Aroma Compounds of Fresh Milk from New Zealand Cows Fed Different Diets

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Volatile compounds were extracted from fresh milk produced by New Zealand cows using the newly developed solvent-assisted flavor evaporation (SAFE) technique. The two samples that were used came from cows that had been fed on different diets and represented the considerably different flavors of Northern hemisphere and New Zealand milk. Using gas chromatography−olfactometry (GC−O), 71 aroma compounds were found from the milk extracts, 66 of which were identified. Nearly all of the aroma compounds were common to both extracts, despite the two milk samples having quite different flavors. Only one compound, γ-12:2 lactone, was significantly odor-active for the extract of milk from cows fed a supplement diet, but was not found for the extract of milk from cows fed a pasture diet. Thus, differences in milk flavor are primarily caused by concentration differences of a common set of flavor compounds, rather than by the occurrence of compounds uniquely associated with a particular feed.

Keywords: *Milk; gas chromatography−olfactometry; aroma compounds; γ-12:2 lactone*

INTRODUCTION

Fresh cow's milk has a distinctive, and yet subtle, delicate flavor. Compared with other dairy products, such as butter or cheese, the concentration of aroma compounds in fresh milk is very low. This makes the task of understanding which compounds give fresh milk its flavor quite challenging, and there have been only a few publications in which gas chromatography−olfactometry (GC−O) has been used to link aroma compounds with the flavor of fresh milk [important examples are the work of Moio et al. (*1*, *2*)].

Recently, the new technique of solvent-assisted flavor evaporation (SAFE) has been described for obtaining extracts from aqueous food samples (*3*). For liquid milk, the SAFE technique gives extracts with sufficiently high concentrations of aroma compounds that the present study was possible.

Also described recently is the nasal impact frequency (NIF) method (*4*). This GC−O method uses a number of sniffers (10 for the present investigation) who report detection of odorants and, if possible, some descriptive information. The greater the percentage of sniffers who detect a compound (NIF-value), the more important that aroma compound is considered to be. The NIF method does not require that sniffers be trained and, if one sniffer has a specific anosmia, the results are not unduly affected (as may happen for other GC−O methods). The NIF method gives a good compromise between repeatability and a minimal number of GC−O runs. A recent comparison of the NIF method with other GC−O methods has shown that there is good correlation with the other methods for the most potent odorants found (*5*).

Although the NIF method is valuable for identifying potentially important aroma compounds, the relationship between the NIF-values and the concentrations of a specific odorant for extracts is not straightforward. Where a compound is present in two extracts at different concentrations, that will not necessarily result in different NIF-values because the distribution of sniffers sensitivities may not increase in a continuous manner, but can contain a plateau. However, NIF-value differences (above a certain statistical margin) for the same compound in two extracts does imply that the compound is present at different concentrations; and NIF-value differences of ≥30% have been regarded as generally indicative of significant concentration differences (*4*). There is one report in which an odorant, at parts per trillion levels, was quantified using GC−O where electronic detection of the compound was not possible (*6*). However, that study used extensively trained sniffers which is a major departure from the usual NIF method.

The following investigation was carried out to gain a better understanding of the compounds that give New Zealand fresh milk its flavor. GC−O, using the NIF method, was used on the SAFE extracts of two milk samples that had considerably different flavors; and the extractions had been completed within 24 h of the cows being milked. The milk samples came from pure-bred Friesian cows that had been fed different diets. One herd was fed a diet of "total mixed rations" (TMR); the other herd was fed a diet of grass pasture. Milks from TMR-fed and pasture-fed cows served as a proxy for Northern hemisphere and New Zealand milks, respectively. Friesian cows were used because they represent the largest proportion of the New Zealand national herd. September milk samples were selected because the flavor difference between the samples was expected to be greatest during the early milking-season. In New Zealand, most dairy cows calve during July or August, and the milking season begins thereafter. Because pasteurized milk is used for the manufacture of all dairy products made by the New Zealand dairy industry, pasteurized milk was also used for this study. Given the extremely dilute nature of the complicated SAFE

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extracts, no attempt was made to quantify the aroma compounds.

MATERIALS AND METHODS

Milk Samples. Two early-season (September 12, 2000) milk samples pooled from TMR-fed and pasture-fed Friesian cows (4 L for each sample) were provided by Dexcel (Hamilton, New Zealand). The TMR diet consisted of 25% maize silage, 19.5% grass silage, 7.5% hay, 10% whole cottonseed, and 38% concentrate which included maize grain, barley, soybean, fishmeal, vegetable oil, protected fat, corn gluten, molasses, minerals, and vitamins. The botanical composition of the pasture was 64.7% ryegrass, 20.1% white clover, 8.5% other grasses (mainly *Poa annua*), 2.6% weeds (mainly plantains), and 4.1% dead matter (*7*).

