

Volatile Compounds of Ascidian, *Halocynthia roretzi*

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About 2.1g of pale yellow flavor concentrate was obtained from 10 kg of chopped fresh ascidians through a Likens-Nickerson steam distillation/solvent extraction. These concentrates could be fractionated to neutral (91.5%), basic (1.0%), phenolic (3.2%), and acidic (4.3%) fractions. Total 65 volatile compounds were identified from those concentrates. The neutral fraction was representative flavor fraction which showed a similar flavor of total steam distillates of ascidian. The major compounds (38.2% of neutral fraction) were identified as carbon atoms 8 to 10 of alcohols. Among these volatile alcohols, 1-octanol, 2,7-decadien-1-ol, 3-octen-1-ol, 7-decen-1-ol, and 1-decanol were the dominant compounds found in neutral fraction. But the basic, phenolic, and acidic fractions differs from ascidian steam distillates flavor.

Key words : ascidian, flavor, steam distillates, volatile alcohols, flavor concentrate

Introduction

Flavors and aromas commonly associated with fish and seafoods have been the subject of many investigations and reviews (Ke et al., 1975; Christensen et al., 1981; Josephson et al., 1984a; Kajiwara et al., 1988; Nettleton et al., 1990; Tanchotikul et al., 1991), but very little information has been reported on ascidian. The ascidian *Halocynthia roretzi* has been esteemed as a palatable seafood in Korea because of its specific flavors. During last 15 years, it has been cultured in the southern and eastern coasts of Korea. According to the Ministry of Kyeongnam Fisheries Statistics (1995), the production of cultured ascidian had been increased from 866 M/T in 1984 to 18,809 M/T in 1994.

This ascidian is well known for its peculiar "ascidian" flavor, which has been found to originated from *n*-alcohols as 1-octanol, 7-decen-1-ol, and 2,7-decadien-1-ol as the major components (Kita, 1957; Suzuki, 1959a; Suzuki, 1959b). The ascidian, being a

plankton feeder, feeds various plant and zooplanktons including species with such excellent (Nevenzel, 1970). Therefore, it can be assumed that the alcohols in the ascidian flavor living with *H. roretzi* such as the sea mussel and scallop do not produce the peculiar smell at all and the alcohol flavor is specific to this ascidian (Fujimoto et al., 1982). Kusaka et al. (1983) also studied the odoriferous compounds in *H. roretzi* using GC-MS and found that the ascidian flavor was composed of a mixture of various unsaturated alcohol isomers besides the major ones mentioned above.

However there was no literature on capillary GC analysis of steam distillation ascidian volatiles. This paper reports on the volatile compounds isolated by a Likens-Nickerson steam distillation/solvent extraction apparatus and identified using a silica capillary GC and GC-MS.

Materials and Methods

Materials

Ascidian, *Halocynthia roretzi*, cultured in Hansan island, Tongyeong, of the southern coast were collected in May, 1992. They were settled on the ropes in December 1989 and cultured there after at the respective farms. Samples were made at a depth of 5 m. While still alive the ascidian were removed from the tunics and the muscles chopped into small pieces.

Sample preparation

Two hundreds gram of chopped ascidian muscle from 10 kg of fresh ascidian and 2 L of distilled water were placed in a 5 liter glass vessel and extracted into diethyl ether using a Likens-Nikerson apparatus for 2hrs. After steam distilled extraction, the ether extract was concentrated using Kudra-Danish (K-D) concentrator. The concentrate was fractionated into neutral, basic, phenolic, and acidic fractions by the usual method. The acidic fraction was treated with diazomethane for methylation of the acids in the fraction. These fractionated volatile components were analyzed by GC and GC-MS.

