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Effect of Sucker Control on the Volatile Compounds in Flue-Cured Tobacco[1]

Willard W. Weeks*[2] and Heinz Seltmann[3]

Flue-cured tobaccos were produced from plants (*Nicotiana tabacum* L. cv. McNair 944) that were not topped (1) or topped with suckers removed as follows: none removed (2); hand-suckered when 30 cm long (3), 20 cm long (4), 10 cm long (5), and less than 1 cm long (6); chemical sucker control treatment using fatty alcohols and potassium maleic hydrazide in a sequential application (7). The concentrations of 58 neutral volatile constituents were determined by capillary gas chromatography following steam distillation of a 10-g sample from the cured tobaccos obtained from each of the seven treatments. In general, increased sucker control increased the concentration of the neutral volatile constituents. A subjective ranking of the treatment means for all compounds gave a positive correlation of 0.96, indicating an association of sucker control and tobacco smoke flavor. Total alkaloids showed a response to topping and sucker control. Concentrations of reducing sugars were inconclusive.

INTRODUCTION

Tobaccos with rather specific chemical and physical characteristics are blended in cigarette manufacturing to perpetuate a brand and to maintain product uniformity. Smokers prefer brands associated with satisfying flavor. Considerable research and development goes into a brand to establish its specific characteristics. Consequently, it is imperative that buyers purchase the tobaccos that meet the brand specifications of blending and manufacturing (Rodgers and Mitchum, 1976).

The relative composition of specific tobaccos among American cigarettes may vary considerably, but they usually contain 30–35% of flue-cured blends (Griese, 1983). The chemical and physical characteristics of the leaf are influenced by genetic factors and by a combination of soil type and agricultural practices during the growing season, weather conditions, and curing (Leffingwell, 1976). Of the controllable agricultural practices, nitrogen fertilization is of considerable importance because the rate of application substantially influences the levels of certain aromatic compounds (Dawson, 1952).

The conventional practice followed by flue-cured growers to increase yield is the removal of the developing inflorescence (topping) and the subsequent control of the axillary buds (suckers). Both practices are important to the manufacturer and the smoker, because these practices bring about certain desirable chemical changes in the cured leaf (Leffingwell, 1976). Physiologically, topping and the control of suckers stimulate the growth of the root system, increase absorption of nutrients, and stimulate the production of alkaloids that accumulate in the leaves. With the removal of suckers, which are metabolic sinks, there is even greater accumulation of organic components in the leaves. Failure to top the plants and reduce sucker growth will increase senescence of the leaves and result in an increase in the sugar to nicotine ratio in the cured leaf (Hawks et al., 1983). The result is a more neutral tobacco that is used as filler in certain brands of cigarettes. Because manual removal of suckers is very time consuming, chemicals that control suckers by contact and systemic action are commonly used (Seltmann, 1970). Chemically controlling suckers affects the physiology of the plant that results in modification of the chemistry of the cured leaf when compared to that from mechanically suckered and hand-suckered plants (Seltmann, 1980).

Since topping and sucker growth affect the chemical and physical characteristics of the cured leaf, we became interested in the possible effect of these practices on those volatile constituents in the cured leaf associated with tobacco smoke flavor. Consequently, we chose to study the effects of the presence of the floral apex, the removal of the floral apex, various amounts of sucker growth, and chemical sucker control as commonly imposed by growers on the neutral volatile compounds of flue-cured tobacco.

MATERIALS AND METHODS

Plot rows of 20 competitive tobacco, *Nicotiana tabacum* L. cv. McNair 944, plants were grown 55 cm in the drill and 122 cm between rows on Wagram sandy loam soil on the Central Crops Research Station, Clayton, NC, during 1983. Cultural practices other than those imposed as treatments were consistent with those recommended for the management of flue-cured tobacco. Seven treatments were replicated three times in a randomized complete block design as follows: (1) plants not topped and not suckered (NT NS); (2) plants topped but not suckered (TNS); (3) plants topped and suckers removed when 30 cm long (TS 30 cm); (4) plants topped and suckers removed when 20 cm long (TS 20 cm); (5) plants topped and suckers removed when 10 cm long (TS 10 cm); (6) plants topped and suckers removed before 1 cm long (TS 1 cm); (7) plants topped and suckers controlled chemically (TS with FA/KMH).

