Volatile Flavor Compounds of Boiled Buckwheat Flour

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Received August 9, 1982

Volatile flavor compounds of boiled buckwheat flour were collected using a Likens and Nickerson apparatus type and were fractionated into acidic, weak acidic, basic and neutral fractions. The neutral fraction was further fractionated by silica gel column chromatography. All fractions were analyzed by gas chromatography and gas chromatography-mass spectrometry.

Two hundred and nine compounds were thus identified. Among them, 168 were newly identified as volatile flavor compounds of buckwheat flour; special note is made of 2-(1'-ethoxyethyl)pyrazine and 2-(1'-ethoxyethyl)-5-methylpyrazine being found which are compounds previously unreported in any literature.

Buckwheat noodles, made from buckwheat (*Fagopyrum esculentum* Moench) flour have a characteristic flavor and pleasant mouth feel differing from other wheat noodles. Generally it is known that the flavor of boiled buckwheat flour has great influence upon the making of appetizing buckwheat foods and their acceptability. Accordingly, we considered it important to clarify the flavor components of boiled buckwheat flour, which have hitherto been insufficiently reported through a few papers,^{1,2)} and have analyzed the volatile flavor components in more detail.

MATERIALS AND METHODS

Materials. Buckwheat seeds, harvested in Gunma prefecture in Japan, were immediately used after hulling and milling.

Methods.

a) *Collection of volatile flavor components.* The volatile flavor compounds of boiled buckwheat flour were collected using a Likens and Nickerson type of apparatus. A mixture of 500 g of buckwheat flour and 3 kg of water was placed in a 5 liter round bottomed-flask. The flask, the mouth of which was left open, was subjected to a 20 minute boil, which initially eliminated the green odor of the freshly milled buckwheat flour and obtained the characteristic boiled aroma. This flask was then connected to the Likens and Nickerson apparatus with a 50 ml

round-bottomed flask containing 30 ml of redistilled ethyl ether. Both flasks were brought to the boil and the volatile flavor compounds were simultaneously steam distilled and extracted with diethyl ether. This process was repeated 180 times and the resulting extract batches were combined and concentrated, giving 1.8 g of flavor concentrate.

b) *Fractionation of flavor concentrate.* As shown in Fig. 1, the flavor concentrate obtained above was separated into acidic, weak acidic, basic and neutral fractions, which were concentrated to approximately 2 ml, 0.1 ml, 0.1 ml and 0.5 ml, respectively. The acidic, weak acidic and basic fractions were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), the acidic fraction being analyzed after methylation. The remaining neutral fraction was further fractionated into seven fractions by the following method.

c) *Fractionation of neutral fraction.* The neutral fraction was subjected to successive column chromatography, using a 10 mm i.d. $\times$ 200 mm column packed with silica gel (Wacogel C-200). This was eluted successively with the following solvent systems (v/v); pentane, pentane-ethyl ether (97:3), pentane-ethyl ether (95:5), pentane-ethyl ether (90:10), pentane-ethyl ether (80:20), ethyl ether and methanol. The volume of each solvent system used was 50 ml. Each eluted fraction was concentrated to approximately 0.1 ml and analyzed by GC and GC-MS. Identification of the flavor compounds was made by comparing and matching the mass spectra and GC retention times of the isolated compounds with those of authentic compounds.

d) *Conditions of GC.* A Hitachi Model 163 gas chromatograph with a flame ionization detector (FID) and a 0.5 mm i.d. $\times$ 100 m glass SCOT column coated with PEG 20M was used. The column temperature was programmed

FIG. 1. Fractionation of the Flavor Concentrate of Boiled Buckwheat Flour.

from 60°C to 190°C at a rate of 3°C/min, and the flow rate of nitrogen carrier gas was 1 ml/min.

e) *Conditions of GC-MS.* A Hitachi Model RMU-6MG mass spectrometer was used. A glass SCOT column (0.28 mm i.d. x 50 m) coated with PEG 20M was used. Other operating conditions were; column temperature, programmed from 50°C to 170°C at 2°C/min; carrier gas, helium; ionizing voltage, 70 eV; accelerating voltage, 3200 V; ion source temperature, 200°C.

