

Aroma Extract Dilution Analysis of Blue Crab Claw Meat Volatiles[†]

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Volatile extracts were prepared from cooked blue crab (*Callinectes sapidus*) claw meat by both atmospheric (A-SDE) and vacuum simultaneous steam distillation-solvent extraction (V-SDE) techniques using dichloromethane as extractant. Serially diluted extracts were analyzed by gas chromatography/olfactometry (GC/O). Eleven and 14 aromas were consistently detected in replicate A-SDE and V-SDE extracts, respectively. Among these, seven aromas were commonly found in both extracts. Four compounds having high \log_2 (flavor dilution factors) (>5) were identified as 2,3-butanedione (sour, creamy); (*Z*)-4-heptenal (potato-like); 2-acetyl-1-pyrroline (nutty, popcorn-like); and 3-(methylthio)propanal (salty, soy sauce-like).

Keywords: *Blue crab; flavor; volatiles; aroma extract dilution analysis*

INTRODUCTION

The volatile flavor of cooked blue crab (*Callinectes sapidus*) has been investigated by various researchers (Rayner et al., 1981; Matiella and Hsieh, 1990; Chung and Cadwallader, 1993). However, compounds essential for authentic crab flavor have not been determined. One procedure used to establish predominant flavor compounds in foods is aroma extract dilution analysis (AEDA) (Gasser and Grosch, 1988; Blank and Grosch, 1991) in which a mixture of flavor compounds, or flavor extract, is separated by gas chromatography and the odor activity of each component is evaluated by olfactometry. The value of odor activity is dimensionless but is expressed as a flavor dilution (FD) factor in AEDA, which is defined as the ratio of the concentration of a compound in the initial extract to that in the most diluted extract in which the odor was detected by gas chromatography/olfactometry (GC/O) (Blank and Grosch, 1991). FD factors are used to compare intensities of odor-active compounds in an extract. The objective of the present study was to identify and compare the predominant odorants in cooked blue crab claw meat by atmospheric (A-SDE) and vacuum simultaneous steam distillation-solvent extraction (V-SDE) and AEDA.

MATERIALS AND METHODS

Materials. A total of 5 lb of freshly cooked blue crab claw meat was purchased in the summer of 1992 from two seafood outlets in Baton Rouge, LA. At the outlets, original samples were kept in ice in a refrigerated shelf. All samples in 1-lb plastic containers were transported on ice to the Louisiana State University Food Science Department and stored (<24 h) at 4 °C until extracted.

All standard compounds were purchased from Aldrich Chemical Co. (Milwaukee, WI), except for 2-acetyl-1-pyrroline (2-AP), obtained from Dr. R. Buttery (USDA, ARS, WRRRC, New Albany, CA), and (*Z*)-4-heptenal, purchased from Alfa (Ward Hill, MA).

Atmospheric Simultaneous Steam Distillation-Solvent Extraction (A-SDE). Five hundred grams of sample plus distilled water [1:2 (w/v)] was extracted for 2 h with redistilled dichloromethane (50 mL) in a Likens and Nickerson (1964) type A-SDE apparatus (catalog no. K-523010-0000, Kontes, Vineland, NJ) as described by Tanchotikol and Hsieh (1989). Extract was dried over 3 g of anhydrous sodium sulfate and concentrated under a stream of ultrahigh purity nitrogen to 1 mL. Extract was stored at -80 °C in a 2-mL amber vial equipped with a Teflon-lined screw cap until analyzed. Duplicate A-SDE extractions were performed.

Vacuum Simultaneous Steam Distillation-Solvent Extraction (V-SDE). The extraction procedure was similar to that described for A-SDE, except that an A-SDE apparatus modified for vacuum extraction was used. The modifications from the standard SDE apparatus were as follows: (1) a vacuum valve replaced the U-joint valve; (2) a liquid nitrogen cold trap and a vacuum valve were installed between V-SDE and the vacuum pump; and (3) a 4-L, three-neck, round-bottom sample flask was modified with one neck for the vacuum valve and one for the thermometer. Vacuum and temperature were maintained at 30 in. of Hg and 45-60 °C, respectively. Extract was collected after 2 h, concentrated under a stream of nitrogen gas to 10 mL, dried over 3 g of anhydrous sodium sulfate, and further reduced to 1 mL. Extract was stored at -80 °C as previously described. Duplicate V-SDE extractions were performed.

