Odor compounds in cheese made from the milk of cows supplemented with extruded linseed and α -tocopherol

F. Sympoura,* A. Cornu,*†¹ P. Tournayre,* T. Massouras,‡ J. L. Berdagué,* and B. Martin†

*UR370 Qualité des Produits Animaux, and

†UR1213 Herbivores, Institut National de la Recherche Agronomique, Theix, F63122 Saint-Genès Champanelle, France

‡Laboratory of Dairy Research, Agricultural University of Athens, Athens, Greece

ABSTRACT

Three diets for cows were used to evaluate the effect of extruded linseed (EL) or extruded linseed plus α -tocopherol (ELVE) supplementation of a maize silage diet (CO) on the odor-active compounds of Saint-Nectaire cheese. Cheese odor and flavor profiles were studied by sensory analysis. The volatile compounds were extracted by purge and trap and separated by gas chromatography. The odor compounds were detected and identified using an 8-way olfactometric device and a mass spectrometer. Twenty-nine volatile compounds were considered as contributing to the odor of Saint-Nectaire cheese. Half the compounds identified were known to be lipid degradation products but not all of them were affected by the diet. Among the markers of unsaturated fatty acid degradation, hexanal was not affected, whereas heptanal was increased more by the ELVE diet (6 times) than by the EL (3 times) diet. The ELVE diet led to cheeses with butanoic acid and heptanal odor peaks that were, respectively, 2 and 6 times higher than with the CO diet, which explained the lower milk odor and flavor scores obtained by sensory analysis on ELVE cheese. Although the cheese-making date had a greater effect than the diet on the aromatic profiles of the cheese, principal component analysis showed that the differences between cheeses obtained on the 3 diets were repeatable. The EL diet successfully enhanced cheese nutritional value without noticeably changing its flavor. α -Tocopherol supplementation was found to be unnecessary, as no oxidized odor was found.

Key words: α -tocopherol, linseed, cheese odor compound, supplemented diet

INTRODUCTION

In the diet of Western populations, milk and dairy products are a major source of dietary lipids, especially

saturated lipids, which are strongly implicated in the rise of coronary heart disease (Kromhout et al., 2002) and cancer but also contain essential nutriment beneficial to human health (Parodi, 1997). Over the last 25 yr, consumer demand for dietary unsaturated fatty acid (UFA) associated with healthy diets has been a major force toward changing the fatty acid (FA) profile of milk fat. The key objective is to reduce saturated fatty acid concentration and to increase monounsaturated and polyunsaturated fatty acid concentrations in milk, especially enhancing the content of some unsaturated fatty acids such as n-3 FA and conjugated linoleic acid (CLA).

Conjugated linoleic acid and n-3 FA contents in milk and in dairy products are influenced by the type of diet offered to the cows. Content of CLA and n-3 FA are higher when cows are fed on pasture compared with concentrates or preserved forages, and when cows are grazed on highland pastures compared with lowland pastures (Collomb et al., 2002; Leiber et al., 2005). Milk CLA and n-3 FA levels may also be increased by supplementing cows with vegetable seed or oils rich in UFA (Chilliard and Ferlay, 2004; Collomb et al., 2004). Linseed is one of the vegetable fat sources with the highest amount of C18:3n-3 (50% of the total FA). Feeding cows with extruded linseed increases milk UFA content and in particular n-3 FA, *trans*-11 C18:1 FA, and total CLA concentrations, and reduces the n-6 to n-3 fatty acid ratio (Fuentes et al., 2008). Milk fat is of prime importance in the genesis of cheese sensorial properties, because cheese texture and flavor largely rely on fatty acids and their degradation products. However, extensive trials on the modification of fat composition indicate that increasing the concentration of UFA promotes undesirable processes of lipolysis and oxidation, leading to degradation of fat and unacceptable changes in the color and flavor of milk and dairy products (Campbell et al., 2003). Although it is well known that a great number of cheese aroma compounds originate from lipid degradation, very few studies have focused on the consequences of lipid supplements on cheese sensorial properties, and to our knowledge this is

Received October 10, 2008.

Accepted February 17, 2009.

¹Corresponding author: agnes.cornu@clermont.inra.fr

the first study dealing with extruded linseed. Texture is invariably the most affected by lipid supplementation, unsaturated fatty acid-enriched cheese being less firm, less friable, and more melting. Flavor may or may not be affected, depending on the cheese variety and probably also on the basal diet of the cows: concentrate-rich diets more readily lead to flavor defects than does pasture. Unsaturated fatty acids may be oxidized and induce undesirable oxidized and rancid flavors. Allred et al. (2006) supplemented cows fed a control hay plus concentrate diet with calcium salts of palm and fish oil alone, or with 5% extruded soybean or 0.75% soybean oil. They observed a decrease in the acceptability of Cheddar cheese made from the supplemented cows' milk and the appearance between 30 and 90 d ripening of oily, oxidized, rancid, and sulfide off-flavors, which disappeared or were less marked after 180 d of ripening. In contrast, extruded soybeans distributed to grazing cows did not modify the sensory properties of the resulting Cheddar (Khanal et al., 2005). Likewise, a mixture of sunflower and fish oil (4.5% of the diet) did not induce off-flavor in Caerphilly cheese (Jones et al., 2005) nor did a soybean oil supplement in Edam (Ryhanen et al., 2005). These last authors, however, reported slightly slower ripening of the cheese resulting from the supplemented diets.