Volatile Separation by Gas Chromatography

A Varian 3400 GC equipped with an FID and a nonpolar fused silica capillary column (60 m×0.32 mm i.d., 0.25 μ m thickness, DB-1, J & W) was used to analyzed the volatile compounds isolated from the steam-distillate concentrates of each fraction. The GC was run with an injector temperature of 270°C, a detector temperature of 300°C, and a helium carrier flow rate of 1 ml/min. The temperature program included an

initial column temperature was 40°C, a temperature increase of 2°C/min from 40 to 260°C and a 60 min isothermal period at the final column temperature. A split ratio of 50 : 1 was used. After steam distillation /solvent extraction, appropriate amounts of *n*-nonane (Sigma Co.) were added to the isolates as the internal standard. The quantity of each component was finally converted into microgram of volatiles. Linear retention indices for the volatile compounds were calculated by using *n*-paraffins (C5-C25, Alltech Associates) as references (Majlat et al., 1974).

Gas Chromatography-Mass Spectromether (GC-MS) Analysis

The fractionated samples were analyzed by GC-MS using a Varian 3400 GC coupled to a Finnigan MAT 8230 high-resolution mass spectrometer, used the same GC program as for the separation. Mass spectra was obtained by electron ionization at 70eV and ion source temperature of 250°C. The filament emission current was 1 mA, and spectra were recorded on a Finnigan MAT SS 300 data system.

Results and Discussion

Total concentrates obtained from ten kilogram of chopped ascidian muscle through the steam-distillation extraction (SDE) was 2.1064 g. It was fractionated to neutral, basic, phenolic, and acidic groups. The yield and flavor description of each fractions obtained from ascidian SDE concentrates are listed in Table 1. Most

Table 1. Yield and flavor qualities of each fraction from ascidian concentrates

Faction	Yield(g) ¹	Flavor description ²
Neutral fraction	1.9276	Ascidian
Basic fraction	0.0205	Pleasant
Phenolic fraction	0.0687	Medicinal
Acidic fraction	0.0896	Rancid, Sweaty

¹ Yield from 10 kg of chopped muscle.

² The ether solution of each fraction was absorbed on a filter paper and the filter paper was air-dried to remove the solvent and then subjected to the sensory test by 10 members of our laboratory.

of the volatile compounds of sample were contained in the neutral fraction (91.5%) and the odor of neutral fraction was almost the same as the odor of the SDE concentrates. Basic (1.0%), phenolic (3.2%), and acidic (4.3%) fractions of concentrates, of which the yields were very low, had a pleasant, medicinal, and rancid odor, respectively. These results indicate that important volatile components of the ascidian odor were contained in the neutral fraction of concentrates.

The quantitatives of volatile compounds identified in this study are listed in Table 2, and a corresponding each fractions of capillary column GC separation is shown in Figure 1. Some peaks of each fractions were contaminated each other using this fractionation method. A total 25 volatile compounds were identified from the neutral fraction. The major compounds of this fraction were 1-octanol (16.3%), 2,7-decadien-1-ol (10.5%), (Z)-3-octen-1-ol and (E)-3-octen-1-ol (4.7%), 7-decen-1-ol (2.7%), and 1-decanol (1.8%), which occupied 38% of the total volatiles. The neutral fraction has a similar flavor of ascidian concentrates and it has character impact compounds of ascidian flavor. Among these alcohols responsible for the ascidian flavor, octanol has already been isolated from a marine tube worm and 2,7-decadien-1-ol and cis-7-decen-1-ol has been isolated from various marine invertebrates such as sea squirts, sea cucumbers, and sea snails (Kita, 1957; Suzuki, 1959b).

Hydroperoxidation of fatty acids by lipoxygenases in mushroom (Tressel et al., 1981) and cucumber and melon fruits (Galliard and Phillips, 1976; Vick and Zimmerman, 1976; Phillips et al., 1979) initiate reactions that lead to the formation of the eight- and nine-carbon volatile aroma compounds, respectively, in these foods. Also, oxidation of the unsaturated acyl chains lipids is a major route to volatile compounds during the cooking of fat-containing food of either seafood or vegetable origin (Frankel, 1983). Since the same compounds occur in ascidian, similar enzyme-mediated and/or thermal oxidation pathways now appear to explain their formation in ascidian. German and Kinse-

lla (1983) reported the identification of a lipoxygenase system in the gill tissue of rainbow trout (*Salmo gairdneri*), and this supports the view for an enzymic involvement in the biogenesis of lipid-derived in fresh fish.