Sample Preparation. The milk samples were pasteurized (heated to 72 °C, held for 20 s), then transferred into brown glass bottles and were kept cool on ice until they were extracted.

Solvent-Assisted Flavor Evaporation. A SAFE apparatus [made by Chris Utton Glassblowing, Auckland, New Zealand, to the design described by Engel et al. (*3*)] was connected to a 5-L distillation flask equipped with a 70 mm × 20 mm diameter Teflon-coated magnetic stirrer bar, and a 500-mL flask as a receiving vessel. Warm water was pumped from a heated reservoir (35 °C) through the jacketed body of the apparatus, and the cold trap was filled with liquid nitrogen. The distillation flask was warmed via a water bath (50 °C), and the receiving vessel was cooled in liquid nitrogen. Then the apparatus was evacuated under high vacuum (1.5×10^{-2} mbar).

Each milk sample (2 L at a time) was added via the sample reservoir into the distillation flask over a 30 min period. This rate was slow enough to prevent excessive foaming of the milk. The vacuum was released after a further 30 min and then the deodorized milk was drained off from the distillation flask. The flask was re-fitted to the apparatus and the vacuum was reapplied so that the second 2-L portion of milk could be distilled as described above for the first 2-L portion.

Finally, the vacuum was again released, and diethyl ether (40 mL) was added to the receiving flask which was then stoppered. The distillate was protected from light and permitted to thaw. Each aqueous distillate was saturated with sodium chloride and then continuously extracted with the diethyl ether for 1 h via a light-phase liquid−liquid extractor. The diethyl ether extract was then reduced in volume to 0.5 mL under a gentle stream of nitrogen. Care was taken not to reduce the extract to too small a volume, as loss of even high-boiling compounds at very small volumes can become significant because of coevaporation (*8*). The extract was immediately analyzed simultaneously on separate GC−flame ionization detection (FID) and GC−mass spectrometry (MS) instruments. The extract was transferred into a brown glass vial and crimp-sealed with a septum cap, and the first GC−O run was undertaken concomitantly with the GC−FID and GC−MS runs. The extracts were stored at −20 °C between GC−O runs.

Gas Chromatography. Gas chromatography was performed on Fisons 8160, Fisons 8060, and Shimadzu GC-9A instruments for GC−FID, GC−MS, and GC−O, respectively. All chromatography used EC-1000 columns (equivalent to FFAP, 30 m × 0.25 mm; 0.25 μm stationary-phase thickness; Alltech, Auckland, New Zealand). The samples were applied by split/splitless injection (230 °C) with the oven temperature held at 35 °C. After 5 min, the oven temperature was raised by 5 °C/min to 230 °C, and then held at 230 °C for 15 min. For GC−O, the end of the column was split using a "Y" Press-Tight connector (Restek Corporation, Bellefonte, PA): one end went to an FID and the other end was passed through a heated tube (230 °C) from which the effluent was swept out via a stream of humidified air (75 mL/min) through a glass nose cone to the sniffer. An air flow-rate greater than 50 mL/min has been shown to be important for GC−O work (*9*). Helium at a flow rate of 1.0 mL/min was used as the carrier gas.

GC−O Analysis of the Milk Extracts. All GC−O data were recorded within a 10-day period to minimize the influence that chemical degradation of reactive compounds might impart to the results. To this end, each GC−O run (recorded from 4 to 55 min) was divided into two sections of about 25 min each, and each section was sniffed by a different sniffer. The sniffers (four female and six male) were time-tabled systematically such that they managed to sniff both sections of a GC−O run (during different sessions) for both extracts.

All GC−O data were plotted so that any patterns over the entire data set could be seen. These patterns were then matched to compounds: (i) where retention times of authentic standards were close (within ≤0.3 min); (ii) whose identities in the extracts had been established by MS; and (iii) where some of the aroma descriptions matched those of authentic standards. The 0.3 min range used for the detection of aroma compounds at the sniffing port made allowances for (i) variations of manual GC injection; (ii) variations of helium flow-rates through the two branches of the capillary column that were at different temperatures after the "Y" connector (*10*); and (iii) variations of sniffers recordings of the elution times for compounds with long-lingering aromas [can vary from 3 to 25 s (*11*)]. Compounds with NIF-values <20% for an extract have been included only if the NIF-value was ≥20% in the other extract; otherwise the data has been discarded as noise.

For some GC−O data, aromas were detected at retention times corresponding to known aroma-active compounds; however, their concentrations from the milk extracts were too low to observe full-scan mass spectra. Tentative identifications for such compounds were therefore made using knowledge that the author has gained from butter and cheese extracts (unpublished results) where it was possible to obtain full-scan mass spectra.

