Flavor Development in the Ripening of Anchovy (*Engraulis encrasicholus* L.)

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Flavor development in anchovy (*Engraulis encrasicholus*) during ripening was investigated by means of aroma extract dilution analysis of samples either commercially processed or ripened on laboratory scale, with and without the addition of microbial inhibitors. Results demonstrated that microbial metabolism contributed no volatiles of sensory significance to the anchovy. The majority of sensorially important volatiles were formed during ripening. Volatiles of importance to anchovy flavor were characterized mainly in two groups: enzymatically generated C_8 alcohols and ketones along with (*E,Z*)-2,6-nonadienal, which contributed plant- and cucumber-like aromas, and autoxidatively derived C_7 to C_{10} conjugated aldehydes, which imparted fatty and fried fat-like aromas.

Keywords: *Anchovy; Engraulis encrasicholus; flavor; ripening; aromagrams; FD chomatogram; microbial; AEDA; 3-methyl-2,4-nonanedione; (E,Z)-2,6-nonadienal*

INTRODUCTION

Cured anchovies are prepared from the fish of the species *Engraulis encrasicholus* (linnaeus) of the clupeid family by a process of salting and maturing. This process goes back to ancient times and is a common tradition in some Mediterranean countries and in Argentina, where the species used is *Engraulis anchoita*. It is not known which particular factors (composition or processing) give the product its distinctive flavor. Experience has, however, demonstrated that the same process applied to sardines (*Clupea pilchardus*) or sprats (*Clupea sprattus*) does not yield a product of a comparable quality (Cheftel, 1965).

In Morocco, the curing of anchovies is now practiced to a large scale in more than 20 manufacturing facilities. The largest part of production is intended for export to the European Economic Community (EEC) and other countries including the United States, Japan, and Australia.

Maturing or "anchoitage" is a complex sequence of reactions that depends on different parameters related to the physicochemical conditions of ripening (temperature, pH, ionic strength, water activity) and to the biology of the fish (fat content, enzymes, bacteria, ...) (Durand, 1982). As a result of these reactions, the finished product acquires a soft consistency along with the development of a pink color and a strong, characteristic flavor. The fish is said to be "anchoite".

The processing of anchovy has been outlined by various authors (Cheftel, 1965; Coullon, 1981; Durand, 1982; Filsinger, 1987). Many authors have also reported on chemical and microbiological changes occurring during the ripening process, but to our knowledge no work related to the flavor of ripened anchovy has been published in the literature.

It is recognized that the ripening of salted anchovy takes place via enzymatic pathways. The importance attributed to tissular enzymes versus microbial enzymes is controversial. Research has indicated that the intestines of the anchovy are particularly important. Partial evisceration, as practiced in the factories, eliminates a part of the digestive enzymes; otherwise, a bitter taste develops in the fish (Durand, 1982). However, a perfect evisceration with rigorous rinsing leads to considerably slower ripening, and the fish does not acquire the characteristic flavor (Voskresensky, 1965; Alm, 1965; Durand, 1982).

The mechanisms of flavor development in freshly harvested fish have been the subject of many papers in the past 10 years. Lipoxygenase enzymes present in the gill and skin of different fresh and saltwater fish species (German et al., 1986; German and Kinsella, 1985; German and Creveling, 1990) are involved in the generation of short-chain carbonyl compounds in these tissues following harvesting. This has been demonstrated by using specific lipoxygenase inhibitors, e.g. esculetin, and by collection of radiolabeled volatile compounds (Hsieh and Kinsella, 1989; German et al., 1991; Josephson et al., 1987). While a major role is ascribed to these enzymes in generating fresh fish volatiles, other enzyme systems such as myeloperoxidase of blood leucocytes (Kanner and Kinsella, 1983) and NADH-dependent oxidase (Slabyj and Hultin, 1984) were also shown to initiate lipid hydroperoxidation in fish tissues.

The present investigation was aimed at providing a better understanding of the mechanisms underlying flavor development in ripened anchovy and whether or not microorganisms play a role in the formation of aroma components. The method of aroma extract dilution analysis (AEDA) (Ullrich and Grosch, 1987) was used to assess the relative contribution of individual volatile compounds to the characteristic flavor that develops during ripening.

