbutanoic acid, δ-hexalactone and hexanoic acid. According to these authors, 3-methylbutanoic acid (cheese-like odour), methional (potato-like), 2,5-dimethyl-4-hydroxy-3-(2H)-furanone (caramel-like) and 2-heptanone (blue-cheese) characterise the odour of heated butter. These compounds were not detected in fresh SwCB. On the other hand, odorants such as 2- and 3-methylbutanal, hexanal, γ-dodecalactone and DMS, found in the fresh SwCB, were not detected in heated butter. Ketones and especially lactones, which are present in fresh butter at subthreshold levels (Beers and Zeijden, 1966) show higher concentrations in heated butter and are hypothesised as being part of the pleasant flavour that is associated with many baked products containing butter. The aroma of heated butter as an ingredient in puff pastries was studied by Gassenmeier and Schieberle (1994a) using AEDA. They reported δ-decalactone, (*E*)-2-nonenal, 4-hydroxy-2,5-dimethyl-3-(2H)-furanone, butanoic acid, 3- and 2-methylbutanoic acid as the most potent odorants. The key odours of heated butter and their concentration, found in the different studies, are summarised in Table 2.2.

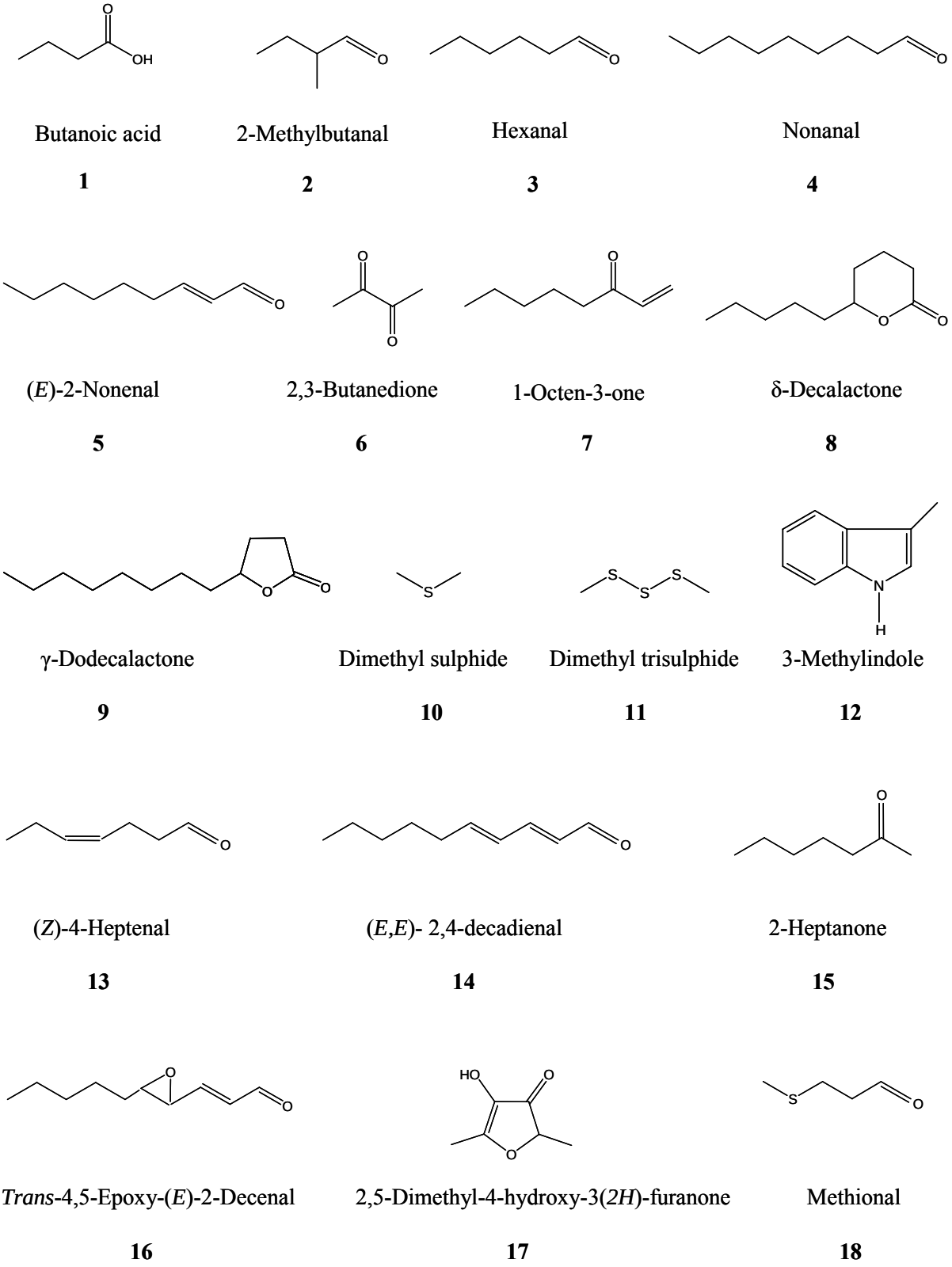


**Fig 2.1** Chemical structures of potent odour-active compounds of butter

**Table 2.2** Odour-active compounds in Heated Cream Sweet Butter as determined by gas chromatography-olfactometry

| No [a] | Compound | Odour quality | Concentration (µg/kg heated butter) |
|---|---|---|---|
| | **Acids** | | |
| 1 | Butanoic acid | Buttery, sweaty, cheesy, rancid | 353[b] |
| | 3-Methylbutanoic acid | Cheesy | 39[b] |
| | Hexanoic acid | Pungent, musty, cheesy, acrid | 1137[b] |
| | **Aldehydes** | | |
| 4 | Nonanal | Waxy, fatty, floral | 83[b] |
| 18 | Methional | Cooked potato | 0.95[b], 2.8[c] |
| 5 | (*E*)-2-Nonenal | Green, fatty, tallowy | 43[b] |
| 14 | (*E,E*)-2,4-Decadienal | Fatty | 13[c] |
| 16 | *trans*-4,5-Epoxy-(*E*)-2-decenal | Metallic | 2.7[c] |
| | **Ketones** | | |
| 6 | 2,3-Butanedione | Buttery | 3.8[b] |
| 15 | 2-Heptanone | Blue cheese | 1294[b] |
| | 1-Hexen-3-one | Vegetable-like | 0.69[c] |
| 7 | 1-Octen-3-one | Mushroom-like | 6 [b], 98.7[c] |
| | **Lactones** | | |
| | δ-Hexalactone | Creamy, chocolate, sweet aromatic | 218[b] |
| | δ-Octalactone | Coconut-like, peach | 258[b], 578[c] |
| 8 | δ-Decalactone | Coconut-like, peach | 2633[b], 5730[c] |
| | **Miscellaneous compounds** | | |
| 17 | 2,5-Dimethyl-4-hydroxy-3-[2*H*]-furanone | Sweet, caramel-like | 233[b], 58.7[c] |
| 12 | 3-Methyl-1H-indole (Skatole) | Mothball, fecal | 24[b], 5[c] |

[a]Numbers refer to Fig 2.1
[b]Concentration determined by the standard addition method according to Peterson and Reineccius (2003b)
[c]Concentration determined by SIDA according to Budin et al. (2001)

## 2.4 Butter off-flavours

The desirable and unique aroma of butter depends on a delicate balance of the concentrations of compounds having a low odour threshold Interactions between volatile and non-volatile compounds and the food matrix are also important. Any distortion of this balance by addition or deletion of aroma components can result in an off-flavour (Urbach et al., 1972). The development of off-flavours can go in parallel with loss of the nutritional quality (loss of vitamins, oxidation of unsaturated lipids) and sensory characteristics of butter and can lead consequently to significant economic losses (Azzara and Campbell, 1992).

Therefore, the identification and the origin of off-flavours in butter and butter oil have been the subject of several investigations (Badings, 1970a, b; Swoboda and Peers, 1977a, b; Widder, 1994a) and reviews (Azzara and Campbell, 1992; Grosch et al., 1992). Off-flavours in butter may have different origin and be related to lipid oxidation, lipolysis and microbial growth, occurring during butter manufacturing, packaging and storage. Transmitted off-flavours, caused by the transfer of substances from feed and environment into the butter, will also be discussed.

### 2.4.1 Oxygen induced off-flavours

Oxidised off-flavours in butter and dairy products have been described as cardboard-like, metallic, oily, fishy, painty and tallowy (Badings, 1960; Collomb and Spahni, 1996). These off-flavours originate from compounds produced during autoxidation of milk fat.

Autoxidation involves the conversion of unsaturated fatty acids, in the presence of oxygen, to hydroperoxides which decompose into various flavourful compounds (Grosch et al., 1992). The autoxidation rate in butter depends on the fatty acid composition (e.g. linoleic acid oxidises 10 times faster than oleic acid), presence of antioxidants (α-tocopherol, ascorbic acid and carotenoids) and pro-oxidants (peroxides and heavy metals). Pro-oxidants, like iron and copper ions, can be naturally present in

butter or originate from the metal equipments used during butter manufacturing. The oxidation rate is also due to external factors, such as oxygen pressure, temperature, light exposure and moisture. The phospholipids that form the fat globule membranes, containing unsaturated fatty acids, are highly susceptible to oxidation. They may come into contact with proxidants such as copper, especially present in the serum phase, because of their position at the fat/water interface (Badings, 1970a,b). In the first phase of the autoxidation, molecular oxygen reacts with unsaturated fatty acids to produce hydroperoxides (primary oxidation products) and free radicals, both of which are very reactive. The primary products of autoxidation are odourless, e.g. linoleic acid hydroperoxides (Belitz et al., 2004). The reactive products of the initiation phase react with additional lipid molecules to form other reactive chemical compounds. The termination phase of the autoxidation leads to the formation of relatively stable compounds such as hydrocarbons, aldehydes and ketones. These compounds are secondary oxidation products and some of them are characterised by an intense odour, which can cause off-flavour at higher concentrations.

Different studies were accomplished about oxidised off-flavours formed in butter during prolonged storage. Badings studied the auto-catalytic oxidation of unsaturated fatty acids that causes flavour defects in butter during cold storage (Badings, 1970a, b). In these studies van der Waarden (1947) is referenced to be the first to present conclusive evidence that cold-storage defects in butter are caused by oxidative degradation of lipid components. Butter samples with “trainy” off-flavour were analysed by Badings who correlated odour thresholds and quantitative data of the potent odorants to explain their contribution to the butter off-flavour. Among the odour compounds present in trainy butter, at concentrations higher than their flavour threshold, there were: hexanal (green), heptanal (oily, putty), (*E*)-2-nonenal (tallowy, cucumbers), (*E*,*E*)-2,4-heptadienal (metallic, fried), (*Z*)-4-heptenal (creamy, putty), (*E*,*Z*)-2,4-decadienal (fried), (*E*,*Z*)-2,6-nonadienal (fresh cucumbers), 1-octen-3-ol (metallic) and 1-octen-3-one (mushroom). Badings explained that the precursors of

these compounds are arachidonic, linoleic and especially linolenic acid, which contributes most to the “trainy” flavour of cold-stored butter.

Hexanal, originating from autoxidation of linoleic acid, often predominates in the volatile fraction of oxidised foods (Belitz et al., 2004) and was chosen as an indicator of lipid oxidation in butter during storage (Christensen and Hølmer, 1996). (E)-2-Nonenal and 1-octen-3-one easily form by oxidation of linoleic acid (Badings, 1970a). The metallic off-flavour in butter can be explained by 1-octen-3-one and 1,5 (Z)-octadien-3-one (Stark and Forss, 1962; Swoboda and Peers, 1977a).

Widder (1994a) studied the off-flavour compound formation in BO by AEDA. This principle proved useful when comparing samples affected by off-notes and samples without odour defects (Schieberle, 1995a). The odour profiles of different samples, stored at 35˚C for 0, 42, 90 and 120 days, respectively, were compared. The results were consistent with the findings of Badings (1970a). The dilution factors of (*E*)-2-nonenal, (*Z*)-2-nonenal, (*Z*)-4-heptenal and 1-octen-3-one increased proportionally with the storage period. The amounts of these compounds increased also in the presence of copper ions acting as pro-oxidants. They decreased when antioxidants, such as α- and γ- tocopherol, BHA and BHT were present.

Another AEDA study (Widder, 1991) showed that nine odour-active carbonyl compounds were responsible for off-flavours in BO, stored for 42 days at room temperature. Hexanal, (*Z*)-3-hexenal, 1-octen-3-one, (*Z*)-1,5-octadien-3-one, nonanal, (*Z*)- and (*E*)-2-nonenal, (*E,E*)-2,4-nonadienal, (*E,E*)-2,4-decadienal and in particular, 1-octen-3-one (FD=1024), (*E*)-2-nonenal (256) and (*Z*)-1,5-octadiene-3-one (256) showed the highest FD factors.

Ullrich and Grosch (1988) demonstrated that (*Z*)-1,5-octadiene-3-one originates from linolenic acid. Widder and Grosch (1997) proved the formation of (*Z*)- and (*E*)-2-nonenal from autoxidised (*Z*)-9-hexadecenoic acid (palmitoleic acid). Their sensory study of BO led to the conclusion that a mixture of (*Z*)- and (*E*)-2-nonenal was

responsible for the cardboard off-flavour (Widder and Grosch, 1994b). The off-note was observed when the OAVs surpassed a value of 0.5 for each of the two nonenals. Further studies with and without antioxidants proved the two nonenals as suitable indicators for the cardboard-like off-flavour of BO.

### 2.4.2 Light-induced off-flavours

Off-flavour in butter and dairy products exposed to light can be generated by protein degradation, which causes burnt, cabbage and mushroom-like odours and by photo-induced lipid oxidation, yielding cardboard, metallic, tallowy and oily off-notes (Azzara and Campbell, 1992). Photo-oxidation takes place when photo-sensitisers such as riboflavine are activated in foods and react with a substrate like an amino acid or lipid, generating substrate radicals in the so-called photo-oxidation Type I reaction. The sensitiser can also activate oxygen to its singlet state, which then starts a photo-oxidation type II chain reaction (Belitz et al., 2004).

Methional, from photodecomposition of methionine, is mainly responsible, together with mercaptanes, for the light-activated flavour in dairy products (Bosset et al., 1993).

Grosch and co-workers (1992) studied BO exposed to fluorescent light for 48 h, using AEDA. Under these conditions, BO developed green, strawy and fatty off-notes, which were mainly due to the formation of 3-methylnonane-2,4-dione derived from furan fatty acids, 4,5-epoxy-(*E*)-2-decenal and high concentrations of (*E*)-2-nonenal and (*E,E*)-2,4-decadienal. It is evident that the packaging of butter has a fundamental role in the protection against light and oxygen.

### 2.4.3 Heating-induced off-flavours

Heating-induced off-flavours have been described in dairy products (Shipe et al., 1978) as cooked, burnt, sulphurous and caramelised. These off-flavours can be formed during pasteurisation at temperatures above 76.7°C (Bodyfelt et al., 1988) or during high temperature treatment leading to a Maillard reaction.

Ellis and Wong (1975) demonstrated that higher levels of lactones in BO are due to increased temperatures at prolonged heating times. Certain lactones can cause an undesirable coconut-like off-flavour (Keeney and Patton, 1956). Lee and co-workers (1991) accomplished a study on SwCB, heated at 100, 150 and 200 °C for 5 h. The highest temperature determined an increase in the number of volatile compounds in butter. In particular aldehydes and ketones increased significantly at 200°C, suggesting that lipid degradation was the major reaction occurring in butter during heating. Heterocyclic compounds including thiazoles, pyrroles and pyridines, were found in butter heated above 150°C. These volatiles contribute significantly to the heated butter flavour because of their low odour thresholds (Shimaboto, 1986). However the long heating period of 5 h used in this study does not compare to usual household or manufacturing processes. Gassenmeier and Schieberle (1994a) found 4,5-epoxy-(*E*)-2-decenal, having metallic odour, and (*E,Z*)-2,4-decadienal, which is fat and green smelling, as most important odorants in puff-pastries prepared with butter, baked for 12 min at 180°C. They suggested that both aldehydes arise from peroxidation of linoleic acid during heating. The same authors reported 4,5-epoxy-(*E*)-2-decenal to be formed from 13-hydroxy-9,11-octadecadienoic acid and 9-hydroperoxy-10,12-octadecanoic acid, which are precursors isolated from thermally treated fat, such as baking shortening (Gassenmeier and Schieberle, 1994b).

### 2.4.4 Lipolysis-induced off-flavours

Lipolysed off-flavours, often described as goaty or soapy, are caused by lipoprotein lipases, enzymes naturally present in the skim part of milk. The lipases are normally occluded by protein, e.g. casein micelles, preventing direct contact with the fat globules. When the double layer membrane protecting the fat globule is disrupted, by agitation or churning, lipolysis can take place causing rancid odour notes. These off-flavours are mainly due to the increase in free fatty acids (Shipe, 1980a; Gonzales-Cordova and Vallejo-Cordoba, 2003).

Schieberle and co-workers (1993) analysed a sour cream butter manufactured traditionally in a farm by AEDA. The sample showed a rancid and sweaty odour, which was due to high concentrations of butanoic and hexanoic acids formed by lipolysis. The presence of lipases in butter can also be caused by external microbial contamination.

Apart from the development of off-flavour compounds, lipolysis can also reduce the churning efficiency of cream (Allen, 1994). A pasteurisation process of at least 76.7˚C for 16 s is in general sufficient to prevent the lipolysis-induced off-flavour (Shipe, 1980b).

### 2.4.5 Microbial off-flavours

Microbial off-flavours in butter are the results of metabolites produced by microrganisms in the raw milk, prior to pasteurisation, or due to successive contaminations, occurring during manufacturing and storage.

Musty off-flavour in cream or butter is often due to 2-methoxy-3-alkylpyrazine produced by *Pseudomonas taetroleus*, which is a psychrotrophic strain (Morgan, 1976). Malty off-flavour is occasionally found in butter caused by the production of 3-methylbutanal and 2-methylbutanal by *Lactococcus lactis* var. maltigenes (Morgan, 1970). The presence of yeasty off-flavour in butter is the evidence that inferior microbiological quality cream was used (Azzara and Campbell, 1992).

The pasteurisation destroys bacteria responsible for microbial off-flavour, nevertheless heat- resistant bacteria lipases may remain active producing off-flavours (Azzara and Campbell, 1992). In butter, the microbiological development is generally limited, due to the presence of a strongly dispersed water phase (Jensen, 1983) and low storage temperature. The microbial-related off-flavours can be prevented by the use of good-quality sweet cream with proper sanitation during storage and processing (Azzara and Campbell, 1992).

### 2.4.6 Taint off-flavours

These off-flavours can be carried over into the butter from the environment, via the milk (Shipe et al., 1962) or directly by external flavour absorption (Azzara and Campbell, 1992).

Off-flavours in dairy products originating from animal feeding can cause serious aroma defects. Among the feeds that are known to transfer off-flavours to milk products are fermented silage, musty hay or silage (Bandler et al., 1976) and alfalfa. They contain (*E*)-2-hexenal, (*E*)-3-hexenals and (*E*)-3-hexenols (Morgan and Periera, 1962) which impart a green flavour to dairy products.