Authentic Standards. The following compounds were sourced from chemical suppliers: from Acros Organics (Geel, Belgium), 2-methylbutanal, ethyl 3-methylbutyrate, hexanal, 2-methylthiophene, hept-*cis*-4-enal, 2-*iso*butyl-3-methoxypyrazine, 4-propylphenol, and phenylacetic acid; from Aldrich (Milwaukee, WI), 3-methylbutanal, pyruvaldehyde, dimethyl disulfide, β-pinene, 2-carene, heptanal, limonene, hex-*trans*-2-enal, acetoin, dimethyl trisulfide, linalool, nona-*trans*-2,*cis*-6-dienal, 3-methylbutyric acid, nona-*trans*-2,*trans*-4-dienal, 4-ethylphenol, indole, and skatole; from Bedoukian (Danbury, CT), hex-*cis*-3-enal, and dec-9-enoic acid; from BDH (Palmerston North, New Zealand), ethyl acetate, acetic acid, valeric acid, benzothiazole, phenol, and ethyl oleate; from Danisco (Brabrand, Denmark), δ-hexalactone, δ-octalactone, γ-decalactone, δ-decalactone, γ-dodecalactone, δ-dodecalactone, γ-tetradecalactone, and δ-tetradecalactone; from ICN (Costa Mesa, CA), pentane-2,3-dione, β-caryophyllene, and ethyl linolenate; from Lancaster (Morecambe, UK), diacetyl, α-pinene, oct-1-en-3-one, methional, butyric acid, and phytol; from Oxford Chemicals (Hartlepool, UK), ethyl 2-methylbutyrate; from Riedel-de Haën (Seelze, Germany), *p*-cresol; from Carl Roth GmbH (Karlsruhe, Germany), α-thujene; and from Soda Aromatic (Tokyo, Japan), γ-dodec-*cis*-6-enolactone.

The following compounds were prepared by following published syntheses: 2-acetyl-1-pyrroline (*12*), non-1-en-3-one (*13*), octa-1,*cis*-5-dien-3-one (*14*), γ-dodec-*cis*-6,*cis*-9-dienolactone (*15*).

S-Methyl thio-3-methylbutyrate was prepared from 3-methylbutyryl chloride and sodium thiomethoxide.

Phyta-*trans*-3,*trans*-5-diene, neophytadiene, and phyta-*trans*-2,*cis*-4-diene were prepared by the acid-catalyzed dehydration of phytol. Phyt-2-ene, phyt-1-ene, and γ-hexadecalactone had previously been isolated and purified from cheese by the author.

3-Methyl-γ-nonalactone was synthesized from hexanal via a 1-ethoxyethyl-protected cyanohydrin, subsequent deprotonation of which, and addition to ethyl crotonate, yielded, after treatments with acid then base and then borohydride reduction, the lactone as product; the physical properties of which matched those described by Takahata et al. (*16*).

Formation of Non-1-en-3-one. A pressure tube containing non-*trans*-2-enal (25 mg) in Milli-Q purified water (25 mL) was

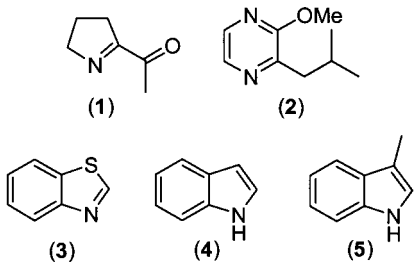

Figure 1. Structures of nitrogen heterocycles with NIF-values ≥50%: 2-acetyl-1-pyrroline (**1**), 2-*iso*butyl-3-methoxypyrazine (**2**), benzothiazole (**3**), indole (**4**), and skatole (**5**).

heated (80 °C) for 90 min, then the tube was cooled to ambient temperature and opened, and the contents were extracted with ether. GC−MS analysis of the ether extract showed a mixture of products including a small amount of non-1-en-3-one.

RESULTS

Overall, 71 different aroma-active compounds were found from the milk extracts by GC−O, 66 of which were identified. These aroma compounds are listed in Table 1, along with their NIF-values for both extracts, their retention indexes, some of the aroma descriptions used by the sniffers, and their published threshold values.

The aroma compounds with the highest NIF-values fell into five major chemical classes: nitrogen heterocycles, linolenic acid oxidation products, γ-lactones, phenolics, and phytol derivatives. Other classes of aroma compounds with lower NIF-values included fatty acids, Strecker esters, sulfur compounds, δ-lactones, terpenes, diacetyl and related compounds, and Strecker degradation products. These classes of aroma compounds are discussed below, and the structures of the aroma compounds with NIF-values ≥50% are shown in Figures 1−8.

