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Volatiles Produced by Microorganisms Isolated from Refrigerated Chicken at Spoilage

L. R. FREEMAN,[1] G. J. SILVERMAN,* P. ANGELINI, C. MERRITT, JR., AND W. B. ESSELEN

Food Sciences Laboratory, U.S. Army Natick Research and Development Command, Natick, Massachusetts 01760, and Department of Food Science and Nutrition, University of Massachusetts, Amherst, Massachusetts 01002*

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Volatile components present at spoilage of refrigerated chicken breasts were identified using high-vacuum-low-temperature distillation techniques followed by analysis with combined temperature-programmed gas chromatography and mass spectrometry. A comparison was made of the compounds detected from both irradiated and non-irradiated muscle stored at 2 and 10°C under both aerobic and anaerobic conditions. Isolates were randomly selected from the spoiled poultry, identified, and evaluated for their ability to produce volatile spoilage notes when grown on radiation-sterilized chicken. Several isolates that produced off-odors on sterile chicken breasts were examined. Twenty-two compounds were associated with spoilage. Some of the compounds found on both irradiated and unirradiated samples were considered to play only a minor role in the spoilage aroma or were present in low concentrations, since the aroma of spoiled irradiated chicken lacked the harsh odor notes typical of spoiled unirradiated chicken. Fifteen of the 22 compounds were considered to be unique to unirradiated, aerobically spoiled samples. Nine of these compounds, hydrogen sulfide, methyl mercaptan, dimethyl sulfide, dimethyl disulfide, methyl acetate, ethyl acetate, heptadiene, methanol, and ethanol, were found on chicken spoiled at both 2 and 10°C. Xylene, benzaldehyde, and 2,3-dithiahexane were detected only in samples stored at 2°C and methyl thiolacetate, 2-butanone, and ethyl propionate were associated with 10°C spoilage. Fifty-eight isolates randomly selected from fresh, radiation-pasteurized, and unirradiated spoiled poultry were classified taxonomically, and 10 of them, which produced spoilage odors on sterilized chicken breasts, were selected for subsequent analysis of their volatiles. Isolates identified as *Pseudomonas putrefaciens* and *Pseudomonas* species that were members of groups I and II of Shewan's classification, as well as *Flavobacterium* and oxidative *Moraxella*, produced a number of the compounds found in the aroma of spoiled chicken. A total of 17 compounds were identified. Whereas no isolate produced all of the aroma compounds found in the aroma of spoiled chicken, together they did produce the nine found in unirradiated samples spoiled at either 2 or 10°C, as well as methyl thiolacetate and xylene. Six compounds were present in the volatiles produced by the isolates but were absent in the volatiles identified from spoiled chicken. These were hydrogen cyanide, methyl isopropyl sulfide, 2-propane thiol, methyl propionate, ethyl benzene, and an unidentified compound.

The spoilage odor of meat, poultry, and fish at refrigeration temperatures is attributed mainly to microbial by-products (2, 10, 13, 15) and not to autolytic products from tissue (10, 13, 17). Whereas spoilage odors have been subjectively described (1, 14, 15), only recently have attempts been made to identify specific volatile compounds present at spoilage and relate them to the causative microorganism. These studies (10, 24-26) have been devoted mainly to fish, and, although meat and poultry have been neglected, a considerable effort has been expended on identifying their spoilage microflora.

Despite the heterogeneous nature of the microflora present on chicken immediately after processing, the microflora during refrigerated storage undergoes a shift in the microbial spectra. At spoilage, the nonpigmented *Pseudomonas* organisms predominate, whereas the larger initial percentage of *Pseudomonas pu-*

[1] Present address: Department of Food Science and Nutrition, Brigham Young University, Provo, UT 84602.

trefaciens, *Achromobacter* (*Acinetobacter-Moraxella*), and the fluorescent pseudomonads form a much smaller proportion of the total flora (1-4, 19). When poultry is subjected to agents capable of selectively altering the microflora, such as pasteurizing doses of radiation, the predominant organisms present at spoilage are the more radiation-resistant psychrotrophic organisms, such as *Acinetobacter-Moraxella* species (13, 14) or *Microbacterium thermosphactum* (3). The refrigerated shelf life of the product is extended, since these organisms are less degradative toward the product and their spoilage aroma is considerably less objectionable.

