Changes in Flavor Profiles with Ripening of Anchovy (*Engraulis encrasicholus*)

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Qualitative and relative quantitative changes in flavor profiles associated with ripening of anchovy (*Engraulis encrasicholus*) were investigated. Samples of commercially processed anchovies were analyzed by gas chromatography (using either a flame ionization, atomic emission, or mass selective detector) and by sniffing of the GC effluent. Qualitative and quantitative differences in aroma profiles, particularly an increase in the concentrations of 2,4-heptadienal and (*E,Z*)-3,5-octadien-2-one, were associated with the development of the typical flavor obtained after anchovy ripening. Results are consistent with lipid autoxidation during ripening being primarily responsible for aroma development.

INTRODUCTION

Anchovy (*Engraulis encrasicholus*) is primarily commercially produced in Spain, Italy, France, Greece, and Morocco (Anonymous, 1992). France and Spain together produced about 17 000 tons of anchovy in 1992 and Morocco about 10 000 tons (FAO, 1992). The anchovy is typically about 12–13 cm in length at maturity but may reach 20 cm as a maximum. It is recognizable by its spindle-shaped body with a green-blue back, silver flanks, and unequal jaws. The anchovy is eaten as a salted and cured (ripened) product.

In Morocco and in other anchovy-producing countries, salting and ripening of anchovy is carried out empirically. There is no reliable objective method of following the ripening process and determining the proper time for sale of the product. Some attempts have been made to develop a suitable method such as using the total ester index (Filsinger et al., 1982), the ratio of free amino acids to total amino acids (FAA/TAA) (Baldrati et al., 1975), the ratio of nonprotein nitrogen to total nitrogen (Mattos et al., 1976), or the estimation of free fatty acids (Roldan et al., 1985). However, these parameters have not been very successful in predicting the quality of the final product or the stage at which anchovy develops optimum flavor and should go to market. As a result, visual inspection and tasting are still the usual methods of following the process (Thackaberry, 1979).

The present study was undertaken to assess the qualitative and quantitative changes in flavor profiles that occur during the ripening of anchovy. The ultimate goal was to provide the basis for developing an objective means of following the ripening of anchovy.

MATERIALS AND METHODS

Materials. This study was carried out on commercially processed anchovies which were purchased from a local processor in the Rabat area. Anchovy samples were collected from the same container at the start of ripening (2 days) and at maturity (90 days). Samples were immediately frozen for later analysis.

Isolation of the Volatiles. Aroma constituents were isolated by vacuum distillation and solvent extraction as previously described (Triqui and Reineccius, 1995), but with the following modifications: For each sample, 200 g of anchovies was weighed into a Waring-type blender, and 1 L of distilled water was added. This mixture was blended at high speed for 1–2 min. The sample was then distilled under vacuum (rotary evaporator) until 150 mL of distillate was collected. The distillate was extracted three times with 25 mL of methylene chloride in a separatory funnel; before concentration, each flavor isolate was spiked with 25 μg of *n*-decane as internal standard, and 50 μg of butylated hydroxyanisole (BHA) was added as an antioxidant.

Gas Chromatographic Analysis. A Hewlett-Packard (HP) Model 5890 gas chromatograph equipped with either an atomic emission detector (AED), a flame ionization detector (FID), or a mass selective detector (MSD) was used. Separation was achieved on a 30 m × 0.25 mm i.d. × 1.0 μm film thickness fused silica capillary column (J&W Scientific, Folsom, CA), coated with cross-linked 5% phenylmethylsilicone (DB-5). The GC column oven was started at 35 °C (2 min hold) and then raised (40 °C/min) to 50 °C, held 1 min isothermally, and then raised at 6 °C/min to 250 °C, and finally held isothermally for 10 min (Triqui and Reineccius, 1995). The injector temperature was 250 °C for all instruments. Detector temperatures were 275 and 300 °C for the FID and AED, respectively. Helium was used as the carrier gas at a column flow rate of 1.25 mL/min and 15 psi of head pressure. A 1-μL sample was injected in the split mode (split ratio of 30:1). The data from the HP 5890 were recorded on a HP integrator (FID signal) and by using the HP Chem Station software for the AED or the MSD. Retention data of the compounds are presented as retention indices (RI) according to the principles of Van den Dool and Kratz (1963).

