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https://doi.org/10.5109/24369

出版情報：九州大学大学院農学研究院紀要. 45 (1), pp.189-197, 2000-11. Kyushu University
バージョン：
権利関係：

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J. Fac. Agr., Kyushu Univ., **45** (1), 189–197 (2000)

Off-flavor in Milk from the Cows Feeded with Swinecress (*Coronopus didymus*)

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(Received July 10, 2000 and accept August 18, 2000)

The effects of swinecress grass feeding on volatile constituents of cow milk were investigated. Volatiles from milk and swinecress grass were separated by simultaneous distillation–extraction under reduced pressure and analyzed by GC and GC–MS. Off-flavored milk indicated the higher concentrations in aldehydes, terpenes, hydrocarbons and miscellaneous volatiles including 6 sulfur containing compounds, on the contrary, the lower concentrations in fatty acids, ketones and lactones. It was supposed that the alteration of volatile composition was caused by the inhibitory actions of benzyl isothiocyanate on microbial and/or enzymatic activities in the rumen of cows. The isothiocyanate comprised 19% of total amount of volatiles from swinecress grass.

INTRODUCTION

Milk flavor which is characterized as sweet, warm, and calm odor is one of the most important quality factors of milk because it makes an impression of safeness and healthiness. Volatile constituents of cow milk are of great interest to food scientists because the constituents contribute to the flavor of dairy products as well as a liquid milk (Badings and Neeter, 1980; Rerkrai *et al.*, 1987; Badings, 1991; Moio *et al.*, 1993a; 1993b; 1994; Shiratuchi *et al.*, 1994). Volatiles can be transferred into milk directly from forage via a rumen and a respiratory tract (Moio *et al.*, 1996; Shipe *et al.*, 1961) or produced by a metabolism (Weidong *et al.*, 1997; Dumont and Adda, 1978). Extensive work has been carried out on the off-flavor in fluid milk. It includes transmitted (Honkanen *et al.*, 1964), microbial (Horimoto *et al.*, 1997a; 1997b), lipolyzed (Shipe *et al.*, 1978; 1980), heated (Contarini *et al.*, 1997), light-activated (Kim and Morr, 1996), oxidized (Kinsella *et al.*, 1967; Stark and Forss, 1962), and miscellaneous off-flavors (Bodyfelt *et al.*, 1988a; 1988b).

An off-flavor, aldehydic flavor lacking in sweet milky-like odor was noticed in the milk after feeding cows with swinecress (*Coronopus didymus*), a naturalized plant,

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which are propagating at pastures in kyushu island, Japan. The objective of our study was to determine the effect of swinecress feeding on volatile constituents of the cow milk.

MATERIALS AND METHOD

Materials

A Holstein–Friesian cow was kept indoors and fed a diet formulated as follows; raw grass of swinecress 4 kg/day, concentrate diet 13 kg/day, and hay 12 kg/day. After the feeding for two days the cow was milked twice a day. The milk sample was frozen in glass bottles just after collection and stored at −20 °C until it was used for the separation of volatiles (the storage time was about 1 week). Ultra–high temperature treated (UHT) cow milk (whole milk) as a reference was purchased from a market.

Swinecress grass was sampled at the Kyushu National Agricultural Experiment Station.

Isolation of Volatile Flavor Compounds

Milk (1 L) was placed in a 2000–mL round bottom flask. Volatiles were separated with 80 mL of diethyl ether by simultaneous distillation–extraction under reduced pressure (approximately 100 mmHg) for 2 h, using a modified Likens–Nickerson apparatus (SDE method). Temperature of the milk was about 62–65 °C during the SDE operation. This SDE procedure was repeated 5 times. After the addition of 2–ethylhexyl acetate (20 ppb) as an internal standard, the ether solution was dried over anhydrous sodium sulfate for 15 h. Ether solution combined was concentrated to about 100 μL. Swinecress grass, 200 g was homogenized with 500 mL of deionized water. Volatiles from the homogenate were separated by SDE method under reduced pressure. The ether extract was concentrated for the following analysis.