The purpose of this study was to identify volatile compounds associated with chicken spoiled aerobically at refrigerated temperatures and relate them to the microorganisms responsible for the production of these compounds. Poultry was also subjected to anaerobic spoilage and to pasteurization dosages of ionizing radiation to study, for comparative purposes, the relationship of altered metabolism and microflora on the production of volatiles.

MATERIALS AND METHODS

Treatment of muscle tissue. Fresh deboned chicken breasts were obtained from a local distributor. For aerobic storage studies, 350 g (±10 g) of fresh muscle was placed into a sterilized sample bottle having a closure suitable for drawing a vacuum (23), and the neck was loosely covered with a sterile 9-cm Pyrex petri dish. The bottle was then enclosed in an oxygen-permeable, medium-density, polyethylene bag to aid in moisture control during storage at 2 and 10°C for 12 and 5 days, respectively.

Anaerobic conditions were established by vacuum evacuation of the container and sealing off the system during storage. Both of the above systems were monitored by GasPak (BBL) disposable anaerobic indicators taped to the inside of the bottle.

Samples irradiated at 0.25 Mrad (±0.025) were heat sealed in laminated polyethylene pouches and irradiated at 0 to 2°C. After irradiation, the samples were removed from the pouch and placed into sample containers for aerobic or anaerobic storage.

Chicken to be radiation sterilized for pure culture studies was placed into laminated polyethylene pouches, the air was evacuated, flushed with N_2, and reevacuated, and the package was heat sealed. The samples were then rapidly frozen, irradiated at −30°C, and stored at −40°C.

Microbiological sampling of surfaces. Aerobic plate counts were obtained from the chicken samples upon arrival and during storage. The aerobic plate counts was obtained by moistening a cotton swab in 4% Trypticase soy broth (BBL) supplemented with 0.5% yeast extract (Difco) (TSY broth) and swabbing a 6.45-cm^2 surface area of the chicken for 15 s. The swab end was then broken into a tube containing 9 ml of TSY broth. The contents were then shaken, serial dilutions were made in additional tubes of TSY broth, and a 0.1-ml sample was uniformly distributed over the surface of prepoured agar plates containing 4.0% Trypticase soy agar (BBL) supplemented with 0.5% yeast extract (TSY agar). The plates were incubated at 2 and 20°C for 11 and 2 days, respectively.

Isolation and classification of pure cultures. Isolates were randomly selected from aerobic plate count plates having 30 to 120 colonies, purified, and taxonomically classified by use of the following tests: Gram stain, motility, catalase, benzidine, cytochrome oxidase, oxidation-fermentation (glucose), pigment production, hippurate hydrolysis, litmus milk, reducing compounds from gluconate, esculin hydrolysis, nitrate reduction, methyl red, Voges-Proskauer, arbutin hydrolysis, growth at 4, 20, and 37°C, phosphatase, starch hydrolysis, skim milk agar proteolysis, Tween 80 hydrolysis, lecithinase, and growth in or on triple sugar iron agar, Sellers differential agar, Herellea agar, and blood agar. Additional tests were conducted on selected isolates using Enterotubes (Roche Diagnostics) and the API Microtube 50 research system (Analytab Products, Inc.). The isolates were broadly classified in a schema, illustrated in Fig. 1, which is partially based upon the study of Shewan (32). Tests were generally conducted at 20°C.

With the exception of *Pseudomonas fragi* and *P. putrefaciens*, which were obtained from R. Levin, University of Massachusetts, the known cultures used in this study were obtained from our culture collection.