MATERIALS AND METHODS

Materials. 3-Methyl-2,4-nonanedione and 2-acetyl-1-pyrroline were respectively, gifts from Dr. H. H. Guth and Dr. P. Schieberle (Deutsche Forchungsanstalt fur Lebensmittelchemie, Garching, Germany). The other chemicals were from Merck (Darmstadt, Germany) and Aldrich (Steinheim, Ger-

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0021-8561/95/1443-0453$09.00/0

many). Fresh anchovies of the species *E. encrasicholus* (Linnaeus) were caught at night (end of June) off the coasts of Agadir (south central Moroccan Atlantic coast). They were approximately 15 cm in length, and about 50–55 fish made 1 kg. The sea salt used was exempt of stain and passed through a grinder having two smooth cylinders with approximately 2 mm spacing.

Two samples of fully ripened anchovies (12 and 16 weeks of ripening) were obtained from a local processor in the Rabat area.

Laboratory Preparation of Anchovies. Anchovies were immersed in a salt brine immediately after being caught and then divided into three lots (I, II, and III) of 20 kg each. They were brought to the laboratory the next day for continued processing. The lots were processed by presalting the anchovies in brine (30% NaCl) for 48 h and then beheading and gutting by hand. The anchovies were then washed in a fresh brine (20% NaCl), drained (or dripped) for 20 min, and packed in plastic containers for final salting as follows (method "carne a carne", Cosnard et al., 1983). A layer of salt (8–10 cm) was first put in the container, then a layer of fish, and so on, finishing with a layer of salt. The ratio of salt/fish was 1.5:10 (w/w). Pressing was done by putting a loose-fitting lid on top of the mass and weighing the lids with drums filled with salt (20 kg total weight). The approximate total pressure was 30–40 g/cm^2 during ripening.

Three lots of fish were prepared as described with the following additional treatments. Lot I (LI) was the control and processed as stated above. Lot II (LII) was processed in the same way except that the salt and brine were sterilized in an autoclave and antibiotics were added to the salt brine in the concentrations of 6 μg of ampicillin/mL and 25 μg of streptomycin/mL. For lot III (LIII), anchovies were immediately beheaded and gutted after catching as opposed to holding for 48 h in salt brine before this operation. They were then processed as LII. For the three lots, the brine was changed three times: 48 h after the presalting step, after 5 weeks of ripening and at the 11th week. Anchovies were ripened in a closed room at 18–24 °C for up to 16 weeks.

Sampling was done at 2, 3, 5, 7, 9, and 11 weeks of ripening. Samples (200 g of fish) were taken from the top layers of anchovies using sterilized materials (gloves, clip, and flask) and preventing any contamination of the containers. Samples were immediately frozen for later analysis.

Isolation of Volatiles. Fish (150–200 g) was mixed with 400 mL of distilled water and blended in a Waring type blender for 1 min. The slurry was then transferred to a 3 L round-bottom flask, and 600 mL of distilled water was added. An aqueous distillate was obtained from the slurry using a flash evaporator (Buchler Instruments) with a vertical condenser under reduced pressure. The temperature of the water bath was set to 57–58 °C. The distillation was stopped upon collection of ca. 200 mL of distillate. The distillate was then extracted three times with 30 mL methylene chloride in a separatory funnel. Extracts were dried over anhydrous magnesium sulfate and then filtered. Concentration of extracts was done at room temperature under a gentle stream of nitrogen and then by microdistillation (Blank et al., 1986).