Gas Chromatography (GC)

Capillary GC analysis was carried out on a Hewlett–Packard Model 5890A gas chromatograph equipped with a flame ionization detector (FID) and connected to a Shimadzu Chromatopak C–R5A integrator. Separation was achieved on a 60 m × 0.25 mm i.d. fused silica capillary column, coated with cross–linked polyethylene glycol 20 M, film thickness 0.25 μm (DB–Wax; J&W Scientific, Folsom, CA). The oven temperature was programmed from 50 to 230 °C at 2 °C/min (40–min hold). The injector and detector temperatures were 240 and 250 °C, respectively. The helium carrier gas flow rate was 28 cm/sec with an injection splitter at a split ratio of 20:1. Retention indices were estimated in accordance with a modified Kovats method (Van den Dool and Kratz, 1963).

Gas Chromatography–Mass Spectrometry (GC–MS)

Electron impact mass spectrometric data were collected on a JEOL Automass 50 mass spectrometer interfaced to a Hewlett–Packard 5890 Series II gas chromatograph. The column and chromatographic conditions were the same as described for GC analysis. The mass spectrometer was operated at an ionization voltage of 70 V and an ion source temperature of 200 °C. The mass spectra of unknown compounds were compared with those in the Wiley/NBS Registry of Mass Spectral Data (Wiley/NBS Registry of Mass

Spectral Data, 1989) by using a computer system.

RESULTS AND DISCUSSION

The recoveries of total volatiles were 97.6 ppb and 118.4 ppb for the normal and off-flavored milk, respectively. The volatile concentrate from the normal milk developed sweet, oily, and warm odors, but from the off-flavored milk had aldehydic and somewhat sickening odors lacking in sweet milky-like odor. Total of 82 peaks from milk samples were definitely or tentatively identified (Table 1). The area of peaks identified represented 94 and 93% of the chromatogram surfaces obtained from the normal and off-flavored milk, respectively (excluding solvent). Figures in Table 1 were only approximate concentration since recoveries from the milk samples and FID response factors were not determined for each compounds (assume all recoveries and response factors of 1.0).

Among esters, acids, aldehydes, alcohols, ketones, lactones, terpenes, hydrocarbons, and miscellaneous compounds chemical groups contributing to the off-flavor seemed to be acids, aldehydes, lactones, hydrocarbones, and miscellaneous compounds. In the off-flavored milk, total amount of short chain fatty acids was extremely small. The contents of octanoic and decanoic acid in the off-flavored milk were less than 14% comparing those in the normal milk, although larger amounts of acetic and nonanoic acid were isolated. Long chain fatty acids could not be isolated by present method. The difference in the composition of these carboxilic acids could be attributed to the alteration in microflora and/or microbiological metabolism in the rumen. With respect to aliphatic aldehydes larger amounts of nonanal and decanal were recovered from the off-flavored milk. These compounds were considered as significant contributors to the off-flavor because of their low threshold values. δ- and γ-Lactones are important contributors to sweet and milky odor (Shiratsuchi *et al.*, 1995). The recovery of lactones from the normal milk was about twice that from the off-flavored milk. In the normal milk, predominant species were δ-nonalactone and δ-decalactone, on the other hand, δ-undecalactone was predominant in the off-flavored milk. It was not cleared whether the difference in the compositions of δ-lactones from the normal and off-flavored milk was attributed to feeding swinecress or not. Lacking in milky-like odor, however, was attributed to the insufficient amount of lactones. Many kinds of hydrocarbons were found in the off-flavored milk. These compounds, as a whole, seemed to be responsible for the off-flavor. These were considered to emigrate into the milk directly from the forage, swinecress. Because large numbers of these compounds were found in the volatile isolate from leaves and stems of swinecress (Table 2). In miscellaneous chemical group many volatiles were found in the off-flavored milk. Of these compounds dimethyl disulfide, benzaldehyde, dimethyl sulfoxide, acetophenone, dimethylphenylmethanol, N,N-dibutylformamide, benzothiazole, and benzyl isothiocyanate were also found in the volatile isolate from swinecress (Table 2). Furthermore, it was noticeable that 6 sulfur containing compounds including 3 kinds of isothiocyanates were found only in the off-flavored milk.