Fujimoto et al. (1982) have been identified as salts of alkyl sulfate with carbon atoms 8 to 10 from ascidian. They reported that the enzyme responsible for the alcohols liberation in the ascidian is alkyl sulfhydrolyase. But no paper on alkylsulfhydrolyase has been available in the marine invertebrates including the ascidian, whereas several other sulfatase were reported from various gastropod (Shaw et al., 1980). Also they did not determine the double bond position of eight- to ten-carbon alcohols.

Eight-carbon alcohols and ketones have been previously identified in crustaceans (Whitfield and Freeman, 1983) and in shellfish (Josephson et al., 1985). Josephson et al. (1984a; 1984b) found 1-octen-3-ol, 2-octen-1-ol, and 1,5-octadien-3-ol, an enzymatic reaction product derived from lipids such as linoleic and arachidonic acid, as one of the volatiles components distributed in fresh and saltwater fish. In this study, we identified the 3-octen-1-ol as one of ascidian flavor compounds from neutral and basic fraction. But the volatile compounds composition of ascidian were different from these fishes.

The minor compounds of neutral fraction as 2-ethyl-1-hexanol, 2,6-dimethyl-5-hepten-1-ol, 3,7-dimethyl-6-octen-1-ol were first separated from the ascidian. These compounds were not reported any other of fish volatile compounds. Ascidian has been reported to contain a relatively high proportion omega-3 fatty acids such as 18 : 3, 20 : 5, 22 : 6 (Vysotskii, et al., 1992) which are susceptible to lipid oxidation. A degradation product of omega-3 polyunsaturated fatty acid, 3,6-nonadien-1-ol (15.0 ppb, threshold = 10 ppb), has been reported to give a cucumber aroma in foods (Kemp et al., 1974). It is probably above the recognition threshold concentration.

