

Volatile Compounds Collected by Simultaneous Steam Distillation-Solvent Extraction from Hong Kong Salt-Dried Croakers

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We compared the quality and quantity of volatile components in salt-dried croakers prepared by different methods and obtained from different locations. In total, 110 compounds were found among regular- and delay-type salt-dried croakers purchased from two locations in Hong Kong. The major chemical classes included miscellaneous compounds (17), pyrazines (16), alcohols (15), and sulfur-containing compounds (13). Fish obtained in different locations but prepared by the same method differed only slightly in the number of identified compounds. In general, fish prepared by the delay method had a larger number of compounds compared to fish prepared by the regular method. Further, a greater number and higher levels of compounds were found in the fish obtained from one of the two locations. Overall, the delay preparation method resulted in a greater number of compounds with stronger intensity compared to the regular method.

Key words: Croaker, GC-MS, Salt-dried fish, SDE, Volatile compounds

Introduction

Among the various processes used to preserve fish, salting and drying has a long history (Carruthers, 1986). In southern China, the marine species Chinese herring, threadfins, croakers, yellow croakers, and white flower croakers are commonly manufactured into salt-dried products (Herklots and Lin, 1960; Cheung, 1994; Yeung et al., 1999). Croakers, of the family *Sciaenidae*, are a popular salt-dried fish (Cheung, 1994; AFCD, 2004). Croaker is the smallest type of salt-dried fish and thus requires as little as one day of salting and five to six days of sun-drying (Yeung et al., 1999). Following its manufacture, salt-dried fish is often pan-fried then steamed (Yeung et al., 1999). In Hong Kong, the method used to dry fish depends on the quality of the fish. Two major production methods exist: regular and delay. In the regular method, freshly caught or refrigerated fish are gutted to maintain freshness, and salt is added to the body cavity. The fish are then layered into vats and dried under the sun for durations ranging from hours to days. The delay method is similar to the regular method in terms of the processing steps; however, the fish, which are often of lower quality, are not immediately gutted and salted after they are caught

(Yeung et al., 1999). Many consumers report that products prepared by the delay method have a much stronger flavor than those prepared by the regular method (Yeung et al., 1999). However, there are no scientific data to substantiate this observation.

Therefore, we examined the effect of production method and purchase location on the quality and quantity of the volatile components in salt-dried croaker to determine how these components contribute to the flavor of the final product.

Materials and Methods

Sample preparation

Two types of salt-dried croaker (regular and delay) were purchased from two dried seafood outlets (locations 1 and 2) in Hong Kong. We divided the products into four groups: A, location 1/delay method; B, location 2/delay method; C, location 1/regular method; and D, location 2/regular method. The bodies of the fish (minus the heads and fins) were cut into 1 cm² pieces with stainless steel scissors for subsequent extraction.

Simultaneous distillation and solvent extraction

Extraction of the volatile compounds in each type of fish was carried out using a simultaneous steam distillation and solvent extraction (SDE) apparatus

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(model no. 523010-0000, Kontes, Vineland, NJ, USA) as described by Chung et al. (2001). Briefly, the samples (100 g) were placed on a stainless steel mesh above double-distilled water (400 mL), and 10 µg of the internal standard (IS) 2,4,6-trimethylpyridine was added. Redistilled dichloromethane (40 mL) was used as the solvent. The SDE apparatus was run for 2 hrs from the time at which the water began to boil. The extracts were concentrated to 10 mL in a solvent flask using a gentle stream of nitrogen (99.999% purity) and then further concentrated to 0.25 mL by passage through anhydrous sodium sulfate (2.85 g).

Gas chromatography-mass spectrometry (GC-MS)

Quantitative analysis of the samples was achieved using a Hewlett-Packard 6890 gas chromatograph coupled with a Hewlett-Packard 5973 mass selective detector (MSD). The GC and MS conditions were similar to those described by Chung (2001). Briefly, the GC conditions were splitless mode, injector temperature of 220°C, and helium carrier gas at 30 cm/s. The temperature program was as follows: initial temperature of 35°C for 5 min, ramp rate of 2°C/min, and final temperature of 195°C for 90 min. The MSD conditions were ion source temperature of 230°C, MS quadrupole temperature of 106°C, interface temperature of 150°C, ionization voltage of 70 eV, mass range from 33–450 amu, scan rate of 6.52 scans/s, and electron multiplier voltage of 1,106 V. All separations were performed on a Supelcowax 10 column (60 m long×0.25 mm i.d.×0.25 µm d_i; Supelco, Inc., Bellefonte, PA, USA).