GC Effluent Sniffing. For sniffing, the final portion of the GC column was passed through an unused heated detector block (275 °C, copper insert) to terminate in a position outside the GC which was comfortable for sniffing. Descriptions of the odors sniffed were recorded with a tape recorder; starting time and duration of each odor were recorded by using a hand-

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Table 1. Volatile Compounds Identified in Commercially Processed Anchovies after 2 Days of Salting

peak[a]	compound	odor quality	RI DB-5[b]	RI SE-54[c]	normalized peak area[d]	identification means[e]
2	2,3-pentanedione	butter-like	702	700	0.019	MS (2)
5	styrene	f			0.265[g]	MS
6	(Z)-4-heptenal	fatty−fishy	904	901	0.002[g]	MS (2)
7	methional	cooked potato	911	909	0.002[g]	MS (2)
8	2-acetyl-1-pyrroline[h]	popcorn-like	928	923	0.002[g]	MS (2)
9	benzaldehyde	almond-like	960	968	0.078	MS (2)
10	1-octen-3-ol	mushroom-like	978	981	0.044	MS (2)
11	(Z)-1,5-octadien-3-one	geranium-like	988	986	0.002[g]	2
13	n-decane (ISTD)	f	1000	1000	1	
14	(E,E)-2,4-heptadienal	fatty	1008	1013	0.103	MS (2)
16	2-phenylacetaldehyde	floral	1046	1052	0.008[g]	MS (2)
19	(E,Z)-3,5-octadien-2-one	fatty−fruity	1098	1095	0.051	MS (2)
20	O-decylhydroxylamine	green	1100		0.003[g]	MS (4)
22	nonanal	fatty/soapy	1109	1105	0.033	MS (2)
23	(E,E)-2,4-octadienal[h]	deep fried fat	1113	1110	0.058	MS (2)
25	(Z)-2-nonenal	fatty/green	1151		0.037	MS (2)
26	(E,Z)-2,6-nonadienal	cucumber-like	1155	1155	0.044	MS (2)
27	4-ethylbenzaldehyde	f	1171		0.035[g]	MS (1)
28	α-terpineol	f	1197	1195	0.074	MS (2)
31	(E,E)-2,4-nonadienal[i]	deep fried fat	1222	1216	0.002[g]	2
33	3-methyl-2,4-nonanedione	fruity/sweet		1253	0.001[g]	MS (1)[j]
35	unknown	sweet anise-like	1278		0.334	
36	unknown	sweet anise-like	1288		0.152	
37	(E,Z)-2,4-decadienal	fatty/green		1295	0.03[g]	MS (1)
39	trans-4,5-epoxy(E)-2-decanal	metallic	1384	1382	0.03[g]	2