Butter tainted by the cruciferous weed Coronopus didymus L. in feedstock is reported to contain sulphur compounds, such as benzylmethyl sulphide, benzyl sulphide, benzyl isothiocyanate, benzyl cyanide, indole and skatole. In particular, benzylmethyl sulphide and benzyl sulphide were considered the principal contributors to the weed off-flavour (Forss, 1971).

# 3. Storage stability of UFA/CLA enriched and conventional butter*

The oxidative stability of UFA/CLA enriched butter was evaluated by chemical, sensory and microbiological analyses during eight weeks of storage at 6 °C and compared to that of conventional butter. The odour-active compounds were analysed 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 to 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.

* This chapter has been published as:
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## 3.1 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 et al., 2002; Jones et al., 2005; Collomb et al., 2006) since they are claimed to have beneficial effects on human health. Milk fat naturally contains UFA in the range of 25% to 35% depending upon feeding regimen, season, breed and period of lactation. More than 95% of UFA in milk fat is in form of oleic acid (21–30% of total fat), linoleic acid (2–2.5%) and α-linolenic acid (1–1.3%) (Collomb et al., 2000a). 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 in winter to 1.20 g/100 g in summer. Similar variation in CLA contents was found in butter by Ledoux and co-workers (2005), who indicated total CLA levels varying from 0.45 g/100 g fat in winter to 0.80 g/100 g fat in summer. The CLA contents in 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 g/100 g milk fat: Kelly et al., 1998; 2.21 versus 0.39 g/100 g milk fat: Dhiman et al., 1999). Collomb and co-workers (2002) found especially high CLA values in milk from mountain pasture, varying from 1.90 to 2.80 g/100 g. 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 et al., 2004a, b; Ryhänen et al., 2005), or with free oils, such as soybean, linseed and fish oils (AbuGhazaleh et al., 2004). Collomb and co-workers (2004b) 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 fat). A dietary supplementation with sunflower seeds led to the highest content of the *cis*-9, *trans*-11 CLA isomer, which is considered a very health promoting fatty acid (FA). It represents 75% to 90% of the total CLA

concentration in milk fat (Baumann et al., 2003) and was reported to show anticarcinogenic (Ha et al., 1990; Ip et al., 1991; Parodi, 1994; Ip et al., 1999), body fat reducing (Pariza et al., 1996) and growth-promoting (Chin et al., 1994) properties.

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 autoxidation 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 autoxidation of the lipids in dairy products and the resulting off-flavours have been comprehensively studied (Badings, 1970; Swoboda and Peers, 1977a, b; Widder et al., 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 autoxidation patterns of CLA are still not well known and only few studies indicate the secondary products of CLA autoxidation (Yurawecz et al., 1995; Eulitz et al., 1999). *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 et al., 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 et al., 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 et al., 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 to 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.

## 3.2 Materials and methods

### 3.2.1 Butter samples and design of storage tests

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 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 per cow of corn silage in the stable. The milk was collected separately after 12 days from the two groups (300 L each) during two 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 pieces of 100 g 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 acids 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 analysed 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.

### 3.2.2 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 butter fat agar incubated for 72 h at 30 °C. Victoria blue butterfat agar consisted of 5 g/L meat extract (Oxoid, Pratteln, Switzerland), 5 g/L bacto-peptone (BD, Basel, Switzerland), 12 g/L agar-agar (Oxoid), 5 % butterfat and 0.004 % Victoria blue B (Fluka, Buchs, Switzerland).

### 3.2.3 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 3 and was calculated from 35 duplicate determinations in accordance with ISO 5725-2 (ISO, 1994).

### 3.2.4 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 3.1.

**Table 3.1**: Chemical composition of UFA/CLA[a] enriched and conventional butter. The results are expressed as absolute values and also reported as relative to dry mass.

| Chemical composition (in absolute values) | Unit | r[b] | UFA/CLA[a] 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 expressed relative to dry mass) | | r[c] | | | | |
| 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]Unsaturated fatty acids/conjugated linoleic acid
[b]Repeatability reported relative to absolute values and calculated according to ISO 5725-2 (ISO, 1994)
[c]Repeatability reported relative to dry mass and calculated by error propagation

### 3.2.5 Determination of copper and iron

For copper determination, 4 g of homogenized butter were 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 analysed 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, atomisation at 1900 °C for 5 s. A calibration curve was obtained measuring different dilutions of a Titrisol copper solution (1 g Cu/L; 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, atomisation at 2100 °C for 3 s. A calibration curve was obtained measuring different dilutions of a Titrisol iron solution (1 g Fe/L; Merck). The repeatability of copper and iron determinations, indicated in Table 3.1, was calculated by 80 and nine duplicate analyses of butter samples, respectively.

### 3.2.6 Analysis of fatty acid composition: short chain fatty acids, UFA and CLA-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 (2000b). Isomers of CLA were analysed and quantified by Ag+ -HPLC according to Collomb et al. (2004a). The repeatability of the fatty acid determination was below 0.49 g/kg for all fatty acids except for C4 (0.65 g/kg), C14 (0.77 g/kg), C16 (1.89 g/kg), C18 (0.66 g/ kg) and C18:1c9 (1.34 g/kg) as determined by 35 duplicate analyses of butter. The repeatability of the CLA isomers was below 0.15 g/kg as determined by 35 duplicate analyses of butter.

### 3.2.7 Extraction and analysis of the odour-active compounds by GC/MS/O

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, then were 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 x 0.25 mm x 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 to 240 °C. Helium was used as the carrier gas at a constant flow of 2.4 mL/min. 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 and 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.

### 3.2.8 Sensory analysis

*Development of methodology*

Ten trained panellists 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 TL40W/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 3.2, 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 panellists 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 samples 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.

*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 and 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 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 panellists evaluated each sample twice, corresponding to 1, 2, 4, 6 or 8 weeks of storage at 6 °C and at -20 °C.

**Table 3.2:** Standardised 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 |
| Oxidised, 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 $H_2O$, 18 ± 2 °C | 10 |
| Oxidised, 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 |

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 3.3, 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 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 3.3). 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.

## 3.3 Results and discussion

### 3.3.1 Microbial status of fresh and stored butter

The results of the microbiological analyses are shown in Table 3.4. 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 3.1. Nevertheless, these samples conformed to the microbiological quality parameters defined by the Swiss Federal hygiene regulation (SR 817.051, 2004).

### 3.3.2 Overall chemical composition of fresh and stored butter

The chemical composition of fresh UFA/CLA enriched butter and conventional butter is shown in Table 3.1. 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 (Chilliard et al., 2001; AbuGhazaleh et al., 2004). Banks et al. (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 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 sunflowers seeds, which contain carotenoids, vitamin E, copper and iron (Souci et al., 2000; Sauvant et al., 2004). All these parameters remained constant during storage, except α-tocopherol which was significantly lower, in both butter types, after 6 weeks of storage.

### 3.3.3 Fatty acid composition of fresh and stored butter

The UFA/CLA enriched butter and conventional butter had a significantly different FA composition. Table 3.5 shows the FA composition of fresh butter and in butter after 8 weeks of storage at 6°C. Compared to conventional butter, the UFA/CLA enriched butter had significantly higher concentrations of monounsaturated FA (MUFA) (+30%), polyunsaturated FA (PUFA) (+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 C18:1 *trans* 9 and C18:1 *trans* 12, 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 3.6. The CLA isomers *cis* 9, *trans* 11 and *trans* 7, *cis* 9 were significantly higher in UFA/CLA enriched butter. The concentration of the most important CLA (*cis* 9, *trans* 11-CLA) 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).

**Table 3.3:** 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 higher peak area found by gas chromatography/mass spectrometry
[c] LRI, linear retention index using DB-5MS column
[d] NS, not significant; *$P$<0.05; **$P$<0.01; ***$P$<0.001
[e] 1 to 8 weeks of storage at 6°C

**Table 3.4:** Microbiological parameters of UFA/CLA[a] enriched and conventional butter (fresh and after 6 weeks of storage at 6°C).

| | Unit | 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

### 3.3.4 Influence of storage on odour-active compounds in UFA/CLA enriched and 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 3.7 summarises 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 3.7. 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 sulphury (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 et al., 2005). Dimethyl disulphide also disappeared during storage. Similar findings were observed by Shooter et al. (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, 1994a). *Trans*-4,5-epoxy-2-decenal was found as an important odour-active compound in puff-pastries prepared with butter (Gassenmeier and Schieberle, 1994a). Both aldehydes arise from the autoxidation of linoleic acid (Widder, 1994a). Interestingly, two unknown compounds (RI= 918 and 1591), 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 3.3). The effect most influencing the results was the storage period (1 to 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, 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. Figure 3.1 shows heptanal as an example for the increase in signal intensity of the volatile aldehydes in UFA/CLA enriched butter during storage, in comparison to conventional samples. Hexanal, a reported indicator of lipid oxidation in food (Christensen and Hølmer, 1996), was surprisingly not significantly higher in UFA/CLA butter compared to 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 et al., 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.

**Table 3.5**: Fatty acid composition (g/100 g 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[a] | Conventional | UFA/CLA[a] | 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[c] | 0.27 | 0.15 | 0.27 | 0.14 | * | NS |
| C16:1 c[d] | 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[e] + 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[f] | 48.55 | 59.50 | 48.54 | 59.74 | ** | NS |
| Sum C18:1[g] | 33.43 | 22.52 | 33.12 | 22.57 | * | NS |

## Table 3.5 continued

| Fatty acid | Fresh | | 8 weeks of storage | | Significance of the effects in the ANOVA[b] | |
|---|---|---|---|---|---|---|
| | UFA/CLA[a] | Conventional | UFA/CLA[a] | Conventional | Butter type | Storage |
| Sum C18:2[h] | 4.92 | 2.96 | 4.70 | 2.92 | * | NS |
| Sum unsaturated FA[i] | 41.50 | 28.94 | 40.97 | 28.90 | * | NS |
| Sum monounsaturated[k] | 35.82 | 25.13 | 35.50 | 25.16 | * | NS |
| Sum polyunsaturated[l] | 5.67 | 3.80 | 5.47 | 3.73 | * | NS |
| Sum C18:1t[m] | 7.74 | 4.33 | 7.57 | 4.39 | * | NS |
| Total CLA (GC)[n] | 1.98 | 0.82 | 1.98 | 0.82 | * | NS |
| Sum C18:2t without CLA t[o] | 1.26 | 0.87 | 1.14 | 0.85 | * | NS |
| Trans total without CLA t[p] | 9.31 | 5.40 | 9.02 | 5.40 | ** | NS |
| Sum omega-3[q] | 0.96 | 0.98 | 0.92 | 0.94 | NS | NS |
| Sum omega-6[r] | 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] trans; [d] cis

[e] methylene interrupted diene

[f] 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

[g] C18 :1 -t4, -t5, -t6-8, -t9, -t10-11, -t12, -t13-14 + -c6-8, -c9, -c11, -c12, -c13, -16 + c14

[h] 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

[i] C10:1, C14:1 ct, C16:1 ct, C17:1 t, Σ C18:1, Σ C18:2, C20:1 t, C18:3 c6,c9,c12, C20:1 c5, C20:1 c9, C20:1 c11, C18:3 c9,c12,c15, C18:2 c9,t11 + t8,c10 + t7,c9, C18:2 t11c13 + c9,c11, C18:2 t9,t11, C20:2 c,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)

[k] C10:1, C14:1 ct, C16:1 ct, C17:1 ct, ΣC18:1, C20:1 t, C20:1 c5, C20:1 c9, C20:1 c11

[l] Σ 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)

[m] 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

[n] Sum (C18:2 c9t11+t8c10+t7c9) + (C18:2 t11c13+c9c11), C18:2 t9t11

[o] C18:2 -ttNMID, -t9,t12, -c9,t13 + -t8,c12, -c9,t12 + -c,c-MID + -t8,c13, -t11,c15 + -t9,c12

[p] C14:1 t, C16:1 t, C17:1 t, C20:1t, C18:1 trans + C18:2 trans (without CLA trans)

[q] C18:2 t11c15 + C18:2c9c15, C18:3 c9c12c15, C20:3 n-3, C20:5, C22:5 and C22:6

[r] C18:1 t12, C18:1 c12, C18:2 t9t12, C18:2 c9t12+(c,c-MID+t8c13), C18:2c9c12, C18:3 c6c9c12, C20:2 cc, C20:3 n-6 and C20:4 n-6

**Table 3.6**: PUFA[a] contents and CLA[b] isomers (g/100 g 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[c] | Conventional | UFA/CLA[c] | 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 |
| Sum 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 3.7**: 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].

| | | | | Fresh /1, 2 weeks | | 4 weeks | | 6 weeks | | 8 weeks | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Compound | LRI[c] | Odour descriptor | Identification[d] | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV |
| **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 | | | + | + | + | + | + | + |

| Compound | LRI[c] | Odour descriptor | Identification[d] | Fresh /1, 2 weeks | | 4 weeks | | 6 weeks | | 8 weeks | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV |

**Table 3.7 continued**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (*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 | + | + | ++ | + | +++ | ++ | +++ | ++ |

| Compound | LRI[c] | Odour descriptor | Identification[d] | Fresh /1, 2 weeks | | 4 weeks | | 6 weeks | | 8 weeks | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV |

**Table 3.7 continued**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (*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-decenalT | 1380 | fatty, metallic | MS, PI | + | + | + | + | +++ | + | +++ | + |
| tridecanal | 1504 | oily | MS, PI, R | | | | | +++ | + | +++ | + |

| Compound | LRI[c] | Odour descriptor | Identification[d] | Fresh /1, 2 weeks | | 4 weeks | | 6 weeks | | 8 weeks | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV |
| **Table 3.7 continued** | | | | | | | | | | | |
| **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-methylpentane | 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 | | | | | +++ | + | +++ | ++ |

| Compound | LRI[c] | Odour descriptor | Identification[d] | Fresh /1, 2 weeks | | 4 weeks | | 6 weeks | | 8 weeks | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV |

## Table 3.7 continued

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | ++ | ++ | | | | | | |

## Table 3.7 continued

| Compound | LRI[c] | Odour descriptor | Identification[d] | Fresh /1, 2 weeks | | 4 weeks | | 6 weeks | | 8 weeks | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV | UFA/ CLA[a] | CONV |
| dimethyl sulfone | 927 | fatty, sulfur | 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 | oxidised 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]Conventional butter; + weak, ++ medium, +++strong odour intensity perceived by two trained panellists

### 3.3.5 Influence of storage on sensory properties of UFA/CLA enriched and conventional butter

The fresh (1-week old) UFA/CLA enriched butter showed a significantly higher creamy aroma and a less intense cooked milk aroma than the fresh conventional butter (Figure 3.2). 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 and co-workers (2005). In their study, differences in spreadability between CLA enriched butter and control butter were observed and were explained by a softer texture of the CLA enriched butter, due to the unsaturated fatty acids present.

During the eight 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 oxidised 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 to results from Krause et al. (2008) and Lozano et al. (2007), who observed the development of a "refrigerator/stale" flavour in butter only after six 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 six 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 two weeks, increasing in the following weeks and decreasing again towards the end of the testing period. Figure 3.3 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 and co-workers (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.

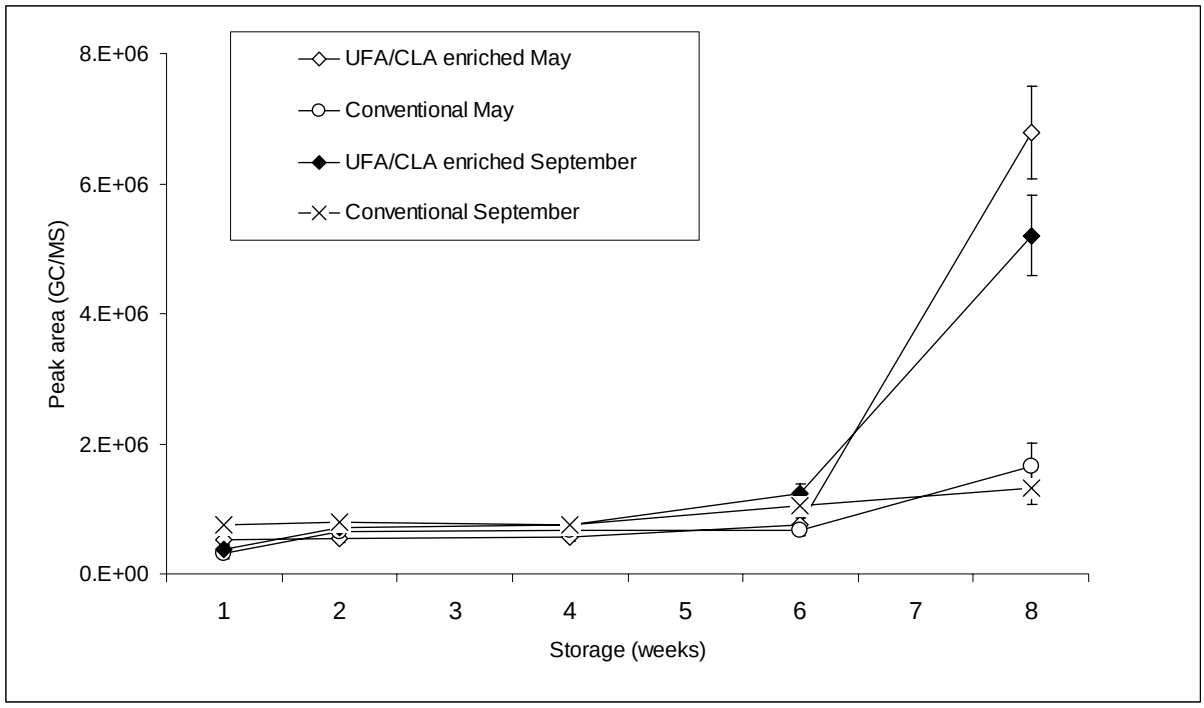


**Fig. 3.1** Heptanal content development during storage at 6 °C in unsaturated fatty acid/conjugated linoleic acid (UFA/CLA) enriched and conventional butter, produced in May and September.