Because modifying milk lipid composition could result in decreased cheese acceptability due to undesirable oxidation products, antioxidants could help prevent this drawback. Milk fat-soluble vitamins such as α -tocopherol (**VitE**), retinol, and β -carotene contribute to protect lipids from oxidative degradation. It could therefore be interesting to determine if adding VitE to the linseed dietary supplement could prevent milk and cheese from becoming oxidized. The aim of the present study was to investigate the effects on the volatile odor-active compounds of Saint-Nectaire, a Protected Denomination of Origin cheese originating from Auvergne (France), of supplementing a control diet with extruded linseed alone or with extruded linseed plus α -tocopherol.

MATERIALS AND METHODS

Experimental Design

Eighteen Holstein ($n = 9$) and Montbéliarde ($n = 9$) cows were divided in 3 equal groups of 6 cows (3 Holstein and 3 Montbéliarde). The trial began on January 29, 2007. During a first 3-wk period all groups were fed the same diet based on hay and corn silage with a concentrate mixture (cereals and soybean meal). Then, for a 5-wk period, group 1 (**CO**; control) was fed a corn silage diet, group 2 (**EL**; extruded linseed) was fed a

corn silage diet supplemented with 3.4 kg of extruded linseed substituted for 3.6 kg of concentrates. This diet was calculated so that the added fat from extruded linseed represented 5% of the DMI. Group 3 (**ELVE**; EL plus VitE) was fed the same diet as group 2 but supplemented with 7,500 IU of VitE per cow and per day. Throughout the entire experiment, the cows were given 200 g/d per cow of a mineral and vitamin additive (phosphorus 5 g/kg, calcium 20 g/kg, magnesium 4.5 g/kg, vitamin A 600,000 IU/kg, and vitamin E 1,500 IU/kg).

During the last 3 wk, the cows were milked twice a day and the milk of each group was collected separately. The milk from the previous evening milking was kept at 4°C, mixed with the following morning's milking to obtain 40 L from each group, and transported to the experimental dairy in Aurillac (Cantal, France).

The raw milk obtained from each group was processed into Saint-Nectaire cheese as described previously (Cornu et al., 2005; Verdier-Metz et al., 2005). The average composition of CO, EL, and ELVE mixed milk was, respectively, 34.6, 31.9, and 31.1 g/L for protein, 39.8, 35.8, and 35.6 g/L for fat, and 66, 288, and 182×10^3 cells/mL for SCC. Spontaneous lipolysis estimated as FFA net production (Jellema, 1991) during 24 h of storage at 4°C was 1.94, 0.91, and 0.85 mEq/100 g of fat, respectively, in CO, EL, and ELVE mixed milk. Cheeses were made from CO, EL, and ELVE milks on 4 dates (March 8, 15, 20, and 22); consequently, a total of 12 cheeses was manufactured, 1 for each of the 3 groups in 4 different days. Cheese was allowed to mature for 8 wk before being cut, wrapped in tinfoil under vacuum in sealed polyethylene bags, and stored at -20°C until analysis. The average CO, EL, and ELVE cheese FA composition was 77, 58, and 60 g/100 g of fat for saturated FA; 18, 33, and 32 g/100 g of fat for monounsaturated FA; and 2, 5, and 5 g/100 g of fat for polyunsaturated FA.

Cheese Sensory Analyses

Cheese sensory analyses were performed by a panel of 12 trained assessors selected 2 yr ago. The assessors first answered a classified advertisement in a local newspaper and were then selected for their ability to detect and recognize basic tastes and odors. These panelists were trained to improve their skills with both diluted pure compounds and cheese. The panelists also had 2 training sessions with the cheeses made in this experiment. During the experimental sessions, panelists gave scores of between 0 and 7 for the intensity of 32 attributes—11 for odor and 6 for flavor. The other attributes related to body, texture, color, and appearance. During one session, 3 cheeses (CO, EL, ELVE)

Table 1. Distribution of vocabulary items used by the 8 panelists in the 9 olfactory classes defined for this purpose