Instrumental Analysis. Gas chromatography was performed using a Carlo Erba gas chromatograph with SE-54 and OV-1701 (each 30 m × 0.32 mm, film thickness 0.3 μm) glass capillary columns. The glass capillaries were deactivated and coated according to the method of Grob (1986). The aroma isolates were analyzed using an "on column injection technique" at 35 °C. The following temperature program was used: After 2 min at 35 °C, the temperature of the GC oven was raised quickly (40 °C/min) to 50 °C, held 1 min isothermally, then raised at 6 °C/min to 250 °C (230 °C for OV-1701), and finally held isothermally for 10 min. The flow rate of the carrier gas (helium) was 2.2 mL/min. At the end of the capillary, the effluent was split 1:1 (by volume) into a flame ionization detector (FID) and a sniffing port using deactivated but uncoated fused silica capillaries (40 cm × 0.3 mm). The FID and sniffing port were held at a temperature of 250 °C. The splitter was flushed with helium for accelerating the split flow to 10 mL/min. Nitrogen was used as makeup gas for the FID (20 mL/min). Retention data of the compounds are presented as retention indices (RI) according to the procedure of Van den Dool and Kratz (1963).

Eluate Sniffing. The method of aroma extract dilution analysis (AEDA) described by Ullrich and Grosch (1987) was used to access the contribution of individual aroma constituents to the aroma of anchovies. Aliquots of each dilution (0.5 μL) were analyzed by GC using the SE-54 capillary column. Sniffing of the original extract was carried out three times. Compound detection and odor description were done by the primary author and confirmed by a person familiar and experienced with the procedure. Results were expressed in the form of FD chromatograms (FD factors of the odorants versus their RI values).

Mass Spectrometry. MS analyses were performed with a Finnigan MS8230 (Bremen, Germany) mass spectrometer using the SE-54 and OV-1701 capillary columns. Mass spectra in the electron impact mode MS(EI) were generated at 70 eV. The scan range was 35–250. Compounds were identified using published spectra (NIH/EPA Mass Spectral Libraries) and in-house-generated libraries as well as gas chromatographic retention properties (cochromatography with known standards).

RESULTS

Instrumental Data. The chromatogram presented in Figure 1 illustrates the volatile profile obtained for anchovy that is ripened (16 weeks) via industrial production. Compound identification is reported in Table 1. Sensory evaluation of fish from this process showed that they exhibited the strong characteristic flavor of fully ripened anchovy. As can be seen in Figure 1 there were many compounds detected through eluate sniffing (those with numbers) that gave little or no discernible GC peak. Compounds identified by GC/MS were mainly C_6–C_{10} aldehydes and ketones (Table 1). 2,4-Alkadienals and 3,5-alkadien-2-ones were a significant portion of the detected volatiles.

To assess the effect of ripening anchovy in a sterile environment (lot II) or after early evisceration (lot III) on the development of flavor constituents, the aroma isolates obtained from these lots at 11 weeks of ripening were analyzed. The resulting GC profiles were essentially identical to the profile obtained for lot I at the same stage of ripening (data not shown), and the same compounds were detected by eluate sniffing.

Odor Profiles and FD Chromatograms. On the basis of their odor assessments through GC eluate sniffing, volatile aroma compounds in anchovy were composed of two distinct groups. The first group included compounds with green, plant-like aromas (mainly the C_8 carbonyls and hexanal). The second group was composed of conjugated carbonyls (2,4-alkadienals) which imparted generally fatty and fried fat-like aromas. (*Z*)-4-Heptenal, at the concentrations present in the eluate of the GC column, had a tallowy character with slight fishy background notes. 3-Methyl-2,4-nonanedione exhibited a pleasant fruity odor. Compound **24** had an odor somewhat similar to anise.

An FD chromatogram of the volatile compounds from a fully ripened (16 weeks) commercial anchovy sample displayed 17 compounds in the flavor dilution range of 16–1024 (Figure 2). Compound **24** showed the highest FD factor. Eight additional compounds appeared with FD factors equal to or higher than 128: (*Z*)-4-heptenal, methional, 1-octen-3-one, (*Z*)-1,5-octadien-3-one, (*E*,*Z*)-2,6-nonadienal, (*E*,*E*)-2,4-nonadienal, 3-methyl-2,4-nonanedione, and (*E*,*E*)-2,4-decadienal. On the basis of their high FD values, these compounds appear to be most important to the aroma of ripened anchovy.

