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Quantitative Analysis of Alkylpyrazines in Snow Crab Cooker Effluents

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Abstract

Alkylpyrazines in snow crab cooker effluent (SCCE) and effluent concentrate (EC) were quantitatively analyzed and compared by simultaneous steam distillation-solvent extraction/gas chromatography/mass spectrometry (SDE/GC/MS). A total of 11 pyrazines were identified in both SCCE and EC. Amounts of tetramethylpyrazine, 2,5-dimethylpyrazine, trimethylpyrazine, and 2,6-dimethylpyrazine were 23.0, 21.1, 13.8, and 13.3 times higher, respectively, in EC than those in SCCE. The total amount of pyrazines in EC (1664.0±171.1ng/g) was 8.1 times higher than that in SCCE (204.5±32.2). The compounds, ethylpyrazine and 2-ethyl-3,6-dimethylpyrazine, were only detected in EC.

Key words : quantitative analysis, alkylpyrazine, snow crab cooker effluent

INTRODUCTION

Snow crab, with its distinctive aroma and taste, has long been favored by consumers throughout the world, and is an important aquatic export of Korea. Most snow crab processing plants are located on the east coast Korea, and are limited to crab meat canning because of the highly perishable character of meat. In processing operations, however, snow crab cooker effluent produced during boiling has been discarded into the waterway causing a potential threat to the marine environment. These aqueous extracts are known to have taste-active flavor compounds such as amino acids, peptides, nucleotides, and organic acids (1,2). Furthermore, the compounds, amino acids and peptides were revealed to act as nitrogen sources in the formation of nitrogen containing heterocyclic compounds having aroma flavor (3,4). An important byproduct produced in snow crab processing, therefore, could be converted into a marketable flavor extract. The objectives of this study were to develop a quantification methods of alkylpyrazines and to determine the levels of alkylpyrazines in snow crab cooker effluents before and after concentration.

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MATERIALS AND METHODS

Materials

Legs, bodies and claws of washed snow crabs (*Chionoecetes japonicus*) were separated using a cutting machine (Daekwang Machine Co., Daegu, Korea) and then boiled in 3% (w/w) saline water for 3~4hr. The cooker effluent was filtered through a No. 14 sieve and concentrated within 3~4hr using a commercial single-pass spray type heat exchanger (Daehu In. Co., Ulgin, Korea) at 170° C. Snow crab cooker effluent (SCCE) and effluent concentrate (EC) were obtained from Namkwang Seafood Inc., Hupo, Korea and transported on ice in polyethylene bottles to the Dept. of Food Sci. & Nutrition, Changwon National University within 5hr and stored at -20° C prior to analysis. Standard flavor compounds were generous gifts from Aldrich Flavor and Fragrance (Aldrich Chemical Co., Milwaukee, WI).

Simultaneous steam distillation-solvent extraction (SDE)

Two liters of SCCE (or EC) and 2.0ml of an aqueous solution containing 90.784μg of internal standard (2,4,6-trimethylpyridine, TMP) were placed in a Likens-Nickerson (5) SDE apparatus (Cat. No. K-523010-

0000, Kontes, Vineland, NJ) and volatile flavor compounds were extracted into 100ml of redistilled diethyl ether. Further details have been reported elsewhere (6,7). Triplicate extractions were carried out for each sample.

Gas chromatography / mass spectrometry (GC / MS)

A 5-μl aliquot of each SDE extract was injected (splitless mode) into an HP 5792A GC/5970B mass selective detector (MSD) (Hewlett-Packard Co., Palo Alto, CA). Separation of volatile components was achieved on a fused silica capillary column (Supelcowax 10 ; 60m length × 0.25mm i.d. × 0.25μm film thickness ; Supelco, Inc., Bellefonte, PA). The linear velocity of the helium carrier gas was 25.7cm/sec. Oven temperature was programmed from 40° C to 175° C at a rate of 2° C/min with the initial and the final hold times of 5 and 30min, respectively ; oven temperature then further increased to 195° C at a rate of 5° C/min and maintained at 195° C for 25min. Electron ionization mass spectra were acquired with a mass range of m/z 33-300, ionization energy at 70eV, the electron multiplier voltage at 2000V, and a scan rate of 1.60 scans/sec. Other details of the MSD procedure have been described by Cha *et al.* (8). Duplicate analyses were performed on each SDE extract. Positive identification of the pyrazines was confirmed by using the authentic standards based on spectral patterns and chromatographic retention data.