Olfactory class	Vocabulary items
Lactic-cheesy	Butter, dirty socks, cream, Emmental, fermented, feet, cheese, vomit, milk, Gruyere, Roquefort, yogurt
Fruity-floral	Citrus fruit, pineapple, banana, grapefruit, strawberry, fruit candy, melon, cherry, lemon, floral, fruity, lilac, orange, apple, pear, green apple, rose, violet
Acid-alcohol	Acid, alcohol, ethanol, sour, vinegar
Earthy-undergrowth	Mushrooms, earth, forest, moss, wood, pine, sawdust
Vegetable-green	Artichoke, carrot, celery, chlorophyll, cabbage, potatoes, hay, grass, onion, vegetable, beans, ivy
Chemical-plastic	Acetone, ammoniac, chemical, chlorine, sulfur odor, formaldehyde, chemical gas, metallic, plastic, rubber, naphthalene, glue
Empyreumatic	Almond, burnt, peanuts, cocoa, chocolate, coffee, caramel, ash, wood fire, smoke, roasted, hazel, peppermint, hot bread, pepper, biscuit
Animal	Dung, fish, salmon, sardine, urea, perspiration, eggs, leather, dog
Not classified	Dust, stone, disagreeable, sugar, baby, hot, cooking, unknown, sweet, chewing gum, shampoo, perfume, paper, fresh, honey, medicament, cement, face cream, musty, piquant, detergent

made on the same day were coded with 3-digit random numbers and presented at 20°C to the panelists in varying order using a monadic sequential method. Four sessions were necessary to analyze the 4 cheese-making dates separately.

GC-Olfactometry and GC-Olfactometry-MS Analysis

Volatile compounds were extracted by purge and trap and independently analyzed using both the 8-way GC-olfactometry (GC-O) device (8W-GC-O) described by Tournayre and Berdagué (2005) and a GC-O-MS device. The 8W-GC-O method maximizes the number of odorous peaks detected from a sample, by allowing a panel of 8 sniffers to detect the same chromatography run. No instrumental detection was performed. The GC-O-MS procedure aims to identify, without ambiguity, the compounds responsible for odors. In this case, detection was carried out simultaneously by a mass spectrometer and by a single sniffer selected for his abilities, allowing olfactograms to be aligned with mass chromatograms. The samples were allowed to thaw at room temperature before being unpacked. The interactions between diet and date of manufacture were nonsignificant for most of the sensory descriptors; therefore, we chose to perform the analyses on a blend of the cheeses made on March 8 and 15, and on a blend of the cheeses made on March 20 and 22 to reduce the number of analyses to 6 runs of the 8W-GC-O. One gram was cut from each of the 2 cheeses and rapidly homogenized with 8 g of anhydrous sodium sulfate (Na_2SO_4) in a mortar. Three grams of the powdery mixture was placed on a 0.2-g glass wool bed in a cylindrical glass extraction cartridge (28 mm diameter $\times$ 47 mm height; Etablissements Maillières Frères, Aubière, France). To compensate for the loss of the most volatile compounds, 2 cartridges containing samplings of the same cheeses were successively used during one analysis. Dynamic headspace extraction was performed using a Tekmar apparatus (Tekmar-Dohrmann, Cincinnati, OH) as follows: purge for 45

min with the first cartridge followed by 15 min with the second cartridge, by a 60 mL/min 99.995% helium flow. Extracted volatile compounds were concentrated on a 180 mm $\times$ 6.35 mm i.d. trap packed with Tenax TA 60-80 Mesh (Supelco, Bellefonte, PA) at 30°C. A dry purge was applied for 3 min with 60 mL/min 99.995% helium before desorption for 10 min at 215°C using 99.9995% helium, cryofocusing at -150°C with liquid nitrogen in the chromatograph injection port and injection by heating 2 min at 215°C. The chromatograph (Hewlett Packard 4890D, Agilent Technologies, Palo Alto, CA) was coupled to a divider that distributed the volatile component outflow synchronously to 8 transfer lines connected to sniffing ports located in separate booths. The separation was performed in an RTX-5 capillary column (60 m $\times$ 0.53 mm i.d. $\times$ 1.5 μm , Restek, Evry, France) with a 99.995% helium flow rate of 8 mL/min. The oven temperature was programmed as follows: 5 min isothermal at 40°C, 4°C/min increase to 215°C, hold for 28 min. At the sniffing ports, 8 panelists indicated the beginning, the end, the intensity from 1 to 5, and the description of each odor they perceived from 0 to 35 min.

The GC-O-MS device comprised a chromatograph (GC 6890, Agilent Technologies), a mass selective detector (MSD 5973 Inert, Agilent Technologies), and a single sniffing port. The extraction conditions were as follows: purge at 20°C with 30 mL/min 99.995% helium, 45 min in a first cartridge and then 15 min in a second cartridge as above, trap on Tenax at 30°C, dry purge for 3 min with 30 mL/min 99.995% helium, desorption for 10 min at 220°C with 1 mL/min 99.9995% helium, and injection 2 min at 220°C. The chromatography column (Supelco SPB5, 60 m $\times$ 0.32 mm id $\times$ 1 μm) was maintained at 40°C for 5 min, increased by 4°C/min to 220°C, and held for 5 min. The divided column outflow was simultaneously directed toward a mass spectrometer monitoring the m/z between 15 and 230 during the 45-min period and an olfaction port where a single assessor detected odors between 5 and 35 min.