In comparing the FD chromatogram of the commercially ripened anchovy (Figure 2) with that of the

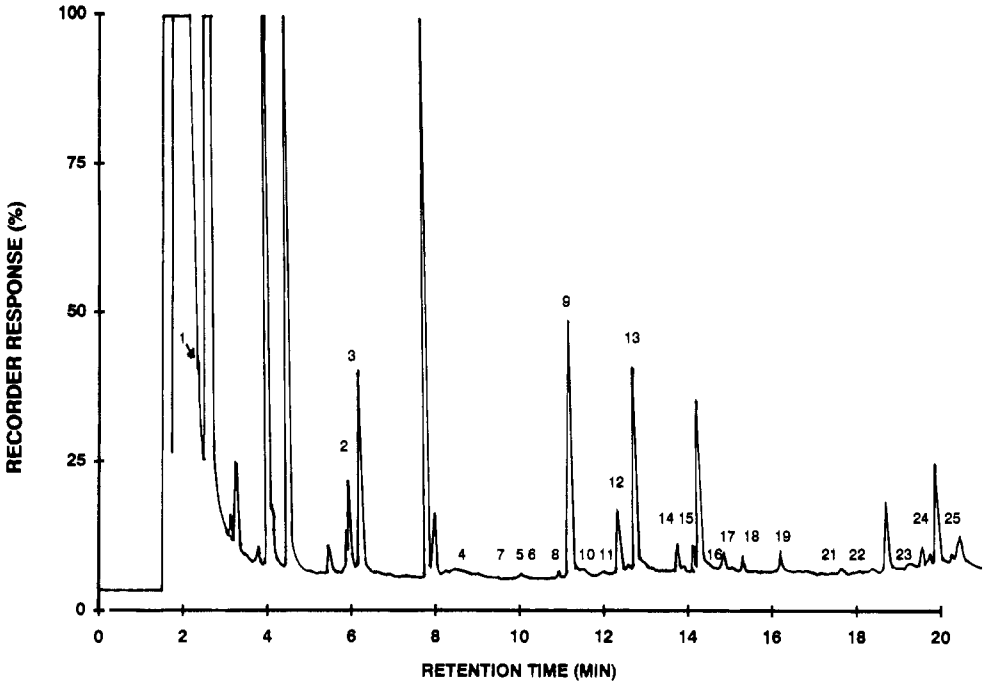

Figure 1. Gas chromatogram (OV-1701 column) of volatiles isolated from commercially ripened anchovy.

Table 1. Volatile Compounds Identified in Anchovy

peak no.[a]	compound	odor quality	RI SE-54[b]	ref RI SE-54[c]	RI OV-1701[b]	ref RI OV-1701[c]	ID means[d]
1	diacetyl	butter-like		595		679	MS[f]
2	hexanal	green grass		801	879	878	MS [1]
3	1-hexen-3-ol	green			888	880	MS [1]
4	(*Z*)-4-heptenal	fatty-fishy	898	901	985	983	MS [1]
5	2,4-hexadienal	*e*	918		1035		MS [2]
6	methional	cooked potato-like	908	909	1044	1042	MS [1]
7	2-acetyl-1-pyrroline	popcorn-like	928	923	1014	1013	MS[f] [1]
8	1-octen-3-one	mushroom-like	977	980	1069	1065	(MS) [1]
9	1-octen-3-ol	mushroom-like		981	1080	1077	MS [1]
10	(*Z*)-1,5-octadien-3-one	geramium-like	988	986	1089	1085	[1]
11	2,4-heptadienal[g]	fatty	999	1000	1116	1117	MS [1]
12	2,4-heptadienal[g]	*e*			1123		MS [2]
13	(*E*,*E*)-2,4-heptadienal	fatty	1012	1013	1137	1137	MS [1]
14	phenylacetaldehyde	floral	1044	1052	1181	1173	MS [1]
15	(*E*,*Z*)-3,5-octadien-2-one	fatty-fruity	1095	1095	1193		MS [1]
16	phenol	*e*			1217	1222	MS [2]
17	3,5-octadien-2-one[g]	*e*			1223		MS [2]
18	(*E*,*E*)-2,4-octadienal	deep fried fat	1111	1110	1244	1247	MS [1]
19	(*E*,*Z*)-2,6-nonadienal	cucumber-like	1158	1155	1275	1271	MS [1]
20	unknown	green	1186				
21	2,4-nonadienal[g]	fatty	1197	1196	1316	1319	[1]
22	(*E*,*E*)-2,4-nonadienal	deep fried fat	1215	1216	1346	1344	(MS) [1]
23	3-methyl-2,4-nonanedione	fruity-sweet	1250	1253	1398	1400	(MS)[f] [1]
24	unknown	sweet anise-like	1269		1430		
25	(*E*,*E*)-2,4-decadienal	deep fried fat	1320	1318	1449	1447	MS [1]