The absolute levels of the volatile compounds from normal and off-flavored milk grouped in chemical classes are shown in Figure 1. A comparison of the levels of chemical classes in both milk samples shows that aliphatic aldehydes, terpenes,

Table 1. Volatile compounds identified in off-flavored and normal milk.

compounds	concentration(μg/kg)	
	off-flavored	normal
aliphatic esters		
ethyl acetate	14.4	20.1
butyl acetate	0.2	
total	(14.6)	(20.1)
acids		
acetic acid	3.3	
n-butanoic acid	0.3	
n-pentanopic acid	0.3	
n-hexanoic acid	0.7	1.9
n-octanoic acid	1.4	10.6
n-nonanoic acid	2.0	0.7
n-decanoic acid	4.9	37.0
total	(12.9)	(50.2)
aliphatic aldehydes		
n-hexanal	1.2	1.4
(*Z*)-2-heptenal	0.4	0.2
n-nonanal	1.5	
(*Z*)-2-nonenal	0.5	
n-decanal	1.9	0.1
n-undecanal	0.3	
total	(5.8)	(1.7)
aliphatic alcohols		
3-methyl-2-butanol	0.9	
2-heptanol	0.3	
3-methyl-2-buten-1-ol[a]	0.8	
2-octenol	0.2	
total	(2.2)	(0)
aliphatic ketones		
2-butanone	0.7	9.0
2-pentanone	1.7	2.1
2-hexanone	0.3	
2-heptanone	6.1	10.5
2-nonanone	7.3	6.4
6-undecanone	0.3	
2-undecanone	4.6	
2-dodecanone	0.2	
2-tridecanone	0.9	2.2
total	(22.1)	(30.2)
lactones		
γ-nonalactone	0.4	0.7
δ-nonalactone	0.4	1.2
δ-decalactone	0.6	3.4
δ-undecalactone	1.3	
total	(2.7)	(5.3)
terpenes		
cumene	0.4	
d-limonene	0.9	0.7
p-cymene	0.4	

Table 1. continued.

3,7–dimethyl–1,6–octadien–3–ol[a]	0.3	
2,6–dimethyl–7–octen–2–ol[a]	0.3	
α–terpineol	0.3	
p–menthan–3–ol[a]	0.3	
p–cymen–8–ol[a]	0.2	
geranyl acetone	0.2	
total	(3.3)	(0.7)
hydrocarbons		
n–decane	0.5	
toluene	0.8	1.7
n–undecane	0.2	
ethylbenzene	0.5	0.4
1,4–dimethylbenzene	1.8	
1,3–dimethylbenzene	0.3	
1,2–dimethylbenzene	0.6	
n–dodecane	0.5	3.2
n–propylbenzene	0.3	0.7
1–ethyl–3–methylbenzene	1.1	1.1
1,3,5–trimethylbenzene	0.7	2.3
1,2,4–trimethylbenzene	3.3	0.4
n–tridecane	1.0	
1,2,3–trimethylbenzene	0.4	
diethylbenzene[a]	1.1	
1,4–bis(1–methylethyl)benzene[a]	0.6	
4–ethyl–1,2–dimethylbenzene[a]	0.6	
2,3–dihydro–1*H*–indene[a]	0.6	
n–tetradecane	2.4	
n–pentylbenzene	1.4	
n–pentadecane	2.2	0.1
n–hexadecane	0.2	
naphthalene	(21.1)	(0.9)
total		
miscellaneous		
dimethyl disulfide	0.3	
N–methylmethanamine[a]	0.3	
n–pentylfuran	0.3	
3–butyl isothiocyanate	0.2	
2–furfural	0.2	
benzaldehyde	0.4	
dimethyl sulfoxide[a]	0.3	
benzonitrile	0.4	
acetophenone	3.2	
furfuryl alcohol	0.2	
cyclohexyl isothiocyanate[a]	1.2	
N–nitrosodibutylamine	0.4	
2,5–dimethylbenzaldehyde	0.3	
dimethylphenylmethanol	1.1	
N,N–dibutylformamide	1.5	
benzothiazole	0.7	0.3
phenol	0.4	
p–cresol	0.4	
benzyl isothiocyanate	0.5	
total	(12.3)	(0.3)

[a] tentative identification by mass spectrum alone.

hydrocarbons and miscellaneous volatiles including 6 sulfur containing compounds at higher amounts in the off-flavored milk; hydrocarbones were found to be as much as twice, and aldehydes, terpenes and miscellaneous compounds were included more than 3 times higher. On the contrary, the concentrations of fatty acids, aliphatic ketones, and lactones were higher in the normal milk sample; especially fatty acids were found significantly higher in the normal one, being more than 4 times.