Figure 1. Total olfactory signal by classes. The breakdown of the total olfactory signal into 9 classes clearly shows the olfactogram zones belonging to a given olfactory class. Labels correspond to the peak numbers given in Table 2.

Identification of all volatile compounds was carried out by comparing their mass spectra and linear retention indices with those in published databases (Kondojoyan and Berdagué, 1996).

Acquisition and Analysis of Olfactometry Data

Olfactometry data were recorded at a rate of 1 scan/s from 0 to 2,100 s and processed using the Acquisniff software according to the VIDEO-sniff method (Berdagué and Tournayre, 2004). This method allows maximized information recovery from quantitative (detection frequency, intensity, perception duration) as well as qualitative odor description data (Tournayre and Berdagué, 2006). The odor descriptive vocabulary items were distributed into 9 olfactory classes as described in Table 1.

The GC-O data from the multi-port system were synchronized with those from the single-port GC-O-

MS by using well-characterized odor signals as internal standards. Because alkanes are odorless, retention indices were calculated using the straight-chain aldehydes naturally occurring in the samples (e.g., hexanal, heptanal, octanal, nonanal). Odor active compounds were identified by comparing their descriptors, mass spectra, and relative retention indices to published databases (Wiley, 1995; Kondojoyan and Berdagué, 1996; NIST/EPA/NIH, 1996; Acree and Arn, 2004) and to those of authentic standards. Pure compounds were obtained from Fluka (Saint-Quentin Fallavier, France) for 2-methyl-propanoic acid and 3-methyl-butanoic acid; from Carlo Erba (Val de Reuil, France) for acetic acid; and from Aldrich (Saint-Quentin Fallavier, France) for ethyl alcohol, 2-propanone, 2,3-butanedione, 3-methyl-butanal, 2-methyl-propanoic acid ethyl ester, toluene, butanoic acid, hexanal, 2-methyl-butanoic acid, styrene, heptanal, 3-(methylthio)-propanal, 1-octen-3-ol, 6-methyl-5-hepten-2-one, 2-octanone, hexanoic acid

Table 2. Identification of the compounds responsible for the main odorous peaks and most representative descriptors given by the judges

Peak number ¹	Main odors given by the panelists	RI ²	ID ³	Chemical name
1	Alcoholic floral note	483	A	Ethanol
2	Acetone	500	A	2-Propanone
3	Lactic-cheesy	509	C	2-Propanol
4	Chocolate	554	B	2-Methyl-propanal,
5	Butter	586	A	2,3-Butanedione
6	Vinegar	628	A	Acetic acid
7	Cheesy-chocolate	683	A	3-Methyl-butanal
8	Feet-vomit	761	A	2-Methyl-propanoic acid
9	Fruity	772	A	2-Methyl-propanoic acid ethyl ester
10	Plastic	774	A	Toluene
11	Vomit-cheesy-feet	793	A	Butanoic acid
12	Green apple	799	A	Hexanal
13	Cheesy	851	A	3-Methyl-butanoic acid
14	Fruity	854	A	2-Methyl-butanoic acid
15	Rubber	896	A	Styrene
16	Fruity	900	A	Heptanal
17	Potato	905	C	3-(Methylthio)-propanal
18	Mushroom	980	C	1-Octen-3-ol
19	Woody-moss	988	C	6-Methyl-5-hepten-2-one or 2-octanone
20	Fruity-floral	993	A	Hexanoic acid, ethyl ester
21	Citrus fruit-fruity	1,000	A	Octanal
22	Mushroom	1,080	C	1-Nonen-3-ol
23	Mushroom	1,087	A	2-Nonanone
24	Citrus	1,100	C	Nonanal
25	Indeterminate			Not identified
26	Rose floral	1,118	A	Phenylethyl alcohol
27	Fruity-floral	1,127	A	Formic acid octyl ester
28	Woody	1,147	B	2-Nonenal
29	Indeterminate			Not identified

¹Peak number as shown in Figure 1.

²Experimental retention indices (RI) calculated from the DB5 capillary column of the GC/MS system.

³Reliability of the identification: A = RI + MS + odor description in accordance with those of standards; B = RI + MS + odor description in accordance with those of databases; C = peak not found in MS or several candidate molecules or data missing in databases.

ethyl ester, octanal, 2-nonanone, nonanal, phenylethyl alcohol, and formic acid octyl ester.