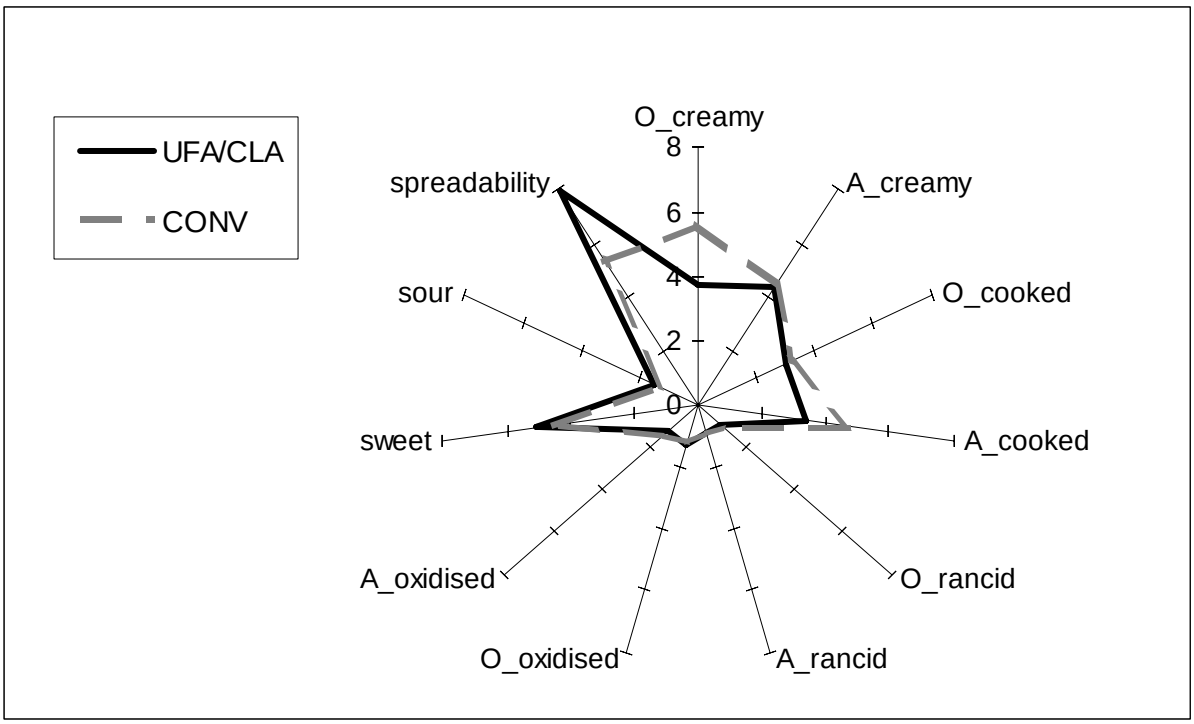


**Fig. 3.2** Odour (O), aroma (A) and texture attributes of the unsaturated fatty acid/conjugated linoleic acid (UFA/CLA) enriched butter and conventional butter after 1 week of storage at 6 °C.

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 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 and Grosch, 1998).

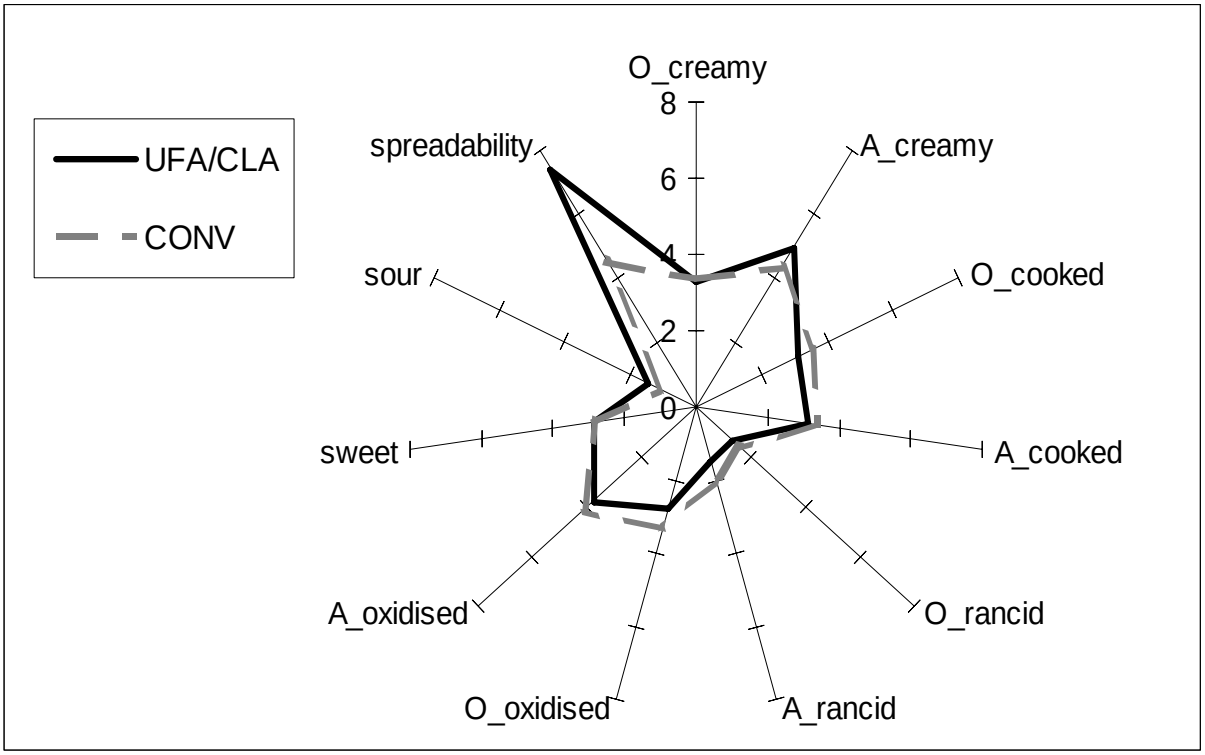


**Fig. 3.3** Odour (O), aroma (A) and texture attributes of the unsaturated fatty acid/conjugated linoleic acid (UFA/CLA) enriched butter and conventional butter after 8 weeks of storage at 6 °C.

A highly significant correlation ($P<0.001$), however, was found between the "oxidized odour" sensory attribute and the peak intensity of hexane ($r2 = 0.80$). Hexane was not perceived by GC/O, probably due to its high odour threshold (Amoore and Hatala,1983). However, hexane could be used as an easily measurable marker of lipid oxidation, which confirms the findings of Christensen and Hølmer (1996).

## 3.4 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.

# 4. Influence of storage and induced oxidation on key odour compounds of UFA/CLA enriched and conventional butter*

Dairy products enriched in unsaturated fatty acids (UFA) and conjugated linoleic acid (CLA) may have higher nutritional value and beneficial health effects. However, these products are susceptible to oxidation and may off-flavors are formed. Our study aimed to compare the aroma profiles of UFA/CLA enriched butter to that of conventional butter during storage and induced oxidation. The volatiles were extracted by solvent-assisted flavour evaporation (SAFE) and identified by gas chromatography-olfactometry coupled to mass spectrometry (GC/MS/O). Aroma extract dilution analysis (AEDA) found eighteen relevant odorants that were quantified by stable isotope dilution analysis (SIDA). Another important odorant, skatole (monthball-like), was quantified by high pressure liquid chromatography (HPLC). After storage, UFA/CLA butter showed higher concentrations of pentanal (fatty odour), heptanal (green), butanoic acid (cheesy), and δ-decalactone (peach-like). Photo-oxidation induced an increase of 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.

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Mallia, S., Escher, F., Dubois, S., Schieberle, P., Schlichtherle-Cerny, H. Characterisation and quantification of odour-active compounds of UFA/CLA (unsaturated fatty acid/conjugated linoleic acid) enriched butter and conventional butter, during storage and induced oxidation.

## 4.1 Introduction

In the last years consumers demand more and more 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) could have these advantages, due to a higher content in essential fatty acids and to potential anticarcinogenic (Islam et al., 2008; Cunningham et al., 1997; Wong et al., 1997), cholesterol lowering (Lee et al., 1994) and body fat reducing effects (Park et al., 1997), attributed to CLA.

Several studies have focused on increasing the amount of UFA/CLA in dairy products by supplementing the ruminant's diet with oils or oleaginous seeds, rich in oleic, linoleic and linolenic acids (Collomb et al., 2006; AbuGhazaleh, 2002; Lawless, 1998). Collomb et al. (Collomb, 2004a, b) showed that higher CLA content in milk fat is obtained supplementing the cow's diet with sunflower seeds especially rich in linoleic acid.

In our study, butter enriched in UFA/CLA was produced feeding the cows with both pasture and sunflower seeds. However, the higher UFA/CLA content could maybe negatively affect the flavor of the butter since unsaturated lipids are more susceptible to auto-oxidation (Grosch, 1987). The effects of UFA oxidation on the flavor of butter have already been described by Badings (1970), who indicated the formation of several odorants during cold storage of butter, such as hexanal (odour of cut grass), heptanal (oily), (*E*)-2-nonenal (tallowy), (*E*,*E*)-2,4-heptadienal (metallic, fried) and (*E*,*Z*)-2,6-nonadienal (cucumber-like). These compounds originated from arachidonic, linoleic and linolenic acids. Another study (Widder et al., 1991), performed aroma extract dilution analysis (AEDA) (Schmid and Grosch, 1986) and showed that nine odour carbonyl compounds, including (*Z*)-3-hexenal (green, apple-like), 1-octen-3-one (mushroom-like), (*Z*)-1,5-octadien-3-one (metallic, geranium-like), (*Z*)- and (*E*)-2-nonenal (fatty, green), were responsible for off-flavors in butter oil that was stored for 42 days at room temperature. These odour compounds are as well formed by oxidation of unsaturated fatty acids. In particular, Ullrich and Grosch (1988) demonstrated that

(*Z*)-1,5-octadien-3-one originates from linolenic acid, whereas Widder and Grosch (1997) concluded that (*Z*)- and (*E*)-2-nonenal can be formed in butter from autoxidized (*Z*)-9-hexadecenoic acid (palmitoleic acid). Off-flavors in butter and generally in dairy products could also be caused by photo-induced lipid oxidation, due to the presence of photosensitizes, such as riboflavin, able to absorb energy and to be shifted to higher energy level, inducing a cascade of oxidation reactions (Borle et al., 2001).

Butter oil exposed to fluorescence light for 48 h developed fatty, green and strawy off-notes, mainly due to high concentrations of (*E,E*)-2,4-decadienal (fried), (*E*)-2-nonenal (tallowy) and *trans*-4,5-epoxy-(*E*)-2-decenal (metallic) (Grosch et al., 1992).

The mechanism of oleic, linoleic and linolenic acid oxidation and their possible secondary odour-active products have since long been extensively studied and the formation pathways are well known (Grosch, 1987). On the other hand, the odour compounds formation from CLA is not yet fully elucidated and further studies are required on this issue. Yurawecz and co-workers (2003) suggested that the auto-oxidation of unsaturated fatty acids via a free radical mechanism is not probable to occur in CLA because higher activation energy is required for separating conjugated double bonds. The authors reported that methyl *cis* 9, *trans* 11 CLA ester, through 1,2 and 1,4 cycloadditions with oxygen could form dioxetane structures possibly leading to the formation of heptanal, lactones and esters, such as methyl octanoate. The oxidation of different CLA isomers also leads to different products: for example *cis* 9, *trans* 11 CLA, kept in glass vials exposed to oxygen and ambient light for 8 days, showed oxidation products such as heptanal, 2-nonenal and methyl 8-(5-hexyl-2-furyl)octanoate; under the same conditions, *trans* 10, *cis* 12 CLA developed mainly hexanal, methyl nonanoate and decadienal. Another study (Chen et al., 1997) examined the stability of CLA in form of free fatty acids, methyl esters and triacylglycerols, respectively. The CLA free fatty acids were extremely unstable and had an oxidation rate considerably higher than linoleic, linolenic and arachidonic acid (Zhang and Chen, 1997). The CLA was also described as having an inhibition activity on lipid oxidation (Ip et al., 1991) whereas other studies reported that CLA might have a pro-oxidant activity (Van den Berg, 1995). It is still not very clear how CLA

behaves during oxidation. Therefore further studies are necessary, including on the storage stability of CLA-containing foods.

Several studies on the oxidative stability of milk, cheese and butter, enriched in CLA, showed no significant differences in flavor compared to the conventional ones (not enriched) (Avramis et al., 2003; Lynch et al., 2005). On the other hand, one sensory study on milk showed that CLA fortified milk was less acceptable than milk without CLA addition, because of its "grassy/vegetable oil" flavor (Campbell et al., 2003).

The objective of the present study was to evaluate the aroma compounds of UFA/CLA enriched butter versus conventional butter during 6 weeks of refrigerated storage. This period of storage was chosen to sufficiently cover the usual shelf life indicated on the label by Swiss butter manufactures (30 days).

The predominant odorants were identified in the two kinds of butter by AEDA coupled to gas chromatography olfactometry (GC/O) and then quantified using stable isotope dilution assay (SIDA). Additionally, oxidation was also induced in the butter samples, to evaluate the stability of UFA/CLA enriched butter under light exposure and in the dark under oxygen atmosphere.

## 4.2 Materials and methods

### 4.2.1 Chemicals

Diethyl ether, sodium carbonate, anhydrous sodium sulfate, sodium chloride, methanol Lichrosolv, potassium phosphate, anhydrous potassium hydrogenphosphate were obtained from Merck (Darmstadt, Germany), acetonitrile was supplied from Romil Pure Chemistry (Cambridge, UK). Demineralized water was obtained using a Millipore (Schwalbach, Germany) system. The compounds listed in Table 4.1 were obtained from commercial sources: 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); (*Z*)-3-hexenal (**16**) was from SAFC

(Hamburg, Germany). (*Z*)-2-nonenal (**17**) and *trans*-4,5-epoxy-(*E*)-2-decenal (**18**) were synthesized according to (Ullrich and Grosch, 1987) and (Schieberle and Grosch, 1991), respectively.

2-Methylindole and 3-methylindole (skatole) were from Aldrich (Buchs, Switzerland). The labeled internal standards were synthesized at the Deutsche Forschungsanstalt für Lebensmittelchemie, by the following published syntheses: [5,6-$^2H_2$]hexanal [**2-d** (Guth and Grosch, 1993)], [2,3-$^2H_2$]-(*E*)-2-octenal [**4-d** (Guth and Grosch, 1993)], [2,3-$^2H_2$]-(*E*)-2-nonenal [**5-d** (Guth and Grosch, 1990b)], [2,3-$^2H_2$]-(*E,Z*)-2,6-nonadienal [**6-d** (Guth and Grosch, 1990b)], [5,6-$^2H_2$]-decanal [**7-d** (Büttner and Schieberle, 2001)], [$^2H_3$]-methional ([$^2H_3$]-3-methylthio-propanal) [**8-d** (Sen and Grosch, 1991)], [8,9-$^2H_2$]-δ-octalactone [**9-d** (Christlbauer, 2005)], [10,11-$^2H_2$]-δ-decalactone [**10-d** (Schieberle et al., 1993)], [12,13-$^2H_2$]-δ-dodecalactone [**11-d** (unpublished synthesis)] was synthesized similarly to [$^2H_2$]-δ-decalactone. [3,4-$^2H_2$]-1-octen-3-one [**12-d** (Guth and Grosch, 1990)], [3,4-$^2H_2$]butanoic acid [**13-d** (Schieberle et al., 1993)], [5,6-$^2H_2$]-hexanoic acid [**14-d** (Guth and Grosch, 1993)], [5,5,6,6-$^2H_4$] nonanal [**15-d** (Kerscher, 2000)], [3,4-$^2H_2$]-(*Z*)-3-hexenal [**16-d** (Guth and Grosch, 1990b)], [7,7,8,8-$^2H_4$]-*trans*-4,5-epoxy-(*E*)-2-decenal [**18-d** (Guth and Grosch, 1990b)].

**Table 4.1:** Compounds quantified in UFA/CLA and conventional butter by stable isotope dilution analysis

| No. | Odorant[a] | Selected[b] ion *m/z* | Labelled standard | Selected ion *m/z* | UFA/CLA[c] and CONV[d] |
|---|---|---|---|---|---|
| **1** | Pentanal[e] | 87 | **2-d** | 103 | Fresh, 6 weeks, oxidized |
| **2** | Hexanal | 101 | **2-d** | 103 | Fresh, 6 weeks, oxidized |
| **3** | Heptanal[e] | 115 | **2-d** | 103 | Fresh, 6 weeks, oxidized |
| **4** | (*E*)-2-Octenal | 127 | **4-d** | 129 | Oxidized |
| **5** | (*E*)-2-Nonenal | 141 | **5-d** | 143 | Fresh, 6 weeks, oxidized |
| **6** | (*E*,*Z*)-2,6-Nonadienal | 139 | **6-d** | 141 | Fresh, 6 weeks, oxidized |
| **7** | Decanal | 157 | **7-d** | 161 | Fresh, 6 weeks |
| **8** | Methional | 105 | **8-d** | 108 | Oxidized |
| **9** | δ-Octalactone | 143 | **9-d** | 145 | Fresh, 6 weeks |
| **10** | δ-Decalactone | 171 | **10-d** | 173 | Fresh, 6 weeks |
| **11** | δ-Dodecalactone | 199 | **11-d** | 201 | Fresh, 6 weeks |
| **12** | 1-Octen-3-one | 127 | **12-d** | 129 | Oxidized |
| **13** | Butanoic acid | 89 | **13-d** | 91 | Fresh, 6 weeks |
| **14** | Hexanoic acid | 117 | **14-d** | 121 | Fresh, 6 weeks |
| **15** | Nonanal | 143 | **15-d** | 147 | Fresh, 6 weeks, oxidized |
| **16** | (*Z*)-3-Hexenal | 81 | **16-d** | 83 | Oxidized |
| **17** | (*Z*)-2-Nonenal | 141 | **5-d** | 143 | Fresh, 6 weeks, oxidized |
| **18** | *trans*-4,5-Epoxy-(*E*)-2-decenal | 139 | **18-d** | 143 | Oxidized |

[a] Odorant quantified using FFAP capillary column

[b] Ion measured in Chemical Ionization (CI) mode

[c] Unsaturated fatty acid/conjugated linoleic acid enriched butter

[d] Conventional butter

[e] Odorant quantified using HS-SPME extraction.

### 4.2.2 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 with 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

(Mallia et al., 2008). The raw milk was collected separately from the two groups (150 L for each), preheated in the pilot plant at 45 °C and then centrifuged to obtain cream containing 35% of fat. The butter-making was already previously described (Mallia et al., 2008). Five kilograms of sweet cream butter were produced, for each kind of milk, wrapped in aluminum foil in pieces of 100 g and stored in the dark at 6 ± 1 °C. Butter samples kept under refrigerated conditions were analyzed for their odour-active composition at 0 (fresh) and 6 weeks of storage.

### 4.2.3 Oxidised butter samples

Fresh butter samples, in pieces of 100 g, were placed in a desiccator and exposed to a continuous flow of pure oxygen for 6 and 12 h at 6 °C, respectively, in the dark.

Fresh samples were also exposed to fluorescence light (Philips TL40W/33RS, 2000 lx) for 6 and 12 h at 6 °C, respectively. The samples were periodically turned to allow uniform light exposure.

### 4.2.4 Chemical composition of UFA/CLA and conventional butter

Moisture, non-fat solids and fat contents were determined according to reference procedures (IDF/ISO, 2002). Retinol and α-tocopherol were quantified using external standards of retinol and α-tocopherol, as described by (Mallia et al., 2008). Copper and iron were determined by atom absorption spectroscopy (Mallia et al., 2008). Short-chain fatty acids and unsaturated fatty acids were separated and quantified as described by Collomb and Bühler (2000). The free fatty acids were determined according to (De Jong and Badings, 1990). Conjugated linoleic acid isomers were analyzed and quantified by Ag+-HPLC according to (Collomb et al., 2004a).