Evaluation of volatiles by isolates. Frozen, radiation-sterilized poultry breasts were thawed at 2°C and inoculated by dipping into a 24-h TSY broth culture of a specific isolate grown at 20°C. The sample was then drained, placed in a sterile petri dish, and incubated at 10°C for 4 days prior to odor evaluation by seven panelists. Isolates were considered positive when at least four of the seven panelists associated a distinct off-odor with a given sample. The volatiles of a number of cultures were also evaluated after growth on TSY agar.

For volatile analysis the inoculated samples were incubated in the sample bottles aerobically at 10°C for 5 days. To eliminate the influence of chicken muscle, selected isolates were inoculated onto TSY agar in a sample bottle enclosed in a polyethylene bag and incubated at 10°C for 5 days, and the head space was analyzed for volatiles.

Collection and analysis of volatiles. The total condensate of each sample was collected at −196°C by using a modification of the low-temperature-high-vacuum distillation technique of Merritt et al. (23). The "center fraction," or that fraction of the total condensate containing compounds exerting vapor pressure between the temperatures of −140 and −80°C, was separated from fractions designated as carbon dioxide and water fractions. Its odor was found to be representative of spoilage aroma even though it represented less than 1% of the total condensate. The center fraction was analyzed by a combined temperature-programmed gas chromatograph and mass spectrometer instrument system (22).

The components in the center fractions were separated on a stainless-steel support, coated open tubu-

lar (SCOT) column (50 feet [ca. 15.2 m] by 0.51 mm [ID]) with 1,2,3-tris(cyanoethoxy)propane as the stationary phase and with a helium carrier gas flow rate of 30 cm/s (5 ml/min). The chromatograph used was a Bendix series 2200 laboratory gas chromatograph, which was coupled directly to a Bendix model MA-2 time-of-flight mass spectrometer. The temperature was programmed at a rate of 6°C/min from −65 to 125°C. This mass spectrometer was equipped with a strip chart recorder to record the gas chromatogram of the sample by monitoring the total ion current of the mass spectrometer, an oscilloscope for displaying the mass spectra, and a recording oscillograph for recording the mass spectra of each gas chromatographic peak as it eluted from the column.

Electron microscopy. Representative motile and nonmotile microorganisms were examined in a Hitachi model HS-7 transmission electron microscope by James V. Allen, Brigham Young University, for the presence of flagella. All cultures were grown at 20°C for 48 h in TSY broth before being prepared for examination in the electron microscope.

RESULTS

Storage temperature and microbial development. The fresh poultry was consistently found to have about 10^5 colony-forming units/cm^2. After 12 days at 2°C or 5 days at 10°C, about 10^8 colony-forming units/cm^2 were present, accompanied by a distinct spoilage odor. For poultry breasts irradiated with 0.25 Mrad and subsequently stored at 2 and 10°C, the microbial population increased to 10^7 to 10^8 colony-forming units/cm^2 after 35 and 14 days, respectively.

Radiation-pasteurized and stored chicken breasts did not display typical spoilage off-odors even when the microbial count increased to 10^8 colony-forming units/cm^2. Instead, a mild sweetish odor developed gradually, making it difficult to precisely decide when the sample was spoiled. This was, in agreement with other studies (12, 14), due to an alteration of the types of microorganisms able to survive radiation and proliferate. Radiation and subsequent storage changed the microflora, consisting mainly of radiation-sensitive *Pseudomonas*, to one of nonoxidative and oxidative *Moraxella*.

General taxonomic classification of the isolates. The isolates were placed in 11 of the 14 alphabetic groups shown in Fig. 1 and their identity, based upon additional tests, is listed alphabetically in Table 1. No organisms were found to belong to groups F, H, and P. Only two gram-positive rods were isolated. One, classified in group A, was a catalase-positive, benzidine-negative rod identical to a culture of *M. thermosphactum*. The other isolate, in group B, was a *Lactobacillus* species, as it was both benzidine and catalase negative. The remaining organisms were either gram-negative rods or coccobacilli. All the gram-negative motile isolates were oxidase positive and could be separated into either groups C and D (group I of Shewan [32]; Shewan et al. [33]), group E (group II [32, 33]), or group G.