Statistical Analyses

Statistical analyses were performed using Statistica software (Statsoft, Maisons-Alfort, France). The GC-O peak areas obtained by summing the individual olfactory signals over the peak duration were submitted to an ANOVA according to the model

$$X_{ijr} = \mu + \text{Diet}_{i=3} + \text{Assessor}_{j=8} + \varepsilon_{ijr=2},$$

where X = variable studied (peak no. x); μ = mean effect; Diet = diet effect with $i = 3$ levels; Assessor = assessor effect with $j = 8$ levels; and ε = residual error with $r = 2$ replicates. Sensory analysis scores were submitted to an ANOVA to study the effects of the cows' diet, cheese-making date, and their interaction according to the model

$$X_i = \mu + \text{Diet}_{i=3} + \text{Assessor}_{j=12} + \text{Date}_{k=4} + \text{Diet} \times \text{Date}_{ik} + \varepsilon_{ijk},$$

where X = variable studied (score of the attribute x); μ = mean effect; Diet = diet effect with $i = 3$ levels; Assessor = assessor effect with $j = 12$ levels; Date = cheese-making date effect with $k = 4$ levels; and ε = residual error with $r = 1$ replicate. Principal component analysis (PCA) was performed using the mean data for cheese-making dates (March 8 and 15 and March 20 and 22). Sensory analysis descriptors (mean scores for the 12 judges and 2 cheese repetitions) significantly affected by the cows' diet (or by the interaction between diet and cheese-making date) were active variables, and the odor compounds (mean areas over the 8 judges) significantly affected by the cows' diet were supplementary variables.

RESULTS AND DISCUSSION

Odor Compound Identification

Summing up the intensities of the odors detected by the 8 panelists on the 6 cheeses in the total olfactogram shown in Figure 1 resulted in a great amount of vocabulary per peak, which helped identify the molecules responsible for odors. Twenty-seven of the compounds

Table 3. Mean odorous peak areas (intensity $\times$ duration) from GC-olfactometry analysis of cheese from the milk of cows fed on hay plus concentrate alone (CO), supplemented with extruded linseed (EL), or with EL plus vitamin E (ELVE)

Peak number	Odor compound	Diet			<i>P</i> -value
		CO	EL	ELVE	
1	Ethanol	4.1 ^b	3.1 ^b	8.4 ^a	*
2	2-Propanone	7.9 ^a	0.8 ^b	2.6 ^{ab}	**
3	2-Propanol	1.9 ^b	14.7 ^a	2.8 ^b	**
4	2-Methyl-propanal	2.2	2.0	1.9	
5	2,3-Butanedione	19.4	13.3	22.6	
6	Acetic acid	27.4	43.1	54.5	
7	3-Methyl-butanol	11.9 ^a	4.6 ^b	10.5 ^a	*
8	2-Methyl-propanoic acid	17.5	21.1	23.9	
9	2-Methyl-propanoic acid ethyl ester	5.4	8.7	16.8	
10	Toluene	12.5	13.6	12.3	
11	Butanoic acid	74.6 ^b	100.1 ^b	157.4 ^a	**
12	Hexanal	9.4	11.9	9.4	
13	Butanoic acid, 3-methyl	41.0	50.1	65.5	
14	Butanoic acid, 2-methyl	19.4 ^b	19.4 ^b	38.9 ^a	†
15	Styrene	1.3	1.6	0.5	
16	Heptanal	2.8 ^b	8.3 ^b	15.7 ^a	**
17	3-(Methylthio)-propanal	22.4	21.4	26.1	
18	1-Octen-3-ol	14.6 ^b	23.1 ^a	19.1 ^{ab}	*
19	6-Methyl-5-hepten-2-one or 2-octanone	0.0 ^b	1.3 ^b	2.9 ^a	†
20	Hexanoic acid, ethyl ester	10.5	13.9	19.4	
21	Octanal	5.4	5.9	10.2	
22	1-Nonen-3-ol	4.8	7.7	9.1	
23	2-Nonanone	3.1 ^{ab}	6.6 ^a	0.1 ^b	*
24	Nonanal	4.9	7.6	12.3	
25	Not identified	4.5	5.6	3.2	
26	Phenylethyl alcohol	2.9	0.9	3.1	
27	Formic acid, octyl ester	2.0	3.9	6.3	
28	2-Nonenal	3.2	7.8	3.9	
29	Not identified	4.3	2.5	4.1	

^{a,b}Means within a row with different superscripts differ significantly.

† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$: effect of the cows' diet on the total olfactive signal.

responsible for the 29 peaks were identified with varying reliability: 5 fatty acids, 3 esters, 4 ketones, 8 aldehydes, 5 alcohols, and 2 miscellaneous compounds were found (Table 2). All of these compounds have previously been identified at different ratios in Salers cheese (Berdagué et al., 2007).

The most abundant odors for Saint-Nectaire cheese were of the lactic and the fruity classes, as described in Table 1. The information given by Figure 1 and Table 2 shows that volatile compounds contributing to the lactic odor were 2-propanol (peak 3), 2,3-butanedione (peak 5), isobutanoic acid (peak 8), butanoic acid (peak 11), and 3-methyl-butanoic acid (peak 13). The volatile compounds contributing to the fruity odor were the esters 2-methyl-propanoic acid ethyl ester (peak 9), hexanoic acid ethyl ester (peak 20), and formic acid octyl ester (peak 27); the aldehydes hexanal (peak 12), heptanal (peak 16), and octanal (peak 21), and possibly ethanol (peak 1), which gives an alcoholic, floral note, phenylethyl alcohol (peak 26), which gives a rose note; and the S-enantiomer of 2-methyl-butanoic acid (peak 14), which gives a fruity note (Leffingwell, 2002).