[a] Peak numbers in Figures 1−6. [b] Calculated retention index on DB-5 capillary column. [c] Reference retention index on SE-54 capillary column. [d] Normalized GC peak area with respect to the internal standard. [e] Compound identified on the basis of the following criteria: MS, mass spectra consistent with NBS/EPA and user generated libraries; (MS), incomplete mass spectra due to low concentration; (1), odor quality perceived at the sniffing port; (2), odor quality and RI on capillary DB-5 column; (3), RI on the capillary DB-5 column; (4), tentative identification based solely on mass spectral characteristics. [f] No odor at sniffing port. [g] Values were visually estimated from the chromatograms. [h] Mass spectra obtained in a separate experiment. [i] Identification confirmed by injection of pure reference compound. [j] Identification based on Guth and Grosch (1989).
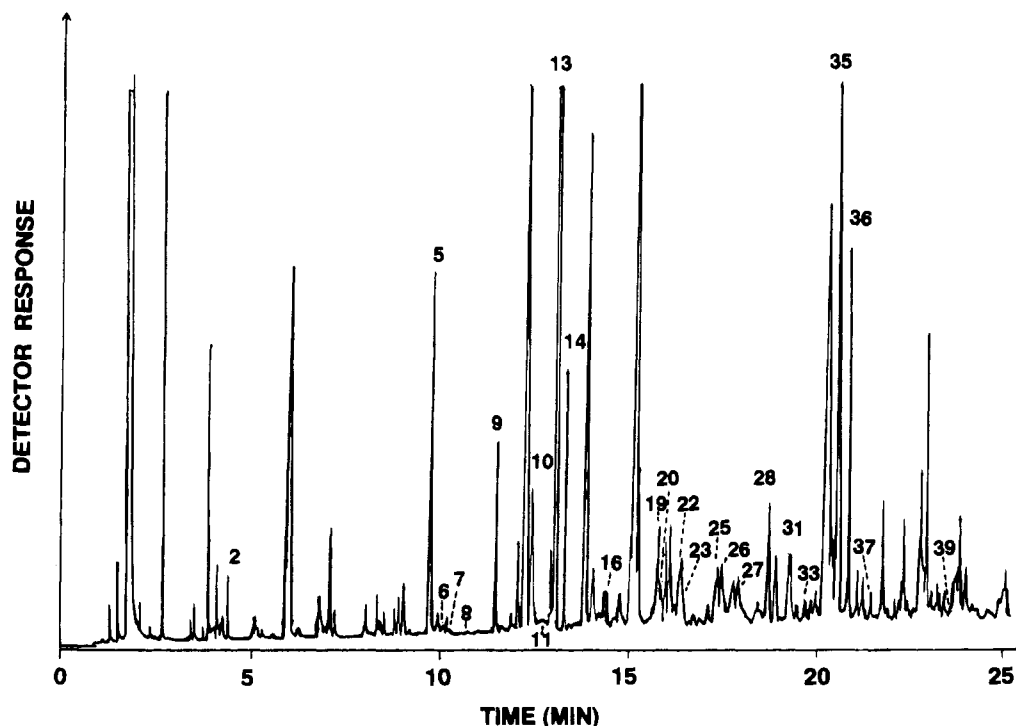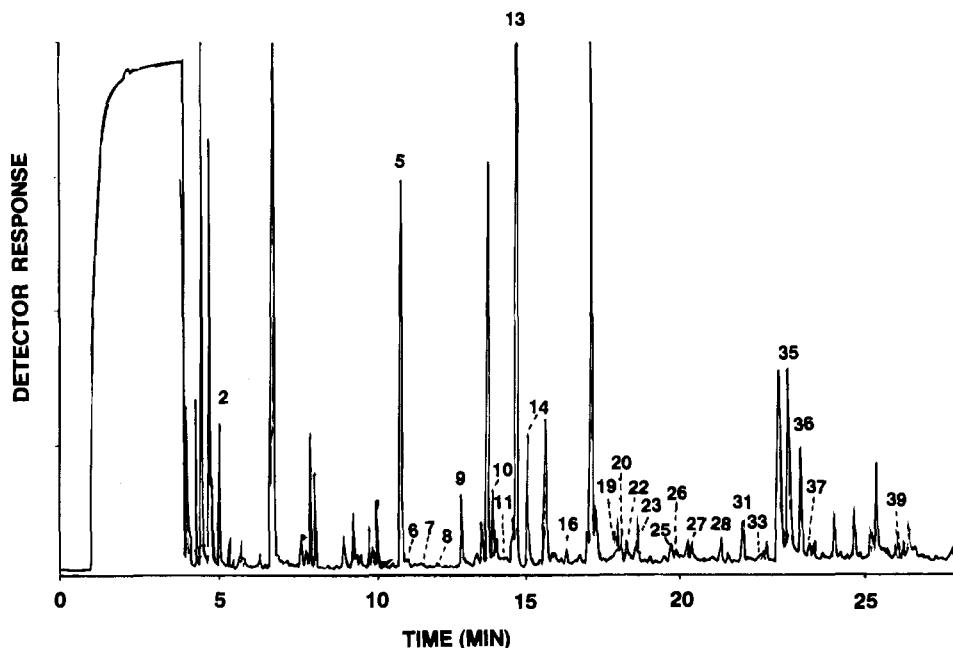

Figure 1. Gas chromatogram of the volatile compounds isolated from commercially processed anchovies after 2 days of salting (FID detector). See Table 1 for identifications.

held button that controlled an external input to the integrator. Once the sniffing run was recorded, the end of the column was removed from the sniffing port and connected to the FID detector. Another sample injection was made to record the GC profile. Chromatographic conditions were the same as those described above.

Mass Spectrometry. Mass spectra in the electron impact mode were obtained using a HP Model 5970 mass detector. The capillary column was interfaced directly to the mass spectrometer. The mass spectra of unknown compounds were compared with those in the NBS/EPA and user-generated libraries by the Chem Station data system and manually checked against literature sources.

Atomic Emission Detection. A HP 5921A atomic emission detector was used in the analysis of the elements C, N, O, and S. Due to the detectable range of the positionable diode array in the AED, C, S, and N were analyzed in the first injection and O was analyzed in a succeeding injection.

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Figure 2. Gas chromatogram of the carbon-containing volatiles isolated from commercially processed anchovies after 2 of days salting (AED detector). See Table 1 for identifications.

Conditions were those of Baloga et al. (1990) and were as follows:

element	wavelength (nm)	scavenger gas	makeup flow (mL/min)
C	193	O_2/H_2	30
S	181.4	O_2/H_2	30
N	174.3	O_2/H_2	30
O	777.3	O_2/H_2CH_4	30

spectrometer purge flow, nitrogen at 2 L/min; window purge flow rate, 40 mL/min; solvent backflush used, yes; transfer line temperature, 300 °C; cavity temperature, 300 °C; water temperature, 65 °C.

Compound Identification. Compound identification was accomplished using a combination of mass spectrometry, elemental analysis by AED detection, odor character, and coelution of standards (when available). The methods used for the identification of each unknown are listed in Table 1.