Quantitative analysis of alkylpyrazines

Mass chromatography (9) was used for quantification of pyrazines. Characteristic m/z (mass/charge) values used to obtain mass chromatograms were as follows : 94 for methylpyrazine, 107 for ethylpyrazine, 108 for dimethylpyrazines, 121 for 2-ethyl-5-methylpyrazine and 2,4,6-trimethylpyridine (internal standard), 122 for trimethylpyrazine and 2-acethylpyrazine, 135 for 2-ethyl-3,5 (and 6)-dimethylpyrazines, and 136 for tetramethylpyrazine. Well resolved peak profiles were obtained and used for area integration, thus reducing the quantification errors from co-eluted compounds. Total ion peak area of a sample pyrazine compound was calculated as follows : total ion peak area of a sample pyrazine compound =total ion peak area of an authentic pyrazine compound × (mass chromatogram peak area of a sample pyrazine compound/mass chromatogram peak area of an authentic pyrazine compound). For each pyrazine compound, a calibration curve was obtained by plotting total ion peak area ratios of an authentic pyrazine compound to TMP, versus amount ratios of an authentic pyrazine compound to TMP (Table 1). Using these calibration curves, amount ratios of pyrazines to TMP present in a sample were calculated. The concentrations of pyrazines in the SCCE and EC were then calculated using the following equation :

$$C=\frac{(A_1/A_2)\times B}{W\times(1-\%\ \text{moisture content})}$$

where : C=concentration (ng/g) of a pyrazine compound present in the sample
A_1/A_2=amount ratio of a pyrazine compound to TMP in the SDE extract.
B=amount (ng) of TMP added to the sample
W=wet weight (g) of sample.

RESULTS AND DISCUSSION

Total ion chromatograms of volatile components in snow crab cooker effluent (SCCE) and effluent concentrate (EC) are shown in Fig. 1. A total of 11 pyrazines were identified. Combined mass chromatogram of these pyrazines identified in SCCE and EC are shown

Table 1. Standard curves of alkylpyrazine compounds

Compound	Equation
Methylpyrazine	Y^a=0.7039X^b − 0.0043
2,5-Dimethylpyrazine	Y=0.6634X + 0.0834
2,6-Dimethylpyrazine	Y=1.1158X + 0.0001
Ethylpyrazine	Y=0.7563X − 0.0046
2,3-Dimethylpyrazine	Y=0.7200X − 0.0029
2-Ethyl-5-methylpyrazine	Y=0.9390X − 0.0051
Trimethylpyrazine	Y=0.9154X + 0.0149
2-Ethyl-3,6-dimethylpyrazine	Y=1.0953X − 0.0076
2-Ethyl-3,5-dimethylpyrazine	Y=1.1522X − 0.0016
Tetramethylpyrazine	Y=0.7711X − 0.0001
2-Acethylpyrazine	Y=0.7281X − 0.0428

[a] Total peak area ratio of an authentic pyrazine compound to TMP
[b] Amount ratio of an authentic pyrazine compound to TMP