In the last part of the olfactogram, many odors remained unidentified or were identified with a low reliability (Table 2). These difficulties were because this part of the olfactogram was very crowded, the description of the panelists was inadequate, and there were many candidate molecules in the corresponding area in the mass chromatograms.

Effect of Diet on the Odor Compounds

Nine odor compounds were significantly affected by the cows' diet (Table 3). Ethanol, butanoic acid, 2-methylbutanoic acid, and heptanal peak areas were equivalent in CO and EL cheese and much higher in ELVE cheese. The amounts of 2-propanone and 3-methylbutanal were lower in EL than in CO cheese; these compounds in ELVE being, respectively, intermediate and equivalent to that in CO. Inversely, the amounts of 1-octen-3-ol and 2-propanol were higher in EL than in CO cheese; these compounds in ELVE being, respectively, intermediate and equivalent to that in CO. 2-Nonanone was higher in EL than in ELVE

Table 4. Sensorial analysis: cheese flavor and odor descriptor scores for the 4 cheese-making dates

Item	CO	EL	ELVE	Effect ¹		
				Diet	Date	Diet × Date
Flavor						
Intensity	4.25	4.33	4.27		***	†
Mushroom	1.00	0.90	0.83		**	
Dried fruit	1.08	1.54	1.42			
Milk	4.33 ^a	2.73 ^b	2.71 ^b	***		
Fish	0.04	0.04	0.04			
Plant	1.71	1.83	1.65		*	
Odor						
Intensity	4.67	4.50	4.46		***	*
Straw	1.15	1.33	1.38			
Mushroom	1.13	0.96	1.15		**	
Silage	0.88	0.67	0.65		***	
Cowshed	1.27	1.21	1.60		***	
Dried fruit	1.21	1.13	0.98			
Milk	3.75 ^a	2.67 ^b	2.63 ^b	**	**	
Musty	0.75	0.75	1.13		***	
Pungent	1.10	1.21	1.17		***	
Fish	0.08	0.35	0.08		*	**
Rancid	0.83	0.81	0.90		**	

^{a,b}Means within a row with different superscripts differ significantly.

¹Effect of the lipid supplement (CO = control, EL = extruded linseed, ELVE = extruded linseed + vitamin E) and cheese-making date according to the model $Y = \text{supplement} + \text{cheese-making date} + \text{assessor} + \text{supplement} \times \text{cheese-making date}$.

† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

cheese, with CO being intermediate. Hexanal, heptanal, 1-octen-3-ol, octanal, 1-nonen-3-ol, and nonanal are known to originate from the degradation of milk unsaturated fatty acids (McSweeney and Sousa, 2000). Of these, EL supplementation only increased 1-octen-3-ol. In contrast, the ELVE diet did increase heptanal. Therefore, the vitamin E ingested by the cows did not protect cheese lipid against oxidation, but in some cases seems to have intensified this oxidation, as if having been oxidized itself.

Seven branched compounds were found in this study: 2-methyl-propanal, 2-methyl-propanoic acid, and 2-methyl-propanoic acid ethyl ester did not vary within the diets; 3-methyl-butanal was lower in EL than in both CO and ELVE; and 3-methyl-butanoic acid gradually increased from CO to EL to ELVE. These branched-chain acids and aldehydes probably originate from the degradation of branched-chain amino acids (McSweeney and Sousa, 2000). Although no evidence can be found in the literature, branched-chain acids and aldehydes might also result from the additional protease activity caused by the somatic cells in the EL and ELVE milks. The increase of 2-methyl-butanoic acid and of the peak 19 tentatively identified as 6-methyl-5-hepten-2-one in the ELVE cheese raises the question of whether these compounds might directly originate from vitamin E degradation. Indeed, in this experiment, the vitamin E content in ELVE milk was more than 2-fold higher

than that in the CO and EL milk (B. Graulet, INRA, Theix, France; personal communication). No literature was found, however, to support this hypothesis.

Cheese Sensorial Analysis

Cheese sensorial analysis revealed strong effects of the cheese-making date but little effect of the cows' diet on the flavor and odor components (Table 4), although significant texture changes were found (data not shown). Lipid supplementation decreased the "milk" note of the cheese flavor as well as odor. In addition, interactions between the cheese-making date and the cows' diet were observed for the intensities of the cheese flavor and odor, and for the "fish" note of the odor. In fact, this fish odor was detected in one of the EL cheeses only (made on March 22) and at a low intensity (mean score 1.42/7), and it can be said that no off-flavor was caused by the lipid supplement. The ELVE cheeses received odor and flavor scores equivalent to those of EL cheeses.

