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Identification and Quantification of Aroma-Active Components that Contribute to the Distinct Malty Flavor of Buckwheat Honey

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Characteristic aroma components of buckwheat honey were studied by combined sensory and instrumental techniques. Relative aroma intensity of individual volatile components was evaluated by aroma extract dilution analysis (AEDA) of solvent extracts and by gas chromatography−olfactometry (GCO) of decreasing headspace samples (GCO−H). Results indicated that 3-methylbutanal, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon), and (*E*)-*β*-damascenone were the most potent odorants in buckwheat honey, with 3-methylbutanal being primarily responsible for the distinct malty aroma. Other important aroma-active compounds included methylpropanal, 2,3-butanedione, phenylacetaldehyde, 3-methylbutyric acid, maltol, vanillin, methional, coumarin, and *p*-cresol.

KEYWORDS: Buckwheat honey; malty aroma; 3-methylbutanal; sotolon; (*E*)-*β*-damascenone; gas chromatography−olfactometry; aroma extract dilution analysis

INTRODUCTION

Flavor varies widely among the different types of floral-source honeys and substantial research has been carried out on the investigation of honey flavor (*1−4*). Aldehydes, ketones, esters, alcohols, hydrocarbons, and sulfur compounds are the common groups of volatiles that have been detected and identified (*2, 3, 5*). Among them, (*E*)-*β*-damascenone, phenylacetaldehyde, phenylacetic acid, 2-phenylethanol, and benzyl alcohol were reported to be important constituents that contribute to honey flavor (*1, 3*).

Buckwheat honey, which has a distinct malty aroma, has found use as a natural preservative because of its high antioxidant content (*6*). The compounds 2- and 3-methylbutanal, methional, (*E*)-*β*-damascenone, and vanillin are some common aroma-active compounds reported to be responsible for malty flavors of various types of malted grains (*7−9*). However, little work has been carried out to study the unique flavor profile of buckwheat honey. The purpose of our study was to identify and quantify the key volatile components that contribute to its unique and characteristic malty aroma.

MATERIALS AND METHODS

Honeys. Two buckwheat honey samples were obtained commercially: Honey 1 from Deer Creek Honey Farms (London, OH), Honey 2 from Pot o' Gold Honey Co. (Hemingway, SC). These honeys are commercialized as "monofloral", meaning that at least 51% of the constituent nectar or 45% of contaminant pollen were from a single floral source (*10*). Thus, the raw honeys of these samples may contain nectars from more than one source with "buckwheat" being the predominant one.

Chemicals. Analytical-grade reference compounds and other reagent-grade chemicals were obtained commercially (Aldrich Chemical Co., St. Louis, Mo), except that (*E*)-*β*-damascenone was provided by Firmenich Co. (Princeton, NJ). Dichloromethane (Aldrich) was purified by distillation prior to use. Odorless-distilled water was prepared by boiling glass-distilled water down to two-thirds of its original volume. Matrix diluent consisted of deodorized aqueous 1.0 M phosphate-citrate buffer containing saturated sodium chloride, with final pH adjusted to 4.0.

Sensory Analysis. Aroma profiling was done by descriptive sensory analysis using a trained panel. The panel was composed of university students and staff (4 males and 9 females, between the ages of 20 and 50). All panelists had previously received extensive training in descriptive sensory analysis (>20 h) and had experience in sensory profiling of various food samples. These panelists were trained for an additional 3 h to identify and define descriptive terms for buckwheat honey aroma and to determine appropriate aroma references. Samples consisted of 1.0 g of honey in 125-mL Nalgene PTFE wash bottles (Fisher, Pittsburgh, PA) with siphon tubes removed from the caps. Bottles were labeled with random 3-digit codes and were covered with aluminum foil to prevent any visual bias. Samples were presented at room temperature (ca. 23 °C). Panelists evaluated each sample by gently squeezing the bottle and sniffing the air emitted from the nozzle. Standard references for "buttery", "vanilla", "burnt sugar", "floral", "fruity/estery", and "malty" were presented at room temperature (**Table 1**). Aroma intensity values were rated on 15-cm universal scales (*11*) anchored on the left with "none" and on the right with "very", which corresponded to the intensity ratings of 0 and 15, respectively. The previously assigned intensity ratings of the standards were used as references for rating the intensities of the honey samples. Rating results from individual panelists were revealed at the end of each sensory analysis session, and final aroma profiles of the honey samples were