Nitrogen Heterocycles. 2-Acetyl-1-pyrroline (**1** in Figure 1) was the only compound found with an NIF-value of 100% (for pasture-derived milk). For TMR-derived milk, the NIF-value was only 50%. This aroma compound has been found previously in Camembert cheese (*17*). 2-Acetyl-1-pyrroline is formed by the interaction of pyruvaldehyde with 1-pyrroline, which is formed from amino acids (*18*). 2-*iso*Butyl-3-methoxypyrazine (**2** in Figure 1) gave very high NIF-values for both milk extracts and has been linked to grassy flavors in drinking water caused by vegetation (*19*). Benzothiazole (**3** in Figure 1) (chemical origin not known) gave a high NIF-value for the pasture-derived milk. Benzothiazole has been found previously as a potent odorant for pasteurized and UHT-treated milk (*2*) and is used to formulate milk and cheese flavorings (*20*). Indole (**4** in Figure 1) and skatole (**5** in Figure 1) were found by GC−O for both extracts, and pasture-derived milk gave high NIF-values. Pasture-derived milk has been reported to have greater concentrations of both indole and skatole than milk from cows fed on a reduced-protein diet (*21*).

Linolenic Acid Oxidation Products. Octa-1,*cis*-5-dien-3-one (**6** in Figure 2), hept-*cis*-4-enal (**7** in Figure 2), hex-*cis*-3-enal (**8** in Figure 2), and hex-*trans*-2-enal (**9** in Figure 2) gave high NIF-values; nona-*trans*-2,*cis*-6-dienal and nona-*trans*-2,*trans*-4-dienal gave low NIF-values. These compounds all arise from oxidation of linolenate (*22*, *23*).

Oct-1-en-3-one [from linoleate oxidation (*24*)] and non-1-en-3-one were also found by GC−O. In a separate

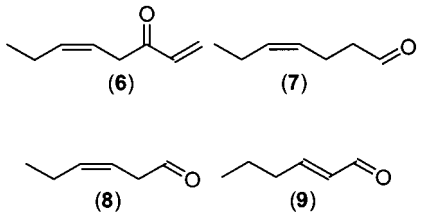

Figure 2. Structures of linolenic acid oxidation products with NIF-values ≥50%: octa-1,*cis*-5-dien-3-one (**6**), hept-*cis*-4-enal (**7**), hex-*cis*-3-enal (**8**), and hex-*trans*-2-enal (**9**).

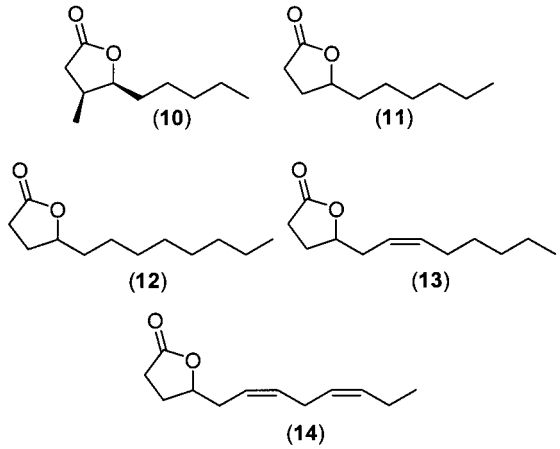

Figure 3. Structures of γ-lactones with NIF-values ≥50%: *cis*-3-methyl-γ-nonalactone (**10**), γ-decalactone (**11**), γ-dodecalactone (**12**), γ-dodec-*cis*-6-enolactone (**13**), and γ-dodeca-*cis*-6,*cis*-9-dienolactone (**14**).

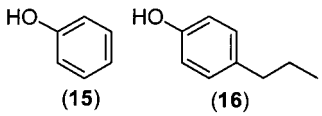

Figure 4. Structures of phenolics with NIF-values ≥50%: phenol (**15**) and 4-propylphenol (**16**).

experiment, a very small amount of non-1-en-3-one was formed by treating non-*trans*-2-enal with hot water. Both oct-1-en-3-one and non-1-en-3-one have been previously identified as aroma compounds of yogurt and milk (*13*).

γ-Lactones. *cis*-3-Methyl-γ-nonalactone (**10** in Figure 3) [known as cognac lactone (*16*)], γ-decalactone (γ-10, **11** in Figure 3), γ-dodecalactone (γ-12, **12** in Figure 3), γ-dodec-*cis*-6-enolactone (γ-12:1, **13** in Figure 3), and γ-dodeca-*cis*-6,*cis*-9-dienolactone (γ-12:2, **14** in Figure 3) all gave high NIF-values. Milk from supplement-fed cows has higher concentrations of γ-12 and γ-12:1 than milk from pasture-fed cows (*25*). γ-Tetradecalactone (γ-14) and γ-hexadecalactone (γ-16) gave low NIF-values.