Topping was performed when plants were in the elongated bud to first flower stage of plant development, and plants were checked for sucker growth every 3–4 days. Suckers of appropriate sizes in treatments 3–5 were removed, counted, and weighed fresh. Suckers on the plants in treatment 6 were destroyed by carefully rubbing out any bud tissue in the leaf axil with a sharpened 10-cm garden

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[2]Associate Professor, Department of Crop Science, North Carolina State University.

[3]Plant Physiologist, USDA, ARS, Southern Regional Research Center. Professor of Botany and Crop Science, Department of Botany, North Carolina State University.

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stake. Chemically controlled suckers, treatment 7, were sprayed over the top with a dual application of fatty alcohols (Off-Shoot T) 5 days apart while in the bud to early flower stage with a 4 and 5% solution, directing 30 mL of the spray solution toward each plant. Maleic hydrazide (Royal MH-30) at the rate of 170 mg/plant in 30 mL of solution was applied 7 days after the second application of fatty alcohols. Leaves were harvested (primed) when considered ripe and cured in a bulk curing barn. Plants in treatment 1 were primed three times and all others four times.

Percent sucker control was calculated from the combined green weight of suckers removed from treatments 3–5, compared to the green weight of suckers removed from the topped and not suckered treatment 2 after the last harvest.

After curing, 25 leaves were taken at random from each priming, the midveins removed, and the lamina dried in a force-draft oven at 55 °C for 12 h and ground in a Wiley mill to pass a 1-mm mesh screen. A combined sample for chemical analyses was prepared proportionally to priming weights for each treatment and each replication. Percent total alkaloids as nicotine and percent reducing sugars were determined with an autoanalyzer (Harvey, 1969). Samples for gas chromatographic analyses were prepared by steam distillation of a 10-g subsample (Lloyd et al., 1976).

Gas chromatographic profiles were obtained on a Varian 3700 gas chromatograph equipped with a fid detector. A CDS 111 data processor was used for data collection. The GC employed a 0.5-mm i.d. glass open tubular SP 1000 wall coated capillary column 60 m long. Helium at 4 mL/min was employed as carrier gas with 30 mL/min makeup gas. Compressed air and hydrogen were maintained at 300 and 30 mL/min, respectively, for flame support. Injector and detector temperatures were at 250 and 275 °C, respectively. The injector was operated in a splitless mode and 1 μL of the prepared steam-distilled sample was injected, which approximated the amount of volatile materials in a single puff from a cigarette under standard conditions as used by a standard smoking machine. The samples were chromatogrammed by using multilinear programming over the temperature range from 60 to 200 °C at 1 °C/min with intermittent lags of 5 min at 125 and 170 °C.

Peak identifications were provided by personnel from R. J. Reynolds Tobacco Co. following gas chromatogramic and mass spectral analysis of a sample prepared in our laboratory from treatment 6. A new capillary column, previously described, was calibrated and checked for reproducibility over the defined range. A steam-distilled sample spiked with several compounds commonly found in the distillate from a flue-cured sample with retention times over the whole temperature range was run. The relative response factor of these compounds and the internal standard, tetradecane, was determined. The peaks from the chromatograms were quantified using internal standardization and RRF.

RESULTS AND DISCUSSION

Many of the compounds in the essential oils of flue-cured tobacco are also found in food and beverages. Since the threshold reported for some of these compounds is very low, we considered a 10-g sample for volatile profile analyses, which was large enough for reproducibility at a detection level of 1 ppm and also representative of each replication. The sample allowed us to focus on the quantity of each compound in the amount of tobacco that would give the same impact as if the tobacco were from a single cigarette. The profiles of the neutral volatile compounds from the treatments were compared qualitatively and quantitatively. To obtain some measure of the number of peaks and their quantities, samples from treatment 1 were prepared and analyzed first. Even though peaks of less than 0.5% of the total area of the chromatogram were not used for comparison, 58 peaks common to all treatments were used. A typical chromatographic profile is shown in Figure 1. The molecular structure and nomenclature of each known peak is illustrated in Table I.

