J. Korean Soc. Food Nutr.
24(3), 420~426(1995)

Volatile Compounds in Oyster Hydrolysate Produced by Commercial Protease

Yong-Jun Cha

Dept. of Food Science & Nutrition, Changwon National University, Changwon 641-773, Korea

Abstract

Volatile compounds in raw oyster and oyster hydrolysate produced with protease were compared by vacuum simultaneous steam distillation-solvent extraction/gas chromatography/mass spectrometry. Sixty-two volatile compounds were detected in both samples. Of these, 57 were positively identified, composed mainly of aldehydes (12), ketones (9), alcohols (14), nitrogen-containing compounds (9), acids (6), terpenes (4), and miscellaneous compounds (8). Levels of acids decreased after hydrolysis, whereas several other compounds such as aldehydes, ketones, and nitrogen containing compounds increased. Pyrazines, found in high abundance, were only detected in oyster hydrolysate.

Key words : volatile compounds, oyster, oyster hydrolysate, commercial protease

INTRODUCTION

Oyster has been known to have a special taste and flavor which are different from those of other marine crustaceans and bivalvia, such as lobster, crab, shrimp, clam and scallop, etc (1). Oyster, having a high level of taurine, has been also recognized as a healthy food for humans (1). However, most of oysters harvested in a summer period is generally processed into a canned product because of the low taste compounds and the potential health hazard. Furthermore, the aqueous waste produced in many oyster plants has been discharged into waterways causing a threat to the environment. An alternative use for unconsumed oyster, therefore, may be a fundamental benefits to both seafood industry and public health in USA. A better understanding of volatile flavor in oyster hydrolysate is needed if alternative products are to be produced.

The objectives of this study were to indentify and compare the volatile flavor compounds in oyster hydrolysate produced via enzymatic hydrolysis which acts a as flavoring agent, compared with raw oyster.

MATERIALS AND METHODS

Materials

Fresh oyster (*Crassostrea virginica*) obtained from Motivatit Seafood Inc.(Homa, LA, USA) were packed in ice and transported within 2 hr to the Food Science Department, Lousiana State University and stored at 4°C. Before analysis, oyster was homogenized using a Waring blender (Waring Products Co., Winsted, CT). Oyster hydrolysate used in this study was produced with a commercial protease, APL™ 440 (Solvay Enzyme Inc., USA) under conditions of pH 8.8, 67°C, 30% substrate concentration (%, w/w), 0.39% enzyme/substrate ratio (%, v/w) and 2.8 hr reaction time. More detail procedures have been described by Cha *et al.* (2,3). Standard flavor compounds were purchased from commercials or were generous gifts from Aldrich Flavor and Fragrance (Aldrich Chemical Co., Milwaukee, WI).

Vacuum simultaneous steam distillation-solvent extraction (Vacuum-SDE)

SDE was carried out under vacuum (ca. 24~26 in. Hg ; b.p. 45~60°C) to minimize artifacts from forming during extraction. Two vacuum valves were installed at U-joint and on the top portion of SDE apparatus (Cat. No. K-523010-0000, Kontes, Vineland, NJ), respectively. Liquid nitrogen cold trap was also set between SDE apparatus and vacuum pump to minimize solvent draining into pump. Details are described by Chung and Cadwallader (4) Extraction was performed on 500ml of oyster hydrolysate (or 500g of raw

oyster homogenate) using vacuum SDE apparatus.

Sample plus 90.784μg of internal standard (2,4,6-trimethylpyridine) were extracted for 3 hr with 100ml of redistilled diethyl ether. The procedures are described elsewhere (5,6). Duplicate extractions were carried out for each sample.

Gas chromatography / mass spectrometry (GC / MS)

GC/MS system consisted of a Hewlett-Packard 5790 GC/HP 5970B mass selective detector (MSD) (Hewlett-Packard Co., Palo Alto, CA). Three μl of each SDE extract were injected in the splitless mode (155° C injector temperature; 30 sec valve delay) into a fused silica open-tubular (FSOT) column (Supelcowax 10, 60m length × 0.25mm i.d. × 0.25μm film thickness; Supelco, Inc., Bellefonte, PA). Oven temperature was programmed from 40° C to 175° C at a rate of 2° C/min with initial and final hold times of 5 and 30min, respectively; oven temperature was then further increased to 195° C at a rate of 5° C/min and maintained for 25 min. Other details of MSD condition and GC/MSD procedure have been described by Cha *et al.* (5,7). Duplicate analysis were performed on each SDE extract.