RESULTS

A typical chromatogram obtained from a sample of anchovy after 2 days of salting is shown in Figure 1. Anchovy at this stage is considered to have a raw fish flavor (compound identification is reported in Table 1). AED elemental profiles from this sample showed a total absence of sulfur- and nitrogen-containing volatiles (data not shown). Thus, any sulfur- or nitrogen-containing volatiles in anchovy at this early stage of ripening are present in extremely low concentrations. 2-Acetyl-1-pyrroline, for example, was detected by smell in this sample even though it was present below the detection level of the AED. The AED-C and AED-O chromatograms of this sample are shown in Figures 2 and 3. Although the sensitivity of the AED in the oxygen mode is low in comparison to the other elements, especially carbon (Baloga et al., 1990), an overlay plot
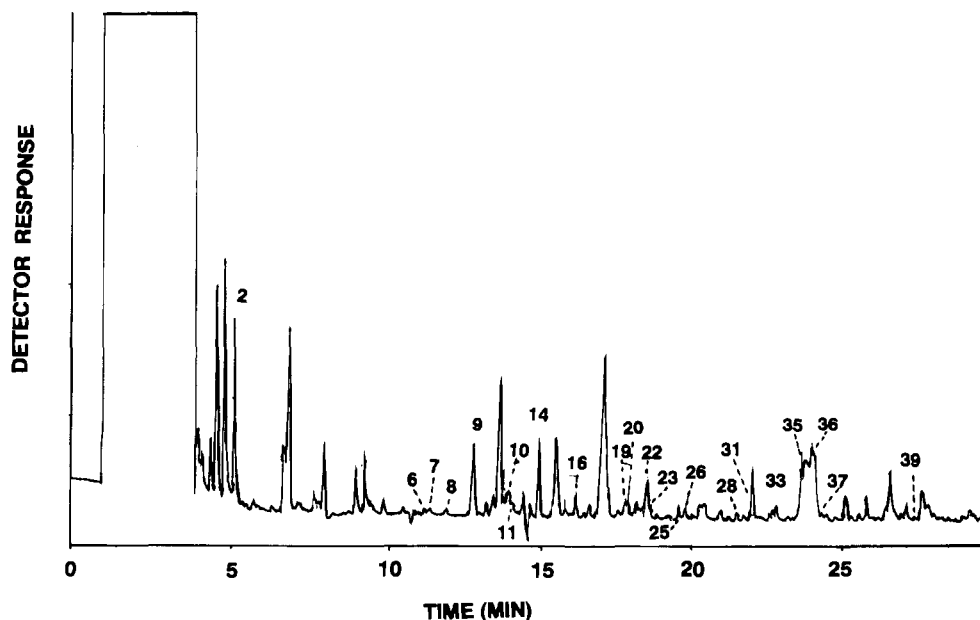
Figure 3. Gas chromatogram of the oxygen-containing volatiles isolated from commercially processed anchovies after 2 of days salting (AED detector). See Table 1 for identifications.

Table 2. Volatile Compounds Identified in Commercially Processed Anchovies after 90 Days

peak[a]	compound	odor quality	RI DB-5[b]	RI SE-54[c]	normalized peak area[d]	identification means[e]
1	diacetyl	butter-like		595	0.02[f]	MS (1)
2	2,3-pentanedione	butter-like	702	700	0.016	MS (2)
3	hexanal	green grass	798	801	0.02[f]	MS (2)
4	(E)-2-hexenal	green	862	856	0.01[f]	MS (2)
5	styrene	g			0.33[f]	MS
6	(Z)-4-heptenal	fatty–fishy	904	901	0.016[f]	MS (2)
7	methional	cooked potato	911	909	0.002[f]	MS (2)
8	2-acetyl-1-pyrroline[h]	popcorn-like	928	923	0.001[f]	MS (2)
9	benzaldehyde	almond-like	960	968	0.06	MS (2)
10	1-octen-3-ol	mushroom-like	978	981	0.32	MS (2)
11	(Z)-1,5-octadien-3-one	geranium-like	988	986	0.025[f]	2
12	2,4-heptadienal	fatty	996	1000	0.31	MS (2)
13	n-decane (ISTD)		1000	1000	1	
14	(E,E)-2,4-heptadienal	fatty	1008	1013	0.94	MS (2)
15	2-ethyl-1-hexenol	g	1035	1029	0.12	MS (3)
16	2-phenylacetaldehyde	floral	1046	1052	0.33	MS (2)
17	(E)-2-octen-1-ol	g	1067		0.081	MS (4)
18	unknown	fatty	1093		0.045	
19	(E,Z)-3,5-octadien-2-one	fatty–fruity	1098	1095	0.195	MS (2)
20	O-decylhydroxylamine	g	1100		0.084	MS (4)
21	dipropyl disulfide	g	1107		0.03[f]	MS (4)
22	nonanal	fatty/soapy	1109	1105	0.093	MS (2)
23	(E,E)-2,4-octadienal[h]	deep fried fat	1113	1110	0.072	MS (2)
24	2-ethylhexanoic acid	g	1128		0.02[f]	MS (4)
25	(Z)-2-nonenal	fatty/green	1151		0.038	MS (2)
26	(E,Z)-2,6-nonadienal	cucumber-like	1155	1155	0.063	MS (2)
27	4-ethylbenzaldehyde	sweet	1171		0.045	MS (1)
28	α-terpineol	floral	1197	1195	0.092	MS (2)
29	verbenone	g			0.055	MS (4)
30	decanal	floral	1208	1208	0.007[f]	(MS) (2)
31	(E,E)-2,4-nonadienal[i]	deep fried fat	1222	1216	0.005[f]	2
32	benzothiazole	g	1224	1227	0.05[f]	(MS) (3)
33	3-methyl-2,4-nonanedione	fruity/sweet		1253	0.005[f]	(MS) (1)[j]
34	unknown	sweet anise-like	1268		0.189	
35	unknown	sweet anise-like	1278		1.124	
36	unknown	sweet anise-like	1288		0.14	
37	(E,Z)-2,4-decadienal	fatty/green		1295	0.02[f]	MS (1)
38	(E,E)-2,4-decadienal	deep fat fried		1318	0.02[f]	MS (1)
39	trans-4,5-epoxy-(E)-2-decanal	metallic	1384	1382	0.005[f]	2