The fluorescent isolates of group C grew at 4°C but not at 37°C, produced fluorescin, and could be further differentiated, on the basis of proteolytic and lecithinase activity (34), into *Pseudomonas fluorescens* and *Pseudomonas putida*.

Group D contained a pigmented nonfluorescent organism that produced a brown diffusable

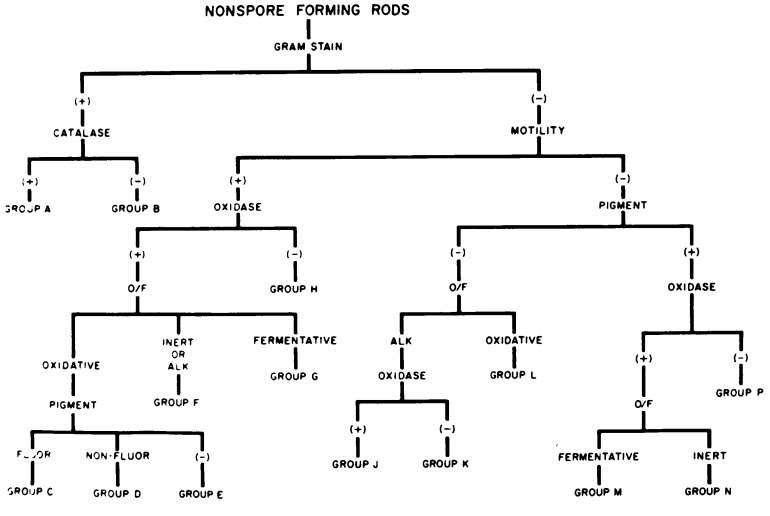

FIG. 1. *Classification of isolates from unirradiated and irradiated, fresh and spoiled poultry. O/F, Reaction in Hugh-Leifson medium (11); ALK, alkaline; FLUOR, fluorescent.*

TABLE 1. *Incidence of isolates on irradiated and non-irradiated poultry*

Group[a]	Organism	Initial no. of isolates	No. of isolates at spoilage	
			Unirradiated	Irradiated (0.25 Mrad [±0.025])
A	*M. thermosphactum*		1 (1)	
B	*Lactobacillus* sp.	1 (1)[b]		
C	*P. fluorescens* and *P. putida*		4 (2)	
D	*Pseudomonas* sp.	1 (1)		
E	*Pseudomonas* sp.	8(4)	18 (12)	
G	*P. putrefaciens*	1	2 (1)	
J	*Moraxella*	1 (1)		2
K	*Acinetobacter*	1	1	
L	*Moraxella*-oxidative	1	2 (1)	8
M	*Flavobacterium*	3 (3)	2 (1)	
N	*Flavobacterium*	1		

[a] These designations correspond to those listed in Fig. 1.
[b] The number in parentheses indicates those isolates producing a pronounced aroma after growth on sterile chicken.

pigment when grown on *Pseudomonas* P agar. Group E consisted of nonpigmented organisms designated as *Pseudomonas* and contained the largest number of isolates, 26 of the 58 studied. It was difficult to subdivide this group on the basis of the tests performed, as no consistent pattern for differentiation was observed. One of the isolates, though, culture 37, was identified as *P. fragi*. The program of differentiation of these nonpigmented pseudomonads has been noted by other investigators (1, 3, 8), and these organisms have been found to be the dominant organisms found on spoiled, proteinaceous foods (8, 19).

The organisms of group G, although fermentative, did not correspond to the fermentative, oxidase-positive, motile rods designated as *Vibrio* and *Aeromonas* by Shewan (32). *Aeromonas* is described as being able to produce gas in addition to being fermentative and *Vibrio* as being nonpigmented and capable of hydrolyzing starch. None of these reactions could be ascribed to the group G isolates of this study.

Group G did contain salmon-to-brown, nondiffusable, pigmented organisms corresponding to the published description of *P. putrefaciens* (18). These isolates reduced litmus milk rapidly, were proteolytic on skim milk agar, reduced nitrates, produced hydrogen sulfide, were capable of fermenting glucose in Hugh and Leifson's medium (11), produced deoxyribonuclease, and were oxidase positive. Under anaerobic conditions these isolates also reduced the indicator in Baird-Parker's medium.