Volatile profile data were analyzed statistically according to the field design. Of the 58 peaks analyzed the f values for 45 peaks were highly significant, 10 were significant, and only 3 were nonsignificant (Table II). Following calculation of f values, the treatments were contrasted by Duncan's multiple-range test for each peak. Unfortunately, this test did not allow us to satisfactorily compare the total profiles from the treatments. However, the treatment mean with the lowest and highest value and the mean of all treatments for each peak are listed in Table II. Treatments 1 and 2 occurred in the low column 17 and 18 times, respectively. In similar fashion, treatments 5–7 occurred in the high column 14, 14, and 12 times respectively. Although there are others, the one outstanding anomaly found in these data is neophytadiene (peak no. 13), a compound that is considered an important component associated with smoothing smoke flavor. Of each sample studied, neophytadiene comprised from 30–50% of the sample. It was higher (245 μg/g) in treatment 3 where suckers were allowed to grow to 30 cm before they were removed. Three other compounds, 4, 8, and 35, were also higher in this treatment.

The volatile profile data were evaluated subjectively by ranking treatments according to increasing quantity present of each of the 58 compounds measured. A rank from 1 to 7 was assigned to the treatment mean value over replication for each peak. Number 1 was assigned to the lowest value and 7 to the highest value. By summing the assigned numbers over all peaks for each treatment, treatments were ranked as in Table III. It was found that the lowest value obtained (170) came from treatment 1, and the highest (280) came from treatment 6. The order from 1 to 6 as indicated in Table III indicated the rank of potential smoking impact from volatile compounds. A positive linear correlation of 0.96 between the sum of rankings of treatments and percent sucker control was found.

The value obtained for the chemically treated plants was an exception to the order of ranking with sucker control. Since the chemically controlled suckers and those completely controlled manually resulted in near perfect control, the differences between treatments 6 and 7 may be attributed to the chemical treatment. It has been suggested that tobaccos coming from plants on which suckers were controlled through the use of maleic hydrazide differed from those controlled manually in total alkaloids, reducing sugars, total ash, filling capacity, and moisture equilibrium content (Seltmann, 1980). Tobacco from chemically treated plants (treatment 7) had 12 constituents as highest in concentration (Table II). Of these, furfural (peak 1) could be attributed to carbohydrate accumulation usually associated with MH treatments.

Most of the compounds found in the neutral volatile fraction are found in tobacco smoke as well as in the tobacco. We are not sure which compounds make the greatest contribution to smoke flavor. It seems probable that most of the compounds found are the results of oxidative changes and that certain oxygen-bearing structures