f) *Sniffing evaluation of flavor compounds emitted at each peak on the gas chromatograms.* The organoleptic evaluation was accomplished by sniffing at the exit port of a Hitachi Model 163 gas chromatograph fitted with an effluent splitter during the elution of samples. The effluent was proportioned by the splitter in a ratio of 1 to 10, the first portion being passed to the FID circuit and the second portion to the exit port, which was heated to 180°C by a small heater to prevent condensation. The gas chromatograph was equipped with a 0.5 mm i.d. x 70 m glass SCOT column coated with PEG 20M. The other operating conditions were the same as mentioned above.

g) *Syntheses of 2-(1'-ethoxyethyl)pyrazine and 2-(1'-ethoxyethyl)-5-methylpyrazine.*

i) *2-(1'-ethoxyethyl)pyrazine.* 2-Ethylpyrazine (10.8 g) was added to a mixture of NBS (19.6 g) and benzoyl peroxide (2.0 g) in CCl_4 (200 ml) at room temperature, and the mixture was refluxed (75°C) for 3 hr. The mixture was then filtered and the filtrate concentrated *in vacuo* to give crude 2-(1'-bromoethyl)pyrazine (25 g). The crude bromopyrazine (25 g) was added to a solution of NaOH (8.0 g) in H_2O (150 ml)/EtOH (150 ml) and the mixture was stirred for 5 hr at 35–38°C. The mixture was extracted with diethyl ether and the ethereal extract was washed with brine and dried over anhydrous MgSO_4 . After removal of the solvent, the residue was distilled to give 2-(1'-ethoxyethyl) pyrazine. (3.1 g, 20% yield, bp 81–83°C/12 torr).

MS m/z ; 152 (M^+ 2), 109 (26), 108 (100), 107 (52), 79 (18), 73 (13), 53 (26), 52 (29), 45 (95).

IR cm^{-1} ; 2880, 1535, 1400, 1110, 1020, 940, 850.

NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$; 1.20 (3H, t, $J=6.5$ Hz), 1.48 (3H, d, $J=6.5$ Hz), 3.47 (2H, q, $J=6.5$ Hz), 4.56 (1H, q, $J=$

TABLE I. YIELDS OF FLAVOR COMPOUNDS OBTAINED FROM BOILED BUCKWHEAT FLOUR

Flavor concentrate	Total 1800 mg* (20 ppm)	Total flavor compounds other than $\text{C}_{14}\sim\text{C}_{18}$ fatty acids 390 mg* (4 ppm)
Acidic Fr.		1 wt%
Weak acidic Fr.		5
Basic Fr.		1
Neutral Fr.		93

* Yield was calculated using the following equation.
Yield (mg) = weight (mg)
 $\times \frac{100 - (\text{solvent peak area\% on gas chromatogram.})}{100}$

6.5 Hz), 8.46 (2H, s), 8.72 (1H, s).

ii) *2-(1'-ethoxyethyl)-5-methylpyrazine.* In the same manner as described above, bromination of 2-ethyl-5-methylpyrazine (18.3 g) with NBS (32 g), and treatment with a solution NaOH (12 g) in H_2O (150 ml)/EtOH (150 ml) gave 2-(1'-ethoxyethyl)-5-methylpyrazine. (5.3 g, 21% yield, bp 106–108°C/16 torr).

MS m/z ; 166 (M^+ 1), 123 (30), 122 (100), 121 (55), 93 (15), 73 (13), 53 (17), 51 (17), 45 (92), 39 (36).

IR cm^{-1} ; 2880, 1480, 1340, 1110, 1035, 940, 900.

NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$; 1.23 (3H, t, $J=6.5$ Hz), 1.52 (3H, d, $J=6.5$ Hz), 2.56 (3H, s), 3.51 (2H, q, $J=6.5$ Hz), 4.58 (1H, q, $J=6.5$ Hz), 8.40 (1H, s), 8.62 (1H, s).

RESULTS AND DISCUSSION

Table I shows the yield of flavor concentrates and each fraction. The yield of flavor concentrates was 1800 mg, and this was found to contain large amounts of $\text{C}_{14}\sim\text{C}_{18}$ higher

FIG. 2. Gas Chromatogram of the Flavor Concentrate of Boiled Buckwheat Flour.

fatty acids which are not necessarily important to the characteristic aroma of boiled buckwheat flour. The yield of total flavor compounds, other than these fatty acids, was 390 mg which is equivalent to only 4.3 ppm of the weight of the raw material used.