Two of the three isolates in group G did not decarboxylate lysine or ornithine and were citrate negative. The third culture was positive for all three tests.

McMeekin and Patterson (20) described isolates of *P. putrefaciens* obtained from spoiled foods that were in many respects dissimilar to the isolates in group G. Their isolates were not fermentative on glucose and did not produce urease, whereas the strains in this study were fermentative on glucose and positive for urease activity.

Using the criterion of Baumann et al. (5, 6), the oxidase-positive group J isolates were designated *Moraxella* and the oxidase-negative group K isolates as *Acinetobacter*. Although the group L isolates were oxidase-positive, nonmotile organisms whose morphology varied from rod to coccobacillus and generally matched the reactions of the group J isolates, they oxidatively attacked glucose. Thornley (36) described *Moraxella* species capable of oxidizing glucose aerobically, as well as *Moraxella* species that did not. In this study group L has been designated *Moraxella*-oxidative to distinguish this characteristic towards glucose.

Groups J, K, and L appear to correspond in some respects to Thornley's (36) phenons 4i, 4ii, and 3, respectively. These groups, however, were not homogeneous and could be further divided into subgroups (Table 2).

Group M and group N were both classified as *Flavobacterium*. Group M was subdivided into two subgroups on the basis of nitrate reduction, litmus milk reactions, hydrolysis of starch, the production of hydrogen sulfide, the production of indole, arbutin hydrolysis, and their reaction in Baird-Parker's medium. With the exception

TABLE 2. *Reactions[a] that subdivide groups J, K, and L*

Test	J		K		L		
	J1	J2	K1	K2	L1	L2	L3
Morphology	CB	CB	CB	CB	R	CB	CB
Nitrate reduction	+	−	+	−	−	−	−
Litmus milk	Alk	Red					
Phosphatase	+	−					
Lactose	Alk	A					
Deoxyribonuclease	−	+					
Tween 80			+	−	−	+	+
Growth at 37°C			+	−			
Blood agar			H	−			
Lysine decarboxylase			−	+			
Ornithine decarboxylase			−	+			
Hippurate hydrolysis					+	−	+
Gluconate					+	−	−
Egg yolk (lecithinase)					−	+	+
Sellers medium							
Slant					Alk	Alk	A
Butt					NC	NC	A

[a] A, Acid; Alk, alkaline; NC, no change; CB, coccobacillus; Red, reduction; R, rod; H, hemolysis.

of motility, one subgroup of group M appeared to be identical to *P. putrefaciens* isolates of group G, and this relationship will be the subject of another publication. Examination in the electron microscope demonstrated that the motile group G isolates, designated *P. putrefaciens*, had a monotrichous polar flagellum and the non-motile *Flavobacterium* sp. were devoid of flagellum.

Evaluation of the spoilage activity of isolates. The ability of isolates to produce a pronounced aroma on chicken is also presented in Table 1. Organisms from 9 of the 11 taxonomic groups found on chicken were capable of producing a distinctive aroma. The aromas most typical of spoilage were associated with gram-negative rods of groups C through M, with the most pronounced belonging to groups C through G. The aroma produced by *M. thermosphactum* and the *Lactobacillus* sp. isolates (groups A and B) had a predominant volatile acid character, whereas 20 of the 34 pseudomonad isolates, 4 out of the 6 *Flavobacterium*, and 2 of the 16 *Moraxella-Acinetobacter* isolates possessed pronounced aromas with typical chill temperature spoilage notes. None of the 10 *Moraxella* isolates (groups J and L) from irradiated chicken produced a distinctive aroma.