### 4.2.5 Aroma extraction

Solvent-assisted flavor evaporation (SAFE), described by Engel et al. (1999) was used to isolate the volatile and semi-volatile aroma compounds from butter. The sample (100 g) was dissolved over 45 min in 250 mL diethyl ether freshly distilled, while stirring at room temperature. The solution was added during 1h ($10^{-5}$ Torr) to the distillation flask (50 °C). The SAFE apparatus was kept at 45 °C.

### 4.2.6 Butter Aroma Fractionation

The aroma extracts were separated into acidic and neutral/basic fractions to facilitate the GC analysis. The distillate (about 250 mL) was extracted twice with 50 mL sodium carbonate solution (0.5 mol/L) and once with 40 mL 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 freshly distilled diethyl ether (25 mL). The diethyl ether extract was then dried over anhydrous sodium sulfate. Both, acidic and neutral/basic fractions were concentrated to 200 μL first using a Vigreux column and then a micro-distillation. The concentrated extracts were finally analyzed by gas chromatography olfactometry (GC/O) and by high-resolution gas chromatography mass spectrometry (HRGC-MS) as described below.

### 4.2.7 GC/O

The analysis was performed on a Trace-GC (Thermo Fisher Scientific, Dreieich, Germany) equipped with a flame ionization detector (FID) and a sniffing port. The column carrier gas was helium with a pressure of 140 kPa for DB-5 column (60 m length, 0.32 mm i.d, 0.25 μm film thickness, JandW Scientific, Folsom, CA) and 75

kPa for a DB-FFAP column (30 m length, 0.32 mm i.d., 0.25 µm film thickness, JandW Scientific). At the end of the column, the flow was splitted through a Y glass splitter (Chrompack, Frankfurt, Germany) 1:1 to FID and sniffing port. The sample (1 µl) was injected cold on column. Both, the sniffing port and the FID were heated at 250 °C. The oven temperature was held at 40 °C for 2 min, then increased to 240 °C at a rate of 6 °C/min, and held at 240 °C for 5 min. Two trained panelists performed the GC/O analyses in duplicate.

The flavor dilution (FD)-factors of the odorants were determined by AEDA, diluting stepwise an aliquot of 50 µl of the SAFE extract with diethyl ether (1:1, v/v). The AEDA was performed using the DB-FFAP column.

### 4.2.8 HRGC/MS

Analysis was performed on an Agilent 5890 Series II gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to a Finnigan MAT 95S mass selective detector (MSD; Thermo Fisher Scientific).

The sample (0.5 µl) was injected manually cold on-column. The carrier gas was helium with a flow of 1.9 mL/min. The volatiles were separated using DB-5 and DB-FFAP capillary columns (both 30 m length, 0.25 mm i.d, 0.25 µm film thickness, JandW Scientific). After 2 min, the oven temperature was raised by 6 °C/min to 240 °C, and then held at 240 °C for 5 min. The MSD operated in scan mode at 2.9 scans/s (*m/z* 29-350) at 70 eV. Mass spectra of unknown compounds were compared with those in the Wiley 138. Retention indices (RI) were calculated in accordance to

(Kovats, 1965). Positive identification was achieved by comparing mass spectra, odours as detected by GC/O analysis, RI in a database or of reference substances.

### 4.2.9 Quantification by stable isotope dilution assay (SIDA) using SAFE extraction

Two butter samples (100 g each) for every butter type were dissolved in diethyl ether (250 mL each) which contained the following labeled internal standards: [3,4-$^2H_2$]butanoic acid (8 µg), [5,6-$^2H_2$]hexanoic acid (9 µg), [5,6-$^2H_2$]hexanal (11 µg), [5,5,6,6-$^2H_4$]nonanal (13 µg), [5,6-$^2H_2$]decanal (14 µg), [2,3-$^2H_2$]-(*E*)-2-nonenal (3 µg), [2,3-$^2H_2$]-(*E,Z*)-2,6-nonadienal (2.5 µg), [8,9-$^2H_2$]-δ-octalactone (10 µg), [10,11-$^2H_2$]-δ-decalactone (24 µg), [12,13-$^2H_2$]-δ-dodecalactone (25 µg). The same procedure was applied to the oxidized samples using [5,6-$^2H_2$]hexanal (18 µg), [5,5,6,6-$^2H_4$]nonanal (13 µg), [2,3-$^2H_2$]-(*E*)-2-nonenal (5.5 µg), [2,3-$^2H_2$]-(*E,Z*)-2,6-nonadienal (2.5 µg), [7,7,8,8-$^2H_4$]-*trans*-4,5-epoxy-(*E*)-2-decenal (0.5 µg), [$^2H_3$]-methional (0.7 µg), [3,4-$^2H_2$]-1-octen-3-one (2 µg), [3,4-$^2H_2$]-(*Z*)-3-hexenal (2 µg) and [2,3-$^2H_2$]-(*E*)-2-octenal (12 µg).

The volatiles of butter and the labeled internal standards were isolated by SAFE. The distillate was separated in an acidic and a neutral/basic fraction and then concentrated, as described above. The quantification was performed by multidimensional gas chromatography mass spectrometry. This system consisted of a Trace 2000 gas chromatograph (Thermo, Egelsbach, Germany) and a CP 3800 gas chromatograph (Varian, Inc., Darmstadt, Germany) connected by a Moving-Column-Stream-Switching system (MCSS, Thermo). In off-line modus the MCSS led the effluent simultaneously to a FID and a sniffing port in the Trace-GC. Retention times (RT)

were determined by analyzing reference compounds by GC-FID and GC sniffing. The compounds in the SAFE extracts were first separated in the Trace GC using a DB-FFAP column, then cryofocused and sent to the second GC, which was equipped with a OV1701 capillary column and a MS system ITD-Saturn 2000 (Finnigan, Bremen, Germany) running in the positive chemical ionization (CI) mode with methane as reagent gas.

Analyses were run in duplicate. The values obtained from the same sample differed by not more than ± 5 %.

### 4.2.10 Quantification by SIDA using Headspace Solid Phase Microextraction (HS-SPME)

The quantification of pentanal and heptanal was performed using [5,6-$^2H_2$]hexanal as internal standard and HS-SPME as described by Roberts et al. (2000). The butter (5 g) was spiked with [5,6-$^2H_2$]hexanal (6.8 µg in ethanol) molten at 40 °C in a water bath, 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 for 30 min and then extracted for 45 min at 45 °C using a 2 cm Divinylbenzene/Carboxen/Polydimethylsiloxane fiber (DVB/CAR/PDMS, Supelco, Bellefonte, USA). All samples were analyzed in triplicate using the same SPME fiber. The analysis was performed using a Trace ultra GC coupled to a Trace DSQ mass spectrometer (Thermo Fisher Scientific). The separation was performed using a DB-FFAP column (30 m length, 0.25 mm i.d., 0.25 µm film thickness, JandW Scientific). The oven program was 40 °C for 2 min, then at 8 °C/min until 150 °C, and then at 20 °C/min to 240 °C. The mass spectrometer operated in CI mode (positive) with isobutane as the reagent gas and in the SIM

mode, monitoring the ions m/z 87 for pentanal, m/z 103 for [5,6-$^2H_2$]hexanal and m/z 115 for heptanal (Table 4.1).

### 4.2.11 Quantification of skatole by high pressure liquid chromatography (HPLC)

Skatole was measured in butter using the following procedure based on different methods previously described (Chen et al., 2007; Verheyden et al., 2007; Hansen-Møller, 1994; Claus et al., 1993). 0.50±0.1 g of butter was introduced into a 2 mL centrifuge micro tube and heated at 45±5 °C in a heating block (Dri-Block, Witec AG, Littau, Switzerland). Then 1 ml of internal standard solution (MeOH: $H_2O$ 95:5, v/v), containing 0.05 mg/L 2-methylindole, was added into the tube and mixed with a vortex during 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 during 20 min at 4 °C. The supernatant was filtered (Chromafil 0-20/15MS PTFE, Pretech Instruments KB, Sollentuna, Sweden) and 5 µl were injected onto the HPLC column (Zorbax Eclipse XDB-C18 4.6 x 50 mm; 1.8 µm, Agilent). The HPLC (Agilent) was equipped with a G1367C SL autosampler (Agilent), column oven (G1316A TTC, Agilent), fluorescence detector (G1321A FLD, Agilent), pump (G1312B Bin pump SL, Agilent), and a degasser (G1379B, Agilent). Data acquisition was performed with the Chemstation Rev. B.0202 SR1 (260) software from Agilent. The mobile phase was 10 mM phosphate buffer pH 6.0 and methanol (55:45) at 1.3 mL/min flow. Fluorescence detection was with excitation at 285 nm and emission at 340 nm.

## 4.3 Results and discussion

### 4.3.1 Chemical composition of UFA/CLA enriched butter and conventional butter

The chemical composition of UFA/CLA enriched butter and conventional butter is reported in Table 4.2.

**Table 4. 2:** Chemical composition of UFA/CLA enriched butter and conventional butter

| Chemical composition | Unit | UFA/CLA[a] | $s_x$[b] | CONV[c] | $s_x$[d] |
|---|---|---|---|---|---|
| Moisture | g/kg | 163 | 11 | 143 | 6.4 |
| Fat | g/kg | 830 | 11 | 852 | 7.4 |
| Non-fat solids | g/kg | 7 | 1 | 5 | 0.9 |
| Retinol | mg/kg | 13 | 0.5 | 11 | 0.5 |
| α-Tocopherol | mg/kg | 36 | 4 | 25 | 0.8 |
| Copper | µg/kg | 33 | 14 | 36 | 14 |
| Iron | µg/kg | 382 | 95 | 132 | 54 |

[a]Mean values for unsaturated fatty acid/conjugated linoleic acid enriched butter (n=6)
[b]Standard deviation for UFA/CLA enriched butter
[c]Mean values for conventional butter (n=6)
[d]Standard deviation for conventional butter

The UFA/CLA enriched butter was characterized by a lower fat content, 83 % versus 85 % fat in conventional butter, and on the other hand, by a higher moisture content. The lower fat content was already observed earlier in UFA/CLA by the authors (Mallia et al., 2008) and in general in dairy products, when the animal's diet is supplemented with oleaginous seeds (Collomb et al., 2004). α-Tocopherol and iron concentrations were significantly higher in UFA/CLA enriched butter, as previously observed (Mallia et al., 2008). The higher content of these components is probably related to the cow's diet supplemented with sunflower seeds, which are rich in vitamin E and iron (Souci et al., 2000).

The UFA/CLA enriched butter showed significantly lower saturated fatty acid levels and significantly higher mono- and polyunsaturated fatty acid content (Table 4.3).

**Table 4.3:** Fatty acid composition of UFA/CLA enriched butter and conventional butter determined by HRGC

| Fatty acids | UFA/CLA[a] | CONV[b] |
|---|---|---|
| | g/100 g fat | g/100 g fat |
| Saturated** | 49 | 62 |
| Butanoic acid C4:0 | 2.7 | 3.0 |
| Hexanoic acid C6:0* | 1.4 | 2.0 |
| Decanoic acid C10:0* | 1.6 | 3.0 |
| Dodecanoic acid C12:0* | 1.8 | 3.0 |
| Tetradecanoic acid C14:0* | 7.6 | 10 |
| Palmitic acid C16:0** | 20 | 27 |
| Stearic acid C18:0* | 9.5 | 8.0 |
| Monounsaturated** | 35 | 25 |
| Oleic acid* (C18:1 c9) | 23 | 17 |
| Polyunsaturated* | 6.0 | 4.5 |
| Linoleic acid* (C18:2 c9c12) | 1.4 | 1.0 |
| α-Linolenic acid (C18:3 c9c12c15) | 0.7 | 0.6 |
| Sum CLA* | 2.0 | 1.2 |
| Sum Omega 3[c] | 1.2 | 1.3 |
| Sum Omega 6[d]* | 3.5 | 2.0 |

*P<0.05, **P<0.01; ***P<0.001

[a] Unsaturated fatty acid/conjugated linoleic acid enriched butter

[b] Conventional butter

[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)

The free fatty acids C14:0, C16:0, C18:0, C18:1, C18:2 and C18:3 were significantly more present in the fresh and stored enriched butters than in the conventional ones, as shown in Table 4.4. Only C12:0 was found at higher level in the conventional butter. The index of lipolysis, defined as the sum of C6:0 until C20:0 fatty acids (FIL-IDF, 1974), was significantly higher in UFA/CLA butter and in particular almost twice as high in the stored enriched samples. The CLA isomers such as *cis* 9, *trans* 11 and *trans* 7, *cis* 9, which are the most prominent in milk fat, were significantly higher in UFA/CLA enriched butter as reported in Table 4.5. This butter is almost twice as rich in CLA as conventional butter: 22.48 mg/g fat versus 12.86 mg/g fat, respectively.

**Table 4.4:** Free fatty acid (FFA) composition in mg/kg butter of UFA/CLA enriched butter

| | Fresh | | 6 Weeks | |
|---|---|---|---|---|
| FFA | UFA/CLA[a] | CONV[b] | UFA/CLA | CONV |
| Butanoic acid C4:0 | 1.8 | 1.8 | 2.0 | 1.9 |
| Hexanoic acid C6:0 | 0.8 | 0.8 | 1.3 | 1.2 |
| Octanoic acid C8:0 | 0.5 | 0.4 | 4.0 | 1.0 |
| Decanoic acid C10:0 | 5.0 | 3.0 | 10 | 7.0 |
| Dodecanoic acid C12:0* | 13 | 20 | 26 | 30 |
| Tetradecanoic acid C14:0* | 64 | 60 | 98 | 84 |
| Palmitic acid C16:0* | 282 | 272 | 396 | 332 |
| Stearic acid C18:0*** | 155 | 90 | 232 | 125 |
| Oleic acid C18:1*** | 653 | 348 | 931 | 418 |
| Linoleic C18:2*** | 77 | 43 | 125 | 58 |
| CLA C18:2 conjugated** | 28 | 14 | 53 | 23 |
| α-Linolenic acid C18:3** | 12 | 8.0 | 32 | 14 |
| Eicosanoic acid C20 | 2.0 | 1.5 | 3.0 | 1.5 |
| Index of lipolysis[c]** | 1345 | 941 | 2034 | 1180 |

*P<0.05, **P<0.01; ***P<0.001

[a] Unsaturated fatty acid/conjugated linoleic acid enriched butter

[b] Conventional butter

[c] Sum of C6 to C20

**Table 4.5:** Conjugated linoleic acid (CLA) isomers in UFA/CLA enriched butter and conventional butter

| CLA isomers | UFA/CLA[a] | CONV[b] |
|---|---|---|
| | mg/g fat | mg/g fat |
| C18:2 t12 t14 | 0.226 | 0.157 |
| C18:2 t11 t13 | 0.420 | 0.470 |
| C18:2 t10 t12* | 0.293 | 0.135 |
| C18:2 t9 t11* | 0.309 | 0.169 |
| C18:2 t8 t10* | 0.167 | 0.020 |
| C18:2 t7 t9* | 0.113 | 0.061 |
| C18:2 t6 t8 | 0.013 | 0.012 |
| C18:2 c / t 12, 14* | 0.098 | 0.076 |
| C18:2 t11 c13 | 0.377 | 0.345 |
| C18:2 c11 t13 | 0.027 | 0.028 |
| C18:2 t10 c12** | 0.156 | 0.049 |
| C18:2 c9 t11** | 18.70 | 10.60 |
| C18:2 t8 c10 | 0.417 | 0.240 |
| C18:2 t7 c9** | 1.201 | 0.469 |
| Sum CLA** | 22.48 | 12.86 |

*P<0.05, **P<0.01; ***P<0.001

[a] Unsaturated fatty acid/conjugated linoleic acid enriched butter

[b] Conventional butter

### 4.3.2 Comparative AEDA of the UFA/CLA enriched butter and conventional butter

Among 58 odorants, detected by AEDA in the butter extracts, 23 had higher FD factors than 4 (Table 4.6). The fresh UFA/CLA enriched butter and fresh conventional butter had similar aroma profiles, characterized by soapy, milky and fruity notes, with high FD factors of nonanal, 2-nonanone, methyl-2-methylbutanoate, 3-methylbutyl acetate, (*E,Z*)-2,6-nonadienal, δ-decalactone and 2-phenylethyl acetate, respectively. Interestingly most of the odorants presented higher FD factors in fresh UFA/CLA butter. Only 2-phenylethyl acetate had a higher FD factor in conventional butter.

The odorants were more intensely perceived at the sniffing port after 6 weeks of storage, in both butter types and especially in UFA/CLA enriched samples. In particular, heptanal (soapy), (*E,Z*)-2,6-nonadienal (cucumber-like), δ-decalactone (peach-like) and δ-dodecalactone (peach-like) had the highest FD factors in stored UFA/CLA butter. Decanal (green), hexanoic acid (animal-like) and *trans*-4,5-epoxy-(*E*)-2-decenal (metallic), weakly or not perceived in fresh butter, became important odorants in stored samples and especially in the enriched butter. Interestingly, 2-phenylethyl acetate that was an important odorant in fresh conventional butter, was not found after 6 weeks of storage. Furthermore the esters, with fruity notes, were less intensely or not perceived after storage.

**Table 4.6:** Most odour-active compounds (FD factor ≥ 4) in fresh and 6 weeks stored (6 °C) UFA/CLA enriched butter and conventional butter

| Odorant[b] | Odour quality[c] | RI DB-5 | RI FFAP | FD factor[a] Fresh | | Stored | |
|---|---|---|---|---|---|---|---|
| | | | | UFA/CLA[d] | CONV[e] | UFA/CLA | CONV |
| 3-Methylbutyl acetate | orange | - | 1116 | 32 | 16 | 16 | 16 |
| Pentanal | fat, green | 738 | 973 | 8 | 8 | 64 | 16 |
| Methyl 2-methylbutanoate | fruit | 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 | 1000 | 1223 | 16 | 8 | 16 | 8 |
| Octanal | almond, fat | 1007 | 1277 | 8 | 4 | 8 | 4 |
| Hexanoic acid | animal-like | 1022 | 1800 | - | - | 64 | 32 |
| 2-Nonanone | milk | 1101 | 1402 | 64 | 64 | 64 | 64 |
| 2-Acetyl-2-thiazoline | cooked | 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 | - | - | 64 | 32 |
| 2-Phenylethyl acetate | fruit | 1250 | 1810 | 16 | 64 | - | - |
| γ-Octalactone | sweet | 1260 | 1878 | 8 | 4 | 8 | 8 |
| δ-Octalactone | fruit | 1290 | 1923 | 8 | 8 | 64 | 64 |
| (*E*)-4,5-Epoxy-2-(*E*)-decenal | fat, metallic | 1380 | 2017 | - | - | 16 | 8 |
| 3-Methyl-1H-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 2 panelists. The FD factors between the 2 panelists differed not more than by a factor of 2.

[b] The compounds were identified by comparing it with the reference substance on the basis of the following criteria: retention index (RI) on two column of different polarity, mass spectra obtained by MS (EI) and odor quality perceived at the sniffing port.

[c] Odour quality perceived at the sniffing port by two trained panelists.