Table I. Structures and Nomenclature of Volatile Compounds

	Name	Structure		Name	Structure		Name	Structure		Name	Structure
1	Furfural		16	2-Methyl-3-pyrrolcar-boxaldehyde		31	4,6,8-megastigmatriene-3-one (Isomer)	Isomer of No. 15	46	Unknown M.W. 190	
2	3-Methyl-4-5-dihydro-furan		17	4,6,8-Megastigmatriene 3-one (Isomer	Isomer of No. 15	32	Unknown M.W. 159		47	3,4-Dihydro-1,5,6-trimethyl-2-hydroxy ethyl naphthalene	
3	2-Keto-4-methyl-furan		18	Iso β, Ionone 4(2,6,6-trimethyl-1,3,cyclo-hexadienyl) butan-2-one		33	Dihydroactinidiolide		48	Phytol	
4	2-Pyrrolidone		19	Cyclohexane		34	Tetrahydroactinidio-lide		49	3-Hydroxy-β-ionol	
5	Solanone		20	M-cresol		35	Farnesyl acetone		50	4-Hydroxy-β-ionol	
6	2-Butenoic lactone		21	4,6,8-Megastigmatriene 3-one (Isomer)	Isomer of No. 15	36	P-vinyl-phenol		51	Unknown	
7	Damascenone		22	2-Methyl-3-pyrrolcar-box-aldehyde (Isomer)	Isomer of No. 16	37	Indole		52	Unknown	
8	Cyclotene		23	Dehydro-β-ionone		38	Tetrahydroactinidio-lide (Isomer)	Isomer of No. 34	53	5,8-Oxido-3,13-duvadiene-9-methylene-1-ol	
9	Geranyl acetone		24	Pseudo-ionone		39	4-Hydroxy-β-dehydroionol		54	Unknown	
10	Solanol		25	Oxysolanone		40	Unknown M.W. 152		55	5-Hydroxy-6,7-dimethyl-Benzo Furan	
11	Benzyl alcohol		26	Farnesyl acetate		41	4-Hydroxy-β-damascone		56	Unknown	
12	Phenyl ethanol		27	O-Methoxy-p-vinylphenol		42	3-Oxo-6-isopropyleno-5,9-dimethyl,bicyclo [4,4,0] dec-4-ene		57	Unknown	
13	Neophytadiene		28	2,2,5,5-tetramethyl cyclohexanone		43	Unknown M.W. 190		58	Unknown	
14	1,3,7,7-Tetramethyl-9-oxo-2-oxabicyclo-(4,4,0)-dec-5-ene		29	Solanascone		44	Methyl linolenate				
15	4,6,8-Megastigmatriene-3-one		30	Methylethylmaleimide		45	4-Hydroxy-β-damascone (Isomers)	Isomer of No. 41			

Table II. Summary of GC Profiles of Cured Tobacco from Seven Sucker Control Treatments Showing the Lowest and Highest Values (mg/g) Obtained for Each Constituent (Peak) and the Treatment in Which Each Occurred

		concentration, mg/g						
peak no.	reten time, min	lowest val	treat. no.	highest val	treat. no.	mean	CV, %	sig f value[a]