Volatile compounds of spoiled commercial chicken. Portions of chicken were allowed to spoil at either 2 or 10°C, and their volatiles were analyzed by gas chromatography-mass spectrometry (Table 3). Fifteen of the 22 compounds identified were common to chicken spoiled at either storage temperature and, although the data is not presented, there was a larger concentration of each of these compounds in the samples stored at 10°C than in those stored at 2°C. Three additional compounds were found only in the volatiles from samples stored at 10°C, and four additional compounds were identified only in those stored at 2°C. Acetone was the sole compound found in the center fraction of fresh, unstored chicken breasts. With the exception of 2,3-dithiahexane, no additional compounds were identified when three center fractions, derived from 1,050 g of chicken breast, were pooled for analysis.

Compounds identified after storage at 2 and 10°C under anaerobic conditions. Chicken stored anaerobically at 2°C for 21 days or at 10°C for 8 days did not develop any typical spoilage aroma. Only four compounds, acetone, ethanol, methanol, and toluene, were detected.

Volatile compounds identified from irradiated chicken breasts. Nine compounds were consistently present in the aroma of chicken analyzed immediately after irradiation with a pasteurizing (0.25 Mrad) or a sterilizing dose (1.5 Mrad) (Table 4). Two additional compounds, 1-propane thiol and dimethyl trisulfide, were only found occasionally. With the exception of the latter two compounds, all of the nine compounds initially present after irradiation persisted throughout anaerobic storage. Dimethyl disulfide disappeared from samples subjected to both levels of irradiation and stored aerobically, whereas benzene was absent only from those samples aerobically stored after subjection to 0.25 Mrad. A stronger irradiation odor persisted in samples receiving a dose of 1.5 Mrad than in samples receiving a dose of 0.25 Mrad.

TABLE 3. *Volatile compounds associated with spoiled chicken breasts stored at 2[a] or 10[b]°C*

Storage temp	Compound
2 or 10°C	Hydrogen sulfide
	Methyl mercaptan
	Dimethyl sulfide
	Dimethyl disulfide
	n-Heptane
	1-Heptene
	Heptadiene
	n-Octane
	Acetone
	Methyl acetate
	Ethyl acetate
	Benzene
	Methanol
	Ethanol
	Toluene
Only at 2°C	Benzaldehyde
	Xylene
	n-Pentane
	2,3-Dithiahexane[c]
Only at 10°C	Methyl thiolacetate
	2-Butanone
	Ethyl propionate

[a] Samples stored for 12 days.
[b] Samples stored for 5 days.
[c] Only found when three center fractions were combined.

Evaluation of the volatiles produced by isolates. Table 5 lists the aroma components of two known cultures, *P. putrefaciens* and *P. fragi*, and 10 isolates representing seven groups listed in Table 1. A total of 16 known compounds, and an unidentified compound isolated as a single gas chromatographic peak, were produced by the 12 cultures. This unknown compound exhibited a mass spectrum whose major mass-to-charge ratios were 86, 70, 43, 42, 41, and 27. It appeared to have a molecular weight of 102. Present but not listed in the body of the table were eight compounds generated by the irradiation used for sterilizing the chicken. The lowest number of compounds produced by a culture was two (cultures 20 and 44, groups A

TABLE 4. *Volatile compounds present during aerobic and anaerobic storage of irradiated chicken breasts*

Storage condition	Compound
Immediately after irradiation	*n*-Pentane
	n-Hexane
	n-Heptane
	1-Heptene
	n-Octane
	Acetone
	Toluene
	Benzene
	Dimethyl disulfide
	1-Propane thiol[a]
	Dimethyl trisulfide[a]
0.25 Mrad[b]	
Aerobic	*n*-Pentane
	n-Hexane
	n-Heptane
	1-Heptene
	n-Octane
	Acetone
	Toluene
Anaerobic	*n*-Pentane
	n-Hexane
	n-Heptane
	1-Heptene
	n-Octane
	Acetone
	Toluene
	Benzene
	Dimethyl disulfide
1.5 Mrad (aerobic)[c]	*n*-Pentane
	n-Hexane
	n-Heptane
	1-Heptene
	n-Octane
	Acetone
	Toluene
	Benzene

[a] Inconsistently found on samples irradiated at 0.25 Mrad.