As can be seen from Table I, the neutral compounds amounted to more than 90% of the total flavor compounds and, therefore, the aroma of boiled buckwheat flour is seen to be made up mainly of hydrocarbons and neutral oxygenated compounds such as alcohols, aldehydes, ketones, esters, ethers, *etc.*

Figure 2 shows a gas chromatogram of the flavor concentrate of boiled buckwheat flour. The chromatogram indicates the presence of over 200 compounds.

Although the flavor concentrate had the characteristic aroma of boiled buckwheat flour, no characteristic specific aroma compounds could be detected by sniffing at the exit port of the gas chromatograph.

1) Acidic and weak acidic fractions

The acidic fraction, from which C2~C9 aliphatic carboxylic acids were identified, had a pungent, acidic odor. But it seems that this acidic fraction is not very important to the characteristic aroma of boiled buckwheat flour.

On the other hand, the weak acidic fraction had a smoky or phenolic odor and eight phenolic compounds such as phenol, guaiacol, 4-vinylphenol and salicylaldehyde were identified from this fraction. Among these phenolic compounds, salicylaldehyde was the major component. Aoki, *et al.* have reported that salicylaldehyde existed in large quantities in roller-milled buckwheat flour consisting mainly of the pericarp, which also has been confirmed by our previous experiments on the pericarp of buckwheat. Salicylaldehyde is seen to be an important flavor component of the pericarp and to contribute to the aroma of the pericarp rather than that of boiled buckwheat flour.

2) Basic fraction

The basic fraction had a nutty and slightly fish-like odor.

Figure 3 shows a gas chromatogram of the basic fraction. The compounds corresponding to peak no. 97 and peak no. 105 had pleasant nutty aromas.

Mass spectra indicated that these compounds were probably 2-(1'-ethoxyethyl)pyrazine and 2-(1'-ethoxyethyl)-5-methylpyrazine, respectively.

Further confirmation was made by comparing and matching the spectra and GC retention times of these compounds with those of authentic compounds synthesized in our laboratory. IR, MS and NMR data of these two synthesized pyrazines are shown in the experiment (g). These two pyrazine derivatives were newly identified here from buckwheat flour and had not been reported in any literature.

In addition, other pyrazines and pyridines such as 2-methylpyrazine, 2-ethylpyrazine, tetramethylpyrazine, 2-methylpyridine and 2,6-dimethylpyridine were identified from the basic fraction. Most of these compounds are thought to have been formed by the Maillard reaction. It seems that the nutty aroma of the basic fraction is due to the pyrazines, with the fish-like odor being due to the pyridines.

3) Neutral fraction

The neutral fraction had a characteristic aroma of boiled buckwheat flour, but with a somewhat green odor.

The gas chromatogram of the neutral fraction is shown in Fig. 4. In order to facilitate analysis of the various minor components, the neutral fraction was further fractionated into seven fractions, using successive silica gel column chromatography. The first fraction consisted of hydrocarbons and the second consisted of mainly BHT, which was thought to be

FIG. 3. Gas Chromatogram of the Basic Fraction of Boiled Buckwheat Flour.

FIG. 4. Gas Chromatogram of the Neutral Fraction of Boiled Buckwheat Flour.

FIG. 5. Gas Chromatogram of the Fourth Fraction Obtained from the Neutral Fraction by Column Chromatography.