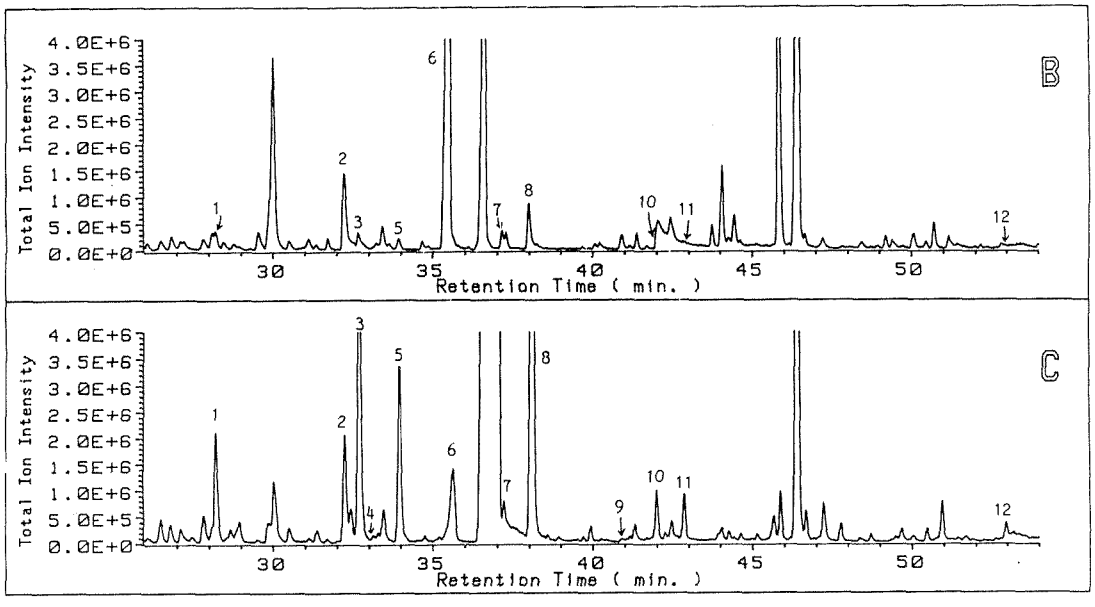

Fig. 1. Total ion chromatograms of volatile components in snow crab cooker effluent (B) and effluent concentrate (C).
1 : methylpyrazine, 2 : 2,5-dimethylpyrazine, 3 : 2,6-dimethylpyrazine, 4 : ethylpyrazine, 5 : 2,3-dimethylpyrazine, 6 : internal standard, 7 : 2-ethyl-5-methylpyrazine, 8 : trimethylpyrazine, 9 : 2-ethyl-3,6-dimethylpyrazine, 10 : 2-ethyl-3,5-dimethylpyrazine, 11 : tetramethylpyrazine, 12 : 2-acethylpyrazine.

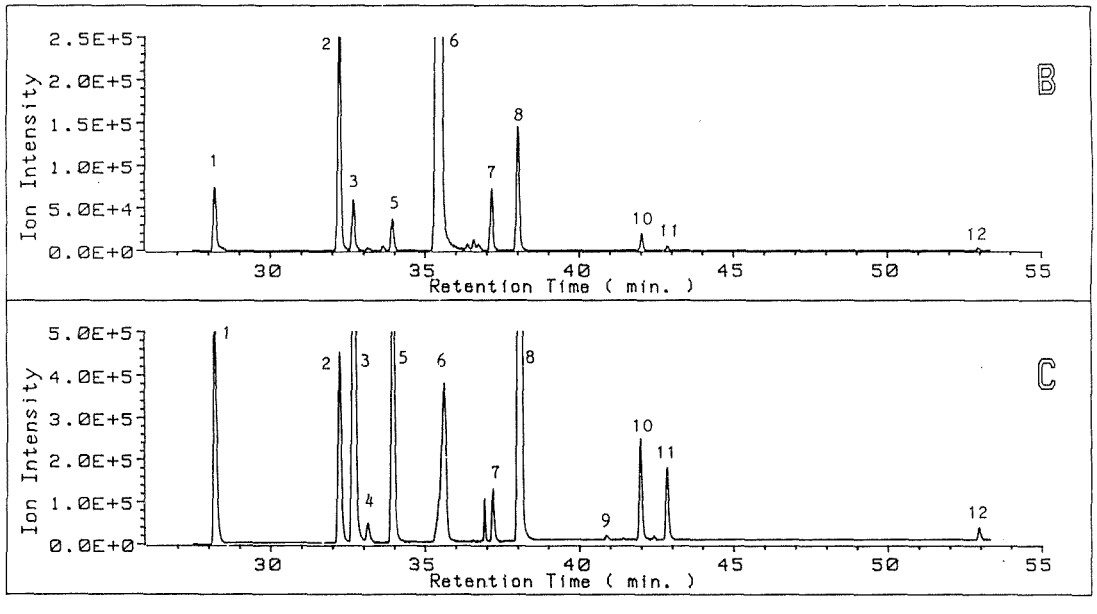

Fig. 2. Combined mass chromatograms of alkylpyrazines in snow crab cooker effluent (B) and effluent concentrate (C).
The peak numbers correspond to those listed in Fig. 1.

in Fig. 2 (B and C). Near baseline mass chromatographic separation was achieved and reduced errors in quantification.