Generally, the volatile carbonyls found in fish ex-

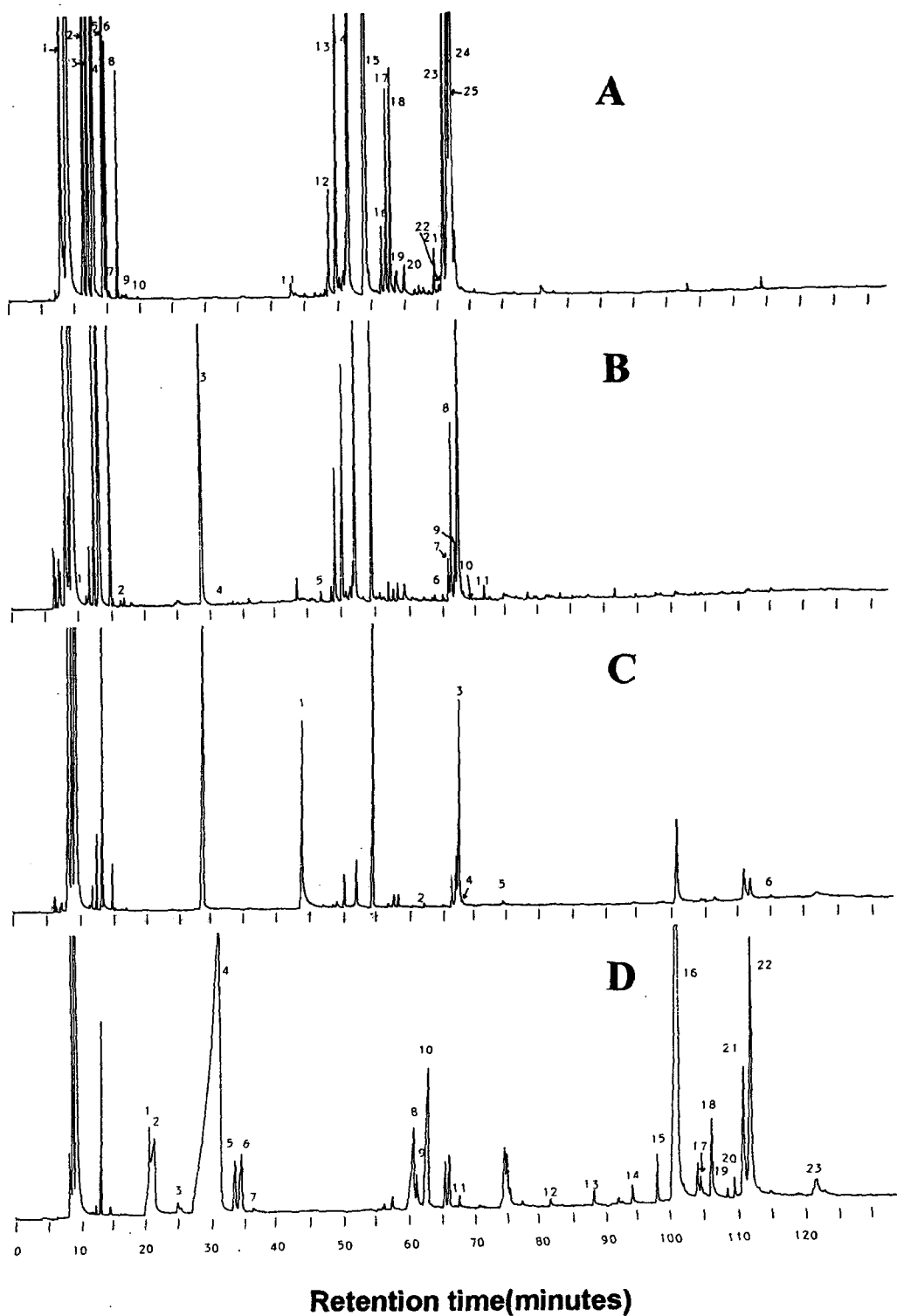


Fig. 1. Capillary gas chromatograms of neutral(A), basic(B), phenolic(C), and acidic fraction(D) of ascidian volatile compounds.

Table 2. Volatile compounds identified from the steam-distillate of ascidian

Peak No ¹	Compounds	RI ²	MW	Amount ppb
Neutral fraction				
1	2-propanone		58	888.7
2	2,3-dimethylbutane	572	86	561.8
3	3-methylpentane	585	86	1914.0
4	2-methylbutanal	598	86	4744.0
5	methylcyclopentane	620	84	1481.6
6	2,4-dimethylpentane	628	100	97.7
7	2,2,3-trimethylbutane	637	100	4.4
8	cyclohexane	655	84	69.4
9	heptane	673	100	1.7
10	3-methylhexane	740	100	0.6
11	2,5,5-trimethyl-1-hexene	964	126	8.9
12	2-ethyl-1-hexanol	1025	130	58.3
13	(Z)-3-octen-1-ol	1038	128	287.6
14	(E)-3-octen-1-ol	1054	128	461.3
15	1-octanol	1082	130	2566.8
16	2,6-dimethyl-5-hepten-1-ol	1111	142	29.4
17	7-methyl-1-octene	1120	126	92.6
18	1-nonanol	1135	144	92.7
19	(E,Z)-3,6-nonadien-1-ol	1147	140	15.0
20	18 isomer	1155	140	14.3
21	3,7-dimethyl-6-octen-1-ol	1210	156	22.8
22	(E)-2-decenal	1227	154	8.3
23	1-decanol	1234	158	291.4
24	7-decen-1-ol	1244	156	425.8
25	2,7-decadien-1-ol	1251	154	1659.2
Basic fraction				
1	tetrahydro-2-methylfuran	562	86	t ³
2	1-hexanol	652	102	t
3	1,3-dioxolane-2-methanol	802	104	1.8
4	2-furanmethanol	835	98	t
5	2-methylpyrazine	1001	94	t
6	4-ethyl-1-octyne-3-ol	1201	154	t
7	isoquinoline	1230	129	0.2
8	4-(1-methylethenyl)-1-cyclohexene-1-methanol	1236	152	0.5
9	8 isomer	1244	152	0.3

Table 2. (Continued)