[a] Peak number in Figure 1. [b] Calculated retention index on capillaries SE-54 and OV-1701. [c] Reference retention index on capillaries SE-54 and OV-1701. [d] Compound identified on the basis of the following criteria: MS, mass spectra consistent with previously reported spectra; (MS), incomplete mass spectra due to low concentration; [1], RI on the capillary SE-54 or OV-1701 (odor quality perceived at the sniffing port); [2], tentative identification based solely on mass spectral characteristics. [e] Odorless at the sniffing port. [f] Mass spectra obtained in a separate experiment. [g] Configuration of isomer not determined.

laboratory-prepared product (lot I at 11 weeks of ripening; Figure 3), differences appear with respect to compounds **25**, **24**, **23**, and **22**, which were present at 2–4-fold lower concentrations in lot I, while compounds **18** [(*E*,*Z*-2,6-nonadienal] and **4** [(*Z*)-4-heptenal] were present at 16-fold lower concentrations in lot I. Additionally, several of the background flavor compounds [i.e. those with FD factors equal to or lower than 64, for example (*E*,*E*)-2,4-heptadienal, (*E*,*Z*)-3,5-octadien-3-one, and (*E*,*E*)-2,4-octadienal] were absent from the laboratory-prepared sample. Sensory evaluation of fish from lot I showed that although the characteristic flavor of ripened anchovy was less pronounced at the 11th week of ripening, it was fully developed after 13 weeks. The FD chromatogram in Figure 3 suggests that the flavor isolate obtained at this stage contained lower concentrations of volatiles. This could be due to small differences in sample size and/or to the fact that the brine was changed just before sampling, resulting in some extraction of volatiles into the brine.

DISCUSSION

Salting of fish has been widely used to produce different types of fermented fish products that are peculiar to a particular region (Beddows, 1985). The influence of microorganisms on the flavor development in such products has received relatively little attention,

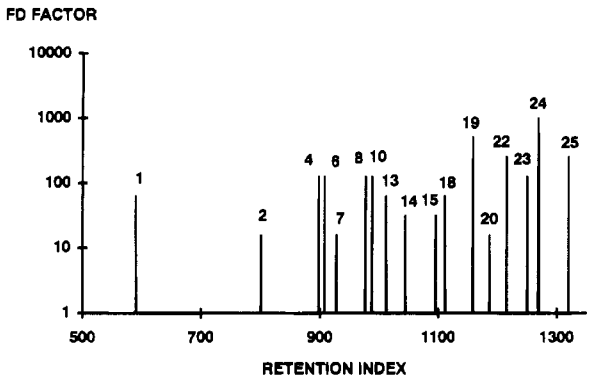

Figure 2. FD chromatogram of volatile compounds isolated from commercially ripened anchovy [*X*-axis is in retention indices units (SE-54 column); numbers in figure refer to compounds listed in Table 1].