Compound identification and quantification

The identification and quantification of the compounds were conducted as described by Chung et al. (2001). We identified compounds by comparing their retention times (RTs) (Van den Dool and Kratz, 1963) and mass spectra with those of authentic standards under identical analytical conditions. All tentative identifications were based on matching the mass spectra of the unknown compounds with those in the Wiley Chemical Database (7th ed., Hewlett-Packard Co., Palo Alto, CA, USA). The amount of each confirmed compound in the samples was determined by the calibration curve method, whereas the relative quantity of each tentatively identified compound was expressed as the ratio of the area of the tentatively identified compound to that of the IS.

Proximate analysis

Moisture was controlled according to the official methods of the AOAC (1980).

Statistical analysis

The compounds from duplicate samples were analyzed by one-way analysis of variance (ANOVA) and compared by Tukey's studentized range test for post-hoc multiple comparisons; significance was assigned at $p=0.05$ (Ott, 1988).

Results and Discussion

Fig. 1 presents typical chromatograms for the four groups (A: location 1, delay method; B: location 2, delay method; C: location 1, regular method, and D: location 2, regular method) of salt-dried fish. Groups A, B, C, and D had moisture contents of 41.03, 36.41, 39.93, and 37.85%, respectively (Fig. 2). Of the 110 compounds recovered, 65 and 45 were positively and tentatively identified, respectively. The major classes of compounds (i.e., those including more than ten components) were miscellaneous compounds (17), alcohols (15), pyrazines (16), ketones (15), and sulfur-containing compounds (13). Samples A, B, C, and D contained 109, 105, 74, and 79 components, respectively. Eighty-four compounds were common to all sample groups. Statistical analysis showed that 75% of the common components were significantly different among the four groups ($P<0.05$, one-way ANOVA). A comparison of the samples produced by the same method but at different locations (A vs. B and C vs. D) revealed that 31 and 24 common compounds were significantly different ($P<0.05$, Tukey's test), respectively. Those compounds that were present in the greatest amounts tended to come from location 2, regardless of the method of preparation. Similarly, a comparison of the samples produced by different methods but at a single location (A and C from location 1 and B and D from location 2) revealed that the delay method produced a greater number and concentration of compounds than did the regular method. As noted above, the samples from location 2 contained a higher number of components at higher levels than did the samples from location 1. This suggests that for the same production method, variations in the actual production steps at the two locations may lead to qualitative and quantitative differences in the finished product.

Comparison of the methods used at a single location revealed that more compounds were present at higher concentrations in delay-method samples than in regular-method samples. For example, in groups A and C (both from location 1), 93 compounds were present at higher levels in the fish prepared by the delay method, whereas 17 compounds were present at higher levels in the fish prepared by