[a] Peak numbers in Figures 4–6. [b] Calculated retention index on DB-5 capillary column. [c] Reference retention index on SE-54 capillary column. [d] Normalized GC peak area with respect to the internal standard. [e] Compound identified on the basis of the following criteria: MS, mass spectra consistent with NBS/EPA and user generated libraries; (MS), incomplete mass spectra due to low concentration; (1), odor quality perceived at the sniffing port; (2), odor quality and RI on capillary DB-5 column; (3), RI on the capillary DB-5 column; (4), tentative identification based solely on mass spectral characteristics. [f] Values were visually estimated from the chromatograms. [g] No odor at sniffing port. [h] Mass spectra obtained in a separate experiment. [i] Identification confirmed by injection of pure reference compound. [j] Identification based on Guth and Grosch (1989).

of the AED-C and AED-O chromatograms was valuable in indicating (or confirming) oxygen-containing compounds in the GC profile. Thus, the AED was useful in supporting identifications. AED profiles were also of value when a volatile could not be identified. For example, we at least know that compounds **34–36** (Table 2) are oxygen-containing volatiles.

A FID chromatogram of the volatile compounds isolated from anchovy after 90 days of ripening is shown in Figure 4. The corresponding compound identifications are reported in Table 2. On the basis of sensory evaluation by trained members, it was concluded that the anchovies after 90 days of ripening had a characteristic fully ripened anchovy flavor.

Compounds from 90-day-ripened anchovy that were able to be sensorially detected on their elution from the GC or tentatively identified are listed in Table 2. We must note that some of the data are contradictory. For example, although dipropyl disulfide and benzothiazole were tentatively identified on the basis of MS data, they were not detected by the AED. Therefore, these compounds are unlikely to be present owing to the excellent sensitivity and selectivity of the AED in the sulfur mode (about 10 pg). Thus, the AED data (i.e. lack of confirmation by AED data) suggest that these library matches

provided by the MS data system are in error. From the AED-C and AED-O profiles (Figures 5 and 6), it appears that the AED is less sensitive than the FID detector. The difference in peak intensity between the AED-C and the FID is due to a dilution of the samples just prior to the AED work (needed additional GC injections) and does not reflect lower sensitivity for the AED-C. However, some compounds such as (E)-2-octen-1-ol and decanal were not detected in the AED-O profile due to the lower sensitivity of the AED to oxygen.

In addition to the qualitative differences observed in the volatile profiles between anchovy just after salting and after ripening, some differences also appear in the relative concentrations (reported as normalized GC peak areas with reference to the internal standard, n-decane) of some volatiles. The main differences appear to reside in the concentrations of carbonyl derivatives of conjugated dienes (Tables 1 and 2). The concentrations of (E,E)-2,4-heptadienal and (E,Z)-3,5-octadien-2-one are approximately 3 and 4 times higher, respectively, after ripening. 1-Octen-3-ol was present at a concentration 7-fold higher after ripening than before ripening. An increase in concentration was also observed for nonanal, with values 3-fold higher. For some other volatiles such