Gas Chromatography/Mass Spectrometry and Flame Ionization Detection/Olfactometry (GC/MS-FID/O). For qualitative analysis, an analytical system consisting of an HP 5790A GC coupled with both a flame ionization detector (FID) and an HP 5970B mass selective detector (MSD) (Hewlett-Packard Co., Palo Alto, CA) was used. Five microliters of each extract was injected (splitless mode; 200 °C injector temperature; 1-min valve delay) simultaneously into dual (closely matched) fused silica open tubular (FSOT) columns (Supelco-wax 10, 60 m length \times 0.25 mm i.d. \times 0.25 μ m film thickness; Supelco, Inc., Bellefonte, PA). Injector effluent, after passing through a FSOT precolumn (uncoated and deactivated, 1 m length \times 0.25 mm i.d.), was split 1:1 to each column using a glass Y-splitter. Column A was connected to the MSD, while the end of column B was split 1:1 to an FID and a sniffing port. Humidified air was mixed with the GC effluent in a transfer line near the outlet to the sniffing port. The temperatures of the FID and the sniffing port transfer line were 250 and 200 °C, respectively. Helium was used as the carrier gas and maintained at a linear velocity of 25 cm/s. It was necessary to partially restrict the flow between column A and MSD (using \sim 1 m length \times 0.1 mm i.d. FSOT column) to achieve the same linear velocity for both columns. Oven temperature was programmed from 40 to 195 °C at a ramp

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rate of 2 °C/min. The initial and final hold times were 5 and 40 min, respectively. MSD conditions were as follows: capillary direct MS interface temperature, 200 °C; ion source temperature, 200 °C; ionization voltage, 70 eV; mass range, 33–290 amu; scan rate, 1.67 scans/s; and electron multiplier voltage, 1800 V.

Aroma Extract Dilutions. Serial dilutions were prepared from the 1.0-mL concentrated extracts in the ratio of 1:1 in dichloromethane. Each dilution was transferred into separate 2-mL amber vials equipped with Teflon-lined screw caps and stored at –80 °C until analyzed.

Gas Chromatography/Olfactometry (GC/O). The GC/O system consisted of an HP 5790A GC (Hewlett-Packard) equipped with an FSOT column (Supelcowax 10; 60 m × 0.32 mm i.d. × 0.25 μm film thickness; Supelco), an FID, and a sniffing port. Each dilution (5 μL) was injected in the splitless mode. GC conditions were the same as described above. GC/O was performed by two panelists familiar with crab flavor and the olfactometric technique.

Data Reduction. The criterion in the reduction process of each detected aroma was based on the number of times it was detected in all of the FD chromatograms from the same extract. Each aroma note was counted on the basis of its detection at the same retention index and with the same aroma attribute under identical GC/O conditions. In the present experiment, an aroma note with a count of two or less from four FD chromatograms was not considered significant and would be dropped from the final calculation of the average log₂ (FD factor). The criterion was arbitrarily set since there is no standard technique in the literature. However, considering that the determination of the odor threshold of a compound requires 50% of the panelists to recognize the compound (ASTM, 1968), a similar criterion was adopted and applied to this experiment.

Estimation of Average FD Factor. To summarize the GC/O results for each odorant, the average FD factor of each aroma note was estimated by the arithmetic mean (N) of the log₂(FD factor). N is readily converted to average FD factor by the equation average FD factor = 2 ^{N} .

Compound Identification. Identifications were based on comparison of GC retention indices (RI) (van den Dool and Kratz, 1963), mass spectra, and aroma properties of unknowns with those of authentic standard compounds analyzed under identical experimental conditions.

RESULTS AND DISCUSSION

Comparison of A-SDE and V-SDE Extracts. The average number of odor-active compounds detected in four original FD chromatograms from A-SDE extracts of claw meat was 39 and ranged from 31 to 44 compounds, while an average number of 29 compounds, ranging from 18 to 41, was detected in V-SDE extracts of claw meat. During both extractions, thermal generation of some artifacts was possible. Since higher temperatures were reached during A-SDE, this might promote the production of a wide variety of aroma compounds, thus resulting in a higher average number of detected aroma notes (39) compared with V-SDE (29). After data reduction, the numbers of odorants that represented a more consistent view of aroma notes isolated by A-SDE and V-SDE were 11 and 14, respectively (Table 1). This seemed to reflect the randomness of the artifact generation, especially during A-SDE.