In the PCA shown in Figure 2a, the cheeses are separated according to their cheese-making date along the first axis, which explains 61.5% of the variability in cheese sensorial characteristics. The cheeses obtained from the milk of supplemented cows are separated from the CO cheeses along the second axis, which explains about 26% of the variability. Adding vitamin E to the

Figure 2. Principal component analysis of the sensory attributes and GC-olfactometry data of the 6 cheeses. a) Sample plot: circles = CO cheeses (control diet), squares = EL cheeses (diet supplemented with extruded linseed), and triangles = ELVE cheeses (diet supplemented with extruded linseed and vitamin E); open symbols = cheeses made on March 8 and 15; solid symbols = cheeses made on March 20 and 22. b) Variable plot: solid lines = sensory attributes as active variables; dotted lines = GC-olfactometry data as additional variables.

lipid supplement had little or no effect. The representation of the variables on the same plane (Figure 2b) shows that the cheeses made on March 20 and 22 were essentially characterized by their higher odor and flavor intensity (*O_int* and *F_int*) compared with those made on March 8 and 15. This effect of cheese-making date probably reflects the variability of the raw milk microbial ecosystem, which is further amplified by differential development during cheese ripening, resulting in the acknowledged high diversity of raw-milk cheese (Duthoit et al., 2005). The cheeses made from the milk of supplemented cows are separated from the control cheeses along a bisecting line opposing milk flavor and milk odor (*F_milk*, *O_milk*) to butanoic acid. Rather

than increasing the overall intensity of cheese flavor, butanoic acid, which is a descriptor for strong cheese, exerts a masking effect on milk odor, a soft cheese descriptor. Trimethylamine, the compound usually responsible for fish odor (*O_fish*) in cheese, was not found by GC-O, whereas 2-nonanone correlated well with this odor on the PCA (Figure 2b). In fish, the fishy odor is mainly due to a combination of aldehydes, alcohols, and ketones (including 2-nonanone) resulting from the sequential actions of lipoxygenase and hydroperoxide lyase on PUFA (Shen et al., 2007). Therefore, it is possible that in cheese, a slight change in the proportions of these different families results in the production of a fishy odor.

CONCLUSIONS

Supplementing cows with extruded linseeds is an efficient way to improve milk fat nutritional quality. However, consumers demand healthier products but with unchanged aromatic properties. In our study, when comparing cheese from the extruded linseed-supplemented diet (EL) to the control, only 4 odor-active compounds were significantly ($P < 0.05$) influenced by the addition of extruded linseed in the cows' diet, 2 of which (2-propanone and 3-methyl-butanol) decreased and 2 of which (2-propanol and 1-octen-3-ol) increased. On the other hand, the major odor compounds [ethanol, butanoic acid, hexanal, methylbutanoic acids, 3-(methylthio)-propanal and octanal] were little affected. Moreover, no fishy off-flavor or rancid note was detected by sensory analysis. The use of an antioxidant in the ELVE diet increased the intensity of some major odor compounds such as butanoic and methylbutanoic acids, but did not seem to prevent unsaturated fatty acid degradation, because markers such as heptanal were much higher in ELVE than in EL. Nevertheless, sensorial characteristics of EL and ELVE cheeses were similar, indicating that adding vitamin E in the diet of dairy cows would be a useless additional cost.

ACKNOWLEDGMENTS

The research was supported by EU project TRUE-FOOD "Traditional United Europe Food," under the 6th Framework Program for RTD (contract no. FOOD-CT-2006-016264) and by a "Leonardo Da Vinci" European fellowship.

REFERENCES

- Acree, T., and A. Arn. 2004. Flavornet and human odor space. <http://www.flavornet.org/> Accessed Jan. 21, 2009.
- Allred, S. L., T. R. Dhiman, C. P. Brennand, R. C. Khanal, D. J. McMahon, and N. D. Luchini. 2006. Milk and cheese from cows