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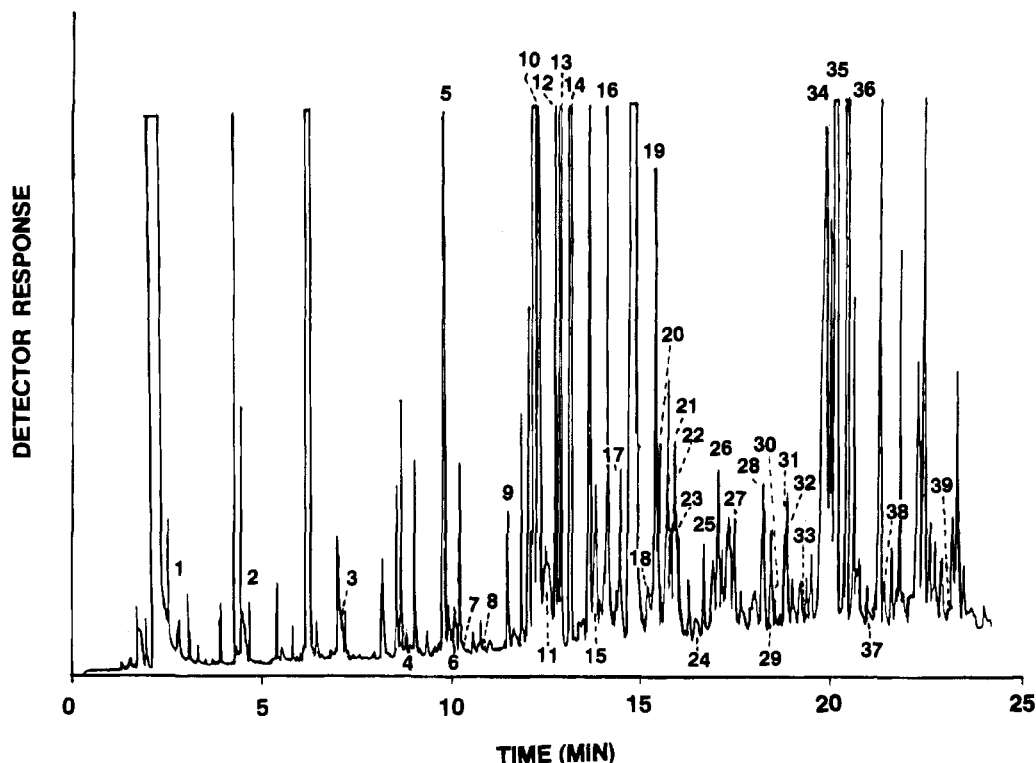
Figure 4. Gas chromatogram of the volatiles isolated from commercially processed anchovies after 90 days of ripening (FID detector). See Table 2 for identifications.
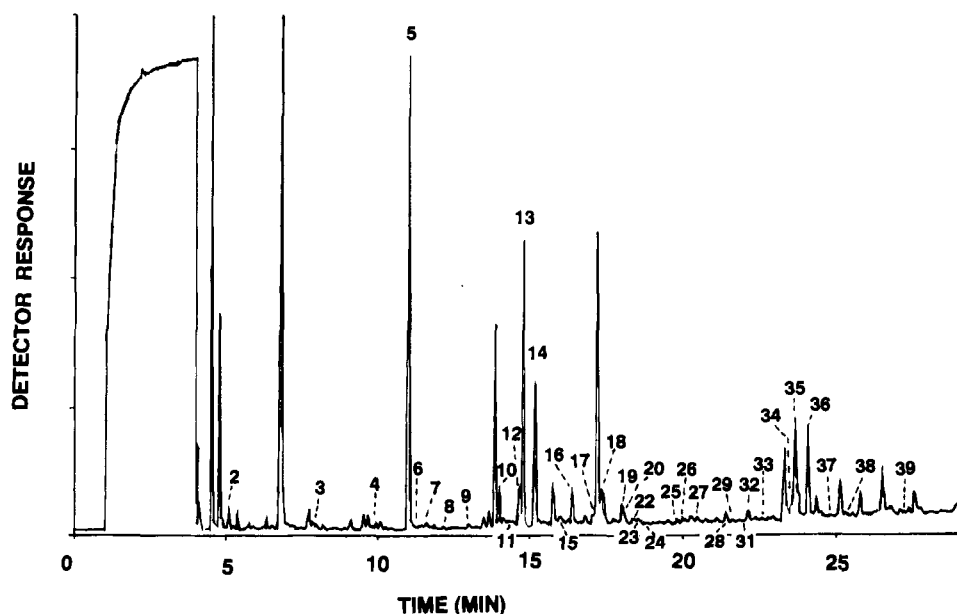
Figure 5. Gas chromatogram of the carbon-containing volatiles isolated from commercially processed anchovies after 90 days of ripening (AED detector). See Table 2 for identifications.

as (E,E)-2,4-octadienal, (Z)-2-nonenal, and (E,Z)-2,6-nonadienal, no differences were observed.