1	52.93	6.37	1	10.21	7	8.68	13	*
2	67.35	1.45	1	3.10	7	2.49	23	*
3	71.43	0.58	2	2.58	6	1.85	10	**
4	82.26	1.49	2	2.64	3	1.83	12	**
5	85.62	19.10	2	30.75	4	25.62	8	**
6	86.42	1.30	2	2.73	4	2.09	22	*
7	96.23	20.52	5	26.70	2	23.62	5	**
8	101.43	3.83	7	8.50	3	5.17	25	*
9	102.95	1.30	2	2.73	7	2.09	22	**
10	105.46	1.21	7	1.80	5	1.52	12	*
11	105.91	3.68	7	4.78	6	4.42	11	ns
12	106.87	9.83	2	19.30	4	15.00	8	**
13	110.88	141.75	1	245.26	3	199.81	5	**
14	115.60	3.61	1	6.72	6	5.14	7	**
15	116.88	2.07	1	10.70	6	4.95	15	**
16	117.31	1.99	5	3.49	7	2.86	9	**
17	118.37	1.45	2	3.27	6	2.12	18	**
18	119.12	1.51	3	2.27	5	1.91	17	ns
19	120.08	7.89	1	13.67	6	11.07	11	**
20	121.79	1.19	7	2.93	6	1.73	15	**
21	124.56	1.35	3	2.01	6	1.65	10	*
22	125.63	1.87	1	3.93	6	3.07	10	**
23	127.49	1.72	1	3.57	4	2.81	12	**
24	128.40	2.77	7	4.17	6	3.39	10	**
25	129.36	1.61	5	6.08	2	3.62	9	**
26	132.13	4.96	7	10.20	1	8.01	13	**
27	135.28	1.41	5	2.49	6	1.98	16	*
28	136.25	12.37	1	18.48	5	15.84	9	*
29	139.28	0.80	2	3.09	5	1.85	14	**
30	140.03	3.02	1	7.78	7	4.90	14	**
31	142.53	3.60	2	7.57	7	6.78	6	**
32	145.47	6.20	1	8.48	7	7.76	9	**
33	147.91	9.47	1	16.86	6	12.53	8	**
34	148.77	2.62	1	7.39	2	5.74	17	**
35	154.11	1.90	1	6.97	3	3.91	15	**
36	154.48	2.75	3	6.80	5	5.01	21	**
37	156.24	1.12	2	4.97	5	3.46	20	**
38	156.88	1.59	2	7.17	7	5.51	14	**
39	157.73	3.23	3	5.35	2	4.38	12	**
40	162.59	1.43	3	2.40	6	1.95	13	**
41	163.60	4.16	2	7.43	7	6.18	13	**
42	165.96	1.50	2	6.33	6	4.05	21	**
43	168.19	2.06	2	4.07	7	3.42	20	**
44	169.95	1.37	1	3.12	4	1.96	15	**
45	170.80	2.87	2	5.35	5	3.95	14	*
46	173.09	1.00	6	2.51	5	1.73	17	**
47	173.73	6.70	2	10.72	7	9.34	11	**
48	175.33	2.43	1	5.84	5	4.18	15	**
49	176.13	5.50	6	7.54	5	6.92	11	**
50	177.84	2.39	2	5.24	7	3.49	10	**
51	178.53	7.45	1	12.39	5	9.34	16	**
52	179.28	1.61	2	5.24	5	4.68	16	**
53	183.49	2.94	6	12.27	5	6.41	11	**
54	185.09	6.66	6	8.49	4	7.65	15	ns
55	187.33	21.80	5	30.65	2	27.15	10	*
56	195.28	1.30	3	2.18	5	1.93	10	**
57	197.95	2.39	3	6.25	2	4.02	7	**
58	204.29	1.52	7	3.46	1	2.28	15	**