Identification and relative abundance of compounds

Volatile compounds were identified by matching retention indices (RI) (8) and mass spetra of unknown with those authentic standard compounds. Tentative identification were based on standard MS library data (Hewlett-Packard Co., 1988) (9). The relative abundance of each compound was expressed by the ratio of its total ion peak area to that of the internal standard (TMP). The peak area co-eluted compounds was calculated as described by Hites and Biemann (10) to minimize chromatographic interference.

RESULTS AND DISCUSSION

Oyster hydrolysate, with raw oyster, was analyzed for volatile flavor components to assess the commercial feasibility of using hydrolysate as a seasoning ag-

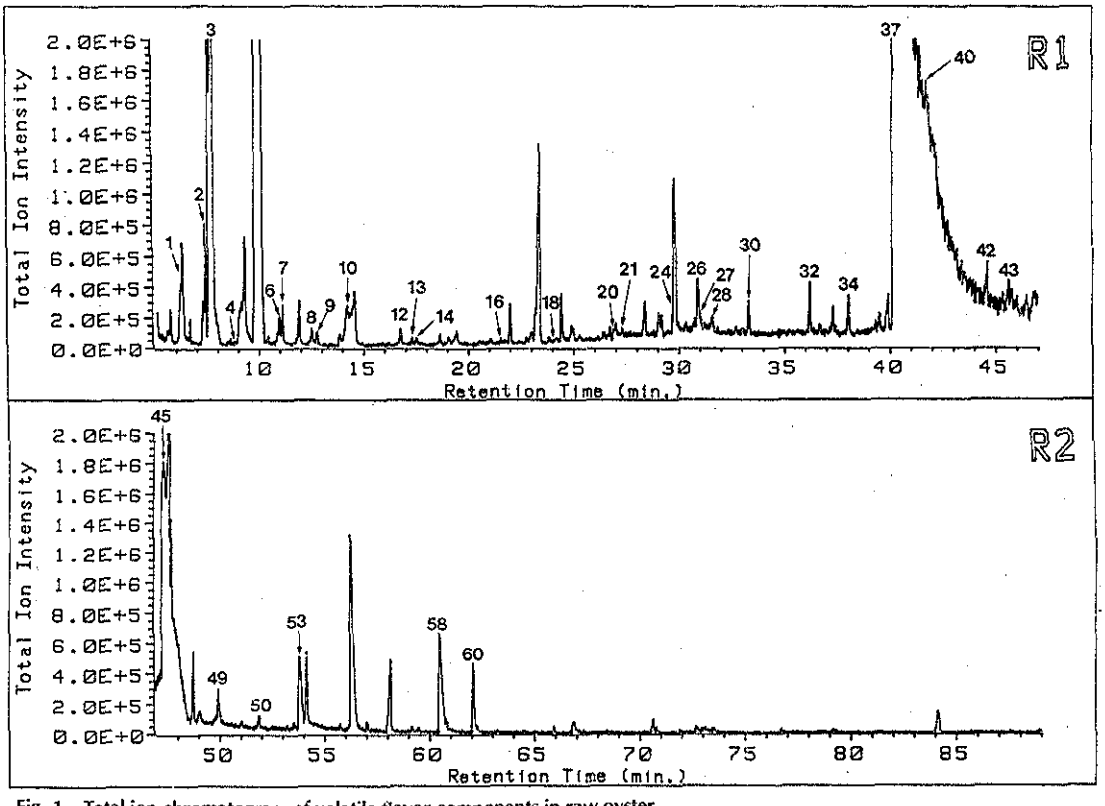

Fig. 1. Total ion chromatogram of volatile flavor components in raw oyster.
The peak number correspond to those listed in Table 1.

ent. Total ion chromatograms of volatile components in oyster hydrolysate and raw oyster are shown in Fig. 1 and 2, respectively. The presence of 62 volatile compounds was established (Table 1), including 12 aldehydes, 9 ketones, 14 alcohols, 9 nitrogen-containnig compounds, 6 acids, 4 terpenes, and 8 miscellaneous compounds. Among these, 56 compounds were positively identified. As shown in Table 1, however, 32 compounds were detected in raw oyster, while 52 compounds were detected in oyster hydrolysate, indicating some volatile compounds formed during hydrolysis.