Since the original crab aroma was thermally generated during cooking, important and representative crab claw meat aroma compounds were assumed to be stable and to exist in each extract. Seven common aroma notes were found in both A-SDE and V-SDE extracts (Table 1). They were described as sour, creamy (no. 4); alkaline, raw egg-like (no. 5); potato-like (no. 7); nutty, popcorn-like (no. 10); nutty (no. 11); salty, soy sauce-like (no. 12); and dry seaweed-like, fishy (no. 16).

Table 1. Odorants Detected in Atmospheric and Vacuum Simultaneous Steam Distillation–Solvent Extracts of Blue Crab Claw Meat

no.	aroma description	RI/RT ^a	av ^b log ₂ (FD factor)		compd
			A-SDE	V-SDE	
1	fishy	(4.2 min)	5.50	– ^c	trimethylamine
2	natural gas	(7.3 min)	–	3.50	unknown
3	solvent	(8.0 min)	–	4.00	unknown
4	sour/creamy	(9.4 min)	7.50	6.25	2,3-butanedione
5	alkaline/ raw egg	1006	3.67	3.00	pyrrolidine
6	burnt/sweet	1081	–	6.00	unknown
7	potato	1238	6.25	6.75	(<i>Z</i>)-4-heptenal
8	paint	1255	2.67	–	unknown
9	herbal	1284	5.25	–	unknown
10	nutty/popcorn	1331	8.50	7.50	2-acetyl-1-pyrroline
11	nutty	1418	5.75	2.50	unknown
12	salty/soy sauce	1453	8.00	6.50	3-(methylthio)-propanal
13	moldy	1486	–	5.25	unknown
14	potato	1535	–	4.00	(<i>E</i>)-4-decenal
15	green	1579	–	3.25	unknown
16	dry seaweed/ fishy	1726	6.75	5.25	unknown
17	fishy	1752	4.75	–	unknown
18	dry seaweed	1963	–	4.00	unknown

^a RI, retention index; RT, retention time. Numbers in parentheses represent average retention times. ^b A-SDE; atmospheric simultaneous steam distillation–solvent extract. V-SDE, vacuum simultaneous steam distillation–solvent extract. ^c –, odorant was not detected.

Except for the potato-like note (no. 7), all other common notes had higher average FD factors in A-SDE extracts than in V-SDE extracts. The nutty, popcorn-like note (no. 10) had the highest average FD factor in each average FD chromatogram. Both extracts contained strong impressions of nutty and raw potato-like aromas mixed with dry seaweed-like aromas. Although Table 1 indicated that only a few potato-like and seaweed-like aromas were detected, the original FD chromatograms (not shown) contained a substantial number. However, many of these were eliminated after data reduction.

Identity of Aroma Notes. Two compounds, identified as (*Z*)-4-heptenal (no. 7) and 2-acetyl-1-pyrroline (2-AP) (no. 10), had potato-like and nutty, popcorn-like aromas, respectively. Chung and Cadwallader (1993) identified (*Z*)-4-heptenal in freshly cooked crab meat. Some investigators have suggested the accumulation of (*Z*)-4-heptenal in certain foods (i.e., cod and soybean oil) was undesirable (Seals and Hammond, 1970; McGill et al., 1974). However, its presence in freshly cooked crab meat might be desirable. McGill et al. (1974) described the aroma of (*Z*)-4-heptenal as being similar to that of boiled potatoes, and its threshold was determined to be 0.04 ppb.

For the first time, 2-AP was identified in blue crab. This compound has been associated with the popcorn, nutty aroma of scented rice (Buttery et al., 1983). These investigators suggested that 2-AP was the most potent compound among the “cracker-like” group of odor compounds and reported its odor threshold in water as 0.1 ppb. Comparison of the presence of 2-AP between raw and cooked scented rice by Buttery et al. (1983) suggested it was thermally generated during cooking. Griffith and Hammond (1989), in an attempt to generate Swiss cheese flavor by reacting amino acids and carbonyl compounds at room temperature, produced 2-AP from several mixtures, e.g. glyoxal and lysine. In the present study, 2-AP had the highest average FD factor

in both types of extracts, which seemed to reflect its importance to claw meat aroma.