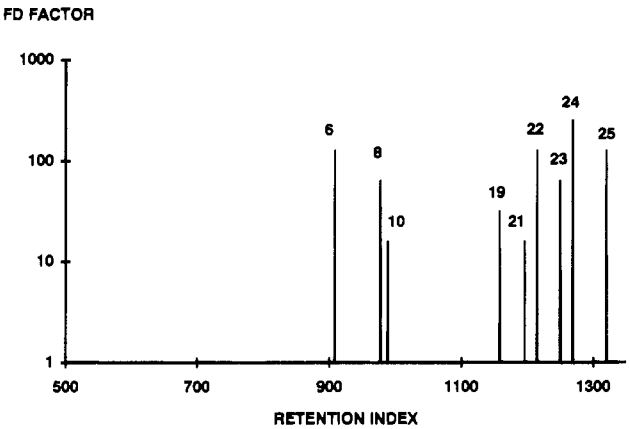

Figure 3. FD chromatogram of volatile compounds isolated from laboratory ripened anchovy [*X*-axis is in retention indices units (SE-54 column); numbers refer to compounds listed in Table 1].

although some authors have emphasized their importance in relation to aroma (Alm, 1965; Shewan, 1965; Beddows, 1985). In this study, we have investigated the ripening of salted anchovy in an attempt to elucidate the mechanisms leading to the development of the typical flavor. On the basis of the GC and the FD chromatograms obtained from the different lots of anchovy ripened in the laboratory or from commercially processed samples, it can be concluded that microorganisms play a minor role in the development of the characteristic flavor of ripened anchovy. This is mainly supported by the fact that the GC chromatograms obtained from lot II (early evisceration) and lot III (ripened in the presence of antibiotics) displayed the same aroma profiles observed as the control. Therefore, it would appear that the high-salt environment creates unfavorable conditions for the activity of the residual halophilic flora (Belemlih, 1986) during ripening of anchovy.

Eight carbon alcohols and ketones were identified at the different stages of the ripening process. These compounds have been shown to occur in various species of fresh- and saltwater fish, in crustaceans, and in shellfish (Josephson and Lindsay, 1986). 1-Octen-3-one and (*Z*)-1,5-octadien-3-one appear with relatively high FD factors in the FD chromatogram of fully ripened anchovy (Figure 3). Therefore, these carbonyls survive the ripening process and contribute plant-like aromas to ripened anchovy. Among the nine-carbon carbonyls that have been reported as volatiles associated with freshly harvested fish, only (*E*,*Z*)-2,6-nonadienal was identified in anchovy. This is not surprising since nine-carbon volatiles have not been identified in all species of seafoods investigated (Josephson, 1991). On the basis of its high FD factor, (*E*,*Z*)-2,6-nonadienal contributes to the flavor of ripened anchovy through its characteristic cucumber-like aroma. Six-carbon alcohols and aldehydes were found at concentrations above their recognition thresholds in fully ripened anchovy. Hexanal appears with a relatively low FD factor (Figure 2) but may contribute some background note to the characteristic aroma of ripened anchovy.

Table 2. Fatty Acid Composition of Mediterranean Anchovy *E. encrasicholus* (Zlatanos and Sagredos, 1993)

fatty acid abbreviation	content, mg/100 g of fat	fatty acid abbreviation	content, mg/100 g of fat
C12:0	0.2	C18:3	1.0
C14:0	7.8	C18:4ω3	1.8
C14:1	0.3	C20:1	1.9
C16:0	19.4	C20:4ω6	1.9
C16:1	4.9	C20:5 3	11.0
C17:0	2.1	C22:1	3.3
C18:0	4.5	C22:5ω3	1.0
C18:1	8.7	C22:6ω3	25.5
C18:2ω6	2.0	C24:1	1.8

Evidence for enzymatic involvement in the oxidation of fresh fish lipids has been steadily mounting (Josephson et al., 1987). In addition to the 12- and 15-lipoxygenase activities that have been characterized in marine teleost fishes (German and Creveling, 1990), a novel linoleate 13(*S*)-lipoxygenase was recently found in the skin of sardine (*Sardinops melanosticus*) (Mohri et al., 1992).