Peak No ¹	Compounds	RI ²	MW	Amount ppb
10	(E)-4-methyloximebenzaldehyde	1291	135	t
11	2-methylquinoline	1301	143	0.1
Phenolic fraction				
1	3,4-dimethylphenol	968	122	6.6
2	naphthalene	1182	128	0.1
3	decahydronaphthalene	1250	138	4.6
4	2,4-dibromophenol	1260	250	0.2
5	γ -cadinene	1388	204	0.1
6	4,4'-methylenebisphenol	2013	200	t
Acidic fraction				
1	butanoic acid methyl ester	708	102	8.4
2	propanoic acid	719	74	12.3
3	butanoic acid ethyl ester	762	116	0.8
4	butanoic acid	830	88	55.2
5	pentanoic acid	857	102	4.7
6	2-methylhexanoic acid	867	130	6.5
7	hexanoic acid	884	116	0.2
8	octanoic acid	1163	144	11.4
9	7-octenoic acid	1167	142	2.7
10	7-methylnonanoic acid methyl ester	1189	186	16.4
11	nonanoic acid	1223	158	3.0
12	undecanoic acid	1446	186	0.4
13	dodecanoic acid	1543	200	1.0
14	tridecanoic acid	1642	214	1.2
15	tetradecanoic acid methyl ester	1704	242	2.5
16	tetradecanoic acid	1754	228	96.1
17	12-methyltetradecanoic acid	1817	242	1.9
18	pentadecanoic acid	1844	242	6.7
19	9-hexadecenoic acid methyl ester	1885	268	0.4
20	14-methylpentadecanoic acid methyl ester	1904	270	1.0
21	9-hexadecenoic acid	1928	254	14.8
22	octadecanoic acid	1949	284	30.0
23	9,12-octadecadienoic acid	2125	280	3.7

¹ Peak numbers refer to Figure 1² RI, linear retention indices were obtained by using *n*-alkanes as standards in non-polar fused silica capillary column [60 m \times 0.32 mm(i.d.); 0.25- μ m thickness; DB-1]³ t, below 0.05 ppb.

hibit coarse, heavy aromas whereas the volatile alcohols contribute smoother qualities. Lower threshold values for the volatile carbonyls, especially 1-octen-3-one (0.005 ppb), 1,5-octadien-3-one (0.004 ppb), (E)-2-nonenal (0.08 ppb), and (E,Z)-2,6-nonadienal (0.01 ppb) (Swoboda and Peers, 1977; Buttery, 1981), result in greater contributions to overall fresh fish like odors than do the corresponding alcohols (1-octen-3-ol, 1,5-octadien-3-ol, and 3,6-nonadien-1-ol) whose threshold values are each 10 ppb (Buttery, 1981; Whitfield et al., 1982).

Hydrocarbons can be formed from autoxidation process through alkyl radicals or from carotenoid decomposition (Pipen et al., 1969). The contribution of these saturated alkanes to ascidian flavor most likely minor.

A total 11 compounds were identified from the basic fraction. The basic fraction have differ from ascidian flavor concentrates, but it has pleasant flavor. The most abundant compounds of this fraction was 1,3-dioxolane-2-methanol and then tetrahydro-2-methylfuran, 2-furanmethanol was also identified. Quinoline and its derivatives are a common organic compounds which is present in coal tar and bone oil and which is used in industry as a reagent as a solvent (Riddick and Bunger, 1970). Since small quantitatives of quinoline must inevitably find their way into effulents and be relased into the enviornment.

Phenolic compounds have been encountered in several food flavors, especially in smoke flavorings (Maga, 1978). The 3,4-dimethylphenol and decahydronaphthalene was detected in very high abundance and gave a strong medicinal odor in the phenolic fraction. The 3-ethylphenol and 3,4-dimethylphenol found to be responsible for the smoky taste of certain cocoa beans (Guoyt, 1986). The uptake of aromatic hydrocarbons in marine animals has been studied (Stegeman and Teal, 1973; Neff et al., 1976; Roubal et al., 1977) due to concerns over the increase and toxicity of petroleum pollutants in aquatic habitat. Lee et al. (1972) reported rapid uptake of naphthalene in

marine fish.

A total 23 compounds were identified from the acidic fraction. The concentration of major even-carbon-numbered were distinctively high (*n*-C4, 55.2 ppb; *n*-C8, 11.4 ppb; *n*-C14, 96.1 ppb; *n*-C18, 30.0 ppb), and their distribution was in the oder *n*-C14 > *n*-C4 > *n*-C18 > *n*-C8 (Table 2). The rancid or sweaty flavor of acidic flavor was may have due to the high proportion of butanoic acid.