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Table 1. Sensory Reference Standards[a]

term	standard reference	rating[b]
buttery	50 μL of Orville Redenbacher's butter-flavored popcorn oil	11
vanilla	5 mL of a 0.1% vanillin aqueous solution	11
burnt sugar	5 mL of a 50 ppm maltol aqueous solution	9
floral	5 mL of a 50 ppm phenylethanol aqueous solution	10
fruity/estery	5 mL of a 50 ppm ethyl butyrate aqueous solution	12
malty	5 mL of a 50 ppm 3-methylbutanal aqueous solution	9.5

[a] Samples were presented in 125-mL PTFE bottles. [b] Aroma intensity values were rated on 15-cm universal scales, with intensity ratings of 0 and 15 corresponding to "none" and "very", respectively.

reported on the basis of discussion and consensus ratings (average of three repetitions) by the panel.

Isolation of Volatiles for Instrumental Analysis. Honey (100 g) was diluted and mixed well with 1200 mL of odorless-distilled water, and spiked with 10 μL of internal standard solution (3.90 μg/μL of 2-ethyl butyric acid in methanol as acidic fraction internal standard; 2.15 μg/μL of 2-methyl-3-heptanone in methanol as neutral/basic fraction internal standard). The honey solution was extracted with 200 mL of dichloromethane in a continuous liquid−liquid extraction apparatus (Kontes, Vineland, NJ) for 16 h. The solvent extract was evaporated to 50 mL using a Vigreux column in a 45 °C water bath. The extract was then subjected to a high-vacuum distillation ($\sim 5 \times 10^{-5}$ Torr operating vacuum level) cleanup step (*12*) for 3 h to further remove nonvolatile residue, with the sample kept at room temperature for the first 1.5 h and then warmed to 45 °C using a water bath.

To separate the acidic volatiles from the neutral and basic volatiles, the extract was washed three times with 5% Na_2CO_3 solution (3 × 20 mL), and the organic layer containing the neutral and basic volatiles (NB) was collected. The aqueous layer was then acidified to pH 3 with 10% aqueous HCl and extracted with dichloromethane (3 × 15 mL). The organic layer containing the acidic volatiles (AC) was collected. Each fraction was then concentrated under a gentle stream of nitrogen gas to 10 mL, dried over 2 g of anhydrous sodium sulfate, and further concentrated to 200 μL under a nitrogen gas stream. Samples were prepared in duplicate and kept at −20 °C until analysis.

Aroma Extract Dilution Analysis (AEDA). Stepwise dilutions (1:3) were prepared with dichloromethane. Each dilution was kept in a 200-μL glass insert put inside a 1-mL amber vial equipped with PTFE-lined screw cap. Dilutions were prepared prior to sniffing and kept at −20 °C until analysis. The aroma-active compounds were evaluated separately by AEDA (*13*), which was conducted on an HP6890 GC (Agilent Technologies, Inc., Palo Alto, CA) equipped with a flame ionization detector (FID), olfactory detection port (DATU Technology Transfer, Geneva, NY), and a DB-FFAP (15 m × 0.32 mm i.d.; 0.25-μm film; J&W Scientific, Folsom, CA) or a DB-5MS (15 m × 0.32 mm i.d.; 0.5-μm film; J&W Scientific) column. The sample (2.0 μL from acidic fractions and 1.0 μL from neutral/basic fractions) was injected using a direct cool on-column injection method (temperature tracking: 3 °C higher than oven temperature). The oven was held at 35 °C for two minutes, then ramped at 10 °C/min to 225 °C and held for 20 min. Column effluent was split 1:1 between FID and olfactory detection port using Siltek deactivated fused silica tubing (1 m × 0.25 mm i.d.; Restek, Bellefonte, PA), with both detector temperatures held at 250 °C. GCO was performed by two trained panelists. Generally, the sensitivity of the assessors varied no more than one dilution order. Therefore, the flavor dilution (FD) factors reported here are based on the results from one of the assessors. Aroma descriptors given in **Table 2** are based on the consensus of both panelists.