[a] Key: **, highly significant; *, significant; ns, not significant.

resulted from degradation of a higher molecular weight precursor (Table I). It is suggested that an altered physiology brought about by the removal of suckers of different sizes translated into different rates of synthesis of precursors to the accumulation of volatiles. These physiological differences are offered as the possibility that good sucker control will result in tobaccos with better smoke flavor than those with poor control. Furthermore, the results point out that the amount of sucker growth may be manipulated culturally to give a particular kind of tobacco and that sucker growth could be used to balance out the effect of environmental factors that influence smoke flavor. This actually was the practice prior to the advent of chemical sucker control.

Concentrations of total alkaloids and reducing sugars have been associated with the quality of flue-cured tobacco, and this study utilized tobaccos of considerable quantitative differences for these constituents. Total alkaloids and reducing sugar data were analyzed according to Duncan's multiple-range test at the 10% level (Table IV). Tobacco from untopped plants differed from all others with respect to total alkaloids. This was expected because the normal cultural practice of topping will stimulate root growth and thus result in increased alkaloid synthesis.

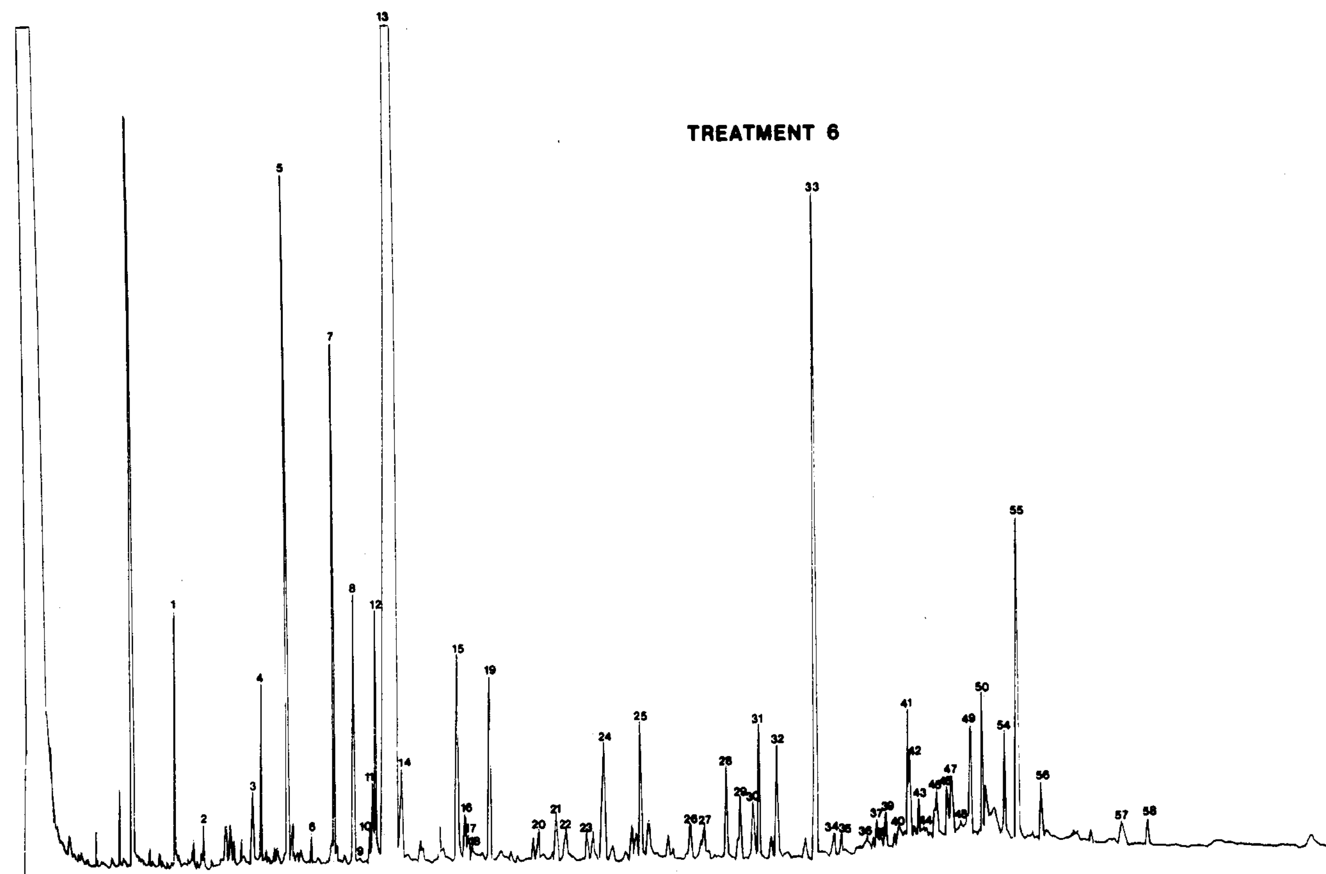

Figure 1. Profile of volatile compounds.

Table III. Comparison of Various Degrees of Sucker Control with Relative Potential Subjective Response[a]

no.	treat. descrip	% sucker control	rel pot. subjective resp
1	NT NS		170
2	TNS	0	198
3	TS 30 cm	29	222
4	TS 20 cm	46	240
5	TS 10 cm	69	267
6	TS > 1 cm	100	280
7	TS with FA/KMH	98	260

[a] Linear correlation 0.96.

Table IV. Percent Total Alkaloids and Reducing Sugars in Flue-Cured Tobacco from Plants with Different Amounts of Sucker Control[a]

no.	treat. descriptn	sucker control	total alkaloids	reducing sugars
1	NT NS		1.74 c	14.5 a
2	TNS	0	2.52 b	9.3 b
3	TS 30 cm	29 d	3.00 ab	12.7 ab
4	TS 20 cm	46 c	3.24 a	13.5 ab
5	TS 10 cm	69 b	3.47 a	13.1 ab
6	TS > 1 cm	100 a	3.16 a	14.1 a
7	TS with FA/KMH	98 a	3.19 a	14.7 a

[a] Values having the same letter are not significantly different from the Duncan multiple-range test at the 10% level.