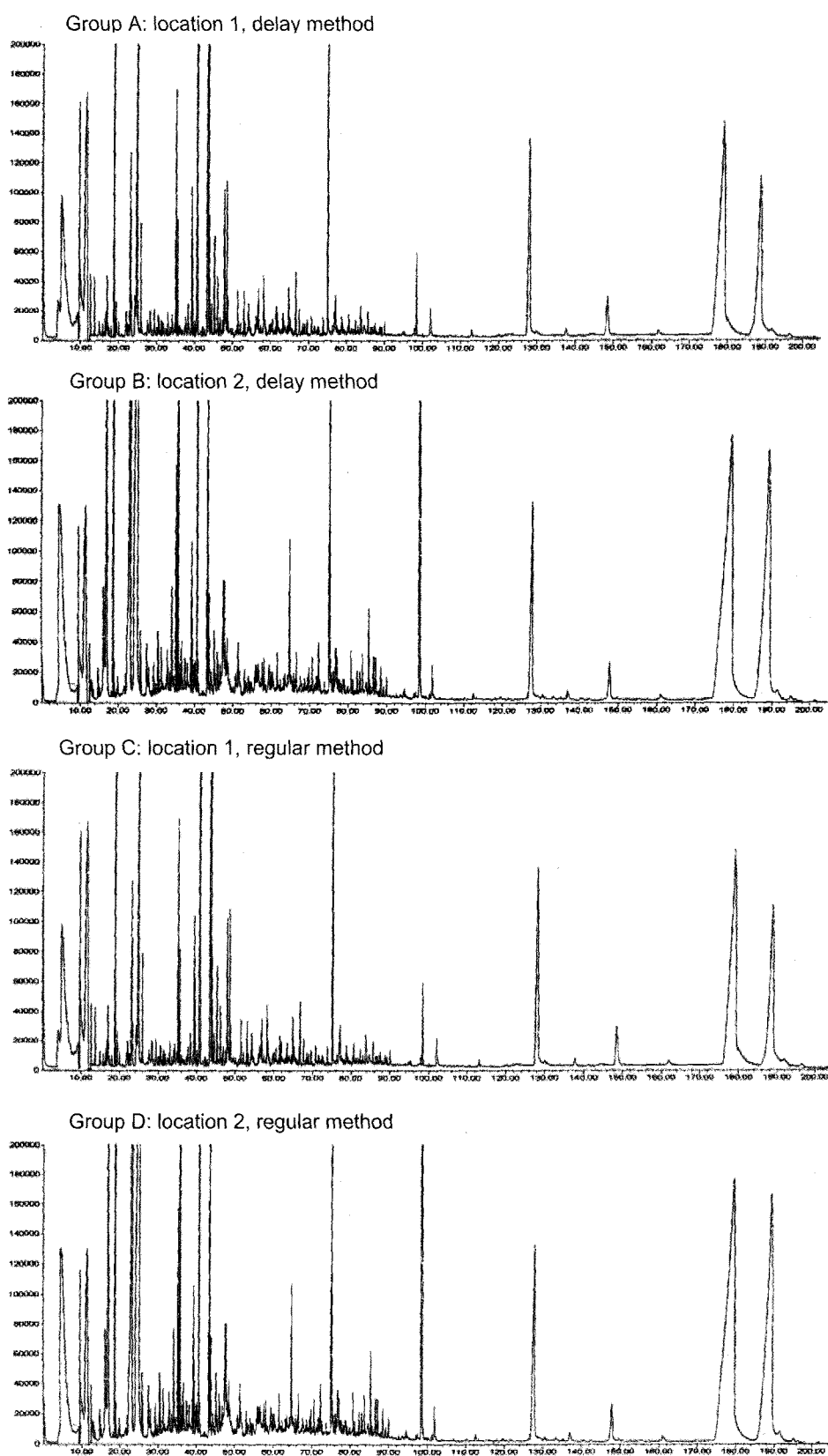


Fig. 1. Typical chromatograms of the four groups (A, B, C and D) of salted-dried croakers.

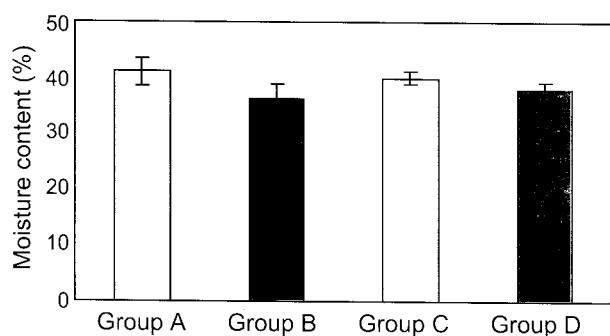


Fig. 2. Moisture contents of four groups (A, B, C and D) of salted-dried croakers.

the regular method. Similar observations were recorded for location 2, with 71 compounds being present at a higher level as a result of the delay method versus 39 for the regular method. All of the compounds except oxygen-containing compounds were present at increased levels following the delay method, in contrast to the regular method. In fact, the concentrations of several esters, pyrazines, and sulfur-containing compounds increased by more than 20 times following the delay method compared to the regular method at both locations (Table 1). Since the delay method is often used for fish of lower quality or fish that are treated to increase their rate of autolysis, the amount of degradation products available for the generation of volatile components in the subsequent production steps should be higher in the delay method than in the regular method (Olley et al., 1988).

Only six odor-impact components found in salt-dried white herring were found in the croaker samples: 3-methyl-butanol, (E)-2-methyl-2-butenal, trimethylamine, 2-ethyl-6-methylpyrazine, dimethyldisulfide, and dimethyltrisulfide (Chung et al., 2007). Among these, trimethylamine, dimethyldisulfide, and dimethyltrisulfide are very potent odorants in white herring. Since these three compounds have a relatively high area ratio per sample weight within their chemical classes in croaker, they likely contribute strongly to the odor of salt-dried products. Trimethylamine, which is produced by the microbial degradation of trimethylamine oxide (Herberd et al., 1985), often contributes to the “fishy” odor to seafood (Sigma-Aldrich, 2007). Both dimethyldisulfide and dimethyltrisulfide reportedly possess a fried garlic-like odor in salt-dried white herring (Chung et al., 2007). The microbial degradation of methanione or the oxidation of methanethiol may contribute to the formation of dimethyldisulfide in croaker products (Christensen et al., 1981). The detection of dimethyltrisulfide in salt-dried croaker could have