Gas Chromatography−Olfactometry of Headspace Samples (GCO−H). GCO−H was conducted on an HP6890 GC (Agilent Technologies, Inc.) equipped with an MPS2 multipurpose (headspace mode) autosampler (Gerstel, Germany), flame ionization detector, and olfactory detector port (ODP2, Gerstel). The determined pH values for Honey 1 and Honey 2 samples were 4.18 and 4.08, respectively. A 1-g portion of honey dissolved in 5 mL of pH 4.0 matrix diluent was placed in a 22-mL headspace vial and sealed with a PTFE-lined septum. The vial was equilibrated at 60 °C for 20 min with agitation (500 rpm, 5 s on, 2 s off). Each headspace volume (2.5, 1.0, 0.5, 0.25, 0.1, 0.05, or 0.025 mL) was injected via a heated (65 °C) gastight syringe into a CIS-4 cooled injection system (Gerstel) operating in the solvent vent mode [vent pressure, 5.35 psi; vent flow, 10.0 mL/min for 0.1 min; splitless time, 1.1 min; initial temperature −120 °C (0.1 min); ramp rate 12 °C/s; final temperature 240 °C (3 min hold)]. Separations were

Table 2. Potent Odorants in Buckwheat Honeys Determined by Aroma Extract Dilution Analysis

no.[a]	odorant	fraction[b]	RI[c]		odor property	Log_3FD[d]	
			FFAP	DB5		honey 1	honey 2
2	methylpropanal[e]	NB	839	572	malty	6	5
4	3-methylbutanal[f]	NB	922	667	malty	7	6
5	ethyl 2-methylpropanoate[f]	NB	967	765	fruity	4	3
6	2,3-butanedione[f]	AC	985	n.a.	buttery	8	3
8	ethyl 2-methylbutanoate[f]	NB	1048	850	estery, fruity	7	5
9	ethyl 3-methylbutanoate[f]	AC	1061	n.a.	fruity, berry-like	3	2
10	dimethyl trisulfide[f]	NB	1359	974	sulfurous	3	2
11	methional[f]	NB	1440	906	cooked potato-like	6	6
12	butyric acid[f]	AC	1613	n.a.	cheesy, fecal	3	2
13	phenylacetaldehyde[f]	NB	1623	1043	floral, rosy	6	6
14	3-methylbutyric acid[f]	AC	1653	n.a.	sweaty, dried fruit-like	7	6
15	(*E*)-β-damascenone[f]	NB	1796	1374	cooked apple/grape-like	6	7
16	2-phenylethanol[f]	NB	1893	1102	rosy, floral	5	3
17	maltol[f]	AC	1953	n.a.	burnt sugar-like	4	3
18	*p*-anisaldehyde[f]	NB	2009	1244	sweet, frangance, floral	4	3
19	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF)[e]	AC	2018	n.a.	caramel/burnt sugar-like	2	5
20	*p*-cresol[f]	AC	2073	1079	cowy, sour, barny	6	4
21	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon)[f]	AC	2186	1107	spicy, seasoning-like	7	7
22	coumarin[f]	NB	2428	1456	wild flower,herbaceous	6	5
23	phenylacetic acid[f]	AC	2555	1263	rosy, floral, sweet	6	6
24	vanillin[f]	AC	2563	1410	vanilla/candy-like	6	7
25	4-(4-hydroxyphenyl)-2-butanone[f]	AC	2893	1555	sweet, candy-like	5	4