References

- Buttery, R.G. 1981. Flavor research recent advances, R. Temanish, R.A. Flath, H. Sugusawa, eds. Marcel Dekker, New York, pp. 193~210.
- Christensen, B.W., A. Kjaer and J.O. Maden. 1981. Volatile sulfur compounds and other headspace constituents of north seafish oils. J. Am. Oil Chem. Soc., 58, 1053~1057.
- Frankel, E.N. 1983. Volatile lipid oxidation products. Prog. Lipid Res., 22, 1~33.
- Fujimoto, K., H. Ohtomo, A. Kanazawa, Y. Kikuchi and T. Kaneda. 1982. Alkyl sulfates as precursors of ascidian flavor in *Halocynthia roretzi*. Bull. Jap. Soc. Sci. Fish., 48, 1327~1331.
- Galliard, T. and D.R. Phillips. 1976. The enzymic cleavage of linoleic to C9 carbonyl fragments in extracts of cucumber (*Cucumis sativas*) fruit and the possible role of lipoxygenase. Biochem. Biophys. Acta., 431, 278~287.
- German, J.B. and J.E. Kinsella. 1983. Abstracts of papers, 186th National Meeting of the American Chemical Society, Washington, D.C., American Chemical Society, Washington, D.C., AGFD 127.
- Guoyt, B., D. Gueule, I. Morcrette and J.C. Vincent. 1986. Coffee, Cocoa, Tea. 113~120.
- Josephson, D.B., R.C. Lindsay and D.A. Stuibler. 1984. a. Variations in the occurrences of enzymatically derived volatile aroma compounds in salt- and fresh-water fish. J. Agric. Food Chem., 32,