resulted from contamination by natural microbial flora, as has been reported in royal red prawns (Whitfield et al., 1981). Sulfur-containing compounds often contribute both pleasant and unpleasant flavors to food overall (Fors, 1983). For the other major chemical classes, including esters and pyrazines, the flavor contribution of the former depends on the amount of the components that are present in the food, although their odor is often described as fruity (Sigma-Aldrich, 2007). Pyrazines are generally considered to be a very important class among flavored compounds, as they are related to desirable flavor properties in foods. They are produced in fish by the Maillard reaction (Fors, 1983), most likely during the sun-drying period. Since more than half of the compounds identified were found to be common components among the four groups of fish, and because most of these compounds were statistically significant, they represent major contributors to the subtle flavor of salt-dried croaker. In contrast, common but statistically insignificant components may contribute more to the background flavor of salt-dried fish. As the levels of the common components in groups A and B were generally higher than those found in groups C and D, the perceived intensity of the fish in groups A and B would likely be higher than that in groups C and D. Therefore, consumers would be more likely to perceive fish in groups A and B as having stronger flavor. In conclusion, although the fish prepared by the same method but obtained from different locations had similar numbers and identities of components, the flavor intensities may vary significantly. Based on our statistical analyses and comparison of the mean levels of the components, both preparation method and location affect the quality and strength of the flavor components in salt-dried croaker.

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Table 1. Volatile compounds in Croaker prepared by difference production methods (Delay and Regular) and purchased from different locations (1 and 2). ^aChemical abstract service registry number. ^bI, Cha and Cadwallader, 1995; 2, Sakakibara et al., 1990; 3, Triqui and Zouine, 1999. ^cMass/charge. ^dAverage retention time calculated from all groups. ^eStandard deviation of the area ratio of a compound on a dry weight basis. ^fArea of a compound not detected. ^gTentatively identified compound by mass spectrum database (6th edition, Wiley Chemical Database, Hewlett-Packard Co., Palo Alto, CA). ^hSignificantly different between two groups at $p < 0.05$, tukey test.

Compounds	cas no. ^a	References ^b	m/z ^c	RT ^d (min)	Delay Method						Regular Method						Statistical results between two groups						
					Group A location 1		Group B location 2		Group C location 1		Group C location 2		Group C location 1		Group C location 2			AB	CD	AC	BD	AD	BC