[a] Numbers correspond to those in **Tables 3** and **4**. [b] NB, neutral/basic fraction; AC, acidic fraction. [c] Retention index was calculated from GCO data. [d] FD factors were determined on DB-5MS column for neutral/basic components, and on DB-FFAP column for acidic components. [e] Compound tentatively identified by comparison of RI values and odor properties with those of authentic reference compound. [f] Compound positively identified by comparison of RI values, odor properties, and mass spectra with those of reference compound.

performed on DB-5MS and DB-FFAP columns (same dimensions as the above columns). Column effluent was split 1:1 between FID and ODP2 using Siltek deactivated fused silica tubing. FID and ODP2 temperatures were maintained at 250 °C. The GC oven temperature was programmed from 30 to 225 °C at a rate of 10 °C/min with initial and final hold times of 2 and 20 min, respectively. GCO was performed as described earlier.

Gas Chromatography−Mass Spectrometry (GC−MS) for Identification. The GC−MS system consisted of an HP6890 GC/5973 mass selective detector (MSD; Agilent Technologies, Inc.). Both NB and AC fractions were further concentrated to 50 μL prior to GC−MS analysis. The selected ion monitoring (SIM) mode also was applied for the identification of trace components. Extracts were injected using cool on-column mode into an HP5-MS (30 m × 0.25 mm i.d.; 0.5-μm film; Agilent Technologies, Inc.) or DB-FFAP (30 m × 0.25 mm i.d.; 0.25-μm film; J&W Scientific, Folsom, CA) column. Helium was the carrier gas at a constant flow of 1 mL/min. The GC oven temperature was programmed from 35 to 225 °C at a rate of 4 °C/min with initial and final hold times of 5 and 30 min, respectively. MSD conditions were as follows: capillary direct interface temperature, 280 °C; ionization energy, 70 eV; mass range, 35−300 amu; electron multiplier voltage (Autotune + 200 V); scan rate, 5.27 scans/s.

For the identification of headspace volatiles, sample was prepared as described for GCO−H except that 5 μL of internal standard solution (0.6 μg/μL of 2-ethyl butyric acid and 19.5 ng/μL of 2-methyl-3-heptanone in methanol) was added, and the sample was preincubated for 10 min with agitation (500 rpm, 5 s on, 2 s off). Then a SPME fiber (DVB/Carboxen/polydimethylsiloxane fiber; Supelco, Bellefonte, PA) was exposed to the vial headspace for an additional 5 min. Immediately after sampling, the fiber was desorbed by splitless injection (injector temperature 260 °C; splitless time 4 min; vent flow 50 mL/min) into the GC−MS system with the same settings as described above. The GC oven temperature was programmed from 35 to 225 °C at a rate of 6 °C/min with initial and final hold times of 4 and 30 min, respectively. Compounds were identified by comparison of their mass spectra, retention indices (*14*), and odor properties with those of authentic standards.

HPLC Analysis of Major Sugars. It is known from the literature that fructose followed by glucose and maltose are the major sugars found in honeys (*15*). Concentrations of these individual sugars were determined by high-performance liquid chromatography (HPLC) analysis using an HP1050 (Agilent Technologies, Inc.) system equipped with a refractive index (RI) detector and a Supelcogel 610H (300 mm × 7.8 mm i.d.; 5 μm d_p; Supelco, Bellefonte, PA) column and a Supelcoge 610H (50 mm × 4.6 mm i.d.; 5 μm d_p; Supelco) guard column. Aqueous phosphoric acid (0.1% v/v) was used as mobile phase at a constant rate of 0.5 mL/min. Diluted honey samples (1:10 in mobile phase) were injected (20 μL) for analysis. Experiments were carried out in duplicate. External standard curves were constructed by analyzing standard solutions containing sugar standards at three concentrations under identical experimental conditions.