Twelve aldehydes were identified in hydrolysate, while only 8 in raw oyster. The amounts of aldehydes increased 4.6 times in hydrolysate compared with raw oyster. Some of aldehydes, such as 3-methylbutanal, nonanal, benzaldehyde and (E)-2-nonenal, were only detected in hydrolysate. Benzaldehyde, having a pleasant almond, nutty and fruit aroma (11), have been known to be thermally generated and partially contributed to characteristic cooked crab flavor (12). The presence of alkanals and alkenals detected in both samples might have been due to oxidation of polyunsaturated fatty acids (13,14). Furthermore, Ho *et al.* (15) reported that certain aldehydes which do not contribute to good flavor could act as important flavor precursors for creating good aromas. Hexanal detected in both samples was reported to have a distinct coarse and green plant-like aroma in the most of fresh fish (16).

Although a total amounts of ketones was not greatly changed, the number of ketones idnetified did increase after hydrolysis reaction. Kubota *et al.* (17) reported that carbonyl compounds, resulting mainly from lipid and amino acid degradation, contributed a fairly strong seaweed-like odor to cooked krill.

Among 14 alcohols identified, 7 compounds were found in raw oyster and 9 in oyster hydrolysate. The amount of alcohols in raw oyster, however, did decrease during hydrolysis. Two alcohols, 1-penten-3-ol and 1-octen-3-ol, were reported to contribute a heavy, plant-like aroma to oyster (18). Alcohols may not

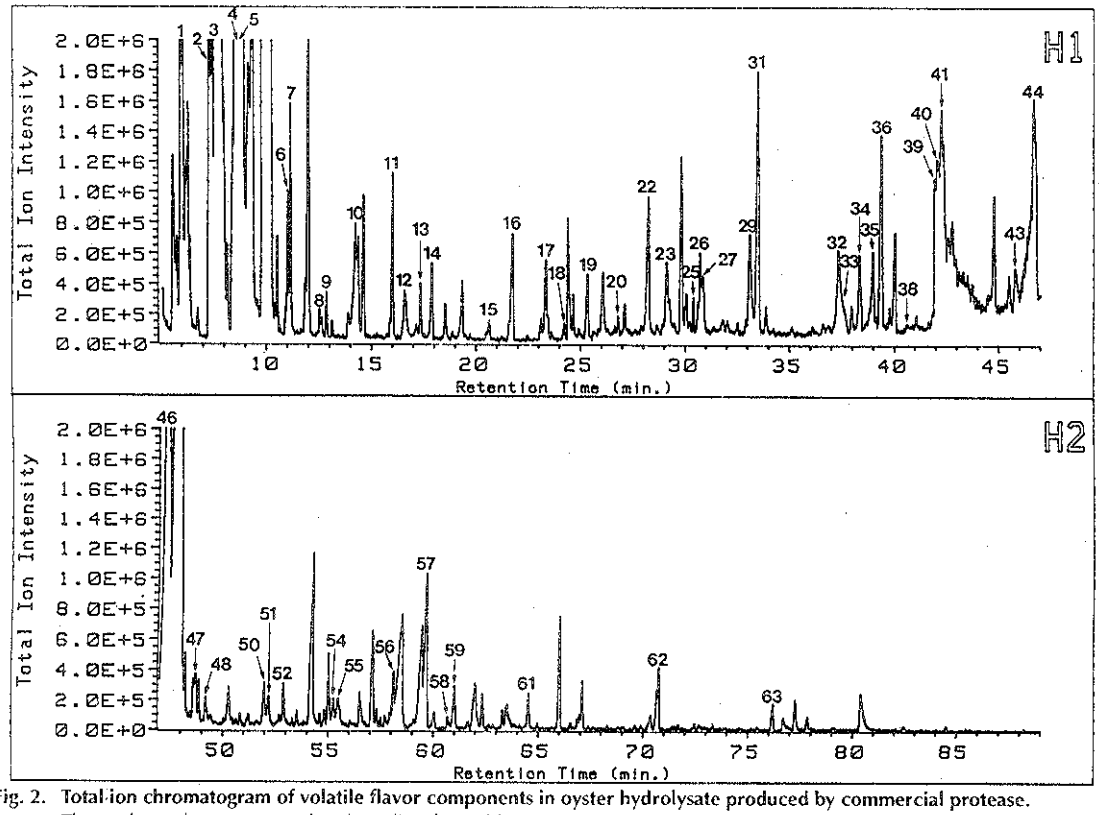

Fig. 2. **Total ion chromatogram of volatile flavor components in oyster hydrolysate produced by commercial protease.** The peak number correspond to those listed in Table 1.