[d] Unsaturated fatty acid/conjugated linoleic acid enriched butter

[e] Conventional butter

### 4.3.3 Quantitative analysis

Fourteen of the most potent odorants were quantified in fresh and stored samples (Table 4.7). Pentanal, heptanal, and δ-decalactone had a higher concentration in UFA/CLA enriched butter. The corresponding odour activity values (OAVs) were calculated by dividing the concentration by its orthonasal odour threshold in sunflower oil (Rychlik et al., 1998). On the basis of OAVs, listed in Table 4.8, δ-dodecalactone and butanoic acid were the most important odorants in both UFA/CLA and fresh conventional butter. After 6 weeks of storage, the highest OAVs were found for δ-decalactone, δ-dodecalactone and butanoic acid in both butter types. In particular δ-decalactone, pentanal and heptanal showed significantly higher OAVs in UFA/CLA enriched butter after storage. A previous study on UFA/CLA butter, performed using SPME coupled to GC-MS-O, also showed heptanal and butanoic acid increasing in UFA/CLA butter after refrigerated storage (Mallia et al., 2008). In the same study, lactones, such as γ- and δ-decalactone, were on the other hand found to have weak odour intensity, probably due to the SPME extraction used that is more suitable for more volatile compounds.

**Table 4.7:** Most important odorants quantified in fresh and 6 weeks stored (at 6 °C) UFA/CLA enriched butter and conventional butter.

| | Fresh | | Stored | |
|---|---|---|---|---|
| Compound[a] | UFA/CLA[b] | CONV[c] | UFA/CLA | CONV |
| | µg/kg | µg/kg | µg/kg | µg/kg |
| Pentanal | 235 | 64 | 661 | 289 |
| Hexanal | 10 | 11 | 10 | 12 |
| Heptanal | 963 | 364 | 1703 | 1140 |
| Nonanal | 68 | 59 | 72 | 73 |
| Decanal | 24 | 12 | 16 | 14 |
| δ-Octalactone | 114 | 137 | 303 | 487 |
| δ-Decalactone | 2858 | 2061 | 7245 | 5293 |
| δ-Dodecalactone | 1314 | 1464 | 1491 | 1536 |
| (*E*)-2-Nonenal | 6.8 | 6.6 | 6.8 | 6.2 |
| (*Z*)-2-Nonenal | 0.07 | 0.10 | 0.36 | 0.32 |
| (*E*,*Z*)-2,6-Nonadienal | 17 | 17 | 17 | 20 |
| Butanoic acid | 1606 | 1568 | 1885 | 1820 |
| Hexanoic acid | 802 | 806 | 1240 | 1237 |
| 3-Methyl-1H-indole | 108 | 111 | 104 | 109 |

[a]Odour compound quantified by stable isotope dilution assay (SIDA), except 3-methyl-1H-indole quantified using an internal standard not labeled by HPLC.
[b]Unsaturated fatty acid/conjugated linoleic acid enriched butter
[c]Conventional butter

**Table 4.8:** Selected important odorants quantified in fresh and stored (at 6 °C) UFA/CLA enriched butter and conventional butter.

| | | OAV[a] | | | |
|---|---|---|---|---|---|
| | | Fresh | | Stored | |
| Compound[b] | Odour threshold[c] | UFA/CLA[d] | CONV[e] | UFA/CLA | CONV |
| | µg/L | | | | |
| Pentanal | 240 | <1 | <1 | 3 | 1 |
| Heptanal | 250 | 4 | 2 | 7 | 5 |
| (*E,Z*)-2,6-Nonadienal | 3.8 | 4 | 4 | 4 | 5 |
| δ-octalactone | 120 | <1 | 1 | 3 | 4 |
| δ-decalactone | 400 | 7 | 5 | 18 | 13 |
| δ-dodecalactone | 120 | 11 | 12 | 12 | 13 |
| butanoic acid | 135 | 12 | 12 | 14 | 13 |
| 3-methyl-1H-indole | 15.6 | 7 | 7 | 7 | 7 |

[a]Odour compound quantified by stable isotope dilution assay (SIDA), except for 3-methyl-1H-indole quantified using an internal standard not labeled by HPLC.
[b]Unsaturated fatty acid/conjugated linoleic acid enriched butter
[c]Conventional butter
[d]Unsaturated fatty acid/conjugated linoleic acid enriched butter
[e]Conventional butter

Schieberle and co-workers (1993) quantified the important odorants in different kinds of butter and found δ-decalactone and butanoic acid as important odorants in sweet cream butter and sour cream butter.

The amount of δ-decalactone found in our study for UFA/CLA enriched butter is higher than the concentrations reported for sweet cream butter in the literature (Schieberle et al., 1993; Peterson and Reineccius, 2001). This could be related to the possible origin of lactones from CLA (Yurawecz, 2003). Interestingly hexanal, which is considered as an indicator of lipid oxidation (Christensen and Hølmer, 1996), remained constant during the storage period and it was present at similar amounts in both butter types. However, the hexanal concentration considerably increased in both UFA/CLA butter and conventional butter after 6 h light exposure as shown in Table 4.9. Surprisingly, for heptanal that had always higher concentration in UFA/CLA enriched butter, higher values were found in conventional butter exposed to fluorescence light.

**Table 4.9:** Important odorants quantified in UFA/CLA enriched butter and conventional butter after 0, 6 and 12 h of fluorescence light exposure

| | Light 0h | | Light 6 h | | Light 12 h | |
|---|---|---|---|---|---|---|
| Compound[a] | UFA/CLA[b] | CONV[c] | UFA/CLA | CONV | UFA/CLA | CONV |
| | µg/kg | µg/kg | µg/kg | µg/kg | µg/kg | µg/kg |
| Pentanal | 235 | 64 | 1699 | 791 | 2590 | 826 |
| Hexanal | 10 | 11 | 5494 | 5032 | 5185 | 4822 |
| Heptanal | 963 | 364 | 1681 | 1905 | 1059 | 1513 |
| Nonanal | 68 | 59 | 360 | 405 | 278 | 379 |
| (*E*)-2-Nonenal | 6.8 | 6.6 | 21 | 23 | 28 | 29 |
| (*E,Z*)-2,6-Nonadienal | 17 | 17 | 13 | 38 | 25 | 30 |
| *(E)*-4,5-Epoxy-(*E*)-2-decenal | 0 | 0 | 27 | 90 | 33 | 90 |
| Methional | 0 | 0 | 0.43 | 0.30 | 0.33 | 0.26 |
| 1-Octen-3-one | 0.06 | 0.05 | 0.31 | 0.36 | 0.39 | 0.40 |
| (*Z*)-3-Hexenal | 0.85 | 0.73 | 0.76 | 0.66 | 0.64 | 0.58 |
| (*E*)-2-Octenal | 7.5 | 2.4 | 88 | 93 | 114 | 101 |
| (*Z*)-2-Octenal | nd[d] | nd | 85 | 91 | 135 | 108 |
| 3-Methyl-1H-indole | 108 | 111 | 93 | 84 | nd | nd |

[a]Odour compound quantified by stable isotope dilution assay (SIDA), except 3-methyl-1H-indole quantified by HPLC using a not labeled internal standard.
[b]Unsaturated fatty acid/conjugated linoleic acid enriched butter
[c]Conventional butter
[d]Not determined

The light induced also an increase of nonanal, (E)-2-nonenal, (E,Z)-2,6-nonadienal and *trans*-4,5-epoxy-(*E*)-2-decenal concentrations in conventional butter. This could be explained by the higher vitamin content (retinol and α-tocopherol) of the UFA/CLA enriched butter. Antioxidative properties of CLA, which might protect the butter from photo-oxidation, have been discussed in the literature (Van den Berg, 1995). Light exposure induced also the formation of aroma compounds not detected in fresh butter, such as methional (potato-like) and *trans*-4,5-epoxy-(*E*)-2-decenal (fatty, metallic). The OAV of most important odorants found in butter exposed to fluorescence light are

listed in Table 4.10. The highest OAVs were found for hexanal and *trans*-4,5-epoxy-(*E*)-2-decenal. In particular the latter was more important in conventional butter. The OAV of pentanal and methional was significantly higher in UFA/CLA enriched samples. All these compounds originate from lipid oxidation, except methional that is formed from photodecomposition of methionine (Borle et al., 2001).The samples exposed to oxygen atmosphere for 6 and 12 h, respectively, developed fatty and metallic notes, due in particular to an increase of aldehydes, such as pentanal, hexanal, heptanal, nonanal and (*E*)-2-octenal (Table 4.11). As observed in the case of light exposure, the conventional butter presented higher concentrations of aldehydes such as heptanal, (*E*)-2-octenal (fatty), and (*Z*)-2-octenal (nutty). 1-Octen-3-one, with a mushroom-like odour, also increased especially in conventional butter exposed to oxygen atmosphere. Table 4.12 shows the odour compounds that had the highest OAVs during the induced oxidation in the dark. In particular, the OAVs of heptanal in conventional butter are the double compared to those of UFA/CLA enriched butter.

**Table 4.10:** Odour thresholds and odour activity values (OAV) of the most important odorants of UFA/CLA enriched butter and conventional butter exposed to fluorescence light

| | | OAV[a] | | | |
|---|---|---|---|---|---|
| | | Light 6 h | | Light 12 h | |
| Compound[b] | Odour threshold[c] | UFA/CLA[d] | CONV[e] | UFA/CLA | CONV |
| | µg/L | | | | |
| 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 |
| (*E*)-4,5-Epoxy-(*E*)-2-decenal | 1.3 | 21 | 69 | 25 | 69 |
| Methional | 0.2 | 2 | 1 | 2 | 1 |

[a]Odour activity value
[b]Odour compound quantified by stable isotope dilution assay (SIDA)
[c]Odour threshold in sunflower oil
[d]Unsaturated fatty acid/conjugated linoleic acid enriched butter
[e]Conventional butter

**Table 4.11:** Most important odorants quantified in UFA/CLA enriched butter and conventional butter exposed to oxygen for 0, 6 and 12 h

| | Oxygen 0 h | | Oxygen 6 h | | Oxygen 12 h | |
|---|---|---|---|---|---|---|
| Compound[a] | UFA/CLA[b] | CONV[c] | UFA/CLA | CONV | UFA/CLA | CONV |
| | µg/kg | µg/kg | µg/kg | µg/kg | µg/kg | µg/kg |
| Pentanal | 235 | 64 | 368 | 166 | 673 | 372 |
| Hexanal | 10 | 11 | 1663 | 1815 | 1577 | 1567 |
| Heptanal | 963 | 364 | 1360 | 2511 | 1000 | 1896 |
| Nonanal | 68 | 59 | 557 | 504 | 712 | 462 |
| (*E*)-2-Nonenal | 6.8 | 6.6 | 17 | 16 | 17 | 18 |
| (*E*,*Z*)-2,6-Nonadienal | 17 | 17 | 16 | 15 | 19 | 20 |
| (*E*)-4,5-Epoxy-(*E*)-2-decenal | 0 | 0 | 0.46 | 0.26 | 0.33 | 0.35 |
| 1-Octen-3-one | 0.06 | 0.05 | 0.20 | 0.36 | 0.31 | 1.05 |
| (Z)-3-Hexenal | 0.85 | 0.73 | 0.79 | 0.43 | 0.59 | 0.66 |
| (E)-2-Octenal | 7.52 | 2.38 | 75 | 92 | 72 | 88 |
| (Z)-2-Octenal | nd[d] | nd | 69 | 112 | 69 | 80 |

[a]Odour compound quantified by stable isotope dilution assay (SIDA)
[b]Unsaturated fatty acid/conjugated linoleic acid enriched butter
[c]Conventional butter
[d]Not determined

**Table 4.12:** Odour thresholds and odour activity values (OAV) of the most important odorants of UFA/CLA enriched butter and conventional butter exposed to oxygen

| | | OAV[a] | | | |
|---|---|---|---|---|---|
| | | Oxygen 6 h | | Oxygen 12 h | |
| Compound[b] | Odour threshold[c] | UFA/CLA[d] | CONV[e] | UFA/CLA | CONV |
| | µg/L | | | | |
| 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]Odour activity value
[b]Odour compound quantified by stable isotope dilution assay (SIDA)
[c]Odour threshold in sunflower oil
[d]Unsaturated fatty acid/conjugated linoleic acid enriched butter
[e]Conventional butter

## 4.4 Conclusion

Cold storage seemed to affect in particular the contents of pentanal and heptanal in UFA/CLA enriched butter, with an increase of fatty and green notes. The higher concentration of free fatty acids in the UFA/CLA enriched butter also contributed to the flavour of this butter type and to its more intense aroma after storage. Surprisingly, the oxidative stability of UFA/CLA enriched butter that we expected to oxidize more easily was instead comparable to the one of conventional butter during induced photo-oxidation and oxidation in the dark under oxygen atmosphere. In particular, aldehydes such as heptanal, (*E*)-2-octenal, (*E*)-2-nonenal and *trans*-4,5-epoxy-(*E*)-2-decenal increased especially in conventional butter when subjected to oxidation. These findings suggest that the higher concentrations in retinol and α-tocopherol of UFA/CLA enriched butter combined with a potential antioxidative activity of CLA, may act as protection, even despite a higher iron content, which is a pro-oxidant, in this kind of butter.

Prior to commercialisation of a butter enriched in UFA/CLA, further studies on the oxidation kinetic and stability of this product are necessary, also in combination with sensory tests to evaluate the acceptability of this butter by the consumers. The chemical formation pathways involved in the potential odour formation from CLA also require further investigation.

# 5. Formation of odour-compounds from 9 cis, 12 cis ethyl linoleate and 9 cis, 11 trans ethyl-CLA ester – a model study*

The odour-active secondary oxidation products of CLA are not yet well known and the chemical pathways generating these compounds are also not fully elucidated. The aim of the present study was to understand which odorants could be generated from both, photo-oxidation and oxidation in the dark of CLA in model systems. These models contained the same proportion of linoleic acid and CLA, found in previous studies in UFA/CLA enriched butter, with the objective to translate the results obtained for the model to the enriched butter. The odour-active compounds in the models were identified by GC/MS/O and their origin from linoleic acid and/or from CLA was investigated by using [$^{13}C_{18}$] labelled linoleate and unlabelled CLA, by monitoring the isotopomers formed after oxidation. (*Z*)-3-Hexenol (fruity odour) and (*Z*)-2-nonenal (green) were found 99 % and 88 % labelled, respectively, after 6 h of light exposure. This indicated their origin mainly from linoleate. On the other hand, hexanal and heptanal were found 66 % and 80 % unlabelled, respectively, after photo-oxidation. This means that these odorants formed mainly from CLA. In addition, the origin of (*E*)-2-octenal, (*E*)-2-nonenal, 2-octanone, furans, ethyl esters and acids was mainly related to CLA oxidation. Pentanal was found 50 % labelled and therefore, was

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formed in equal proportions from both, linoleate and CLA. The majority of the odour compounds identified in the butter models were also previously found in UFA/CLA enriched butter, under the same oxidation conditions. The results obtained for the model systems can be translated to the UFA/CLA butter, but it is necessary to take in account important factors, such as vitamins and metal ions, which might influence the oxidation rate in butter. Finally, some chemical pathways for the formation of odour compounds from CLA are proposed.

## 5.1 Introduction

Oxidation can easily occur in foods containing unsaturated fatty acids during storage, resulting eventually in potential off-flavour development.

The formation of odour-active compounds from the autoxidation of oleic, linoleic, linolenic and arachidonic acids and their esters has been the subject of many studies (Badings, 1970; Frankel et al., 1986; Ullrich and Grosch, 1987) and reviews (Grosch, 1987; Collomb and Spahni, 1996). In particular, due to great abundance in foodstuffs and their high susceptibility to autoxidation, linoleic acid and its esters are among the most important precursors of aroma-active compounds such as aldehydes, ketones, alcohols, acids and esters (Ullrich and Grosch, 1987).

On the other hand, little is known about the initial stages of the autoxidation of CLA and their primary and secondary oxidation products. Zhang and Chen (1997) indicated that the oxidation rate of CLA, as free fatty acid, mainly consisting in a mix of 9-*cis*, 11-*trans*/9-*trans*, 11-*cis* and 10-*trans*, 12-*cis*/10-*cis,* 12-*trans* CLA, was greater than those of linoleic, linolenic and arachidonic acid.

A recent study (Luna et al., 2007) compared the oxidation kinetics of methyl 9-*cis*, 11-*trans* linoleate to methyl 9-*cis*, 12-*cis* linoleate at 30 °C in the dark. The results showed that conjugated methyl linoleate oxidised slower than its non-conjugated counterpart, and indicated also different oxidation pathways. Eulitz and co-workers (1999) and Yurawecz et al. (2003) suggested that the radical oxidation mechanism with formation of hydroperoxides, generally occurring in unsaturated fatty acids, is less probable in CLA, since more activation energy is required to separate the conjugated double bonds. Instead they postulated a 1,2 and 1,4 cycloaddition of oxygen. The first mechanism leads first to dioxetane and then to aldehydes and esters. The 1,4 cycloaddition forms endoperoxides, which give eventually furan fatty acids. The authors assumed that only a small part of secondary oxidation products of CLA are accounted for by the breakdown of the primary autoxidation products 9-hydroperoxide (9-LOOH) and 13-hydroperoxide of linoleic acid (13-LOOH). In contrast to these hypotheses, Hämäläinen et al. (2001) and Pajunen et al. (2008) suggested that the hydroperoxides are primary products of CLA oxidation. The authors oxidised *cis*-9, *trans*-11 CLA methyl ester and *trans*-10, *cis*-12 CLA methyl ester for 16 days, in the presence of α-tocopherol (20 % per weight), under atmospheric oxygen at 40 °C in the dark. They concluded that hydroperoxide pathway is one of the reaction pathways of CLA oxidation in the presence of a good hydrogen atom donor. The authors proposed a mechanism for predicting the hydroperoxides and their isomeric distribution formed during autoxidation of CLA.

In a recent study (Garcia-Martinez et al., 2009) the volatiles formed from the oxidation of oil and triacylglycerols rich in CLA were investigated and compared to those

formed from oil and triacylglycerols rich in linoleic acid. The authors quantified the volatile compounds formed in the two cases and found hexanal and heptanal as the most abundant compounds formed from CLA.

The present study was undertaken as a model to understand which odour-active secondary oxidation products could be formed from CLA in UFA/CLA enriched butter.

In our previous work, butter enriched with UFA/CLA showed more intense fatty, green and metallic notes after storage compared to conventional butter, due in particular to an increase in pentanal, hexanal, heptanal, (*E*)-2-octenal and (*E*)-2-nonenal (Mallia et al., 2008). After Photo-oxidation and oxidation in the dark of the UFA/CLA enriched butter, several volatile compounds, such as *trans*-4,5-epoxy-2-decenal, (*E*)- and (*Z*)-2-octenal, (*E*)-2-nonenal, 1-octen-3-one, showed higher concentrations than before oxidation.