TABLE II. VOLATILE FLAVOR COMPOUNDS IDENTIFIED
FROM BOILED BUCKWHEAT FLOUR

Peak no.	Compounds	Peak no.	Compounds
<i>Hydrocarbons</i>		110	1-Nonanol
1*	Octane	124*	1-Decanol
6*	Nonane	129*	Nerol
13*	Decane	133*	Geraniol
17*	α -Pinene	137	Benzyl alcohol
19	Toluene	142	Phenethyl alcohol
22*	Camphene	<i>Aldehydes</i>	
24	Undecane	8*	3-Methylbutanal
27*	β -Pinene	12	Pentanal
28*	Sabinene	22	Hexanal
30	Ethylbenzene	24*	(<i>E</i>)-2-Methyl-2-butenal
32*	Myrcene	35*	Heptanal
39*	Limonene	38*	(<i>E</i>)-2-Pentenal
48*	Styrene	40*	(<i>E</i>)-2-Hexenal
51*	<i>p</i> -Cymene	53	Octanal
56*	Tridecane	59*	(<i>E</i>)-2-Heptenal
59*	2,2,6-Trimethylcyclohexane	65*	2,4-Hexadienal
67	Tetradecane	71	Nonanal
86*	α - <i>p</i> -Dimethylstyrene	77*	(<i>E</i>)-2-Octenal
90	Pentadecane	87*	2,4-Heptadienal
107	Hexadecane	88	Decanal
107*	β -Elemene	92	Benzaldehyde
121	Heptadecane	93*	(<i>E</i>)-2-Nonenal
124	Naphthalene	102*	2,4-Octadienal
137	Octadecane	102*	2,6-Nonadienal
140*	Calamenene	105*	Undecanal
142*	1-Methylnaphthalene	108*	β -Cyclocitral
151*	2-Methylnaphthalene	110	(<i>E</i>)-2-Decenal
155*	Nonadecane	111*	Phenylacetaldehyde
164*	Diphenyl	118*	2,4-Nonadienal
168*	Eicosane	120*	Dodecanal
175*	Heneicosane	120	Salicylaldehyde
180*	Docosane	125*	(<i>E</i>)-2,(<i>Z</i>)-4-Decadienal
184*	Tricosane	132*	(<i>E</i>)-2,(<i>E</i>)-4-Decadienal
187*	Tetracosane	134*	Tridecanal
189*	Pentacosane	140*	Cinnamic aldehyde
190*	Hexacosane	145*	2,4-Undecadienal
195*	Heptacosane	146*	2-Phenyl-2-butenal
<i>Alcohols</i>		149*	Anisaldehyde
8*	Ethanol	<i>Ketones</i>	
18*	1-Propanol	12	2-Pentanone
21*	2-Methylpropanol	15*	4-Methyl-2-pentanone
26	1-Butanol	22*	2-Hexanone
28*	1-Penten-3-ol	25*	3-Penten-2-one
36	3-Methylbutanol	28*	4-Methyl-3-penten-2-one
43	1-Pentanol	34	2-Heptanone
56*	2-Heptanol	43*	6-Methyl-2-heptanone
61	1-Hexanol	47*	3-Octanone
67*	3-Octanol	52	2-Octanone
78*	1-Octen-3-ol	55*	1-Octen-3-one
80*	6-Methyl-5-hepten-2-ol	55*	Cyclohexanone
85	2-Ethylhexanol	60*	6-Methyl-5-hepten-2-one
89*	2-Nonanol	64*	3,3-Dimethylcyclohexanone
92	Linalool	70	2-Nonanone
96	1-Octanol	73*	Isophorone
104*	4-Terpineol	82*	Menthone

TABLE II. (continued)