High contents of polyunsaturated $\omega-3$ fatty acids (C22:6 and C20:5) have been found in the fat of the Mediterranean anchovy (*E. encrasicholus*) (Table 2; Zlatanos and Sagredos, 1993), and these fatty acids are probably precursors of the six- and eight-carbon alcohols and ketones along with (*E*,*Z*)-2,6-nonadienal found in anchovy, assuming the existence of 12- and 15-lipoxygenase activities. A mechanism for the biogenesis of these fresh fish volatiles has been proposed by Josephson and Lindsay (1986).

Various conjugated carbonyl compounds were identified in anchovy. Owing to their relatively high FD factors, these volatiles impart general fatty and fried fat-like aromas to ripened anchovy flavor. 2,4-Heptadienal and 3,5-octadien-2-one arise from autoxidation of $\omega-3$ PUFA, e.g. eicosapentaenoic acid (Josephson, 1991), while $\omega-6$ PUFA are precursors of 2,4-nona- and 2,4-decadienal (Grosch, 1987). 2,4-Hepta- and decadienal along with 3,5-octadien-2-one were detected at low levels in harvested fish held for a day on ice (Josephson and Lindsay, 1986). (*E*,*E*)-2,4-Octadienal has not been previously reported as a fish volatile, while 2,4-nonadienal was identified in oxidized whitefish (*Coregonus clupeaformis*) (Josephson et al., 1984).

On the basis of its FD factor, (*Z*)-4-heptenal appears as a potent odorant in anchovy flavor. Although (*Z*)-4-heptenal was not considered to elicit a fishy character (Karahadian, 1988; Josephson and Lindsay, 1987), we found this compound to exhibit some fishy background notes when evaluated through GC effluent sniffing. Early information in the literature related the formation of this compound to lipid autoxidation, even though no satisfactory mechanism of formation was suggested. More recently, Josephson and Lindsay (1987) demonstrated that (*Z*)-4-heptenal was formed by a water-mediated, retro-aldol condensation of (*E*,Z)-2,6-nonadienal. Furthermore, heating an aqueous model system (pH 7.4) containing (*E*,*Z*)-2,6-nonadienal caused an enhanced formation of (*Z*)-4-heptenal. On the basis of

these findings, it appears that while some conversion of (*E*,*Z*)-2,6-nonadienal to (*Z*)-4-heptenal may occur after the harvesting of anchovy, formation of (Z)-4-heptenal was most likely enhanced during the distillation of volatiles through the combined effect of time and temperature (1 h at 57–58 °C) in an aqueous environment.

To our knowledge, 3-methyl-2,4-nonanedione has never been reported as a fish volatile. Guth and Grosch (1989) first identified this compound along with nonane-2,4-dione in reverted soybean oil. They described the odor of 3-methyl-2,4-nonanedione as "lard-like, strawy, and fruity" and found its odor threshold to be very low (0.01 ng/L of air) as compared to nonane-2,4-dione (3.9 ng/L of air). On the basis of the results of an aroma extract dilution analysis, it was concluded that this compound contributed very strongly to the reversion odor of soybean oil. In the present investigation, 3-methyl-2,4-nonanedione was found to exhibit a pleasant fruity aroma when evaluated through GC effluent sniffing. On the basis of its high FD factor, this volatile appears to be highly contributing to anchovy flavor. Guth and Grosch (1990, 1991) investigated the origin of this compound in reverted soybean oil and found small amounts of branched furanoid fatty acids to be precursors of this volatile. The participation of singlet oxygen ($^1O_2$) in the oxidation of these furanoid fatty acids was concluded on the basis of model experiments. Furanoid fatty acids have been previously shown to occur in the lipids of several fish species (Gunstone et al., 1978), and our results suggest their occurrence in the lipids of anchovy. Because 3-methyl 2,4-nonanedione was identified in anchovy just after salting (Triqui and Reineccius, 1995), it must have been formed following evisceration of anchovy. Natural sensitizers in fish (heme compounds and probably residual chlorophyll from the stomach) may, in an oxygen environment, generate singlet oxygen species which may then react with furanoid acids present in anchovy. However, the low concentrations of 3-methyl-2,4-nonanedione found in anchovy suggest that termination reactions probably occur in the absence of light during the final salting of anchovy, therefore limiting further formation of this compound during ripening.