In the current study, a model system, which mimics UFA/CLA enriched butter, was produced. It contained the same proportion of linoleic acid and CLA (1.5 versus 2), both as ethyl esters, as the one found previously in the UFA/CLA enriched butter (Mallia et al., 2008). In this model system only the *cis* 9, *trans* 11 CLA isomer was used because it represents almost 90% of the total CLA in UFA/CLA enriched butter (Mallia et al., 2008). Isotope labelled ethyl linoleate was used to study the origin of the carbon atoms in the odour compounds formed in these models during oxidation. The model samples were oxidised under the same conditions (photo-oxidation and induced oxidation with oxygen in the dark at 6 °C) previously reported for the UFA/CLA

enriched butter (cf. 4.2.3). The odour-active compounds were identified by GC/MS/O and their origin from linoleic acid ethyl ester and/or from CLA ethyl ester was studied by monitoring the isotopomers formed during oxidation. To our knowledge, this is the first study aiming to identify odour compounds originating from CLA.

## 5.2 Materials and methods

### 5.2.1 Chemicals

Chemicals were of analytical grade. *Cis* 9, *trans* 11 CLA ethyl ester was from Larodan Fine Chemicals AB (Malmö, Sweden); *cis* 9, *cis* 12 ethyl linoleate was from Nu-Chek Prep (Elysian, MN, USA); [$^{13}C_{18}$]*cis* 9, *cis* 12 ethyl linoleate (98% enrichment) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Miglyol® 812 neutral oil SASOL used as lipid matrix was from Hänseler AG (Herisau, Switzerland).

### 5.2.2 Samples

Model UFA/CLA enriched systems were prepared on the basis of the same proportions of linoleic acid and CLA than the ones found in butter enriched with UFA/CLA. Three models were prepared as described in table 5.1. One model contained only CLA ethyl ester (EtCLA), the second one only ethyl linoleate (EtLn) and the third one a combination of both, CLA ethyl ester and $^{13}C_{18}$ labelled ethyl linoleate (EtCLA+[$^{13}C_{18}$]EtLn). Miglyol®, a neutral oil with neutral odour, used for farmaceuticals and cosmetics, was used as a matrix. This is a stable oil free of antioxidants, solvent or catalyst residues. Its composition is indicated in table 5.2. Triplicate samples of the three models were placed in open glass vessels (height 3 cm, diameter 4.5 cm). The three model systems were oxidised by exposure to light (2000

lx) at 6 °C and in the dark under an oxygen atmosphere for 0, 2, 4 and 6 h, respectively, as previously described for the oxidation of UFA/CLA butter (4.2.3).

**Table 5.1:** Composition of the three model systems used for the oxidation experiments of CLA and linoleate

| Model system | Composition of model system (amount in mg) | | | |
|---|---|---|---|---|
| | Miglyol | 9c, 11t CLA ethyl ester | 9c, 12c ethyl linoleate | [$^{13}C_{18}$]9c, 12c ethyl linoleate |
| 1. Et CLA | 5000 | 100 | - | - |
| 2. Et Ln | 5000 | - | 75 | - |
| 3. Et CLA + [$^{13}C_{18}$]Et Ln | 5000 | 100 | - | 75 |

**Table 5.2:** Fatty acid composition of the neutral oil Miglyol® used as matrix in the model systems as analysed by HRGC

| Composition of Miglyol® | |
|---|---|
| **Fatty acid** | **%** |
| Hexanoic acid C6:0 | <0.5 |
| Octanoic acid C8:0 | 59 |
| Decanoic acid C10:0 | 40 |
| Dodecanoic acid C12:0 | 0.5 |
| Tetradecanoic acid C14:0 | <0.2 |

### 5.2.3 Analysis

All samples were analysed by headspace solid-phase microextraction (HS-SPME) combined with gas chromatography mass spectrometry and olfactometry (GC/MS/O). The analyses were carried out using a Combi PAL Autosampler (CTC Analytics,

Zwingen, Switzerland). The SPME fiber (2 cm DVB/CAR/PDMS, Supelco, Bellefonte, USA) was exposed for 30 min at 45 °C into the headspace above the samples in closed 20 ml glass vials. Then the fiber was placed in the GC injector, heated at 250 °C, for 12 min, and equipped with a 0.75 mm i.d. liner (Supelco). GC/MS/O analyses were performed using an Agilent HP 5890 Series II gas chromatograph (Agilent, Palo Alto, CA, U.S.A.) coupled to a mass selective detector (MSD; HP 5971A) and a sniffing port (Sniffer 9000 system, Brechbühler, Schlieren, Switzerland), and equipped with a HP-5MS column (30 m length, 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies). GC/O analyses were carried out in duplicate by one trained sniffer. The same samples were also analysed on a Trace GC coupled to a Trace DSQ mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) and equipped with a FFAP column (30 m length, 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). The oven temperature was, in both of cases, held at 40 °C for 2 min, then increased to 240 °C at a rate of 6 °C/min, and held at 240 °C for 5 min. Mass spectra in the electron impact mode (EI) were obtained at 70 eV and a scan range from m/z 29 to 350. The volatile compounds were identified by comparing the mass spectra with the Wiley 138 mass spectra library (Wiley & Sons, Inc., 1990) and with the spectra of authentic reference compounds. The retention index, calculated running a series of n-alkanes under the same working conditions, were also compared to those of the reference compounds and to published data. The same odour perception as the one obtained for the reference compounds was another criterion for identification.

## 5.3 Results and discussion

The three model systems in table 5.1 were oxidised for 0, 2, 4 and 6 h, respectively, and then analysed by GC/MS/O. The unoxidised samples (0 h) presented very weak odour. These odour notes were due in particular to a compound tentatively identified as 3-heptanol (metallic) and to heptanal (fatty, oily) (Table 5.3). After 6 h of oxidation (photo-oxidation and oxidation in the dark under an oxygen atmosphere) the number and the intensity of odour compounds increased, especially in the mixed EtCLA/[$^{13}C_{18}$]EtLn samples.

In the photo-oxidised samples, hexanal (oily/green), 3-heptanol (metallic), heptanal (fatty), 2-pentanol (oily/green) and 2-hexyl furan (painty) were more intensely perceived than in the samples oxidised in the dark. 2-Pentyl furan, described as plastic-like and nutty, and 2-hexyl furan were found especially in the mixed EtCLA/[$^{13}C_{18}$]EtLn samples. Some volatiles were found only by GC/O in the photo-oxidised samples, such as (*E,Z*)-2,4-nonadienal (green) and (*E,E*)-2,4-decadienal (fried), in particular in the EtLn model system. Other compounds, tentatively identified as 4-ethoxy cyclohexanone or heptyl cyclohexane (not shown in Table 5.3), which were not odour-active, were found only in the three model systems subjected to oxidation in the dark.

γ-Nonalactone and δ-undecalactone, with fruity notes, were detected only by olfactometry in the mixed EtCLA/[$^{13}C_{18}$]EtLn samples subjected to photo-oxidation and oxidation in the dark, respectively.

Table 5.4 compares the odour compounds found in the oxidised EtCLA/[$^{13}C_{18}$]EtLn model with those identified in oxidised UFA/CLA enriched butter. The majority of the odour compounds found in the model systems had already been previously found in UFA/CLA enriched butter (Chapter 3 and 4). However, *trans*-4,5-epoxy-2-decenal, methional, δ-decalactone and δ-dodecalactone, detected after (photo)-oxidation in UFA/CLA enriched butter, were not found in the oxidised models. It is concluded that they are not formed from CLA nor from [$^{13}C_{18}$]EtLn under the oxidation conditions applied. The model did not contain methionine, as source for methional. Thus, methional could not be found in the models.

**Table 5.3:** Selected odour-active compounds detected by GC/O in model samples

| Compound | Odour quality | RI[a] | 0 h | | | Light 6 h | | | $O_2$ 6 h | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | EtCLA[b] | EtLn[c] | EtCLA + [$^{13}C_{18}$]EtLn[d] | EtCLA | EtLn | EtCLA + [$^{13}C_{18}$]EtLn | EtCLA | EtLn | EtCLA + [$^{13}C_{18}$]EtLn |
| Pentanal | Green, painty | 738 | 1[f] | 1 | 1 | 2[f] | 2 | 2 | 2 | 2 | 2 |
| Hexanal | Oily, green | 805 | 1 | 1 | 2 | 2 | 3 | 3 | 1 | 1 | 2 |
| (*Z*)-3-Hexenol | Sweet, fruity | 858 | nd[f] | nd | 2 | nd | 1 | 2 | nd | 2 | 3 |
| 3-Heptanol[e] | Metallic | 877 | 2 | 2 | 1 | 1 | 2 | 2 | 1 | nd | 3 |
| Heptanal | Fatty | 906 | 1 | 2 | 2 | 2 | 1 | 2 | 1 | 1 | 2 |
| 2-Pentanol | Oily, green | 951 | 1 | 1 | 1 | 2 | 2 | 1 | nd | nd | 1 |
| 1-Octen-3-one | Mushroom | 967 | nd | nd | nd | nd | nd | nd | nd | 2 | 3 |
| 2-Ethyl-2-hexenal | Paint, soapy | 988 | nd | nd | 2 | nd | 2 | 2 | 1 | 2 | 1 |
| 2-Pentyl furan | Plastic, nutty | 991 | nd | nd | 2 | 1 | nd | 2 | 2 | nd | 3 |
| 2-Octanone | Soapy | 1002 | nd | nd | 2 | nd | nd | 1 | nd | nd | 1 |
| Hexanoic acid ethyl ester | Orange, fruity | 1007 | 2 | nd | nd | nd | nd | 2 | nd | nd | 2 |
| (*E*)-2-Octenal | Flower, sweet | 1057 | 1 | 1 | 1 | 1 | nd | nd | 1 | nd | nd |
| 2-Hexyl furan | Painty | 1136 | nd | 2 | 2 | 2 | 1 | 2 | nd | nd | 2 |
| Heptanoic acid ethyl ester | Orange | 1139 | nd | nd | nd | 1 | 1 | 1 | 1 | nd | 3 |
| (*E*)-2-Nonenal | Green, metallic | 1170 | 1 | nd | 3 | 1 | nd | 2 | nd | nd | 3 |
| (*Z*)-2-Nonenal | Green | 1189 | 2 | nd | 1 | 1 | 2 | nd | nd | 3 | nd |
| (*E,Z*)-2,4-nonadienal | Green | 1219 | nd | nd | nd | nd | 2 | 1 | nd | nd | nd |
| Octanoic acid ethyl ester | Flower | 1295 | 1 | nd | 1 | nd | nd | 1 | nd | nd | nd |
| *(E,E)*-2,4-decadienal | Fried oil | 1319 | nd | nd | nd | nd | 2 | 1 | nd | nd | nd |
| γ-Nonalactone | Fruity | 1366 | nd | nd | nd | nd | nd | 2 | nd | nd | 1 |
| δ-Undecalactone | Fruity | 1610 | nd | nd | nd | nd | nd | 2 | nd | nd | 1 |

[a]Retention index on HP-5MS; [b]Model system consisting of *c*9, *t*11 CLA ethyl ester in mglyol®; [c]Model system consisting of *c*9, *c*12 linoleate in myglyol®; [d]Model system consisting of a mix of *c*9, *t*11 CLA ethyl ester and [$^{13}C_{18}$]*c*9, *c*12 linoleate in miglyol®; [e]Compound tentatively identified; [f]1, weak odour intensity; 2, medium odour intensity; nd, not detected

**Table 5.4:** Comparison between odour compounds found in the oxidised model system EtCLA/[$^{13}C_{18}$]EtLn and oxidised UFA/CLA enriched butter

| Compound | Odour | Photo-oxidised mixed model | Photo-oxidised UFA/CLA butter | Oxidised[a] mixed model | Oxidised[a] UFA/CLA butter |
|---|---|---|---|---|---|
| Pentanal | Green, painty | + | + | + | + |
| Hexanal | Oily, green | + | + | + | + |
| (*Z*)-3-Hexenol | Sweet, fruity | + | + | + | + |
| 3-Heptanol[b] | Metallic | + | nd | + | nd |
| Heptanal | Fatty | + | + | + | + |
| 2-Pentanol | Oily, green | + | nd | + | + |
| 1-Octen-3-one | Mushroom | + | + | + | + |
| 2-Ethyl-2-hexenal | Paint, soapy | + | nd | + | nd |
| 2-Pentyl furan | Plastic, nutty | + | nd | + | nd |
| 2-Octanone | Soapy | + | nd | + | nd |
| Hexanoic acid ethyl ester | Orange, fruity | + | + | + | + |
| (*E*)-2-Octenal | Flower, sweet | + | + | + | + |
| 2-Hexyl furan | Painty, perfume | + | nd | + | nd |
| Heptanoic acid ethyl ester | Orange | + | nd | + | nd |
| (*E*)-2-Nonenal | Green, metallic | + | + | + | + |
| (*Z*)-2-Nonenal | Green | + | + | + | + |
| Octanoic acid ethyl ester | Flower | + | + | + | + |
| *(E,E)*-2,4-decadienal | Fried oil | + | + | + | + |
| (E,Z)-2,4-nonadienal | Green | + | nd | + | + |
| *trans*-4,5-Epoxy-2-decenal | Metallic | nd | + | nd | + |
| Methional | Potato-like | nd | + | nd | + |
| γ-Nonalactone | Fruity | + | nd | + | + |
| δ-Decalactone | Peach-like | nd | + | nd | + |
| δ-Undecalactone | Fruity | + | + | + | + |
| δ-Dodecalactone | Peach-like | nd | + | nd | + |

[a]Oxidation in oxygen atmosphere in the dark; [b]Compound tentatively identified; nd, not detected

The effects of photo-oxidation and oxidation in the dark on the formation of odour compounds in model system EtCLA +[$^{13}C_{18}$]EtLn are illustrated in table 5.5. The GC-TIC (GC-total ion current) peak area showed that pentanal clearly increased during oxidation. The peak of hexanal increased 1.5 times after 6 h of oxidation. However, this compound was already present before oxidation in the EtCLA model. Heptanal and (*Z*)-3-hexenol increased faster under light exposure. Photo-oxidation seemed also to cause the degradation of esters, such as hexanoic acid ethyl ester and octanoic acid ethyl ester, which were found partly hydrolised after 6 h of light exposure. (*Z*)-2-Nonenal significantly decreased after 6 h of photo-oxidation and oxidation in the dark, respectively. These results are only an indication of the decrease/increase of odour-active compounds in the model systems during oxidation. To provide more precise data, quantitative methods should be applied and other extraction techniques, complementary to SPME, should also be used to extract the less volatile compounds, formed during oxidation. However, the semi-quantitative results obtained during this study allowed some comparisons with UFA/CLA enriched butters. Pentanal, hexanal, heptanal and (*E*)-2-nonenal were also found as important compounds during the oxidation of UFA/CLA butter (Chapter 4). In particular, these volatiles increased after 6 h of light exposure and under oxygen atmosphere, as found in the model systems under the same oxidation conditions.

The GC/MS chromatograms of the three model systems after 4 h of photo-oxidation and oxidation in the dark are shown in Figures 5.1 and 5.2, respectively. In both cases, the numbers and the abundances of volatile compounds generated in the EtCLA samples, after 4 h of oxidation, was higher than in EtLn samples. Therefore, we

conclude that EtCLA, under our oxidation conditions, oxidised faster than EtLn. These findings are in agreement with previous observations (Chen et al., 1997; Zhang and Chen, 1997; Yang, 2000) suggesting that the conjugated double bonds of CLA may be more vulnerable to autoxidation than the non-conjugated double bonds.

Table 5.6 shows the proportions of isotopomers formed in the mixed model system during oxidation. In particular, the carbon atoms of (*Z*)-3-hexenol were found labelled to more than 99 %. This means that (*Z*)-3-hexenol is a secondary oxidation product of ethyl linoleate and, under the experimental conditions applied, not formed from EtCLA. (*Z*)-2-Nonenal was also found labelled to 88 % and 82 % after 6 h of photo-oxidation and oxidation in the dark, respectively. Surprisingly, (*E*)-2-nonenal behaved differently from (*Z*)-2-nonenal and the percentage of the labelled carbon atoms was about 56 % (mean value) in photo-oxidised samples and 63 % (mean value) in the models oxidised in the dark. Pentanal was formed likewise from EtCLA and EtLn. The proportion of unlabelled/labelled carbon atoms in this compound was 50/50, after 6 h of oxidation in both experiments. On the other hand, other aldehydes, such as hexanal and heptanal, were found unlabelled to about 75 % (mean value) and therefore, originated mainly from EtCLA. However, the proportion of the labelled isotopomers increased in hexanal during oxidation, meaning that the formation of hexanal from [$^{13}C_{18}$]EtLn increased during the oxidation experiment.

These findings confirm the study of García-Martínez et al. (2009), who found hexanal and heptanal as main secondary oxidation products in oil and triacylglycerols, containing equal amounts of *cis* 9, *trans* 11 CLA and *trans* 10, *cis* 12 CLA isomers.

The formation of pentanal and hexanal is predictable from the expected major 13-hydroperoxide, formed from *cis* 9, *trans* 11 CLA (García-Martínez et al., 2009). However, hexanal can also be formed from a tertiary reaction, e.g., during the autoxidation of 2,4-decadienal (Belitz et al., 2004).

The origin of heptanal, in oxidised oil containing CLA, can be explained by β-scission of the alkoxyl radical formed from 12-hydroperoxy-*trans*-8, *trans*-10-octadecadienoate (García-Martínez et al., 2009), which was reported to originate from oxidised *cis* 9, *trans* 11 CLA (Hämäläinen et al., 2002).

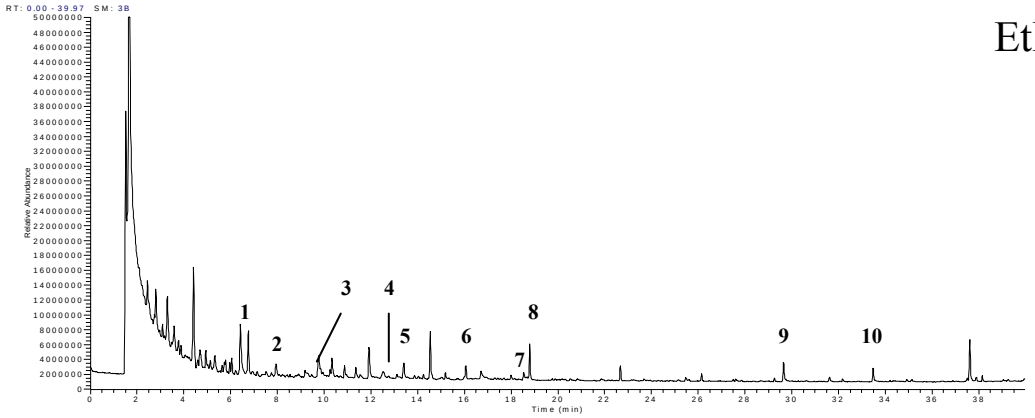
(*E*)-2-Nonenal could originate from two hydroperoxides, 10-hydroperoxy-*trans*-8, *trans*-11-octadecadienoate and 10-hydroperoxy-*trans*-8, *cis*-11-octadiecanoate, formed in oxidised *cis*-9, *trans*-11 CLA (García-Martínez et al., 2009).