Quantification of Major Volatile Compounds. An aqueous mimic matrix was prepared based on results from HPLC sugar analysis. Results showed that the major sugar contents of these two honeys were similar. Therefore, average values were used for the individual sugars when preparing the matrix, which was composed of 7.2% w/w maltose, 29.7% w/w glucose, and 39.3% w/w fructose in odorless water with pH adjusted to 4.0 using gluconic acid (major acid found in honey (*3*)). MS response factor (f_i) for each positively identified odorant was determined by adding known amounts of authentic standards into the matrix solution. Both H−SPME and solvent extraction methods were applied for the quantification. Sample preparation and GC−MS analysis were performed in the same way as described above for the honey samples, with the assumption that the extraction rates of individual volatile components in this matrix were similar to those in the honey samples. MS response factor − f_i [compound (i) relative to internal standard (IS)] was used to determine the concentration for each compound, calculated as follows:

$$\text{concn}_i = \text{concn}_{\text{IS}} \times f_{\text{I}} \times \text{area}_i/\text{area}_{\text{IS}}$$

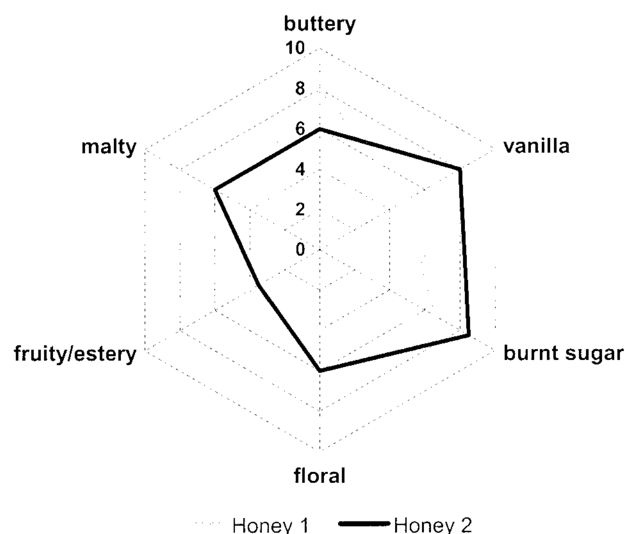

Figure 1. Descriptive aroma profiles of buckwheat honeys.

RESULTS AND DISCUSSION

Sensory Evaluation. Results of descriptive aroma analysis are summarized in **Figure 1**. Malty, burnt sugar, vanilla, buttery, floral, and fruity/estery were the predominant aroma notes detected by the sensory panel in both honey samples. Compounds such as 3-methylbutanal have been found to be responsible for malty flavors (*7*−*9*). Maltol (3-hydroxy-2-methyl-4-pyrone) and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone possess caramel/burnt sugar notes (*1, 7, 9, 16*). Vanilla aroma is commonly associated with vanillin (*1, 9*), whereas the fruity/estery note is associated with esters (*16*−*18*), and a floral note is linked to 2-phenylethanol, (*E*)-β-damascenone, and phenylacetaldehyde (*4, 16, 19*).

The two samples differed in intensities of all attributes. However, the overall aroma profiles of the two honey samples from different commercial sources were similar, with Honey 1 having a slightly stronger buttery note and Honey 2 having a stronger vanilla aroma (**Figure 1**).

AEDA. Components of intermediate and low volatility were isolated from the honey matrix through continuous liquid−liquid solvent extraction, followed by a high-vacuum distillation cleanup step. Twenty-two volatile compounds with Log_3FD factors higher than 2 were detected, and their relative aroma intensities were established by AEDA (**Table 2**). Among them, 3-methylbutanal (**4**, malty), (*E*)-β-damascenone (**15**, grape/cooked apple-like), and sotolon (**21**, spicy, seasoning-like) were the predominant odorants. Other important odorants included methylpropanal, 2,3-butanedione, phenylacetaldehyde, 3-methylbutyric acid, maltol, vanillin, methional, coumarin, and *p*-cresol. Results also indicated that several aroma-impact compounds made major contributions to the malty flavor of buckwheat honey, and their combination with some other aroma-active components accounted for the distinct flavor of the honey.