Phenolics. Phenol (**15** in Figure 4), *p*-cresol, 4-ethylphenol, and 4-propylphenol (**16** in Figure 4) were found by GC−O. Phenolic compounds have long been known as the agents responsible for the smell of cow urine (*26*), and they can impart flavor to milk upon liberation from conjugate precursors (*27*).

Phytol Derivatives. Phytol (**17** in Figure 5), phyt-1-ene (**18** in Figure 5), and phyt-2-ene (**19** in Figure 5) gave high NIF-values. Lower NIF-values came from neophytadiene, phyta-*trans*-3,*trans*-5-diene, and phyta-*trans*-2,*cis*-4-diene. Phytol is a product of the degradation of chlorophyll *a* and chlorophyll *b* (*28*). Dehydration of phytol gives phytadienes which can hydrogenate in the rumen to give phyt-1-ene and phyt-2-ene (*21*, *29*).

Table 1. Aroma Compounds Found by Gas Chromatography−Olfactometry

compound[a]	NIF-values TMR/pasture	retention index	some aroma descriptions used by sniffers	reported threshold[b]
ethyl acetate	20%/10%	890	sweet ester; solvent	5
2-methylbutanal	30%/20%	920	musty	0.9
3-methylbutanal	30%/30%	935	musty; wet dog	0.25
pyruvaldehyde	40%/30%	970	sweet; earthy	5000[c] ?
diacetyl	30%/40%	1005	butter; pastry	5
α-pinene	20%/10%	1035	solvent	120
α-thujene	30%/20%	1045	cooked; nutty	
ethyl 2-methylbutyrate	60%/50%	1055	juicy-fruit chewing-gum; berry-like	0.006
ethyl 3-methylbutyrate	40%/20%	1075	ester; EMB; green ester	0.03
pentane-2,3-dione	40%/20%	1090	milky	5
dimethyl disulfide[d]	20%/20%	1100	rubber	12
hexanal	20%/20%	1105	cooked	10.5
β-pinene	30%/40%	1125	soapy, fragrant; green	
2-methylthiophene	20%/20%	1130	milky; cooked vegetables	
2-carene	30%/20%	1150	rolled oats; ethereal	
hex-*cis*-3-enal	60%/40%	1160	grassy; fresh milk; warm milk	0.03
heptanal	40%/20%	1210	cheesy; caramel	3
limonene	20%/20%	1215	orange	200
S-methyl thio-3-methylbutyrate	30%/10%	1225	cooked milk; lactone, cherry; shortbread	0.04[c]
hex-*trans*-2-enal	40%/50%	1240	wet grass; rancid fat; sour cream	27
hept-*cis*-4-enal	50%/80%	1260	fresh milk; biscuity; apple shortcake	0.06
oct-1-en-3-one	60%/60%	1300	mushroom; vegetable; dry hay	0.01
acetoin	10%/30%	1325	caramel; butterscotch	800
2-acetyl-1-pyrroline	50%/100%	1330	mouse-dirt; crackers; burnt chocolate pudding	0.0073(starch)
dimethyl trisulfide[d]	50%/30%	1385	cheesy; rolled oats	0.008
non-1-en-3-one[d]	40%/40%	1410	paint	0.000008[f]
octa-1,*cis*-5-dien-3-one[d]	80%/90%	1430	herbaceous, strong; warm milk; green grassy	0.0004
acetic acid	30%/30%	1455	vinegar	22000
methional[d]	20%/nil	1470	compost; cooking	0.04
2-*iso*butyl-3-methoxypyrazine[d]	90%/80%	1545	vegetable capsicum; woody/green; grassy	0.005
linalool	40%/40%	1560	floral; fruity	1.5
nona-*trans*-2,*cis*-6-dienal	20%/30%	1595	green; fresh milk; green apple/tomato	0.01
β-caryophyllene	30%/40%	1615	berry-fruit; orange	
butyric acid	30%/50%	1630	vomit; fetta cheese	1000
3-methylbutyric acid	10%/40%	1680	slight grassy; cooked vegetables	250
nona-*trans*-2,*trans*-4-dienal[d]	20%/30%	1705	fatty; old cardboard	0.06
valeric acid	20%/20%	1740	acidic; bready; cheese, dog	2100
phyt-2-ene	30%/50%	1775	grass; hay	
δ-hexalactone[d]	40%/40%	1830	fruity; rolled oats	
phyt-1-ene	50%/40%	1850	grassy; cardboard	
phyta-*trans*-3,*trans*-5-diene	30%/30%	1880	wood polish; paint	
neophytadiene	40%/20%	1915	wood polish; burnt	
phyta-*trans*-2,*cis*-4-diene	20%/30%	1945	faecal; burnt	
benzothiazole	40%/70%	1955	dead match-head; popcorn, strong; musty	50[g]
δ-octalactone	30%/30%	1990	lactone fruity; sweet	400
phenol	40%/50%	2015	leather; paint; shortbread	250[h] ?
cis-3-methyl-γ-nonalactone	70%/40%	2070	sweet lactone; buttery; toast	
p-cresol	40%/40%	2100	soap; old milk	2[h]
γ-decalactone	50%/40%	2145	lollies; milky	11
4-ethylphenol[d]	30%/30%	2180	barny; burnt paper; dirty faecal	100[h] ?
δ-decalactone	20%/20%	2215	coconut; hot milk; fruity	100
4-propylphenol[d]	50%/30%	2275	wet hair; bitter	
dec-9-enoic acid	40%/50%	2305	wet paper; burnt dust	
unknown	20%/50%	2350	strong; brothy; rubbery	
γ-dodecalactone	40%/50%	2395	lactone; fruity; sweet floral	7
γ-dodec-*cis*-6-enolactone	40%/50%	2425	fruit; dishcloth; burnt sugar	0.1 (alc. soln.)
δ-dodecalactone	20%/20%	2455	sweet fruity	1000
ethyl oleate	10%/20%	2470	ester	
indole	20%/60%	2490	hot rubber; jonquils; earthy	90
γ-dodec-*cis*-6,*cis*-9-dienolactone	50%/nil	2505	muesli with honey; baby powder	
skatole	40%/60%	2530	melted/baked butter; garden dirt; lamb	3
phenylacetic acid	20%/40%	2565	sweet peachy; old cotton; oats	1000
ethyl linolenate[d]	10%/30%	2590	fruity; sweet lactone	
phytol[d]	50%/40%	2610	bitter; oats	
γ-tetradecalactone[d]	20%/40%	2645	hot milk	
δ-tetradecalactone[d]	30%/50%	2675	dry hay	
unknown	10%/30%	2710	musk, perfume; burnt; cheesy	
unknown	20%/30%	2730	dung; milky; stilton cheese	
unknown	40%/20%	2745	burnt rubber; brothy; cheese	
unknown	20%/30%	2775	burnt vegetables; coffee; gravy	
γ-hexadecalactone[d]	10%/40%	2810	lactone; hot; smoky	