					Area ratio per dry wt (kg ⁻¹)	SD ^e	Area ratio per dry wt (kg ⁻¹)	SD	Area ratio per dry wt (kg ⁻¹)	SD	Area ratio per dry wt (kg ⁻¹)	SD	Area ratio per dry wt (kg ⁻¹)	SD	Area ratio per dry wt (kg ⁻¹)	SD							
Acids (7)																							
Undecanoic acid	112-37-8		60	93.94	0.001	0.001>	0.001	0.001	0.001	0.001>	0.001>	0.001>	0.004	0.006									
Dodecanoic acid	143-07-7		73	102	0.016	0.019	0.016	0.007	0.007	0.003	0.003	0.03	0.031		*								
Tetradecanoic acid	544-63-8	2	73	128.1	0.253	0.231	0.302	0.153	0.153	0.106	0.066	0.757	0.614		*								
Pentadecanoic acid	1002-84-2		73	148.3	0.06	0.052	0.05	0.019	0.019	0.037	0.019	0.162	0.119										
Hexadecanoic acid	57-10-3	2	73	179.4	0.765	0.126	1.331	0.49	0.49	0.864	0.389	1.555	0.649		*								
9-Hexadecenoic acid ^f	2091-29-4		55	189.1	0.647	0.644	0.946	0.419	0.419	0.348	0.232	2.3	1.814										
9-Hexadecenoic acid ^f	2091-29-4		55	192	0.028	0.036	0.016	0.009	0.009	0.007	0.003	0.049	0.039										
Alcohols (15)																							
2,4-Hexadien-1-ol ^f	111-28-4		41	15.19	0.001	0.001>	0.002	0.001	0.001	0.002	0.001	0.003	0.001						*				
1-Pentene-3-ol	616-25-1	1	57	24.25	0.065	0.011	0.18	0.02	0.02	0.177	0.04	0.699	0.298		*				*				
3-Methyl-1-butanol	123-51-3	1	55	27.63	0.008	0.002	0.035	0.013	0.013	0.061	0.023	0.047	0.012		*				*				
1-Pentanol	71-41-0	1	42	30.58	0.022	0.003	0.065	0.005	0.005	0.044	0.012	0.121	0.049		*				*				
1-Hexanol	111-27-3	1	56	37.67	0.012	0.001	0.025	0.003	0.003	0.028	0.005	0.059	0.015		*				*				
5-Ethylcyclopent-2-en-1-one ^f	34094-63-8		82	42.24	0.005	0.001	0.004	0.001	0.001	0.004	0.001	0.011	0.005		*				*				
1-Octen-5-ol ^f	53907-72-5		57	43.93	0.119	0.011	0.178	0.02	0.02	0.082	0.025	0.228	0.097		*				*				
1-Heptanol	111-70-6	1	41	44.47	0.006	0.001	0.007	0.001	0.001	0.005	0.001	0.019	0.012		*				*				
(Z)-1,5-Octadien-3-ol ^f	50306-18-8		57	46.13	0.042	0.004	0.041	0.021	0.021	0.017	0.005	1.775	4.232		*				*				
1-Octanol	111-87-5		55	50.9	0.002	0.001	0.005	0.001	0.001	0.002	0.001	0.005	0.003		*				*				
3-Methyl-2-cyclohexen-1-one	1193-18-6		82	52.82	0.002	0.001	0.001	0.001>	0.001>	0.001	0.001>	0.001	0.001>		*				*				
(Z)-2-Octen-1-ol ^f	26001-58-1		110	54.25	0.001	0.001>	0.001	0.001>	0.001>	0.001>	0.001>	0.001	0.001>		*				*				
Benzyl alcohol	100-51-1	1	108	68.72	0.004	0.001>	0.005	0.002	0.002	0.003	0.001	0.018	0.01		*				*				
Phenol	108-95-2	1	94	75.13	0.19	0.18	1.017	0.78	0.78	0.066	0.075	0.107	0.157		*				*				
4-Methylphenol	106-44-5		53	78.93	0.001	0.001>	0.001	0.001>	0.001>	0.001>	0.001>	0.001>	0.001>		*				*				
Aldehydes (4)																							
(E)-2-Methyl-2-butanal	497-03-0	1	55	20.05	0.006	0.001	0.011	0.002	0.002	0.002	0.001	0.017	0.009		*				*				
(E)-2-Hexenal ^f	505-57-7	1	2	3	41	28.35	0.004	0.001	0.003	0.003	0.004	0.022	0.009		*				*				