Although alkaloid data from topped but not suckered plants (treatment 2) differed significantly from the topped plants where suckers were controlled from an intermediate to high degree, as in treatments 4–7, it did not differ in tobacco where suckers were allowed to develop to 30 cm before they were removed.

Tobaccos from topped, not suckered plants or from plants with substantial sucker growth generally contained low sugar concentrations. Surprisingly, however, the MH-treated tobacco did not show increased reducing sugars.

ACKNOWLEDGMENT

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Registry No. $PhCH_2OH$, 100-51-6; $Ph(CH_2)_2OH$, 60-12-8; m-HOC_6H_4Me, 108-39-4; $Ac(CH_2)_2CH(Pr$-$i)(CH_2)_2Ac$, 55023-57-9; p-$HOC_6H_4CH{=}CH_2$, 2628-17-3; furfural, 98-01-1; 2-keto-4-methylfuran, 1679-49-8; 3-methyl-4,5-dihydrofuran, 34314-83-5; 2-pyrrolidone, 54036-77-0; solanone, 1937-54-8; 2-butenoic lactone, 497-23-4; damascenone, 23726-93-4; cyclotene, 765-70-8; geranyl acetone, 3796-70-1; solanol, 40525-38-0; neophytadiene, 504-96-1; 1,3,7,7-tetramethyl-9-oxo-2-oxabicyclo[4.4.0]dec-5-ene, 20194-67-6; 4,6,8-megastigmatrien-3-one, 13215-88-8; 2-methyl-3-pyrrolecarboxaldehyde, 103002-58-0; cyclohexane, 110-82-7; dehydro-β-ionone, 1203-08-3; pseudoionone, 141-10-6; farnesyl acetate, 29548-30-9; 2,2,5,5-tetramethylcyclohexanone, 15189-14-7; solanascone, 68690-84-6; (methylethyl)maleimide, 20189-42-8; dihydroactinidiolide, 17092-92-1; tetrahydroactinidiolide, 16778-27-1; farnesylacetone, 1117-52-8; indole, 120-72-9; 4-hydroxy-β-dehydroionol, 31162-45-5; 4-hydroxy-β-damascone, 35734-61-3; 3-oxo-6-isopropyleno-5,9-dimethylbicyclo[4.4.0]dec-4-ene, 102977-86-6; methyl linolenate, 301-00-8; 3,4-dihydro-1,5,6-trimethyl-2-(hydroxyethyl)naphthalene, 102977-87-7; 3-hydroxy-β-ionol, 27185-80-4; 4-hydroxy-β-ionol, 33759-63-6; 5,8-oxido-3,13-duvadiene-9-methylene-1-ol, 102977-88-8; 5-hydroxy-6,7-dimethylbenzofuran, 60026-12-2; o-methoxy-p-vinylphenol, 7786-61-0; 4-(2,6,6-trimethyl-1,3-cyclohexadienyl)butan-2-one, 20483-36-7.

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Supercritical Carbon Dioxide Extraction of Oils from Antarctic Krill

Katsumi Yamaguchi,* Masahiro Murakami, Hiroshi Nakano, Shoji Konosu, Tsunehiko Kokura, Hiroshi Yamamoto, Masahiko Kosaka, and Kazuhiko Hata

Supercritical carbon dioxide extraction of the Antarctic krill yielded oils that were composed solely of nonpolar lipids, largely triglycerides, without phospholipids. The extracted oils were fluid and of red color due to astaxanthin, which tended to be decomposed at temperatures higher than 60 °C and a fixed pressure of 250 kg/cm^2. Analyses of the fatty acids showed a comparatively high proportion of eicosapentaenoic acid (11%). These results indicate that supercritical carbon dioxide is effective in obtaining nonpolar lipids from the krill by only one-step extraction and in excluding phospholipids that interfere with the utilization of krill oils.

Organic solvent extraction of oils from raw materials is a well-developed technology. However, after the extraction process, further purification steps are generally required to remove impurities and gum-forming compounds from the extracted oil, especially in foodstuffs intended for human use.