Several compounds (**2**−**4**) that have malty notes contribute significantly to the overall malty flavor of buckwheat honey, with 3-methylbutanal being the most important one. This compound had been previously described as malty in milk, in wheat and rye bread crusts, in various malts, and in some other foods (*7*−*9, 20*−*22*). During analysis of solvent extracts, it coeluted with the solvent peak and with 2-methylbutanal. In addition, because its odor property is similar to that of 2-methylbutanal, to identify them clearly from the solvent extract would be difficult. However, because of their high volatility,

Table 3. Potent Odorants in Headspace of Buckwheat Honeys

no.[a]	odorant	RI[b]		odor property	FD[c]	
		FFAP	DB5		honey 1	honey 2
1	dimethyl sulfide[d]	706	<500	pumpkin/sweet corn-like	5	5
2	methylpropanal[d]	770	546	malty	25	25
3	2-methylbutanal[d]	908	654	malty	5	1
4	3-methylbutanal[d]	915	642	malty	100	50
5	ethyl 2-methylpropanoate[d]	955	763	fruity	10	2.5
6	2,3-butanedione[d]	980	582	buttery	50	2.5
7	ethyl butanoate[d]	1020	800	fruity	50	25
9	ethyl 3-methylbutanoate[d]	1059	857	fruity, berry-like	50	n.d.
10	dimethyl trisufide[e]	1359	969	sulfurous	5	2.5
11	methional[e]	1445	910	cooked potato-like	1	1
13	phenylacetaldehyde[d]	1637	1053	rosy, floral	2.5	n.d.
15	(*E*)-β-damascenone[d]	1819	1408	cooked apple-like	5	5

[a] Numbers correspond to those in **Tables 2** and **4**. [b] Retention index was calculated from GCO data. [c] FD factors were determined on DB-FFAP column, FD factors of 1, 2.5, 5, 10, 25, 50, and 100 corresponding to headspace volumes of 2.5, 1, 0.5, 0.25, 0.1, 0.05, and 0.025 mL, respectively. [d] Compound positively identified by comparison of RI values, odor properties, and mass spectra with those of reference compound. [e] Compound tentatively identified by comparison of RI values and odor properties with those of authentic reference compound.

Table 4. Concentrations of Potent Odorants in Buckwheat Honeys

		threshold		solvent extract[b]				H–SPME[d]			
				honey 1		honey 2		honey 1		honey 2	
no.[a]	odorant	(ng/g, in water)	ref.	concn (ng/g)	OAV[c]	concn (ng/g)	OAV	concn (ng/g)	OAV	concn (ng/g)	OAV
1	dimethyl sulfide	0.3	*27*	–[e]	n.a.	–	n.a.	822	2740	1390	4633
2	methylpropanal	1	*28*	–	n.a.	–	n.a.	10	10	5	5
3	2-methylbutanal	1	*29*	–	n.a.	–	n.a.	4652	4652	1092	1092
4	3-methylbutanal	0.2	*27*	–	n.a.	–	n.a.	15315	76575	6256	31280
5	ethyl 2-methylpropanoate	0.1	*30*	–	n.a.	–	n.a.	21	210	6	60
6	2,3-butanedione	15	*31*	–	n.a.	–	n.a.	7095	473	1780	119
7	ethyl butanoate	1	*32*	–	n.a.	–	n.a.	6	6	2	2
8	ethyl 2-methylbutanoate	0.3	*30*	–	n.a.	–	n.a.	59	197	12	40
9	ethyl 3-methylbutanoate	0.2	*33*	–	n.a.	–	n.a.	136	680	12	60
10	dimethyl trisulfide	0.01	*34*	17	1727	6	600	–	n.a.	–	n.a.
11	methional	0.2	*28*	36	180	30	150	–	n.a.	–	n.a.
12	butyric acid	240	*35*	5663	24	5087	21	2623	11	1312	5
13	phenylacetaldehyde	4	*28*	1527	382	1995	499	2422	606	2112	528
14	3-methylbutyric acid	250	*27*	11367	45	4699	19	12621	50	2238	9
15	(*E*)-β-damascenone	0.002	*23*	7	3686	10	4808	4	2000	9	4500
16	2-phenylethanol	1000	*27*	708	0.71	614	0.61	498	0.50	245	0.25
17	maltol	35000	*36*	791	0.02	754	0.02	–	n.a.	–	n.a.
18	p-anisaldehyde	n.a.		47		38		–	n.a.	–	n.a.
19	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF)	31	*37*	–	n.a.	–	n.a.	–	n.a.	–	n.a.
20	*p*-cresol	55	*38*	181	3	61	1	264	5	30	0.55
21	sotolon	0.001	*24*	41	40615	20	20078	–	n.a.	–	n.a.
22	coumarin	25	*39*	32	1	26	1	–	n.a.	–	n.a.
23	phenylacetic acid	1000	*40*	3725	4	4056	4	–	n.a.	–	n.a.
24	vanillin	25	*41*	126	5	5657	226	–	n.a.	7740	310
25	4-(4-hydroxyphenyl)-2-butanone	5	*42*	24	5	21	4	–	n.a.	–	n.a.