Table 1. continued

Compounds	cas no. ^a	References ^b	m/z ^c	RT ^d (min)	Delay Method				Regular Method				Statistical results between two groups	
					Group A location 1		Group B location 2		Group C location 1		Group C location 2			
					Area ratio per dry wt (kg ⁻¹)	SD ^e	Area ratio per dry wt (kg ⁻¹)	SD	Area ratio per dry wt (kg ⁻¹)	SD	Area ratio per dry wt (kg ⁻¹)	SD		
Benzaldehyde	100-52-7	1	2	106	48.57	0.039	0.023	0.041	0.028	0.034	0.006	0.054	0.036	
2-Phenyl-2-butenal	4411-89-6			115	71.54	0.003	0.001	0.004	0.002	-	-	0.001	> 0.001	>
Esters (9)														
Ethyl butanoate	105-54-4			71	16.71	0.005	0.001	0.023	0.014	0.001	> 0.001	> 0.001	> 0.001	>
Methyl tetradecanoate	124-10-7			74	75.75	0.004	0.004	0.006	0.002	0.001	0.001	0.005	0.003	
Methyl hexadecanoate	112-39-0			74	85.62	0.025	0.017	0.039	0.018	0.013	0.008	0.027	0.015	*
Methyl (Z)-9-hexadecenoate	1120-25-8			55	86.83	0.003	0.003	0.006	0.003	0.002	0.001	0.007	0.005	
Ethyl hexadecanoate	628-97-7			88	87.46	0.006	0.006	0.024	0.002	0.001	0.001	0.006	0.004	*
Ethyl (Z)-9-hexadecenoate [†]	56219-10-14			55	88.74	0.002	0.002	0.007	0.002	0.001	0.001	0.004	0.001	*
Methyl heptadecanoate	1731-92-6			74	91.03	0.001	0.001	-	-	0.001	> 0.001	> 0.001	> 0.001	*
Methyl octadecanoate	112-61-8			74	98.01	0.004	0.002	-	-	0.002	0.001	0.003	0.002	*
Methyl (Z)-9-octadecenoate	112-62-9			55	99.49	0.002	0.001	0.001	0.001	0.001	0.001	0.002	0.001	*
Hydrocarbons (4)														
Bicyclo [4.2.0]octa-1,3,5-triene	694-87-1			104	31.07	0.003	0.001	0.004	0.002	0.001	0.001	> 0.001	> 0.001	>
(Z)-2-Pentadecen-4-yne [†]	74646-33-6			79	31.8	0.003	0.001	-	-	0.002	0.001	0.01	0.005	*
1-Cyclopentyl-3-ethoxy-2-propanone [†]	51149-71-4			83	83.79	0.0132	0.002	0.022	0.006	0.019	0.007	0.04	0.025	*
5-Methyl-2-phenyl-2-hexenal	21834-92-4			104	78.72	0.001	0.001	> 0.002	0.001	0.001	> 0.001	-	-	*
Miscellaneous compounds (17)														
Trimethylamine	75-50-3			58	5.41	1.36	0.653	2.1	0.752	0.959	0.528	2,795	1,921	
1-Vinylaziridine [†]	5628-99-9			41	14.81	0	0.001	> 0.002	0.001	-	-	-	-	*
2-Methyl-N-(2-methylbutylidene)-1-butanamine [†]	54518-97-7			98	23.21	0.224	0.259	0.619	0.452	-	-	-	-	*
N-isopentylidene isopentyl amine [†]	35448-31-8			98	25.03	0.319	0.283	1.164	0.61	-	-	-	-	*
limonene	138-86-3			68	27.21	0.001	0.001	>	-	-	-	-	-	*
(2-Chloroethyl)-benzene	622-24-2			91	53.46	0.001	0.001	0.002	0.002	-	-	-	-	*
1-Aminoadamantanet	768-94-5			94	54.51	0.004	0.001	0.008	0.002	0.001	0.001	-	-	*
3,4-Dihydro-2,5-dimethyl-2H-pyran-2-carboxaldehyde [†]	1920-21-4			111	59.74	0.006	0.001	0.007	0.002	0.006	0.003	0.019	0.013	*
3-Methylbutyl-2-phenylethylidene amine [†]	-			118	64.73	0.03	0.033	0.075	0.079	-	-	-	-	*
N-(3-methylbutyl)acetamide [†]	13434-12-3			73	67.67	0.009	0.012	0.033	0.038	-	-	-	-	*
3,5-di-tert-Butyl-4-hydroxytoluene (BHT)	128-37-0			205	70.75	0.009	0.004	0.023	0.006	0.002	0.001	0.015	0.01	*
2-Propyl-3-cyclohexyl-1,3,2-oxaazaborinane [†]	-			166	74.7	0.001	0.001	0.001	0.001	-	-	-	-	*