Table 2 lists the identification and composition of volatiles from swinecress grass. As

Table 2. Volatile compounds identified in swine cress grass

compounds	content[a] (%)
dimethyldisulfide	0.9
n-hexanal	0.8
n-undecane	0.7
1,4-dimethylbenzene	1.0
1,3-dimethylbenzene	1.1
2-propylfuran	1.8
(1-methylethyl)benzene	0.7
1,2-dimethylbenzene	1.0
n-tridecane	0.9
n-propylbenzene	0.8
(*E*)-2-hexenal	1.3
1,3,5-trimethylbenzene	1.4
1,2,4-trimethylbenzene	1.0
methyl isothiocyanate	1.2
1,2,3-trimethylbenzene	2.6
(*Z*)-2-penten-1-ol	1.9
6-methyl-5-hepten-2-one	1.2
1-ethyl-4-methylbenzene	1.5
3-hexenol	2.0
dimethyltrisulfide	1.7
n-tetradecane	3.5
acetic acid	3.7
2-ethylhexanol	0.9
2-sec-butyl-3-methoxypyrazine	0.9
benzaldehyde	8.0
dimethylsulfoxide	0.7
acetophenone	2.6
ethyl benzoate	0.8
benzylmethylsulfide	1.0
n-heptadecane	1.6
neral	1.1
phenylmethanol	1.5
N,N-dibutylformamide	1.7
benzylalcohol	1.5
benzothiazole	1.9
benzyl isothiocyanate	19.1
phytol	5.9
butyl 2-ethylhexyl phthalate	5.3
di-*n*-octyl adiphate	13.5
total	(100)

[a] peak area percentage

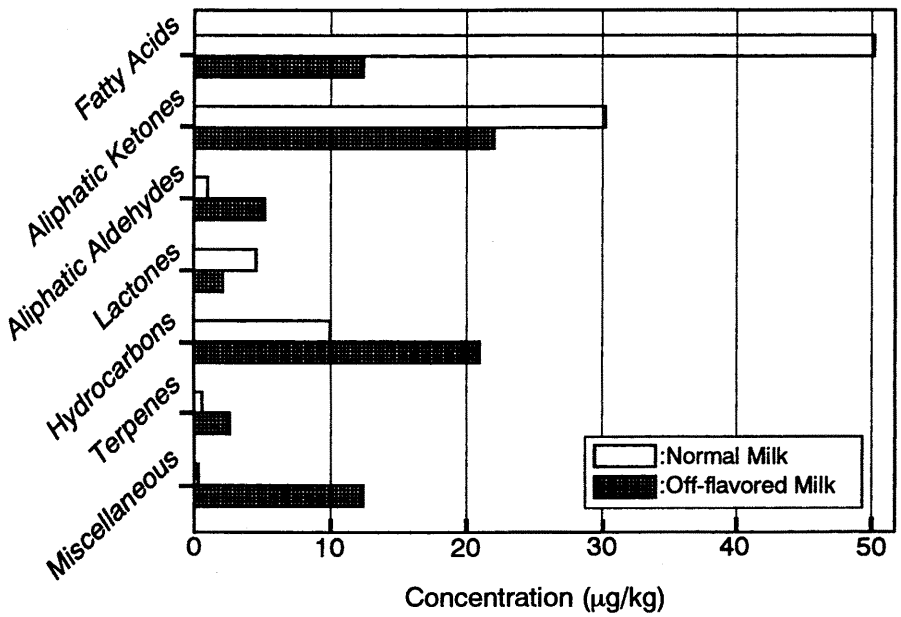

Fig. 1. Levels of volatile compounds grouped according to chemical class in the normal and off–flavored milk.

mentioned above, it was suggested that many compounds of hydrocarbons and miscellaneous chemical class in Table 1 emigrated from swinecress grass to the milk. The milk from cows on feeds such as alfalfa demonstrated the increase in dimethylsulfide content (Reddy *et al.*, 1967). Unpleasant odors of cow milk were produced when cows were fed on beet by–products, onion pulp, cruciferae, some gramineae and legumes, citrus meal, poor quality silage, land cress, and dry feed such as lucerne hay (Walker and Gray, 1970). Table 2 indicated benzyl isothiocyanate, which was a typical natural toxicant occurred in cruciferous vegetable, comprised 19% of total amount of volatiles in swinegrass. Kroll *et al.* (1994; 1996) have reported on the chemical reactions of various isothiocyanates with proteins, and benzyl isothiocyanate was the most reactive compound among many isothiocyanates (Rawel and Kroll, 1995). Therefore, benzyl isothiocyanate was supposed to exert inhibitory actions on microbiological and/or enzymatic activities in the rumen of cows fed on swinecress grass. Further studies are necessary to make clear the influence of this compound on the odor of cow milk.

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