[a] Numbers correspond to those in **Tables 2** and **3**. [b] Concentration (average, $n = 2$) was determined from solvent extracts prepared by solvent extraction–high vacuum distillation. [c] Odor active value (OAV) was calculated by dividing compound concentration by its published threshold. [d] Concentration (average, $n = 2$) was determined from headspace sample using solid-phase microextraction (H–SPME) sampling method. [e] Concentration was not determined because the method was not suitable for that compound or the compound was present in trace amount.

headspace sampling made the identification easier. 3-Methylbutanal was detected even for the 25-μL headspace sample (**Table 3**). Subsequent quantification (**Table 4**) also showed that 3-methylbutanal had an extremely high odor activity value due to its relatively high concentration and its very low detection threshold.

Another highly volatile compound that was important to the malty aroma of buckwheat honey was methylpropanal. It has been reported to be important to the flavor of malt (*8*−*9*). Its importance in buckwheat honey was also confirmed through headspace sampling.

(*E*)-β-Damascenone, which had a grape/cooked apple-like odor, is another odorant found in the NB fraction with very high potency. It has been reported to be an important odorant of honey flavor (*1*, *4*). Its extremely low threshold of 0.002 ppb in water (*23*) also indicated its significance in the aroma of buckwheat honey even though it was present at a very low concentration.

Sotolon was an odorant detected from the AC fraction with the highest Log_3FD factor; its extremely low threshold (0.001 ppb in water (*24*)) also suggested the important role it plays in the flavor of buckwheat honey. This compound has been found

to impact the flavor of many foodstuffs due to its high aroma potency (*24−26*). Its presence in buckwheat honey might be desirable because of its seasoning-like odor.

Vanillin belonged to another group of odorants that had intermediate potency and were important to the flavor of buckwheat honey. It had a high Log_3FD factor, and its pleasant sweet and vanilla-like aroma might play an important role in the flavor profile of buckwheat honey. 2,3-Butanedione was another compound from this group.

Some odorants could be readily associated with the aroma of buckwheat honey because of their pleasant odor characteristics, for example, phenylacetaldehyde with a floral note and the esters which contribute fruity/estery notes. Some compounds had odor properties that were totally different from the perceived aroma of buckwheat honey. However, because of their low thresholds and/or high concentrations in buckwheat honey, they also contribute to the overall aroma of buckwheat honey with high Log_3FD factors. Methional, coumarin, *p*-cresol, and 3-methylbutyric acid belonged to this group.