Table 1. continued

Compounds	cas no. ^a	References ^b	m/z ^c	RT ^d (min)	Delay Method				Regular Method				Statistical results between two groups					
					Group A location 1		Group B location 2		Group C location 1		Group C location 2		AB	CD	AC	BD	AD	BC
					Area ratio per dry wt (kg ⁻¹)	SD ^e	Area ratio per dry wt (kg ⁻¹)	SD	Area ratio per dry wt (kg ⁻¹)	SD	Area ratio per dry wt (kg ⁻¹)	SD						
2,4-Dimethylquinazoline [†]	703-63-9		158	78.81	0.005	0.003	0.006	0.004	-	-	-	-	-	-	-	-		
4-Methylquinazoline [†]	700-46-9		144	79.23	0.003	0.001	0.009	0.006	-	-	-	-	-	-	-	-		
Piperidinone [†]	27154-43-4		99	80.7	0.01	0.008	0.021	0.012	-	-	-	-	-	-	-	-		
2,4-bis(1,1-Dimethylethyl)phenol	96-76-4		191	90.06	0.011	0.003	0.017	0.003	0.002	0.001	0.005	0.005	0.005	*	*	*		
1H-indole	120-72-9	2	117	98.46	0.216	0.185	1.136	0.96	0.029	0.04	0.059	0.099	0.099	*	*	*		
Nitrogen-containing compound (4)																		
Pyridine	110-86-1	1	79	25.6	0.004	0.001 ^{>}	0.007	0.001	0.002	0.001	0.005	0.002	0.002	*	*	*		
2-Pentylfuran	3777-69-3	1	81	29.43	0.018	0.003	0.03	0.004	0.068	0.025	0.1	0.062	0.062	*	*	*		
2,3-Dihydro-2-(2-methylpropyl)-3,5-di(1-methylethyl)pyridine [†]	92900-69-1		149	60.05	0.001	0.002	0.006	0.008	-	-	-	-	-	-	-	-		
2-Phenyl-4-tertbutylpyridine [†]	53911-36-7		196	90.36	0.001 ^{>}	0.001 ^{>}	0.001	0.001 ^{>}	-	-	-	-	-	-	-	-		
Oxygen-containing compounds (3)																		
2-Ethylfuran	3208-16-0		81	12.62	0.021	0.001	0.019	0.003	0.085	0.029	0.156	0.09	0.09					
5-Ethylidihydro-2(3H)-furanone	695-06-7		85	59.12	0.004	0.002	0.008	0.003	0.005	0.003	0.017	0.014	0.014					
5-Ethyl-2(5H)-furanone [†]	2407-43-4		83	62.29	0.008	0.004	0.012	0.004	0.015	0.01	0.048	0.051	0.051					
Oxazole (1)																		
5-Ethyl-3-methylisoxazole	31301-34-5		111	62.09	0.001	0.001	0.002	0.003	-	-	-	-	-	-	-	-		
Ketones (15)																		
2-Pentanone	107-87-9		43	13.69	0.009	0.001	0.011	0.001	0.311	0.034	0.352	0.164	0.164	*	*	*		
2-Hexanone	591-78-6		58	19.26	0.002	0.001 ^{>}	0.002	0.001 ^{>}	0.001	0.001 ^{>}	0.002	0.001	0.001	*	*	*		
3-Methyl-3-buten-2-one	814-78-8		41	22.08	0.008	0.001	0.012	0.003	0.006	0.001	0.011	0.005	0.005	*	*	*		
2-Heptanone	110-43-0	1	43	25.96	0.075	0.01	0.109	0.004	0.028	0.006	0.045	0.022	0.022	*	*	*		
2-Octanone	111-13-7	1	43	33.01	0.016	0.004	0.034	0.006	0.01	0.002	0.019	0.009	0.009	*	*	*		
2-Methyl-2-cyclopentenone	1120-73-6		67	38.43	0.002	0.001 ^{>}	0.002	0.001	0.001	0.001 ^{>}	0.002	0.001	0.001	*	*	*		
2-Nonanone	821-55-6	1	58	40.06	0.008	0.001	0.015	0.003	0.007	0.002	0.011	0.007	0.007	*	*	*		
2-Cyclohexen-1-one	930-68-7		96	42.76	0.001	0.001 ^{>}	0.001	0.001 ^{>}	0.001	0.001 ^{>}	0.001	0.001 ^{>}	0.001 ^{>}	*	*	*		
2-Decan-2-one	693-54-9		58	46.87	0.01	0.002	0.017	0.005	0.013	0.02	0.007	0.004	0.004					
(E)-3,5-Octadien-2-one [†]	30086-02-3	3	95	51.44	0.015	0.002	0.028	0.002	0.019	0.007	0.037	0.013	0.013	*	*	*		
2-Undecanone	112-12-9	1	58	53.31	0.005	0.001	0.017	0.003	0.012	0.005	0.024	0.018	0.018	*	*	*		
3-Ethyl-2-cyclopenten-1-one [†]	19780-56-4		110	54.75	0.003	0.001	0.003	0.003	0.001	0.001	0.004	0.002	0.002	*	*	*		
1-Phenylethanone	98-86-2		77	56.23	0.005	0.001	0.01	0.002	0.003	0.001 ^{>}	0.003	0.001	0.001	*	*	*		
3-Ethylcyclohexanone [†]	22461-89-8		126	57.85	0.001	0.001 ^{>}	0.001	0.001 ^{>}	-	-	-	-	-	*	*	*		
2-Pentyl-2-cyclopenten-1-one [†]	25564-22-1		96	61.77	0.002	0.001 ^{>}	0.001	0.001 ^{>}	0.001	0.001 ^{>}	0.002	0.001	0.001	*	*	*		