- 1344~1347.
- Josephson, D.B., R.C. Lindsay and D.A. Stuibler. 1984
b. Biogenesis of lipid-derived volatile aroma compounds in the emerald shiner (*Notropis atherinoides*). J. Agric. Food Chem., 32, 1347~1352.
- Josephson, D.B., R.C. Lindsay and D.A. Stuibler. 1985. Volatile compounds characterizing the aroma of fresh Atlantic and Pacific oysters. J. Food Sci., 50, 5~9.
- Kajiwara, T., A. Hatanaka, T. Kawai, M. Ishihara and T. Tsuneya. 1988. Study of flavor compounds of essential oil extracts from edible Japanese kelps. J. Food Sci., 53, 960~962.
- Ke, P.J., R.G. Ackman and B.A. Linke. 1975. Autoxidation of polyunsaturated fatty compounds in mackerel oil; Formation of 2,4,7-decatrienals. J. Am. Oil Chem. Soc., 52, 349~353.
- Kemp, T.R., D.E. Knavel, L.P. Stoltz and R.E. Lundin. 1957. 3,6-Nonadien-1-ol from *Citrullus vulgaris* and *Cucumis melo*. Phytochemistry, 13, 1167~1170.
- Kita, M. 1957. Isolation of 7-decen-1-ol from an ascidian. J. Org. Chem., 22, 436~438.
- Kusaka, H., H. Narita, K. Iwata and S. Ohta. 1983. Gas liquid chromatographic determination of flavor component from ascidian. Bull. Jap. Soc. Sci. Fish., 49, 617~620.
- Lee, R.F., R. Sauerheber and G.H. Dobbs. 1972. Uptake, metabolism, and discharge of polycyclic aromatic hydrocarbons by marine fish. Marine Biol., 17, 201~208.
- Majlat, P., I. Erdos and J. Takacs. 1974. Calculation and application of retention indices in programmed temperature gas chromatograph. J. Chromato., 91, 89~92.
- Ministry of Kyeongnam Fisheries. 1995. Statistical yearbook of fisheries, Kyeongnam, 222 pp.
- Neff, J.M., B.A. Cox, D. Dixit and J.W. Anderson. 1976. Accumulation and release of petroleum-derived aromatic hydrocarbons by four species of marine animals. Marine Biol., 38, 279~289.
- Nettleton, J.A., W.H. Allen Jr., L.V. Klatt, W.M.N. Ratnayake and R.G. Ackman. 1990. Nutrients and chemical residues in one- to two-pound mississippi farm-raised channel catfish (*Ictalurus punctatus*). J. Food Sci., 55, 954~958.
- Nevenzel, J.C. 1970. Occurrence, function and biosynthesis of wax esters in marine organisms. Lipids, 5, 308~319.
- Phillips, D.R., J.A. Mathews, J. Reynolds and G.R. Fenwick. 1979. Partial purification and properties of a cis-3: trans-2-enal isomerase from cucumber fruit. Phytochemistry, 18, 401~404.
- Pipen, E.L., E.P. Mecchi and M. Nonaka. 1969. Origin and nature of aroma in fat of cooked poultry. J. Food Sci., 34, 436~442.
- Riddick, J.A., W.B. Bunger and T.K. Sakano. 1986. Physical properties and methods of purification. In Organic Solvents, 4th ed. Techniques of Chemistry, Vol. II. D.R. Perrin, John Wiley & Sons, New York. pp. 651~653.
- Roubal, W.T., T.K. Collier and D.C. Malins. 1977. Accumulation and matabolism of carbon-14 labeled benzen, naphthalene, and anthracene by young coho salmon (*Oncorhynchus kisutch*). Arch. Environ. Contamin. Tox., 5, 513~529.
- Shaw, D.J., K.S. Dodgson and G.F. White. 1980. Substrate specificity and other properties of the inducible S3 secondary alkylsulphohydrolase purified from the detergent-degrading bacterium *Pseudomonas* C12B. Biochem. J. 187, 181~190.
- Stegeman, J.J. and J.M. Teal. 1973. Accumulation, release and retention of petroleum hydrocarbons by the oyster *Crassostrea virginica*. Marine Biol., 22, 37~44.
- Suzuki, Y. 1959a. Biochemical studies of the ascidian, *Cynthia roretzi*. V. Drasche. I. On the nitrogen extracts. Tohoku J. Arg. Res., 10, 85~89.
- Suzuki, Y. 1959b. Biochemical studies of the ascidian, *Cynthia roretzi*. II. Isolation of *n*-octanol,

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- n*-decanol and *n*-decadienal. *Tohoku J. Arg. Res.*, 10, 391~395.
- Swoboda, P.A.T. and K.E. Peers. 1977. Volatile od-
ble for metallic, fish taint formed in butterfat by
elective oxidation. *J. Sci. Food Agric.*, 28, 10
10~1018.
- Tanchotikul, U. and T.C.-Y. Hsieh. 1991. Analysis of
volatile flavor components in steamed. rangia
clam by dynamic headspace sampling and
simultaneous distillation and extraction. *J. Food
Sci.*, 56, 327~331.
- Tressel, R., D. Bahri and K. Engel. 1981. Lipid oxida-
tion in fruits and vegetables. In *Quality of
selected fruits and vegetables of North America*,
R. Temanishi, H. Barrera-Benitez, eds. American
Chemical Society, Washington, D. C., pp. 213~
232.
- Vick, B.A. and D.C. Zimmerman. 1976. Lipoxy-
genase and hydroperoxide lyase in germinating
watermelon seedlings. *Plant Physiol*, 57, 780~
788.
- Vysotskii, M.V., T. Ota and T. Tagaki. 1992. *n*-3
Polyunsaturated fatty acids in lipids of ascidian
Halocynthia roretzi. *Nippon Suisan Gakkaishi*,
58, 953~958.
- Whitfield, F.B., D.J. Freeman, L.H. Last, P.A. Bannister
and B.J. Kennett. 1982. Oct-1-en-3-ol and (5Z)-
octa-1,5-dien-3-ol, compounds important in the
flavor of prawn and sand-lobstes, *Aust. J.
Chem.*, 35, 373~383.
- Whitfield, F.B. and D.K. Freeman. 1983. Off flavors
in crustaceans caught in Australian coastal wa-
ters. *Wat. Sci. Tech.*, 15, 85~95.

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