[a] Compounds are listed in order of their GC retention index, using an EC-1000 column (equivalent to FFAP). Each compound was identified by comparing it with an authentic standard based on the following criteria: (i) matching retention time on the same column; (ii) mass spectrum; (iii) descriptions of its aroma attribute. [b] All threshold values are expressed as ng g^{-1} and, unless otherwise stated, were measured in water as reported by Rychlik et al. (*48*). [c] From Palamand et al. (*49*). [d] Compounds tentatively identified only on the basis of (i) matching retention time of an authentic sample on the same column; (ii) descriptions of its aroma attribute. The signal from the mass spectrometer was too weak to support an identification in these milk extracts, but a full-scan mass spectrum had previously been observed by the author for extracts from butter or cheese (unpublished results). [e] From Cuer et al. (*50*). [f] From Ott et al. (*13*). [g] From Mestres et al. (*51*). [h] From Ha and Lindsay (*52*).

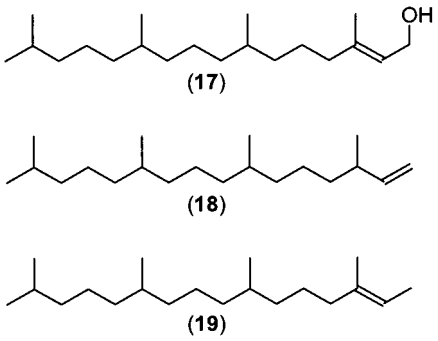

Figure 5. Structures of phytol derivatives with NIF-values ≥50%: phytol (**17**), phyt-1-ene (**18**), and phyt-2-ene (**19**).

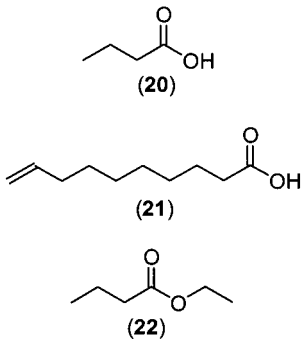

Figure 6. Structures of fatty acids, and Strecker esters, with NIF-values ≥50%: butyric acid (**20**), dec-9-enoic acid (**21**), and ethyl 2-methylbutyrate (**22**).

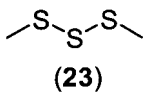

Figure 7. Structures of sulfur compounds with NIF-values ≥50%: dimethyl trisulfide (**23**).

Phytol derivatives have been implicated previously by both GC−O and chemical studies as causal factors of the pasture-associated flavors of cooked meat (*30*).