While we do not have very accurate data for the differences in the concentrations of some influential volatiles including (Z)-4-heptenal, (Z)-1,5-octadien-3-one, (E,E)-2,4-nonadienal, 3-methyl-2,4-nonanedione, and (E,E)-2,4-decadienal, they all appear to have increased considerably (3–10-fold increases) after 90 days of ripening. In most of these cases, the peaks were not integrated but their concentrations visually estimated from the chromatogram, which may introduce some error. The concentrations of hexanal, (E)-2-hexenal, and (E,E)-2,4-decadienal likely increased during ripening

since they were not detected by eluate sniffing after 2 days of salting but were noted after 90 days of ripening.

DISCUSSION

We previously reported on volatile compounds that contributed significantly to the aroma of ripened anchovy (Triqui and Reineccius, 1995). In the present investigation, some additional volatile compounds were positively or tentatively identified in anchovy (Tables 1 and 2). Some of these compounds were found at concentrations above their sensory recognition thresholds at the GC effluent, such as 2,3-pentanedione, (E)-2-hexenal, nonanal, (Z)-2-nonenal, and decanal, and

Figure 6. Gas chromatogram of the oxygen-containing volatiles isolated from commercially processed anchovies after 90 days of ripening (AED detector). See Table 2 for identifications.
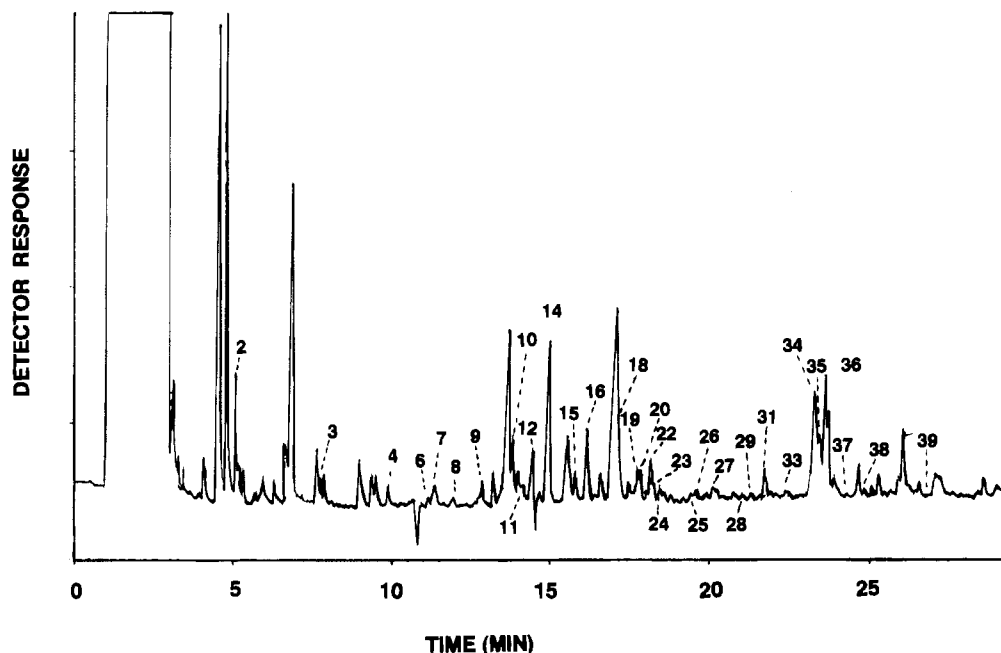
thus may make a contribution to the aroma of anchovy. The source of many of the volatiles can be suggested. The terpene compounds (α-terpineol and verbenone) are probably from the diet of the anchovy. Styrene is likely a contaminant from the plastic barrels (Heath and Reineccius, 1986) used for the packing of anchovy. This compound did not contribute to the GC odor profile at the concentrations present in the GC effluents. Trace amounts of trans-4,5-epoxy-(E)-2-decenal were also detected during GC-eluant sniffing of flavor isolates from anchovy. Because of its very low threshold (0.0005–0.005 ng/L of air; Guth and Grosch, 1990), this compound was identified only on the basis of its characteristic metallic odor and the RI value. The mechanism of formation of this epoxydecenal has been discussed by Guth and Grosch (1990).

More compounds were identified after 90 days of ripening (Table 2) as compared to the early salting stage (Table 1). On the basis of normalized GC peak areas, a 7-fold increase in concentration was observed for 1-octen-3-ol. This increase may be accounted for through further formation of this alcohol via lipid peroxidation. A substantial increase in concentration was observed for the 2,4-heptadienals after ripening (Tables 1 and 2). A parallel increase was also observed for (E,Z)-3,5-octadien-3-one. Similarly, (E,E)-2,4-decadienal and hexanal were not detected at 2 days of salting, while they were shown to be important odorants in the flavor of fully ripened anchovy (Triqui and Reineccius, 1995). A small increase was also observed in the concentration of nonanal, while decanal was identified only after ripening.