Table 1. continued

Compounds	cas no. ^a	References ^b	m/z ^c	RT ^d (min)	Delay Method			Regular Method			Statistical results between two groups							
					Group A		Group B		Group C		Group C		AB	CD	AC	BD	AD	BC
					location 1	Area ratio per dry wt (kg ⁻¹)	SD ^e	Area ratio per dry wt (kg ⁻¹)	location 2	Area ratio per dry wt (kg ⁻¹)	SD	location 1						
Pyrazines (16)																		
Pyrazine	290-37-9	1	80	27.69	0.006	0.001	0.005	0.002	0.006	0.001	0.01	0.004			*	*		
2-Methylpyrazine	109-08-0	1	94	31.4	0.009	0.001	0.02	0.008	0.008	0.001	0.012	0.005			*	*		
2,5-Dimethylpyrazine	123-32-0	1	108	35.26	0.086	0.013	0.19	0.038	0.003	0.001	0.004	0.002	*		*	*		
2,6-Dimethylpyrazine	108-50-9	1	108	35.68	0.043	0.005	0.399	0.285	0.006	0.001	0.009	0.003			*	*		
2-Ethyl-6-methylpyrazine [†]	13925-03-6	2	121	39.53	0.042	0.009	0.075	0.012	0.002	0.001	> 0.003	0.002	*		*	*		
Trimethylpyrazine	14667-55-1	1	122	40.79	0.376	0.121	0.342	0.107	0.008	0.002	0.013	0.006	*		*	*		
3-Ethyl-2,5-dimethylpyrazine [†]	13360-65-1		135	43.56	0.622	0.182	0.704	0.356	0.001	0.001	> 0.005	0.009	*		*	*		
2,6-Diethylpyrazine [†]	13067-27-1		135	44.76	0.005	0.001	0.012	0.003	0.002	0.001	0.003	0.002	*		*	*		
2-Methyl-6-propylpyrazine [†]	29444-46-0		108	44.76	0.001	0.001	> 0.006	0.002	0.001	> 0.001	> 0.001	> 0.001	*		*	*		
Tetramethylpyrazine	1124-11-4	1	136	45.34	0.021	0.01	0.041	0.027	0.002	0.001	0.006	0.009	*		*	*		
2-Ethyl-6-methylpyrazine [†]	13925-09-2		120	46.35	0.002	0.001	> 0.005	0.002	0.001	> 0.001	> 0.001	> 0.001	*		*	*		
2,3,5-Trimethyl-6-ethylpyrazine [†]	17398-16-2		149	47.87	0.032	0.015	0.065	0.018	0.001	> 0.001	> 0.003	0.006	*		*	*		
2-(3-Methylbutyl)-3,5-dimethylpyrazine [†]	111150-30-2		122	56.61	0.013	0.004	0.027	0.006	-	-	-	-						
2-Butyl-3,5-dimethylpyrazine [†]	50888-63-6		122	59.45	0.002	0.001	0.006	0.002	-	-	-	-						
2,5-Dimethyl-3-n-butylpyrazine [†]	40790-29-2		122	84.69	0.003	0.001	0.006	0.004	-	-	-	-			*	*		
2,5-Dimethyl-3-(phenylmethyl)pyrazine [†]	73570-44-2		197	89.21	0.003	0.001	0.005	0.003	-	-	-	-			*	*		
Pyrroles (2)																		
1H-Pyrrole	109-97-7	1	67	48	0.01	0.003	0.014	0.003	0.003	0.001	0.01	0.006	*		*	*		
2-Acetylpyrrole	107-83-9		94	73.39	0.001	0.001	> 0.001	0.001	0.001	0.001	> 0.001	0.001	*		*	*		
Sulfur-containing compounds (13)																		
Dimethyldisulfide	624-92-0	1	94	18.86	0.213	0.169	0.906	0.513	0.004	0.003	0.011	0.021			*	*		
Dimethyltrisulfide	3658-80-8	1	126	39.35	0.043	0.025	0.228	0.15	0.001	0.002	0.008	0.007			*	*		
4-Methyl-pentan-2-one-4-thiol [†]	19872-52-7		132	47.49	0.001	0.001	> 0.001	0.001	-	-	> 0.001	> 0.001			*	*		
4-(Methylthio)-2-butanone [†]	34047-39-7		118	48.81	0.003	0.001	0.004	0.001	0.001	0.001	0.001	0			*	*		
1-(Methylthio)-3-pentanone [†]	66735-69-1		61	53.09	0.006	0.003	0.011	0.002	0.002	0.001	0.004	0.001			*	*		
2-Acetylthiazole	24295-03-2	1	127	55.99	0.001	0.001	> 0.001	0.001	0.001	0.001	> 0.001	> 0.001			*	*		
1,1-Bis(methylthio)ethanone [†]	7379-30-8		122	56.37	0.002	0.002	0.006	0.007	-	-	-	-			*	*		
Methyl methylthiomethyl disulfide [†]	42474-44-2		140	56.83	0.02	0.017	0.028	0.02	-	-	-	-			*	*		
Methyl 2-methyl-3-furyl disulfide [†]	65505-17-1		160	57.38	0.002	0.001	0.002	0.001	-	-	-	-			*	*		
3-(Methylthio)propanol	505-10-2		106	60.05	0.003	0.001	> 0.007	0.002	0.001	> 0.001	> 0.001	> 0.001			*	*		
1,2,4-Trithiolane	289-16-7		124	61.87	0.01	0.003	0.001	0.001	-	-	-	-			*	*		
N,N-Dimethylmethanethioamide	758-16-7		89	65.79	0.005	0.003	0.007	0.005	0.008	0.003	0.016	0.011	*		*	*		
2-Benzothiazole	95-16-9	1	135	72.75	0.003	0.001	0.006	0.002	0.001	> 0.001	> 0.002	0.001	*		*	*		

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