- fed calcium salts of palm and fish oil alone or in combination with soybean products. *J. Dairy Sci.* 89:234–248.
- Berdagué, J. L., and P. Tournayre. 2004. The VIDEO-Sniff® method, a new approach for the “Vocabulary-Intensity-Duration” study of “Elementary Odours” perceived by gas chromatography-olfaction. Pages 514–519 in *Flavour Research at the Dawn of the Twenty-First Century*. Lavoisier Editions, Cachan, France.
- Berdagué, J. L., P. Tournayre, and S. Cambou. 2007. Novel multi-gas chromatography-olfactometry device and software for the identification of odor-active compounds. *J. Chromatogr. A* 1146:85–92.
- Campbell, W., M. A. Drake, and D. K. Larick. 2003. The impact of fortification on conjugated linoleic acid (CLA) on the quality of fluid milk. *J. Dairy Sci.* 86:43–51.
- Chilliard, Y., and A. Ferlay. 2004. Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. *Reprod. Nutr. Dev.* 44:467–492.
- Collomb, M., U. Bütikofer, R. Sieber, B. Jeangros, and J. O. Bosset. 2002. Composition of fatty acids in cow's milk fat produced in the lowlands, mountains and highlands of Switzerland using high-resolution gas chromatography. *Int. Dairy J.* 12:649–659.
- Collomb, M., R. Sieber, and U. Bütikofer. 2004. CLA isomers in milk fat from cows fed diets with high levels of unsaturated fatty acids. *Lipids* 39:355–364.
- Cornu, A., N. Kondjoyan, B. Martin, I. Verdier-Metz, P. Pradel, J. L. Berdagué, and J. B. Coulon. 2005. Terpene profiles in Cantal and Saint-Nectaire-type cheese made from raw or pasteurised milk. *J. Sci. Food Agric.* 85:2040–2046.
- Duthoit, F., C. Callon, L. Tessier, and M. C. Montel. 2005. Relationships between sensorial characteristics and microbial dynamics in “Registered Designation of Origin” Salers cheese. *Int. Dairy J.* 103:259–270.
- Fuentes, M. C., S. Calsamiglia, C. Sánchez, A. González, J. R. Newbold, J. E. P. Santos, L. M. Rodríguez-Alcalá, and J. Fontecha. 2008. Effect of extruded linseed on productive and reproductive performance of lactating dairy cows. *Livest. Sci.* 113:144–154.
- Jellema, A. 1991. Determination of free fatty acids in milk and milk products. *Bull. Int. Dairy Fed.*, Brussels, Belgium.
- Jones, E. L., K. J. Shingfield, C. Kohen, A. K. Jones, B. Lupoli, A. S. Grandison, D. E. Beever, C. M. Williams, P. C. Calder, and P. Yaqoob. 2005. Chemical, physical, and sensory properties of dairy products enriched with conjugated linoleic acid. *J. Dairy Sci.* 88:2923–2937.
- Khanal, R. C., T. R. Dhiman, A. L. Ure, C. P. Brennand, R. L. Boman, and D. J. McMahon. 2005. Consumer acceptability of conjugated linoleic acid-enriched milk and Cheddar cheese from cows grazing on pasture. *J. Dairy Sci.* 88:1837–1847.
- Kondojoyan, N., and J. L. Berdagué. 1996. A compilation of Relative Retention Indices for the Analysis of Aromatic Compounds. Edition du Laboratoire Flaveur, INRA de Theix, Clermont-Ferrand, France.
- Kromhout, D., A. Menotti, H. Kestleloot, and S. Sans. 2002. Prevention of coronary heart disease by diet and lifestyle: Evidence from prospective cross-cultural, cohort, and interventional studies. *Circulation* 105:893–898.
- Leffingwell, J. C. 2002. Chirality and Odour Perception. <http://www.leffingwell.com/chirality/chirality.htm> Accessed Jan. 21, 2009.
- Leiber, F., M. Kreuzer, D. Nigg, H. R. Wettstein, and M. R. L. Scheeder. 2005. A study on the causes for the elevated n-3 fatty acids in cows' milk of alpine origin. *Lipids* 40:191–202.
- McSweeney, P. L. H., and M. J. Sousa. 2000. Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. *Lait* 80:293–324.
- NIST/EPA/NIH. 1996. Mass Spectral Database, version 1.5 PC. Standard Reference Database Program of the National Institute of Standards and Technology.
- Parodi, P. W. 1997. Cow's milk fat components as potential anticarcinogenic agents. *J. Nutr.* 127:1055–1060.
- Ryhanen, E. L., K. Tallavaara, J. M. Griinari, S. Jaakkola, S. Mantere-Alhonen, and K. J. Shingfield. 2005. Production of conjugated linoleic acid enriched milk and dairy products from cows receiving grass silage supplemented with a cereal-based concentrate containing rapeseed oil. *Int. Dairy J.* 15:207–217.
- Shen, C., J. Xie, and X. Xu. 2007. The components of cuttlefish (*Sepiella maindroni* de Rochebruns) oil. *Food Chem.* 102:210–214.
- Tournayre, P., and J. L. Berdagué. 2005. Gas chromatography-olfaction analyses device and method. International Patent no. WO 2005/001470 A2.
- Tournayre, P., and J. L. Berdagué. 2006. AcquiSniff® software: A tool for a complete analysis of GCO data. Registered trademark No. 023166185, May 28 2002, class 9 and 42 and APP reference: IDD. FR.001.210006.001R.P.2003.000.30000.
- Verdier-Metz, I., B. Martin, P. Pradel, H. Albouy, M. C. Montel, and J. B. Coulon. 2005. Effect of grass-silage vs. hay diet on the characteristics of cheese: Interactions with the cheese model. *Lait* 85:469–480.
- Wiley. 1995. Wiley 275K. 6th ed. Palisade Corp., Newfield, NY