The above results are consistent with aroma development resulting from lipid oxidation (mainly of $n-3$ polyunsaturated fatty acids). The rate of lipid oxidation in anchovy during aging may be affected by many factors. Of these factors, the effect of sodium chloride is controversial. It has been reported to act either as a prooxidant or as an antioxidant depending upon its concentration and/or the food system composition. Recently, Kanner et al. (1991) demonstrated that the catalytic effect of NaCl was due to its capability to

displace iron ions from binding macromolecules, therefore enhancing their activity toward lipid peroxidation. It has also been postulated that halide ions (Cl^-) may activate the myeloperoxidase–H_2O_2–halide system associated with blood components in fish (Kanner and Kinsella, 1983), therefore initiating lipid oxidation. In the processing of anchovy, presalting was initially done to prevent spoilage. However, manufacturers now feel it also improves the flavor of the final product. After evisceration, blood and organic matter are spread over the surface of the fish, which may then activate the peroxidase of blood leucocytes in the presence of NaCl and oxygen. Additionally, sea salt is also a good source of trace heavy metal catalysts which both decrease the induction period and increase the rate of lipid oxidation (Grosch, 1982).

Access to oxygen is another important factor for lipid oxidation. Low oxygen pressures are probably encountered in the containers where anchovy ripen. Marcus and Frederickson (1968) stated that termination reactions in the course of lipid oxidation could be different and produce different oxidative products at different oxygen concentrations. This is because the alkyl-type radical is predominant at oxygen pressures of <100 mmHg, while at oxygen pressures >100 mmHg, the alkoxy radical is the major species (Hsieh and Kinsella, 1989). This could explain the fact that 2,4,7-decatrienals were not identified in anchovy following ripening.

We do not have enough information to positively conclude which volatile compounds are the most important to the characteristic aroma of ripened anchovy. No single volatile appears as a character impact compound. Therefore, this characteristic flavor may be explained in terms of a balance of several volatiles. It is likely that the increase with aging in the concentrations of some oxidatively derived volatiles may modify the green, plant-like aromas of fresh anchovy by providing more fatty and fried-like aromas. Compounds such as (E,Z)- and (E,E)-2,4-decadienal have been reported to suppress the aromas of green compounds through subthreshold interactions (Karahadian, 1988). Other compounds identified in anchovy at low concentrations and/or

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exhibiting higher thresholds may contribute to overall flavor as well.

Trimethylamine has often been associated with fishiness in seafood flavors. Karahadian (1988) found that when 1–5 ppm of trimethylamine was added to fresh steam-deodorized fish oils, an unusual fish oil aroma was noted that was reminiscent of fishy odors observed in canned sardines. In a recent study, parallel determinations of trimethylamine in commercially processed samples showed a 15-fold increase in this amine, with values around 9 mg/100 g being observed after ripening (El Rhaffari, 1992). Thus, trimethylamine and its combined effects with certain lipid oxidation products (Karahadian, 1988) appear to impart a general fishy character to ripened anchovy.

Flavor development in anchovy is concomitant with other biochemical changes occurring in the lipid and protein components. It is known that interactions of volatile flavor compounds with the different nonvolatile food components affect the concentrations of volatiles in the gas phase above or around a food (Solms, 1985). As an example, proteolysis has been shown to decrease the amount of hexanal and *n*-hexanol retained by soy protein during enzymatic hydrolysis (Solms, 1985). Such interactions could also influence the volatility of flavor compounds in anchovy through the ripening process.

CONCLUSIONS

The development of the characteristic anchovy flavor during ripening is apparently related to both qualitative and quantitative differences in flavor profiles between raw and ripened anchovy. Oxidation (mainly of *n*–3 polyunsaturated fatty acids) in anchovy is likely to be responsible for flavor development during ripening. While a major role can be ascribed to lipoxygenase enzymes in initiating lipid peroxidation in anchovy, other enzyme systems such as myeloperoxidase of blood leucocytes may be active, especially after salting. The rate of autoxidation is probably affected by the presence of salt and the low oxygen concentrations.

Sensory assessment is the usual method of following the process of ripening. However, in the context of contemporary industry practices, there is a demand for methods to objectively measure fish aroma quality (Josephson et al., 1986). The development of oxidized flavors in anchovy during ripening is a common problem observed in the industry. While the present study has provided information on the chemical basis of anchovy flavor and a relative estimation of the changes in concentration of some volatiles with aging, a more concerted study aimed at determining one or a few influential volatiles that could be monitored to assess both the progress of ripening and the quality of the final product is required.

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