In recent years supercritical fluid extraction has received much attention, though its fundamental principles were known over 100 years ago (Hannay and Hogarth, 1879). The theory and practice of the supercritical fluid extraction process have been reviewed by Paul and Wise (1971), who predicted its application to foods, pharmaceuticals, fine chemicals, petrochemicals, mineral extraction, and fuel- and waste-processing technologies. Various kinds of supercritical fluids have been studied (Wilke, 1978), but most work done so far has used carbon dioxide as the extractant. Carbon dioxide has the advantages of nontoxicity, incombustibility, low critical temperature (31 °C) and pressure (75 kg/cm^2), and low price, all of which meet the recent energy and health concerns.

The application of supercritical carbon dioxide (SC-CO_2) extraction to foods has had limited success, as exemplified by decaffeination of green coffee beans in large-scale industrial plants (Zosel, 1974) and production of hop extract (Hubert and Vitzthum, 1978). Further, SC-CO_2 has been used to extract oils from soybean (Friedrich and List, 1982), coconut palm (Brannolte et al., 1983), butter (Kaufmann et al., 1983), etc. Applications of SC-CO_2 extraction to animal sources, in particular seafoods, are still more limited.

Lipids of aquatic organisms are generally rich in highly unsaturated fatty acids and phospholipids, which are readily deteriorated. The Antarctic krill, *Euphausia superba*, possesses an especially high proportion of phospholipids (Mori and Hikichi, 1976), which hampers the effective utilization of krill oils. We applied SC-CO_2 extraction to krill samples and proved that the extracted oils were composed solely of nonpolar lipids without contamination by phospholipids and their deteriorated lipids.

The present paper deals with the extraction and characterization of the extracted oils and residual lipids.

Materials and Methods Commercial preparations by Nippon Suisan Kaisha, Ltd., of frozen Antarctic krill and its meal were sampled. A freeze-dried sample was prepared by lyophilization of frozen krill. The samples were kept below −25 °C until used. Standard commercial preparations of lipid reagent were used without further purification.

Proximate composition of the samples was analyzed according to the AOAC procedures (No. 7.003, 7.009, 7.015, and 7.060; 1984).

The extraction with SC-CO_2 was carried out using a test plant instrument manufactured by Mitsubishi Kakoki, Co., Ltd. This equipment has a 75-mL extraction vessel with upper limits of pressure and temperature of 500 kg/cm^2 and 100 °C, respectively.

Ground freeze-dried krill (20 g) and krill meal (25 g) were put into the extraction vessel, gaseous CO_2 of which pressure was increased to a supercritical state by a pressure pump was introduced, and extraction was carried out at different pressures and temperatures. Gaseous CO_2 of commercial purity (Iwatani & Co., Ltd.) was used. Average flow rate was 0.6 kg/h. The extracted oils were measured gravimetrically.

The fractionation of the oils (150 mg) was carried out on a column (1 × 8.5 cm) using silica gel (Wakogel C-200, 100–200 mesh) and 50 mL of chloroform and then 50 mL of methanol. The eluted oils were measured gravimetrically after evaporation of the solvent.

The method of Bligh and Dyer (1959) was applied to the extraction of whole lipids from the krill samples and the residues after SC-CO_2 extraction. After evaporation of the solvent, the oils and lipids were measured gravimetrically.

The oils and lipids extracted with SC-CO_2 and by the method of Bligh and Dyer (1959) were analyzed by TLC on silica gel $60F_{254}$ (Merk, 0.25 mm thick) with petroleum ether–diethyl ether–acetic acid (90:10:1) or chloroform–methanol–water (65:25:4) as solvents and 50% aqueous sulfuric acid or Dragendorff reagent as indicator.

Laboratory of Marine Biochemistry, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan (K.Y., M.M., H.N., S.K.); Iwatani & Company, Limited, Higashi-ku, Osaka 541, Japan (T.K., H.Y.); Osaka Hydrogen Industries, Limited, Amagasaki 660, Japan (M.K.); and Central Research Laboratory, Nippon Suisan Kaisha, Limited, Hachioji, Tokyo 192, Japan (K.H.).

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