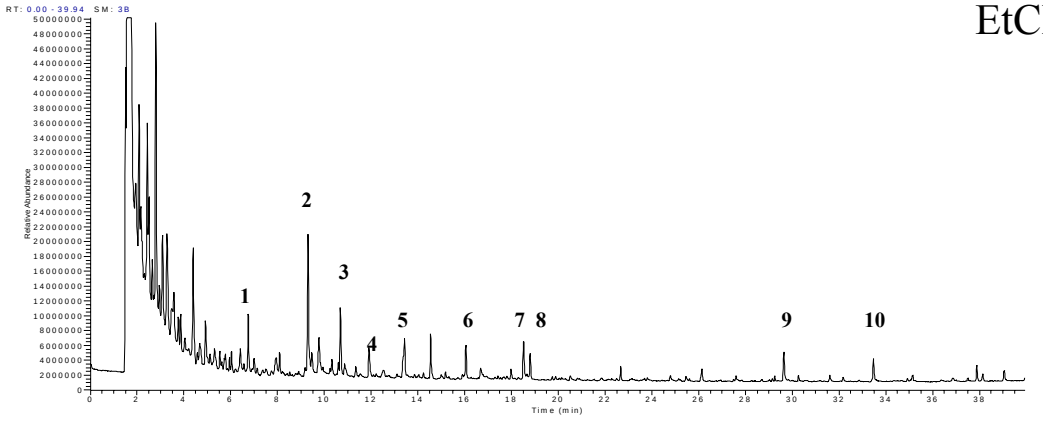
**Table 5.5**: Effects of the oxidation on the formation of odour-compounds in the model system formed by CLA ethyl ester (EtCLA) and [$^{13}C_{18}$] ethyl linoleate ([$^{13}C_{18}$]EtLn) (GC-TIC Peak Areas x $10^4$)[a]

| | | | | Light | | | | $O_2$ | | |
|---|---|---|---|---|---|---|---|---|---|---|
| No.[b] | Compound[c] | RI[d] | RI[e] | 0 h | 2 h | 4 h | 6 h | 2 h | 4 h | 6 h |
| | Pentanal | 975 | 738 | 4 | 9 | 12 | 13 | 8 | 12 | 13 |
| 1 | Hexanal | 1082 | 805 | 217 | 266 | 360 | 385 | 283 | 323 | 392 |
| 2 | Heptanal | 1170 | 906 | 67 | 124 | 195 | 184 | 99 | 128 | 140 |
| | 2-Octanone | 1218 | 1002 | 14 | 24 | 15 | 14 | 25 | 14 | 32 |
| 3 | Hexanoic acid ethyl ester | 1222 | 1007 | 77 | 66 | 59 | 39 | 72 | 74 | 82 |
| | (*E*)-2-Octenal | 1278 | 1057 | 81 | 70 | 70 | 64 | 67 | 67 | 92 |
| 4 | 2-Ethyl-2-hexenal | 1305 | 988 | 69 | 66 | 70 | 66 | 64 | 66 | 81 |
| 5 | Heptanoic acid ethyl ester | 1330 | 1139 | 65 | 56 | 58 | 54 | 59 | 60 | 75 |
| | (*Z*)-3-Hexenol | 1395 | 858 | 13 | 20 | 52 | 52 | 15 | 18 | 20 |
| 6 | Octanoic acid ethyl ester | 1430 | 1295 | 70 | 61 | 59 | 52 | 60 | 57 | 86 |
| 7 | (*Z*)-2-Nonenal | 1510 | 1189 | 34 | 33 | 49 | 19 | 41 | 48 | 14 |
| 8 | (*E*)-2-Nonenal | 1529 | 1170 | 38 | 35 | 48 | 43 | 36 | 39 | 52 |
| 9 | Octanoic acid | 2060 | 1290 | 22 | 25 | 26 | 22 | 23 | 20 | 23 |
| 10 | Decanoic acid | 2280 | 1380 | 14 | 16 | 16 | 12 | 17 | 11 | 14 |

[a]Standard deviation of the GC-TIC peak area was between 0.1 and 0.8 (x $10^4$)
[b]Number refers to the peak of the compound in the chromatograms (figures 5.1 and 5.2)
[c]Odour-active compounds found by GC/O
[d]Retention index on FFAP
eRetention index on HP-5MS

**Figure 5.1** Chromatograms of the three model systems, ethyl linoleate (EtLn), CLA ethyl ester (EtCLA) and the mixture EtCLA/[$^{13}C_{18}$]EtLn, after 4 h of photo-oxidation at 6 °C.



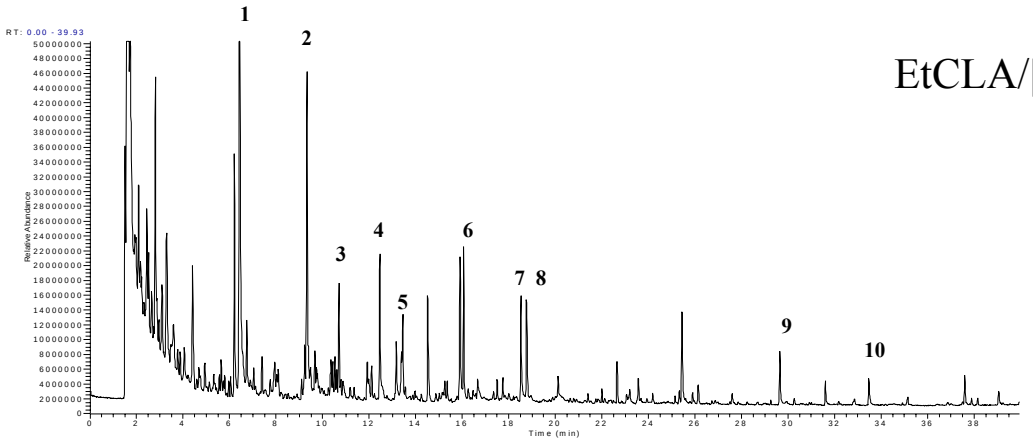
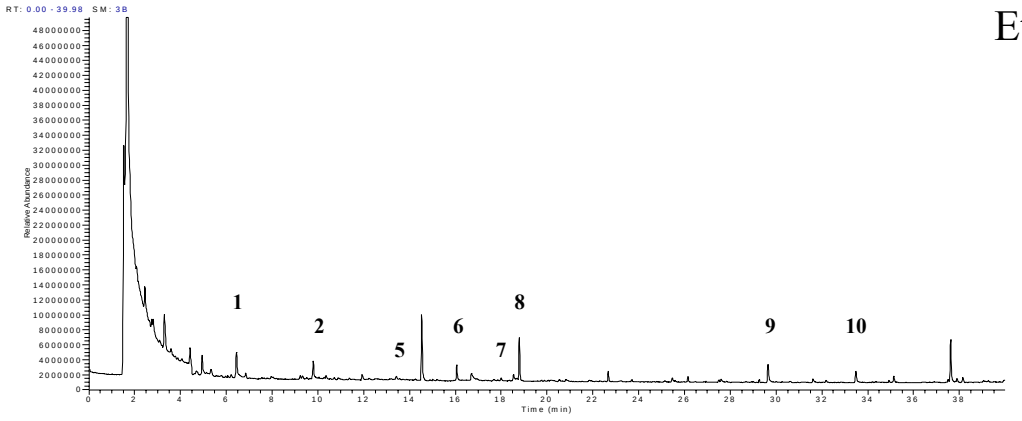
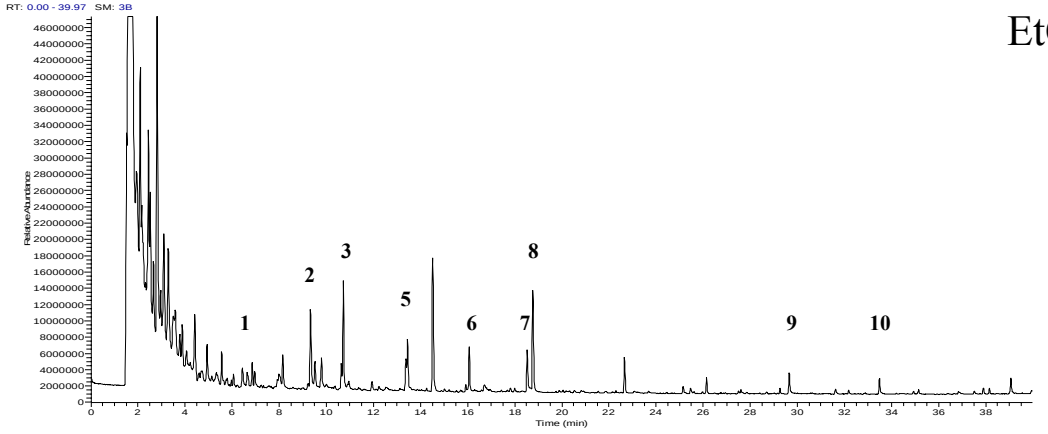



**Figure 5.2** Chromatograms of the three model systems, ethyl linoleate (EtLn), CLA ethyl ester (EtCLA) and the mixture EtCLA/[$^{13}C_{18}$]EtLn,after 4 h of oxidation under oxygen atmosphere at 6 °C in the dark.



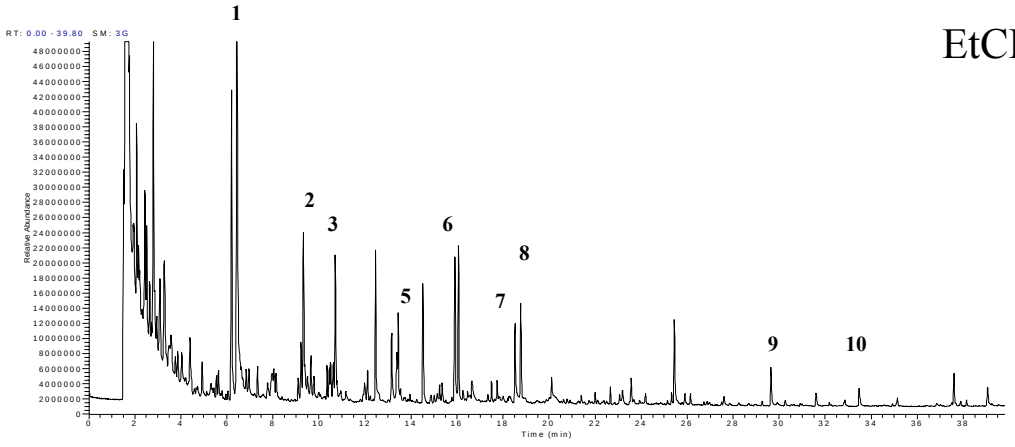



**Table 5.6** Proportion of isotopomers formed in the model system consisting of CLA ethyl ester (EtCLA) and [$^{13}C_{18}$]ethyl linoleate ([$^{13}C_{18}$]EtLn)

| | | | Proportion of unlabelled/labelled carbon atoms (%)[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Compound | *m/z* (M+) | *m/z* (analysed | Light and $O_2$ | Light | | | $O_2$ | | |
| | | | 0 h | 2 h | 4 h | 6 h | 2 h | 4 h | 6 h |
| Pentanal | 86 | 86; 91 | nd/nd | 67/33 | 50/50 | 50/50 | 58/42 | 50/50 | 50/50 |
| Hexanal | 100 | 100; 106 | 83/17 | 77/23 | 70/30 | 66/34 | 76/24 | 75/25 | 74/26 |
| (*Z*)-3-Hexenol | 102 | 102; 108 | nd/nd | nd/>99 | nd/>99 | nd/>99 | nd/>99 | nd/>99 | nd/>99 |
| Heptanal | 114 | 114; 121 | 73/27 | 75/25 | 81/19 | 80/20 | 75/25 | 76/24 | 75/25 |
| 2-Ethyl-2-Hexenal | 126 | 126; 134 | nd/nd | 98/2 | 89/11 | 86/14 | 98/2 | 76/24 | 97/3 |
| 2-Pentyl furan | 138 | 138; 147 | 58/42 | 57/43 | 57/43 | 51/49 | 60/40 | 59/41 | 55/45 |
| 2-Octanone | 128 | 128; 136 | nd/nd | 71/29 | 69/31 | 66/34 | 70/30 | 71/29 | 73/27 |
| Hexanoic acid ethyl ester | 144 | 144; 152 | >99/<1 | >99/<1 | >99/<1 | >99/<1 | >99/<1 | >99/<1 | >99/<1 |
| (*E*)-2-Octenal | 126 | 126; 134 | >99/<1 | >99/<1 | >99/<1 | >99/<1 | >99/<1 | >99/<1 | >99/<1 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Proportion of unlabelled/labelled carbon atoms (%)[a] | | | | | |
| Compound | *m/z* (M+) | *m/z* (analysed | Light and $O_2$ | Light | | | $O_2$ | | |
| **Table 5.6 continued** | | | | | | | | | |
| 2-Hexyl furan | 152 | 152; 162 | >99/<1 | >99/<1 | >99/<1 | >99/<1 | >99/<1 | >99/<1 | >99/<1 |
| Heptanoic acid ethyl ester | 158 | 158; 167 | >99/<1 | >99/<1 | >99/<1 | >99/<1 | >99/<1 | >99/<1 | >99/<1 |
| (*E*)-2-Nonenal | 140 | 140; 149 | 20/80 | 43/57 | 47/53 | 40/60 | 44/66 | 28/72 | 50/50 |
| (*Z*)-2-Nonenal | 140 | 140; 149 | nd/nd | 43/57 | 3/97 | 12/88 | 4/86 | 3/87 | 18/82 |
| Octanoic acid | 144 | 144; 152 | 100/nd | 100/nd | 100/nd | 100/nd | 100/nd | 100/nd | 100/nd |
| Octanoic acid ethyl ester | 172 | 172; 182 | >99/<1 | >99/<1 | >99/<1 | >99/<1 | >99/<1 | >99/<1 | >99/<1 |
| Decanoic acid | 172 | 172; 182 | 100/nd | 100/nd | 100/nd | 100/nd | 100/nd | 100/nd | 100/nd |

[a]The values represent the proportion between unlabelled and labelled isotopomers and are based on the abundance of the respective analysed ions

Among the other odour compounds formed during oxidation of the mixed model system EtCLA/[$^{13}C_{18}$]EtLn, 2-ethyl-2-hexenal and 2-octanone were mainly found unlabelled, from 76 % to 98 % and from 66 to 73 %, respectively. 2-Octanone originates from EtCLA and 2-ethyl-2-hexenal may be formed as an aldol product of butenal.

The proportion of unlabelled/labelled carbon atoms was between 51/49 % and 55/45% for 2-pentyl furan, whereas 2-hexyl furan was found unlabelled to more than 99 % in all samples. Our results point to the formation of these compounds mainly from EtCLA. However, in the literature, 2-pentyl furan and 2-hexyl furan are described as oxidation products of free linoleic acid (Min et al., 2003; Lee et al., 2003). To date, only furan fatty acids, such as 8,11-epoxy-8,10-octadecadienoic or 9,12-epoxy-9,11-octadecadienoic acid, are reported as products of CLA oxidation. The furan fatty acids could arise from cyclic peroxides formed by a 1,4 addition mechanism (Eulitz et al., 1999; Yurawecz et al., 2003). The furan fatty acids themselves are susceptible to oxidation (Eulitz et al., 1999) which may lead to other products, including alkyl furans.

In the oxidation experiments, (*E*)-2-octenal, acids and esters were found almost completely unlabelled (>99 %). This means that these compounds probably originate from EtCLA oxidation. On the other hand, García-Martínez et al. (2009), who compared the oxidation products of a CLA-containing oil to a linoleic acid-containing oil, concluded that (*E*)-2-octenal was mainly formed from linoleic acid. This different

result might be explained by the different oxidation conditions and the different matrix used in this study. However, (*E*)-2-Octenal has been also reported to form from 11-hydroperoxy-9,12-octadecadienic fatty acid, thus from *cis* 9, *trans* 11 CLA (García-Martínez et al., 2009).

The degradation of hydroperoxides is one possible pathway leading to volatile compounds from CLA. However, the low temperature used in our oxidation experiment (6 °C) probably reduces or even inhibits the formation of the hydroperoxides. Luna et al. (2007) observed that the formation of hydroperoxides was negligible at 30 °C, whereas Hämäläinen et al. (2001) and Pajunen et al. (2008) obtained hydroperoxides from CLA methyl esters, oxidised under atmospheric oxygen pressure, at 40 °C in the dark.

Thus, in our study, the formation of volatile compounds from the oxidation of *cis* 9, *trans* 11 CLA, has probably to be attributed to a cycloaddition of oxygen, as proposed by Eulitz et al. (1999) and Yurawecz et al. (2003). The authors found hexanal, heptanal, octanoic acid methyl ester, 2-heptenal and 2-nonenal as secondary oxidation products in photo-oxidised *cis* 9, *trans* 11 CLA methyl ester. They suggested that *cis* 9, *trans* 11 CLA methyl ester undergoes a 1,2 cycloaddition of oxygen, resulting in 11,12-dioxetane or 9,10-dioxetane, leading to heptanal and 2-nonenal by scission, respectively.

## 5.4 Conclusion

The results obtained in this study allow a better understanding of the role of CLA in odour compound formation. The main chemical pathways leading to volatile formation from CLA oxidation are either a 1,2 or 1,4 cycloaddition or a hydroperoxide mechanism, respectively. Under our experimental conditions, low temperature and a relatively short oxidation time, the two cycloaddition mechanisms seem to be the most plausible pathway for the formation of the volatile compounds which have been detected in the model. The origin of hexanal, heptanal, (*E*)-2-octenal, (*E*)-2-nonenal, 2-octanone, furans, ethyl esters and acids, in the model system EtCLA /[$^{13}C_{18}$]EtLn, is mainly attributed to EtCLA oxidation. However, to provide more precise data on odour formation from CLA, quantitative studies are needed. Besides, other aroma extraction techniques, complementary to SPME, should also be applied to identify the less volatile compounds formed from CLA oxidation.

These findings may also be translated to UFA/CLA enriched butter studied previously (Mallia et al. 2008). The odour compounds found in this butter, during storage, photo-oxidation and oxidation in the dark, respectively, are the same ones as found in the model system EtCLA /[$^{13}C_{18}$]EtLn. Therefore, the odorants found in UFA/CLA butter may also be formed by CLA oxidation and not only by oxidation of linoleic, linolenic and arachidonic acid as previously described in the literature (Badings, 1970; Widder, 1994a, b).

However, our model systems do not take into account the influence of antioxidants, such as α-tocopherol, or pro-oxidants, such metals ions. The α-tocopherol and retinol contents of UFA/CLA enriched butter were always higher than in conventional butter (Mallia et al., 2008). Therefore, these vitamins could partly protect the UFA/CLA butter from oxidation. On the other hand, the iron content, which was always higher in UFA/CLA enriched butter, might negatively affect the oxidative aroma formation of this butter because of its pro-oxidant activity. Further studies are needed to better understand the odour formation from CLA, using model systems with pro-oxidants and anti-oxidants. Moreover, the formation of odour compounds from CLA should further be investigated under various oxidation conditions, including temperature, matrix, and interactions with other food components, such as water. The water content of about 12-15 % in butter may change the stability of CLA during oxidation and lead to different odorants. Seo et al. (1999) demonstrated, for example, that CLA was more stable than linoleic acid in an aqueous system. The aroma formation of different CLA isomers and different CLA forms, such as CLA esters or CLA free fatty acids, should also be further studied.