Table 1. Volatile flavor components in oyster hydrolysate produced with commercial protease

Peak No.	Compound name by class	Retention index		Raw		Hydrolysate	
		Spl	Std	Mean area ratio	S.D.	Mean area ratio	S.D.
	Aldehydes (12)						
1	2-Methylpropanal	813	810	3.07	2.17	1.45	0.92
2	Butanal	856	858	3.75	2.62	3.83	1.94
4	2-Methylbutanal	903	905	0.17	0.08	12.42	6.67
5	3-Methylbutanal	911	914			16.02	7.74
7	Pentanal	975	976	0.64	0.22	1.01	0.35
10	(E)-2-Butenal	1037	1037	1.68	1.22	1.50	0.29
12	Hexanal	1077	1079	0.52	0.37	0.37	0.31
13	2-Methyl-(E)-2-butenal	1090	1093	0.17	0.11	0.23	0.10
27	Octanal	1286	1290	0.15	0.12	0.18	1.17
35	Nonanal	1403	1399			0.56	0.44
46	Benzaldehyde	1526	1526			8.25	3.07
47	(E)-2-Nonenal	1543	1543			0.20	0.12
	Ketones (9)						
6	2-Pentanone	970	971	0.80	0.23	0.61	0.34
8	4-Methyl-2-pentanone	1008	1007	0.69	0.41	0.19	0.03
17	2-Heptanone	1179	1182			0.35	0.37
26	3-Hydroxy-2-butanone	1284	1290	1.17	0.31	0.49	0.35
34	2-Nonanone	1394	1389	0.67	0.29	0.64	0.34
50	2-Undecanone	1594	1594	0.17	0.17	0.24	0.19
54	Acetophenone	1647	1648			0.11	0.14
61	2-Tridecanone	1803	1810			0.23	0.14
63	2-Tetradecanone*	2011				0.36	0.19
	Alcohols (14)						
15	Butanol	1139	1140			0.12	0.04
16	1-Penten-3-ol	1156	1156	0.17	0.11	0.76	0.35
21	2-Hexanol	1234	1224	0.14	0.16		
22	Pentanol	1249	1250			1.00	0.52
24	1-Hexen-3-ol	1268	1261	0.43	0.25		
28	(Z)-2-Penten-1-ol	1300	1309	0.43	0.41		
30	(E)-2-Penten-1-ol	1321	1317	0.53	0.31		
40	1-Octen-3-ol	1448	1449	1.19	1.21	0.52	0.59
42	2-Ethyl-1-hexanol	1487	1484	2.16	3.33		
48	Octanol	1552	1554			0.10	0.08
52	2-Decanol	1608	1618			0.16	0.15
57	2-Undecanol	1719	1723			1.10	0.67
59	Decanol	1742	1744			0.42	0.21
62	2-Phenylethanol	1915	1910			0.36	0.22
	N-Containing compounds (9)						
19	Pyrazine	1208	1208			0.54	0.20
23	Methylpyrazine	1262	1261			0.44	0.35
29	2,5-Dimethylpyrazine	1318	1317			1.88	0.53
31	2,6-Dimethylpyrazine	1324	1324			0.87	1.38
32	2,4,6-Trimethylpyridine (I.S.)**	1367	1366	1.00	0.00	1.00	0.00
36	Trimethylpyrazine	1409	1402			1.11	0.83
39	2-Ethyl-3,6-dimethylpyrazine	1446	1443			0.65	0.54
41	2-Ethyl-3,5-dimethylpyrazine	1451	1455			0.46	0.70
44	1H-Pyrrole	1515	1516			1.20	1.38
55	3-Butyl-2,5-dimethylpyrazine*	1650				0.15	0.21