As shown in Table 2, trimethylpyrazine was the most abundant among the 11 pyrazines detected in both samples, and followed by 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, methylpyrazine, and 2,5-dimethylpyrazine in EC. Similar results have been repor-

Table 2. Concentrations of pyrazine compounds in snow crab cooker effluent (SCCE) and effluent concentrate (EC)

Compound	SCCE		EC		Ratio[d]
Methylpyrazine	46.5[a]± 9.8[b]	22.7[c]	158.0[a]±11.8[b]	9.5[c]	3.4
2,5-Dimethylpyrazine	7.1 ± 3.0	3.4	149.4 ± 16.0	9.0	21.1
2,6-Dimethylpyrazine	20.4 ± 2.8	10.0	271.2 ± 20.5	16.3	13.3
Ethylpyrazine			14.6 ± 0.8	0.9	-
2,3-Dimethylpyrazine	26.3 ± 3.2	12.8	252.3 ± 24.6	15.2	9.6
2-Ethyl-5-methylpyrazine	37.7 ± 1.9	18.4	21.8 ± 0.5	1.3	0.6
Trimethylpyrazine	50.4 ±10.4	24.7	698.3 ± 86.7	42.0	13.8
2-Ethyl-3,6-dimethylpyrazine			2.7 ± 0.1	0.1	-
2-Ethyl-3,5-dimethylpyrazine	9.9 ± 1.0	4.8	41.9 ± 4.2	2.5	4.2
Tetramethylpyrazine	2.1 ± 0.0	1.0	46.8 ± 5.6	2.8	23.0
2-Acethylpyrazine	4.2 ± 0.1	2.1	7.2 ± 0.3	0.4	1.7
Total	204.5 ±32.2	99.9	1664.0 ±171.1	100.0	

[a] Concentration (ng/g)
[b] Standard deviation (n=6)
[c] Ratio of each compound to total alkylpyrazines
[d] Ratio of EC/SCCE

ted by Tanchotikul and Hsieh (10) in the dynamic headspace of crayfish waste. These pyrazines, with the lower weight aldehydes, enals, dienals, and ketones, may be significant contributors to the desirable flavor in aqueous extract of snow crab (6,11). Furthermore, trimethylpyrazine and 2,5-dimethylpyrazine have been known to be contributed to characteristic flavors in potato chips, roasted nuts, cocoa beans, baked potato, and peanut (12). The total concentration of alkylpyrazines was 8.1 times higher in EC than that in SCCE. The concentrations of compounds, e.g., trimethylpyrazine, methylpyrazine, 2-ethyl-5-methylpyrazine and 2,3-dimethylpyrazine were high in SCCE, while trimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine were in EC. Shibamoto and Bernhard (4) have reported that reaction temperature influenced total yield of pyrazines in model systems. It was supposed that many of these alkylpyrazines were thermally generated during concentration processing. The amount of pyrazines in EC was significant comparing to pyrazines in roasted cocoa beans (13) and potato chip (14).

The concentration ratio of each pyrazine compound in EC to that in SCCE showed an interesting pattern (Table 2). For example, the ratios of some pyrazines ranged from 1.7 to 4.2. Certain pyrazines, however, notably increased in concentration, in particular, tetramethylpyrazines, 2,5-dimethylpyrazine, trimethylpyrazine and 2,6-dimethylpyrazine (range : from 13.3 to 23.0). This result suggested that the formation of these pyrazines may be more favorable than that of other pyrazines at higher temperatures. The pyrazines, such as ethylpyrazine and 2-ethyl-3,6-dimethylpyrazine, were detected only in EC. Wong and Bernhard (15) reported that the distribution of pyrazines formed in the reactions containing ammonium hydroxide, ammonium formate, ammonium acetate, glycine, and monosodium glutamate depended strongly on the nature of the nitrogen source. Our distribution pattern was considered very similar to glucose-glycine model system, since the formation of trimethylpyrazine was related with the content of glycine among the nitrogen source (15). Glycine was reported most abundant amino acid in snow crab (1). Based on the amounts of alkylpyrazines, it was concluded that SCCE concentrate could be produced as a marketable flavor extract.

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홍게 자숙농축액 중의 Alkylpyrazines의 정량적 분석

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요 약

우리나라 동해안에서 생산되는 홍게 가공부산물인 자숙액을 열교환기로 농축하여 농축 전 후에 생성되는 고소한 향기성분인 alkylpyrazine 화합물들을 GC/MSD로 동정 및 정량분석하였다. 11종의 pyrazine이 동정되었는데 자숙액에서의 총 pyrazine 함량은 204.5±32.2ng/g이였고 농축 후의 총 함량은 1664.0±171.1ng/g으로 8.1배의 증가하였다. 자숙액에서는 trimethylpyrazine, methylpyrazine, 2-ethyl-5-methylpyrazine의 함량이 높았으나 농축 후에는 trimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine의 함량이 높았다. 그리고 ethylpyrazine 및 2-ethyl-3,6-dimethylpyrazine은 농축과정에서 생성된 새로운 pyrazine 화합물이었다.