The compound 2,3-butanedione was among the strongest notes detected in both extracts, with a threshold of 2.6 ppb in water (Fors, 1983). It is a characteristic product in cooked food and is thermally generated through the Maillard reaction (Hodge, 1967). Chung and Cadwallader (1993), using A-SDE, detected high average concentrations of 2,3-butanedione in both freshly cooked blue crab meat and its processing byproduct (CPB) at 30 and 29 ng/g, respectively. However, Hsieh et al. (1989) and Matiella and Hsieh (1990) did not detect 2,3-butanedione when using dynamic headspace sampling (DHS). The discrepancy might be due to the type as well as the conditions of the extraction procedures employed.

Pyrrolidine (no. 5), having an alkaline, raw egg-like aroma, was identified for the first time in blue crab; however, its aroma was among the weakest found in both samples. Its odor threshold is 0.15 ppm (Devos, 1990). Pyrrolidine has been detected in seawater (Yang et al., 1993) and can be formed biologically by bacteria, e.g. *Clostridium perfringens*, under low sugar conditions, as well as chemically by decarboxylation of proline via the Strecker degradation (Allison and MacFarlane, 1989; Griffith and Hammond, 1989). This compound was reported as a volatile component in whiskey and alfalfa (Viro, 1984; Srinivas, 1988). Recently, Okumura et al. (1993) proposed the use of pyrrolidine and other additives for prolonging the alkali and amine odor in Chinese noodles.

A salty, soy sauce-like aroma (no. 12) was detected that had a characteristic crab meat quality. The compound responsible for this aroma was identified as 3-(methylthio)propanal (3-MP), and its existence is being reported for the first time in blue crab. 3-MP has a low odor threshold of 0.2 ppb (Guadagni et al., 1972) and could be formed via the Maillard reaction from methionine (Morton, 1960).

(*E*)-4-Decenal was detected in V-SDE extracts only and was described as potato-like. Although this compound contributed to the potato-like aroma in V-SDE extracts, its importance to the overall aroma of crab meat is questionable since it was absent in the A-SDE extracts. Ames and MacLeod (1984) detected (*E*)-4-decenal in unflavored textured soy protein and proposed that the compound originated from either lipid oxidation or lipid degradation. Hsu et al. (1982) also detected this compound in the neutral fraction of roasted beef.

Trimethylamine (TMA) has been described in the literature as ammonia-like and fishy (Dravnieks, 1985). This odorant was the earliest to elute during GC/O and was detected only in the A-SDE extracts. Since the volatility of TMA is high, extraction of it by V-SDE might not be as efficient as that by A-SDE. Chung and Cadwallader (1993) reported that similar amounts of TMA were found in the A-SDE of both fresh claw meat and CPB. Its odor threshold is 2.40 ppb (Devos et al., 1990). TMA is not present in fresh muscle but will increase during the postmortem bacterial reduction of trimethylamine oxide (TMAO) (Yamada, 1967). Blue crabs contain about 65 mg/100g of TMAO in their muscle (Yamada, 1967).

Two unidentified and seemingly predominant aromas were detected in both extracts and were described as nutty (RI = 1418, no. 11) and dry seaweed-like (RI = 1726, no. 16). These compounds could not be identified due to their low concentrations.

On the basis of the results of this study, the aroma or volatile flavor of blue crab meat could be defined based principally on seven individual compounds including 2,3-butanedione, pyrrolidine, (*Z*)-4-heptenal, 2-acetyl-1-pyrroline, 3-(methylthio)propanal, and two unknowns. Many of these compounds are identified for the first time in crab claw meat, which seems to indicate that previous attempts by others, such as Hsieh et al. (1989) and Matiella and Hsieh (1990), at defining crab aroma failed to recognize those important compounds responsible for much of the characteristic crab claw meat aroma. Most of these compounds have extremely low threshold values and are more effectively detected by GC/O. Their low concentrations probably contributed to their lack of detection by other GC detectors.

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