Strecker aldehydes (methional and phenylacetaldehyde) were also identified in our flavor isolates. These aldehydes are known to be formed under mild heating conditions (Pokorny, 1980; Chan and Reineccius, 1994a,b). Whether these volatiles are components of anchovy flavor or artifacts of aroma isolation awaits further study.

2-Acetyl-1-pyrroline was detected in trace amounts in anchovy just after salting (Triqui and Reineccius, 1995). This compound has been shown to be a potent volatile of various foods including cooked rice (Buttery et al., 1983), popcorn (Schieberle, 1991), and bread (Grosch and Schieberle, 1991). On the basis of labeling and model experiments, Schieberle (1990) concluded that in bread crust 2-acetyl-1-pyrroline was formed in the yeast cells from ornithine or proline and dihydroxyacetone phosphate, with 1-pyrroline and 2-oxopropanal as intermediates, and released from the yeast cells during baking. 2-Acetyl-1-pyrroline is unlikely to be a component of fresh anchovy flavor. It was probably formed during the isolation procedure from 1-pyrroline generated by the Strecker degradation of proline (Tressl et al., 1985) and 2-oxopropanal, which has been shown to occur in glycolyzing tissues (Riddle and Lorenz, 1968). The higher concentrations observed at the end of ripening are likely due to the higher content of free proline generated by proteolysis.

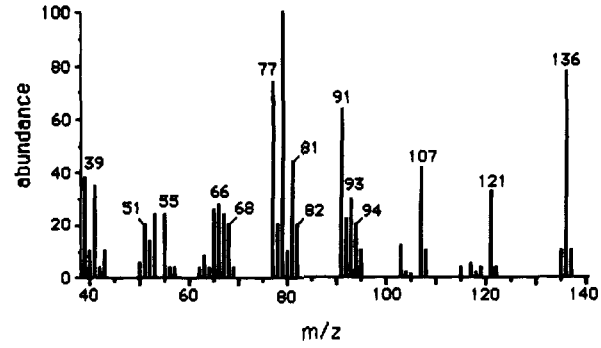

Figure 4. Mass spectrum of unknown compound (has highest FD value).

Compound **24** (Table 1) was the volatile showing the highest FD factor in the FD chromatograms of anchovy. This unknown exhibited an odor somewhat reminiscent of anise. Mass spectral data suggested that there are four isomers of this volatile—the other four isomers having substantially lower FD values. The molecular weight was determined as 136 on the basis of mass spectra obtained in the chemical ionization mode (data not shown), but our attempts at identification were unsuccessful. The mass spectra and the odor characteristics suggest a terpene-like structure (Figure 4).

CONCLUSIONS

The present investigation has provided insight into the chemical basis of ripened anchovy flavor. Our results indicated that there was no evidence for the microbial formation of volatiles in the ripening process. Anchovy ripened in the presence of microbial inhibitors had the same aroma profile as compared to the control. Volatile compounds identified in anchovy were composed of two groups based on their mechanism of formation. Lipoxygenase-derived volatiles included eight-carbon alcohols and ketones and (*E*,*Z*)-2,6-nonadienal, which contributed plant- and cucumber-like aromas to anchovy flavor. Autoxidatively derived volatiles were composed of various conjugated aldehydes which imparted general fatty and fried fat-like aromas, such as 2,4-heptadienal, 3,5-octadien-2-one, and 2,4-decadienal. Two volatiles, not previously reported to occur in fish, 3-methyl-2,4-nonanedione and an unknown with an odor reminiscent of anise, were also important components of anchovy flavor. The changes in flavor profiles associated with ripening will be the subject of a later publication (Triqui and Reineccius, 1995).

ACKNOWLEDGMENT

We gratefully acknowledge Pr. W. Grosch and Dr. H. H. Guth from Deutsche Forchungsanstalt fur Lebensmittelchemie (Garching, Germany) for their guidance, their assistance in the method of aroma extract dilution analysis, and the use of their facilities.

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Received for review April 11, 1994. Accepted November 10, 1994.[⊗]

JF940181M

[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1994.