Fatty Acids and Strecker Esters. Butyric acid (**20** in Figure 6) and dec-9-enoic acid (**21** in Figure 6) gave high NIF-values. Butyric acid has been reported as a potent odorant for butter (*31*). The extremely potent flavor compounds ethyl 2-methylbutyrate (EMB, **22** in Figure 6) and ethyl 3-methylbutyrate were also found by GC−O and are produced by the esterification of 2-methylbutyric acid and 3-methylbutyric acid with ethanol.

Sulfur Compounds. Dimethyl trisulfide (**23** in Figure 7) is particularly potent and gave a high NIF-value for the TMR-derived milk. Formation of dimethyl trisulfide starts from the amino acid methionine, which forms dimethyl sulfide and methanethiol; and methanethiol can further oxidize to form dimethyl disulfide and dimethyl trisulfide (*32*). Dimethyl trisulfide has been reported previously as a flavor compound of both yogurt and milk (*13*). Dimethyl disulfide, which gave a low NIF-value by GC−O, is much less potent than dimethyl trisulfide (see Table 1 for reported threshold). Neither methanethiol nor dimethyl sulfide was found by GC−O because they would have coeluted with the ether solvent before sniffing commenced. 2-Methylthiophene (origin unknown) gave a low NIF-value and has been found previously in both yogurt and milk (*13*).

***δ*-Lactones.** The even-carbon-numbered δ-lactones, δ-6 to δ-14, were all found by GC−O, but only δ-tetradecalactone (**24** in Figure 8) gave a high NIF-value. δ-Decalactone gave low NIF-values, a surprising fact, as it is an important aroma compound of butter flavor, particularly when in comparison with margarine for baked products (*31*, *33*).

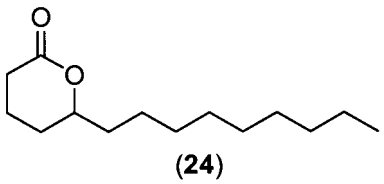

Figure 8. Structures of δ-lactone with NIF-values ≥50%: δ-tetradecalactone (**24**).

Terpenes. Terpenes originate from plant material and several of these compounds were found by GC−O. Although their individual NIF-values were all low, collectively they could make an important contribution to the aroma of milk. Terpenes are considered to impart significant flavor to cheeses (particularly Swiss Alpine) via the milk, and investigations into the role of terpenes in fodder and cheese flavor have been reported (*34*−*36*).

Diacetyl and Related Compounds. Pyruvaldehyde and pentane-2,3-dione gave the highest NIF-values in this class of compounds. Diacetyl, acetoin, and acetic acid gave lower NIF-values. Diacetyl has been reported as a potent aroma compound of butter (*31*) and also, along with pentane-2,3-dione, of yogurt (*13*). There are several biochemical means by which these compounds can be produced (*18*, *37*, *38*).

Strecker Degradation Products. The Strecker aldehydes 2-methylbutanal and 3-methylbutanal were found by GC−O. They arise from the Strecker degradation of the two amino acids isoleucine and leucine, respectively. Of the Strecker acids 2-methylbutyric acid and 3-methylbutyric acid, only the latter was found by GC−O. Aldehydes are the main products of the Strecker degradation, with acids being formed as side products (*39*, *40*). *S*-Methyl thio-3-methylbutyrate (a thioester) gave a low NIF-valve, and was formed by reaction of 3-methylbutyric acid with methanethiol. Phenylacetic acid and methional, produced from the amino acids phenylalanine and methionine, respectively, were also found by GC−O.

DISCUSSION

A striking feature of the results is that, with only one significant exception, all aroma-active compounds found for TMR-derived milk were also found for pasture-derived milk. The differences between the two milk samples were in the magnitudes of the NIF-values. However, as NIF-values are a measurement of the *frequency* of detection, rather than *intensity*, some caution is required when relating an aroma compound's NIF-values to the contribution it makes to the overall flavor of each milk sample.

γ-12:2 Lactone was the only compound found that gave a high NIF-value for one extract (TMR-derived milk) but a zero NIF-value for the other extract. The biosynthesis of this compound from linolenic acid by a yeast has been reported (*15*). However, to the author's knowledge, γ-12:2 has never previously been identified as an aroma compound from any food. As milk from pasture-fed cows has more linolenic acid than milk from supplement-fed cows (*41*), the absence of γ-12:2 from