# 6. Conclusions and outlook

## 6.1 Effects of storage on UFA/CLA enriched butter compared to conventional butter

As expected, the present investigation showed that UFA/CLA enriched butter has a significantly lower percentage of SFA and, thus, a significantly higher percentage of MUFA and PUFA, including CLA, compared to conventional butter. The feeding regime of the dairy cows, based on pasture and sunflower seeds is particularly rich in unsaturated fatty acids. It is responsible for the different FA composition and for the higher content of antioxidants, such as α-tocopherol, in UFA/CLA enriched butter. This result supports the findings of earlier investigations by Chilliard et al. (2001), AbuGhazaleh et al. (2004) and Collomb et al. (2004). The UFA/CLA enriched butter is also characterised by a lower fat content, which is 1-2 % lower than in conventional butter. A lower fat content of dairy products was also observed by AbuGhazaleh et al. (2004) and Chilliard et al. (2001), when the diet of the cows contained a source of UFA. This phenomenon seems to be related to an increase in *trans* fatty acids, such as *trans* 10, *cis* 12 CLA, which inhibit the milk fat synthesis (Baumgard et al., 2000). The mechanisms of fat secretion inhibition are not yet clear and need further investigation. However, the inhibition of milk fat synthesis does not yet explain the particularly low fat content (80 %) and the subsequently higher moisture content (19

%) found in the UFA/CLA enriched butter sample, which was produced in September 2006 (cf. Chapter 3). This could be due to the butter-making process, which was non-continous and partly carried out manually, such as in the kneading step. The complex organisation of the feeding experiments and the restricted availability of the cows made a replication of this trial impossible. A continous industrial butter production process could avoid similar experimental deficiencies, as automated adjustments of target fat and water contents would be controlled.

The UFA/CLA enriched butter also contained significantly more *trans* fatty acids than conventional butter (9 versus 5 g/100 g fat). The *trans* CLA isomers are not included in the sum of the total *trans* fatty acids, as recommended by the Swiss Regulation of FDHA (2008), because of their potential beneficial effects. At any rate, in fact, CLA have been reported to show potential anticarcinogenic and cholesterol lowering effects.

The role of *trans* fatty acids of animal origin in the human diet is still dibated in literature. The intake of *trans* fatty acids of animal origin and the occurrence of coronary heart diseases is negatively correlated according to Willet et al. (1993), Bolton Smith et al. (1996) and Pietinen et al. (1997). On the other hand, a decrease of coronary heart diseases was positively correlated with an intake of *trans* fatty acids from animal sources (Danish Nutritional Council, 2003).

Other differences in the chemical composition of UFA/CLA enriched butter compared to conventional butter are found in a higher content of essential fatty acids and vitamins.

Furthermore, the higher content in UFA causes a better spreadability of the butter, a further advantage for the consumer.

## 6.2 Effects of oxidation on UFA/CLA enriched butter and conventional butter

The aroma of UFA/CLA enriched butter was compared to that of conventional butter to investigate the oxidation behaviour of UFA/CLA during storage. The GC/MS/O findings showed no significant differences in the aroma composition between the two types of butter, when fresh (0 days until 2 weeks of cold storage). Their odour profiles, characterised by milky, soapy and sulphury notes in the beginning, were different after 2 to 8 weeks of storage. The UFA/CLA enriched samples developed more intense fatty, green and fruity notes. Quantification by stable isotope dilution analysis revealed significantly higher amounts of pentanal (fatty, green), heptanal (soapy) and δ-decalactone (peach) in the stored UFA/CLA enriched butter. Pentanal and heptanal are reported to form from oxidation of unsaturated fatty acids (Grosch, 1987). Delta-decalactone was already identified as an important aroma compound in sweet cream butter (Schieberle et al., 1993; Peterson and Reineccius, 2001a). However, our investigation showed a concentration of 2858 µg/kg in fresh UFA/CLA enriched butter, which was more than two times higher than the 1193 µg/kg found by

Peterson and Reineccius (2001a). Storage seemed to play an important role in the δ-decalactone concentration which was more than twice as high in the stored UFA/CLA butter than in the fresh UFA/CLA samples. The significantly higher amount of δ-decalactone in the UFA/CLA enriched butter could stem from CLA oxidation and not only from 5-hydroxy fatty acids already present in cream, as previously reported (Kinsella et al., 1967; Alweijn et al., 2007; Morán Hernández, 2007). By 1,2 cycloaddition of oxygen, CLA isomers can first form an instable intermediate, dioxetane, which gives, by scission, aldehydes and aldehyde esters (figure 6.1). The latter can form lactones by an intramolecular reaction of the aldehyde group with the carboxy group (Yurawecz et al., 2003). However, the chemical reaction pathway from CLA to lactones is not yet fully understood.

A sensory panel evaluated the samples and rated the two kinds of butter as very similar in aroma during storage. The sensory results contradict the GC/MS/O findings. It can be explained by the two different methodologies used. During the GC/O analysis the odour compounds are separated and perceived individually, one after another. The sensory panel perceive the odours simultaneously as an overall sensory impression, including complex odour-matrix and interactions between odorants.

The effects of storage on the aroma of UFA/CLA butter confirm the hyphothesis that UFA are prone to oxidation and produce aroma compounds by oxidation reactions. However, the higher concentrations of aldehydes and lactones in UFA/CLA enriched butter did not impair the overall butter flavour as demonstrated by the sensory

analyses of the stored samples. These results have to be considered in the context of the storage conditions chosen for this study. The results may change with different conditions.

When the butter samples were subjected to photo-oxidation, the GC/MS/O analyses allowed the identification of new compounds, such as methional (potato-like) and *trans*-4,5-epoxy-(*E*)-2-decenal (metallic), previously not found in the fresh and stored samples. Light exposure of butter induces different oxidation pathways and involves reactions with vitamins, such as riboflavin (vitamin B2), a photosensitiser. The photo-oxidation leads to losses of vitamins and amino acids, to discolouration of foods, as well as to formation of strong off-flavours, such as aldehydes, ketones and sulphur-containing-compounds (Borle et al., 2001). Light exposure provoked a stronger metallic off-flavour in the conventional samples, due to a higher amount of *trans*-4,5-epoxy-(*E*)-2-decenal compared to UFA/CLA enriched butter. The higher contents of α-tocopherol and retinol in the UFA/CLA enriched butter may protect it from photo-oxidation. A potential antioxidant activity of CLA on lipid oxidation has been described in the literature (Ha et al., 1990; Ip et al., 1991), but it is disputed by contradictory studies indicating CLA as prooxidant (van den Berg et al., 1995; Banni et al., 1998).

Exposure of the samples to a saturated oxygen atmosphere in the dark produced some different volatiles in the two types of butter. The concentration of heptanal (fatty), 1-octen-3-one (mushroom-like), (*Z*)-3-hexenal (green), (*E*)- and (*Z*)-2-octenal (fatty and

nutty, respectively) significantly increased in conventional butter exposed for 12 h to the oxygen atmosphere. α-Tocopherol might play an important role in the protection of UFA from oxidation in this case, too.

## 6.3 Comparison of UFA/CLA butter with a butter model: origin and mechanisms of odour compounds formation during oxidation

An oxididation experiment with three model systems containing labelled EtLn, EtCLA and a mixture of both, respectively, under the same oxidation conditions as the ones used previously for butter showed that EtCLA oxidised faster than [$^{13}C_{18}$]EtLn. This result suggests that a conjugated double bond is more susceptible to oxidation than a nonconjugated double bond under these experimental conditions. This is in agreement with observations published by van den Berg et al. (1995), Chen et al. (1997), Zhang and Chen (1997) and Yang et al. (2000). However, results obtained by Seo et al. (1999) and Suzuki et al. (2001) show that CLA may be more stable against oxidation than linoleic acid. These contradictions are most probably due to different oxidation conditions used in the experimental setups.

Monitoring the carbon atoms of the compounds formed after oxidation of the mixed model (EtCLA/[$^{13}C_{18}$]EtLn) allowed to track the origin of the volatiles from [$^{13}C_{18}$]EtLn or EtCLA. Some compounds, such as pentanal, were found 50 % labelled, indicating that it is equally formed from both precursors. (*Z*)-3-Hexenol, found labelled more than 99 %, was, on the other hand, formed from [$^{13}C_{18}$]EtLn. Hexanal and heptanal were found mainly unlabelled and, therefore, stem predominantly from

EtCLA. These results are in agreement with a previous study from Garcia-Martinez (2009). Table 6.1 summarises the likely origin of the odorants found in UFA/CLA enriched butter from oleic, linoleic, linolenic, or from CLA. The compounds originating from UFA can be formed by a radical mechanism leading first to hydroperoxides and then, after breakdown of these, to odour-active carbonyl compounds (Grosch, 1987). On the other hand, the formation of odorants from CLA follows different chemical pathways and a radical oxidation mechanism with hydroperoxide formation is less probable than with UFA (Eulitz et al., 1999). In addition, the low temperature used in our oxidation experiment probably slowed down the formation of hydroperoxides. The volatile compounds are probably mainly formed by a 1,2 or 1,4 cycloaddition of oxygen. In particular, the 1,4 cycloaddition leads to the formation of cyclic peroxides and subsequently to furan fatty acids, which can form dioxoenes, formylfurans or α-oxo-furans (Figure 6.1). A radical mechanism can also lead, in particular, to aldehydes, such as 2,4-decadienal and hexanal, formed from 9-LOOH and 13-LOOH, respectively (Figure 6.1). Yurawecz et al. (2003) reported that 14% of the oxidation products of *cis* 9, *trans* 11 CLA result from the breakdown of 9- and 13-LOOH.

The majority of odour compounds detected in the mixed model system by GC/MS/O were the same as the ones found in UFA/CLA enriched butter. This confirms that the mixture of [$^{13}C_{18}$]EtLn and EtCLA in miglyol was a suitable model to study the formation of individual aroma compounds and to compare it to the ones found in

UFA/CLA enriched butter. However, these models did not take into account the influence of antioxidants, such as α-tocopherol or pro-oxidants like metal ions. The emulsion nature of the butter was also not considered in the simplified model. The presence of water (12-15 % as in butter) might play an important role in the oxidation and can influence the speed of oxidation. A previous study (Seo et al., 1999) demonstrated that CLA was more stable than linoleic acid in an aqueous system. Another limitation of the model systems is the absence of other UFA, such as oleic, linolenic and arachidonic acids, which are also potential precursors of aroma compounds. A study with more complex butter models could further explain aroma formation by possible interactions with more different UFA.

**Table 6.1:** Origin of important odour compounds found in UFA/CLA enriched butter and conventional butter during oxidation

| Compound | Odour quality | Oleic acid[a] | Linoleic acid[a] | Linolenic acid[a] | CLA[b] |
|---|---|---|---|---|---|
| Hexane | Chemical | | + | | |
| Pentanal | Green, fatty | | + | | + |
| Hexanal | Green | | + | | + |
| Heptanal | Oily, fatty | + | + | + | + |
| Octanal | Soapy | + | + | | + |
| Nonanal | Soapy, citrus | + | + | | |
| Decanal | Soapy | + | | | |
| 2-Octanone | Soapy | | + | | + |
| 1-Octen-3-one | Mushroom | | + | | |
| (*E*)-2-Octenal | Nutty, fruity | | + | + | + |
| (*Z*)-2-Octenal | Fatty, nutty | | + | | + |
| (*E*)-2-Nonenal | Cut grass | | + | | + |
| (*Z*)-2-Nonenal | Hay | | + | | + |
| (Z)-3-Hexenal | Green | | | + | |
| (*Z*)-3-Hexenol | Fruity | | + | | |
| (*E,E*)-2,4-Nonadienal | Green, oily | | + | | + |
| (*E,Z*)-2,6-Nonadienal | Cucumber-like | | | + | |
| (*E, Z*)-2,4-Decadienal | Fried oil | | + | | + |
| *trans-4,*5-Epoxy-(*E*)-2-decenal | Metallic | | + | | |
| Hexanoic acid ethyl ester | Orange | | + | | + |
| Octanoic acid ethyl ester | Fruity | | + | + | + |
| γ-Nonalactone | Fruity | | | | + |
| δ-Undecalactone | Fruity, flower | | | | + |
| 2-Pentyl furan[c] | Nutty | | + | | + |
| 2-Hexyl furan[d] | Painty | | + | | + |

a The origin of volatile compounds from oleic, linoleic and linolenic acid is reported according to Grosch (1987) and Belitz et al. (2004)

[b]The origin of volatile compounds from CLA is based on the results reported in chapter 5 and on the literature (Yurawecz et al., 2003)

[c]The origin of this compound is based on the results reported in chapter 5 and on the literature (Grosch, 1987; Min et al., 2003)

[d]The origin of this compound is based on the results reported in Chapter 5

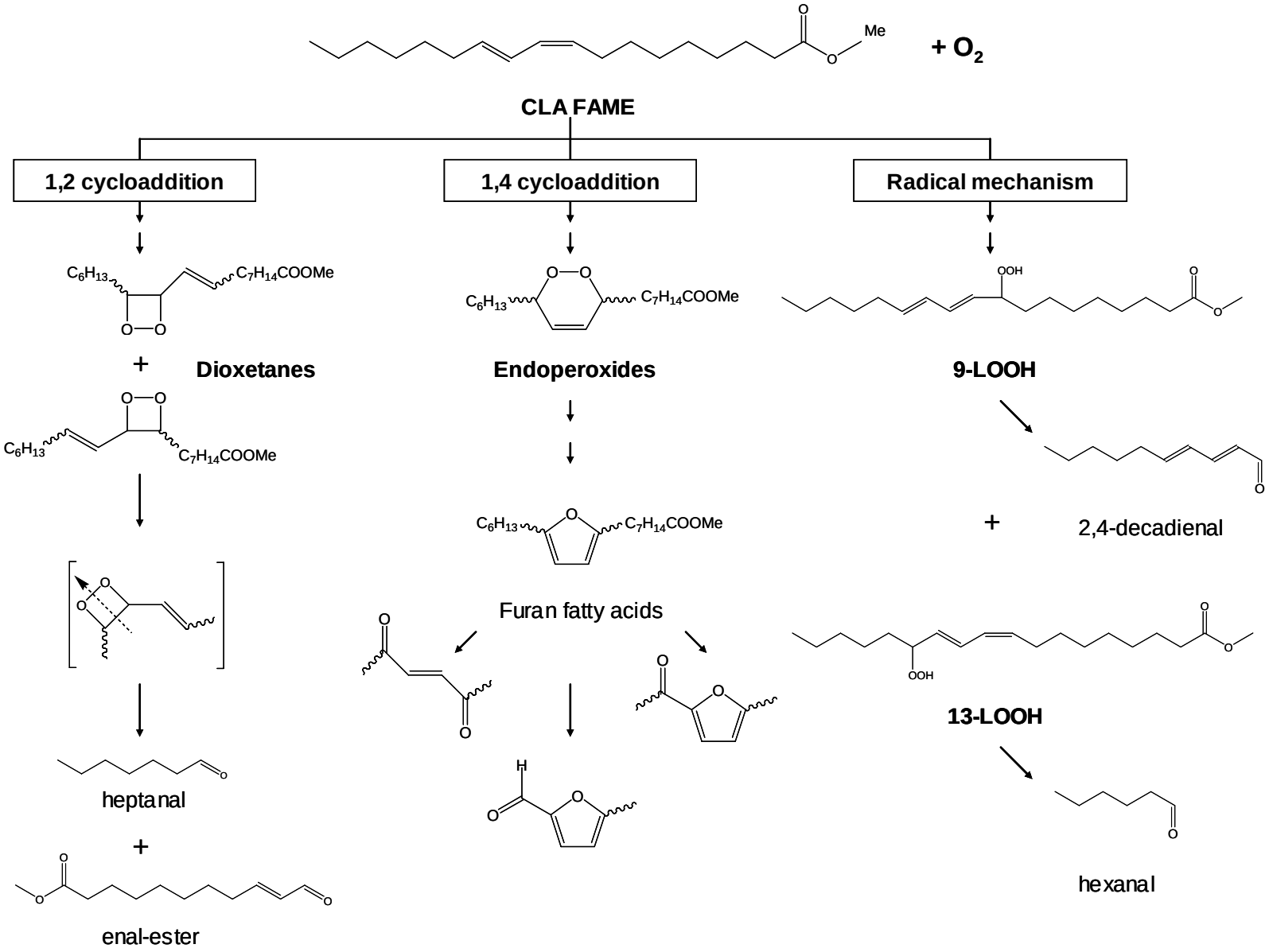



**Figure 6.1:** Formation of volatile compounds from CLA, according to Eulitz et al. (1999)

## 6.4 Concluding remarks and perspectives

Although our findings allow to conclude that the enrichment of milk with UFA/CLA does not impart detectable off-flavours to butter during 8 weeks of cold storage, further studies on storage stability of UFA/CLA enriched dairy products would be advisable, to evaluate the possible flavour changes of these products during longer storage periods and at different temperatures.

The role of pro- and antioxidants during storage of UFA/CLA enriched milk products also needs to be clarified. The pro-oxidative effect of iron ions, found in high amount in UFA/CLA enriched butter, was probably balanced by the antioxidative properties of its high α-tocopherol content. CLA could also play an important role in protecting UFA/CLA enriched butter against oxidation. However, additional knowledge on the antioxidative properties of CLA is required. The antioxidative activity of other milk micro-nutrients, such as ascorbic acid, β-carotene, lutein, zeaxantine, and in addition the chelation of transition metals by milk proteins, such as albumin and lactoferrin, should also be taken in account in future investigations.

To date, the classical lipid oxidation mechanism fails to satisfactorily explain the oxidation pathways of CLA. Knowledge of the kinetics and mechanisms involved on these reactions would be important to develop strategies for the storage of CLA-enriched foods.

It would also be interesting to compare a natural CLA enrichment by animal feeding to a direct CLA-fortification in dairy products. The latter may not yield the same CLA isomers or isomer ratios as via animal feeding (Linch et al., 2005). This could have

consequences on the flavour quality and on the potential beneficial effects of the CLA in dairy products. A study by Campbell et al. (2003) on direct CLA-fortification with CLA oil found a “grassy/ vegetable oil” flavour in the fortified milk, which was less acceptable than the flavours of not fortified milk. In this case, off-flavours could be avoided by microencapsulation of CLA in dairy products. Kim and al. (2000) showed that it is possible to protect CLA from oxidation by encapsulation in cyclodextrins. Similar studies could also be applied to dairy products, which may be considered functional foods.

There is a lack of detailed sensory profiles and analyses of CLA-enriched dairy products. The sensory results available are mainly based on triangle tests (Linch et al., 2005; Jones et al., 2005) and sometimes indicated significant differences and sometimes no significant differences in flavour between UFA/CLA enriched dairy products and conventional samples. Thus, before commercialising UFA/CLA enriched dairy products, additional sensory tests would be recommended, not least to assess the acceptability of these products by the consumer.

Further clinical studies are still necessary to demonstrate and confirm the beneficial health effects claimed for the different CLA isomers, and to clarify the necessary daily CLA intake that would result in these beneficial health properties.

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