Highly volatile compounds that may be overlooked because of loss during various preparation steps were evaluated by the GCO technique of decreasing headspace samples (GCO−H). A total of 12 odorants were detected. Among them, dimethyl sulfide and ethyl butanoate were not detected by AEDA of the solvent extracts (**Table 3**). These compounds were identified by comparing their retention indices and odor qualities with those identified from the solvent extracts and also by headspace-SPME/GC−MS. Stepwise reduction of the headspace volume showed that 3-methylbutanal (**4**, malty), ethyl butanoate (**7**, fruity), methylpropanal (**2**, malty), and 2,3-butanedione (**6**, buttery) had very high aroma potencies.

Quantification. Quantitative data were consistent with the AEDA results, and are summarized in **Table 4**. Individual compound concentrations were associated with published threshold values by calculating odor activity values (OAVs). All compounds except maltol were present at levels above their thresholds, and their OAVs correlated well with the determined FD factors. Maltol has a relatively high threshold value (36000 ppb in water (*36*)) which might be due to its high solubility in water. But for AEDA, it was extracted out from the sample matrix with solvent prior to GCO, and this might have affected its detection level.

Headspace−SPME method was applied mainly for the quantification of highly volatile compounds, whereas the liquid−liquid solvent−high vacuum distillation extraction method was employed to quantify compounds with medium/high boiling points. Some compounds (**12**−**16**, **20**, **24**) were quantified by both methods, and generally the results from both methods were in good agreement (**Table 4**). H−SPME is a valuable tool for the estimation of highly volatile compounds that are not easily determined by traditional solvent extraction methods due to solvent peak interference or loss during tedious sample preparation. However, several critical factors such as polarity of the fiber and fiber exposure time should be carefully selected to minimize any bias. On the other hand, solvent extraction followed by high-vacuum distillation is a more exhaustive technique especially suitable for highly water-soluble compounds (e.g., maltol) or compounds that have high boiling points (e.g., 4-(4-hydroxyphenyl)-2-butanone). However, for compounds that are present at trace levels (e.g. 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone), stable isotope dilution techniques might be a better choice.

Most of the odorants detected were not unique to buckwheat honey. For example, phenylacetaldehyde, phenylacetic acid, and (*E*)-β-damascenone have been suggested to contribute to honey flavors (*1−3*), and 3-methylbutanal is a very important odorant found in malts (*7−9*). However, it was the complex and unique combination of those odorants that rendered buckwheat honey with its distinct malty aroma to be different from other honeys. The compounds 2- and 3-methylbutanal are commonly found in barley malt (*9, 43*). They are known to be Strecker aldehydes, and their presence in honeys is usually associated with the Maillard browning reactions (Strecker pathway) (*2, 4, 44−45*). As the processing of honey involved several heating steps (*3−4*), and it is assumed that commercialized honeys generally undergo similar heat treatments, the extremely high amounts of compounds 2- and 3-methylbutanal presented in buckwheat honeys compared with some other honeys (*2, 4*) suggested that buckwheat honey might contain a higher abundance of Strecker degradation precursers, such as amino acids (for example, leucine for 3-methylbutanal), which would result in a honey with an aroma resembling that which develops upon heat-promoted chemical reactions that occur during the malting of barley (*44*). The presence of other Maillard reaction products such as methylpropanal, phenylacetaldehyde, methional, and dimethyl trisulfide supports this hypothesis.

In general, the sensory evaluation results correlated well with the instrumental analysis. The similar flavor profiles of these two honeys indicate that there are some major components that contribute to the distinct buckwheat honey flavor, independent of their commercial sources. The slight difference between these two samples might be due to different processing and storage conditions, as well as natural variation.

Results showed that the complex and unique combination of several aroma-active compounds contributed to the flavor of buckwheat honey, with 3-methylbutanal being primarily responsible for the distinctive and characteristic malty aroma. The relatively high abundance of Strecker aldehydes (**2**−**4**, **11**, **13**) and their corresponding acids (**14** and **23**), as well as furanones (**17**, **19**, **21**), suggested that Maillard reaction was a key pathway for aroma generation in buckwheat honey. Further study on factors that affect the flavor profile of buckwheat honey may be useful for product quality control processes, as well as for honey floral source identification.

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