Continued

Table 1. Continued

Peak No.	Compound name by class	Retention index		Raw		Hydrolysate	
		Spl	Std	Mean area ratio	S.D.	Mean area ratio	S.D.
	Acids (6)						
37	Acetic acid*	1421		22.79	10.41		
45	Propionic acid*	1522		10.59	7.94		
49	Isobutyric acid	1567	1569	0.57	0.35		
53	Butyric acid	1624	1628	1.64	0.64		
58	Valeric acid	1737	1737	2.89	1.31	0.04	0.04
60	2-Methylpentanoic acid*	1764		1.08	0.44		
	Terpenes (4)						
9	alpha-Pinene	1014	1010	0.41	0.29	0.16	0.09
14	beta-Pinene	1098	1088	0.29	0.05	0.36	0.21
18	Limonene	1192	1197	0.19	0.09	0.10	0.05
25	alpha-Terpinolene	1279	1276			0.15	0.08
	Miscellaneous compounds (8)						
3	Ethylacetate	874	880	52.41	21.74	10.17	56.92
11	Dimethyldisulfide	1068	1069			0.78	0.23
20	2-Pentylfuran	1228	1230	0.07	0.02	0.11	0.08
33	Dimethyltrisulfide	1379	1381			0.41	0.42
38	Dimethyloctanylacetate*	1427				0.07	0.05
43	Pentadecane	1501	1500	3.46	4.87	2.40	1.52
51	Hexadecane	1597	1600			0.25	0.15
56	Heptadecane	1696	1700			1.19	1.05

* Tentatively identified by MS library data

** *I.S.=Internal standard*

Mean area ratio=Compound peak area/I.S. peak area from the average of 2 vacuum SDE extractions, and 2 injections of each extract

S.D.=Standard deviation of mean area ratio

contribute to the overall flavor of oyster hydrolysate because of their high threshold values (19).

Eight alkylpyrazines except 1 H-pyrrole were only detected in hydrolysate. These heterocyclic compounds might have to be formed by Maillard and pyrolysis reactions during hydrolysis at 67° C (2,13,20). 2,5-dimethylpyrazine was the highest concentration in hydrolysate, followed by trimethylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine, pyrazine and 2-ethyl-3,5-dimethylpyrazine. Pyrazines were reported to contribute to characteristic cooked flavor of snow crab meat flavor to (12). Pyrazines also have been known to contribute to nutty, roasted, and toasted characteristics to foods (21).

Among acids detected in both samples, 6 acids were found in raw oyster and only 1 in hydrolysate. Acetic acid, in particular, was the highest amount in raw oyster, followed by propionic acid, valeric acid, butyric acid, 2-methylpentanoic acid and isobutyric acid. However, most acids except valeric acid were not found after hydrolysis. Further research is needed to determine the action of acids in oyster flavor.

Small amounts of 4 terpenes were identified in both samples. These compounds, generally derived from essential oils of plants, might have originated from algae via the food chain. Two surfur-containing compounds, dimethyldisulfide and dimethyltrisulfide, were only identified in oyster hydrolysate. These compounds, having strong, sulphurous, cooked cabbage odors in vegetables, meats, and marine products (22), were also found in Pacific oyster (18).

REFERENCES

1. Nakamura, S. : Extraction and utilization of oyster extract. *New Food Industry*, 25, 62 (1983)
2. Cha, Y. J., Baek, H. H. and Cadwallader, K. R. : Volatile flavor components in oyster hydrolysate produced with protease. Book of Abstracts, 1995 IFT Annual