Peak no.	Compounds	Peak no.	Compounds
87*	2-Decanone	*	Eicosanyl acetate
90	Camphor	*	Benzyl salicylate
104	2-Undecanone		<i>Lactones</i>
118*	6,10-Dimethylundecan-2-one	145*	4-Octanolide
118*	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	159*	4-Nonanolide
126*	<i>p</i> -Methylacetophenone	188*	Dihydroactinidiolide
136*	α -Ionone		<i>Ethers</i>
136*	Geranyl acetone	40*	1,8-Cineol
142*	β -Ionone	79*	<i>p</i> -Cresyl methyl ether
154*	β -Ionone epoxide	83*	Linalool oxide
161*	2-Pentadecanone	115*	1-Methoxy-4-vinylbenzene
170*	6,10,14-Trimethylpentadecan-2-one	120	1,2-Dimethoxybenzene
172*	2-Heptadecanone	131*	1,2-Dimethoxy-4-methylbenzene
179*	Farnesyl acetone	133*	Anethole
	<i>Acids</i>	154*	Caryophyllene epoxide
78*	Acetic acid	157*	Diphenyl oxide
92*	Propionic acid	158*	Methyleugenol
98*	2-Methylpropionic acid	161*	1,2-Dimethoxy-4-vinylbenzene
116*	Butanoic acid		<i>Phenols</i>
118*	3-Methylbutanoic acid	136	Guaiacol
122*	Pentanoic acid	154	Phenol
134*	Hexanoic acid	154*	<i>o</i> -Cresol
148*	Heptanoic acid	164*	<i>p</i> -Cresol
162*	Octanoic acid	168*	Thymol
*	Nonanoic acid	168*	Eugenol
*	Benzoic acid	184*	4-Vinylphenol
*	Myristic acid		<i>Pyridines</i>
*	Pentadecanoic acid	35*	Pyridine
207*	Palmitic acid	40*	2-Methylpyridine
*	Oleic acid	46*	2,6-Dimethylpyridine
208*	Stearic acid	52*	2-Ethylpyridine
209*	Linoleic acid	55*	4-Methylpyridine
*	Linolenic acid	103*	2-Acetylpyridine
	<i>Esters</i>		<i>Pyrazines</i>
3*	Ethyl formate	40*	Pyrazine
6	Ethyl acetate	48*	2-Methylpyrazine
77	Ethyl octanoate	60*	2-Ethylpyrazine
102*	Bornyl acetate	84*	Tetramethylpyrazine
109*	Methyl geranate	97*	2-(1'-Ethoxyethyl)pyrazine
118*	Terpinyl acetate	105*	2-(1'-Ethoxyethyl)-5-methylpyrazine
159*	Methyl myristate		<i>Thiazoles</i>
169*	Methyl pentadecanoate	47*	Thiazole
181*	Methyl palmitate	111*	2-Acetylthiazole
183*	Isopropyl palmitate	149*	Benzothiazole
184*	Ethyl palmitate		<i>Miscellaneous</i>
*	Methyl stearate	19*	Dimethyl sulfide
*	Methyl oleate	42	2-Pentylfuran
*	Ethyl stearate	118*	2-Formylthiophene
*	Ethyl oleate	130*	Aniline
*	Methyl linolate	137*	Nicotine
*	Octadecyl acetate	143	B.H.T
*	Ethyl linolate	147*	Cinnamyl nitrile
*	Methyl linolenate	182*	Indole
*	Ethyl linolenate	189	Dibutyl Phthalate
*	Benzyl benzoate	*	Diphenylamine

* Newly identified.

a contaminant. The third fraction, having a green odor, consisted mainly of alkanals. The fourth fraction had part of the characteristic aroma of boiled buckwheat flour. Figure 5 shows a gas chromatogram of this fraction. The many peaks indicate the complexity of its composition. From these peaks, 55 compounds, most of which were carbonyl compounds such as alkenals, alkadienals and ketones, were identified. However, none of compounds individually could be claimed to have the characteristic aroma of boiled buckwheat flour by sniffing at the exit port of the gas chromatograph. Therefore, it is thought that the characteristic aroma of the fourth fraction was due to the combined aroma of all the compounds appearing on the gas chromatogram.

1-Octen-3-ol, having a musty odor, was the major compound in the fifth fraction. This has also been identified as one of major flavor components of freshly-milled buckwheat flour in our previous experiments. Thiazoles, having cereal-like aromas, were also identified in this fraction, and seem to contribute in part to the characteristic aroma of boiled buckwheat flour. These thiazoles are considered to have been produced by various reactions of sulfur-containing amino acids with carbonyl compounds.

Table II lists the compounds that were identified from the flavor concentrate of boiled

buckwheat flour. The aldehydes such as alkanals, alkenals and alkadienals, which were the major components in the neutral fraction, are thought to have been formed through the oxidative degradation of lipids and fatty acids and/or the Maillard reaction of various amino acids with carbonyl compounds. In conclusion, 209 compounds were identified from the flavor concentrate of boiled buckwheat flour. Among these, 168 were identified from buckwheat flour for the first time. None of them, however, were found individually to have the characteristic aroma of boiled buckwheat flour. The aroma of boiled buckwheat flour is very complex in its composition and seems to be made up from the combined effect of aromas of the identified and possibly unidentified compounds.

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