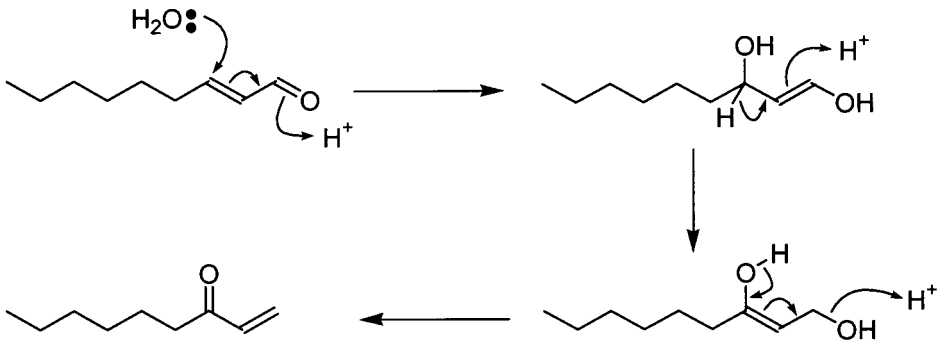

Figure 9. Formation of non-1-en-3-one from non-*trans*-2-enal.

the pasture-derived milk cannot be related to a lack of precursor, but must instead be related to either a decrease in nutritional energy or to microbiological factors within the rumen. Urbach and Stark (*25*) have suggested that the lower rumen pH of cows fed on a concentrate diet might facilitate the production of lactones.

Skatole and indole are strongly associated with the unpleasant smell of cow's feces (*42*). They are produced from the amino acid L-tryptophan in the cow's rumen (*43* and references therein). Cows fed on a pasture diet receive more protein than cows fed on a supplement diet; however, as the pasture diet has less energy, much of the protein is broken down so that the gluconeogenic amino acids can be used as an energy source (*41*). Because L-tryptophan is not a gluconeogenic amino acid, the rumen of a pasture-fed cow will have to cope with far more free L-tryptophan than the rumen of a supplement-fed cow. More skatole and indole will be produced and a small proportion of these powerful aroma compounds will enter the milk. Thus, the milk of pasture-fed cows has higher concentrations of skatole and indole than the milk of supplement-fed cows (*21*). Lane and Fraser (*44*) have extended similar reasoning to phenolic compounds that are produced from the amino acid L-tyrosine.

Faster rates of autoxidation and shorter induction times with the increasing unsaturation of a fatty acid have been reported (*45*). Thus, it was anticipated that, for fresh milk, oxidation of linolenic acid (C18:3) should be more important for flavor than oxidation of either linoleic acid (C18:2) or oleic acid (C18:1). The GC−O results showed that linolenic acid oxidation products (particularly octa-1,*cis*-5-dien-3-one, hept-*cis*-4-enal, and hex-*cis*-3-enal) were more important to the NIF profiles than linoleic acid oxidation products (oct-1-en-3-one and non-1-en-3-one).

Non-1-en-3-one was first reported as an aroma compound of both yogurt and milk, and has one of the lowest flavor thresholds reported to date (*13*). Non-1-en-3-one was formed to a small extent by treatment of non-*trans*-2-enal with hot water, and a proposed mechanism for its formation is shown in Figure 9. Considering the extreme potency of non-1-en-3-one, such transformation need occur to only a very small extent to allow it to make a contribution to milk flavor. Non-*trans*-2-enal is known to come from linoleate oxidation (*24*).

Milks from TMR-fed and pasture-fed cows have considerably different flavors. From these results, it is readily apparent that milk flavor is derived from the combination of a wide variety of different compounds that have many origins. GC−O is an indispensable tool for finding the potent aroma compounds of a food. However, because gas−liquid partition coefficients are not usually taken into account, flavor dilution values [which correlate with NIF-values (*5*)] can over-emphasize or under-emphasize the role of some compounds to a large extent (*46*).

The NIF-values give a tentative indication of the importance of individual compounds to milk flavor. And significant concentration differences between milk extracts might exist for hept-*cis*-4-enal, 2-acetyl-1-pyrroline, 3-methylbutyric acid, benzothiazole, *cis*-3-methyl-γ-nonalactone, indole, γ-12:2, and γ-16 because those compounds have NIF-value differences of ≥30%. However, milk samples from cows fed on different diets are also known to have significant concentration differences of γ-12, γ-12:1, and skatole (*25*, *21*), yet these compounds did not have NIF-value differences ≥30%. So caution is required when using NIF-value differences, as they do not completely reveal concentration differences. A fuller understanding of milk flavor will come only from the accurate quantification of aroma compounds, and from sensory studies of individual compounds within a milk matrix. Bendall and Olney (*47*) have already accomplished such work for hept-*cis*-4-enal.

The concordance of the inventories of aroma compounds, identified by GC−O for two considerably different flavored milk samples, leads to the conclusion that quite dramatic differences in milk flavor can be caused primarily by the concentration differences of a common set of flavor compounds, rather than by the occurrence of compounds uniquely associated with a particular feed.

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