Meeting at Anaheim, CA, USA, p.166 (1995)
3. Cha, Y. J. and Kim, E. J. : Response surface methodology in processing of oyster hydrolysate. *J. Korean Soc. Food Nutr.*, **24**, 427 (1995)
4. Chung, H. Y. and Cadwallader, K. R. : Aroma extract dilution analysis of blue crab claw meat volatiles. *J. Agric. Food Chem.*, **42**, 2867 (1995)
5. Cha, Y. J., Baek, H. H. and Hsieh, T. C.-Y. : Volatile components in flavor concentrates from crayfish waste. *J. Sci. Food Agri.*, **58**, 239(1992)
6. Tanchotikul, U. and Hsieh, T. C.-Y. : Analysis of volatile flavor components in steamed rangia clam by dynamic headspace sampling and simultaneous distillation and extraction. *J. Food Sci.*, **56**, 327 (1991)
7. Cha, Y. J., Cadwallader, K. R. and Baek, H. H. : Volatile flavor components in snow crab cooker effluent and effluent concentrate. *J. Food Sci.*, **58**, 525 (1993)
8. van den Dool, H. and Kratz, P. D. : A generalization of the retention index system including linear temperature programmed gas liquid partition chromatography. *J. chromatogr.*, **11**, 463 (1963)
9. Hewlett-Packard Co. : Wiley/NBS Database (PBM format). Palo Alto, CA, (1988)
10. Hites, R. A. and Biemann, K. : Computer evaluation of continuously scanned mass spectra of gas chromatographic effluents. *Anal. Chem.*, **42**, 855 (1970)
11. Vejaphan, W., Hsieh, T. C.-Y. and Williams, S. S. : Volatile flavor components from boiled crayfish (*Procambarus clarkii*) tail meat. *J. Food Sci.*, **53**, 1066 (1988)
12. Hayashi, T., Ishii, H. and Shinihara, A. : Novel model experiment for cooking flavor research on crab leg meat. *Food Reviews International*, **6**, 521 (1990)
13. Bailey, M. E. and Einig, R. G. : Reaction flavors of meat. In "*Thermal Generation of Aromas*" Parliment, T. H., McGorrin, R. J. and Ho, C. T. (eds.), ACS, p.421 (1989)
14. Karahadian, C. and Lindsay, R. C. : Role of oxidation in the formation and stability of fish flavors. In "*Flavor Chemistry : Trends and Developments*" Teranishi, R., Buttery, R. G. and Shahidi, F. (eds.), ACS, p.60 (1989)
15. Ho, C. T., Bruechert, L. J., Zhang, Y. and Chiu, E. M. : Contribution of lipids to the formation of hetrocyclic compounds in model systems. In "*Thermal Generation of Aromas*" Parliment, T. H., McGorrin, R. J. and Ho, C. T. (eds.), ACS, p.105 (1989)
16. Josephson, D. B., Lindsay, R. C. and Stuiber, D. A. : Variations in the occurrences of enzymically-derived volatile aroma compounds in salt- and freshwater fish. *J. Agric. Food Chem.*, **32**, 1344 (1984)
17. Kubota, K., Kobayashi, A. and Yamanishi, T. : Basic and neutral compounds in cooked odor from Antarctic krill. *Agric. Biol. Chem.*, **46**, 2835 (1982)
18. Josephson, D. B., Lindsay, R. C. and Stuiber, D. A. : Volatile compounds characterizing the aroma of fresh Atlantic and Pacific oysters. *J. Food Sci.*, **50**, 5 (1985)
19. Heath, H. B. and Reineccius, G. : Off-flavors in foods. In "*Flavor Chemistry and Technology*" Heath, H. B. and Reineccius, G. (eds.), MacMillian Pub. LTD, Basingstoke, England, p.121 (1986)
20. Shibamoto, T. and Bernhard, R. A. : Effect of time, temperature, and reactant ratio on pyrazine formation in model systems. *J. Agric. Food Chem.*, **24**, 847 (1976)
21. Maga, J. A. and Sizer, C. E. : Pyrazines in foods. A review. *J. Agric. Food Chem.*, **21**, 22 (1973)
22. Shankaranarayana, M. L., Raghavan, B., Abraham, K. O. and Natarajan, C. P. : Sulphur compounds in flavors. In "*Food Flavours, Part A. Introduction*" Morton, I. D. and Macleod, A. J. (eds.), Elsevier, New York, p.169 (1989)

(Received June 5, 1995)

굴단백질 가수분해물의 휘발성 향기성분

차 용 준
창원대학교 식품영양학과

요 약

상업용 단백질 분해효소로 제조한 굴 가수분해물의 휘발성 향기성분을 생굴(미국산)을 대조구로 하여 Vacuum-SDE/GC/MS를 이용하여 분석 비교하였다. 총 62개의 휘발성 성분이 검출되었으며 이 중에서 57개의 성분은 표준품으로 확인 동정되었다. 이는 주로 알데히드류(12), 케톤류(9), 알콜류(14), 질소함유화합물류(9), 산류(6), 테르펜류(4)와 기타 화합물류(8)로 구성되어 있었다. 생굴에서는 산류의 함량이 가장 많았으나 가수분해함으로서 거의 검출되지 않았고 반면에 질소 함유 화합물은 가수분해물에서만 검출되었다. 그리고 가수분해함으로서 질소 함유 화합물, 알데히드류 및 케톤류의 함량은 증가하는 경향이었다.