[b] Stored for 19 days at 2°C or 14 days at 10°C.

[c] Stored at 2°C for 20 days or at 10°C for 5 days.

and M) and the highest number of compounds, six, was present in the head space of *P. fluorescens* (culture 60), a *Pseudomonas* (culture 48), and *P. fragi* (culture 37). These cultures produced different combinations of their six compounds. Hydrogen cyanide (9) was identified from cultures of *P. fluorescens* grown either on TSY agar or on chicken breasts. In fact, 12 of these compounds were produced in detectable amounts by cultures grown either on chicken or on TSY agar.

Four compounds, benzaldehyde, 2,3-dithiahexane, 2-butanone, and ethyl propionate, were isolated from the aroma of spoiled chicken but were not found in the pure culture studies.

The volatile produced by the largest number of cultures tested was dimethyl disulfide, followed by ethanol, methanol, and ethyl acetate.

Of the 17 compounds, 11 were also found on spoiled chicken. The remaining six compounds, methyl propionate, hydrogen cyanide, methyl isopropyl sulfide, 2-propanethiol, ethyl benzene, and the unidentified compound, were associated with five organisms, cultures 59, 60, 18, 14, and 48.

DISCUSSION

A recent study of McMeekin (19) verified the importance of the contribution of *Pseudomonas*, especially those of Shewan's groups I and II and, to a minor extent, IV (32), to the spoilage aroma of chicken breast muscle. Of the 250 cultures isolated by him from samples stored at 2°C, 80% produced off-odors. Refrigerated storage favored the growth of organisms able to generate spoilage aroma, and, at spoilage, the predominate microflora was found to be organisms belonging to *Pseudomonas* group II. Although the strains of pseudomonads included in a comprehensive study by Stanier et al. (34) were isolated from a variety of environments, organisms corresponding to group II of Shewan were omitted. Davidson et al. (8) has concluded that the majority of commonly occurring pseudomonads on meat and meat products do not conform with the species studied by Stanier et al. (34).

In this study, involving a smaller number of isolates, groups C and E, corresponding to groups I and II, respectively, were the predominant organisms in unirradiated fresh and spoiled chicken. Group E, though, was the most numerous organism isolated at both test periods. Half or more of the isolates from these two groups were capable of producing obvious spoilage aromas. McMeekin did not recover *P. putrefaciens* in his initial study (19), although it was recovered in a subsequent study by the use of a selective medium (20). In this study, *P. putrefaciens* as well as *Moraxella* and *Acinetobacter* were isolated. Whereas all of the isolates of *P. putrefaciens* studied by McMeekin and Patterson (20) produced off-odors, only one out of three cultures isolated in this study demonstrated this ability when grown on sterile chicken muscle.

The following compounds are considered to be responsible for or associated with the characteristic off-odor of chicken breasts undergoing normal spoilage at refrigerated temperatures: hydrogen sulfide, methyl mercaptan, dimethyl sulfide, heptadiene, methyl acetate, ethyl acetate, methanol, ethanol, dimethyl disulfide, benzaldehyde, xylene, 2,3-dithiahexane, 2-bu-

TABLE 5. *Volatiles*[a] *produced by cultures grown on irradiation-sterilized chicken for 5 days at 10°C*

Volatile	Known cultures		Isolates[b]									Present on spoiled chicken
	P. putrefaciens	*P. fragi*	*M. thermosphactum* (20, A)	*P. putida* (14, C)	*P. fluorescens* 59, C	*P. fluorescens* 60, C	*Pseudomonas* sp. (48, D)	*P. fragi* (37, E)	*Pseudomonas* sp. (15, E)	*P. putrefaciens* (36, G)	*Moraxella-oxidative* (18, L)	*Flavobacterium* sp. (44, M)	
Hydrogen cyanide					+[c]	+[c]							
Hydrogen sulfide	+[c]								+	+			+
Methanol		+	+	+	+			+		+			+
Ethanol		+	+	+	+[c]			+		+		+	+
Heptadiene				+									+
Methyl acetate		+					+	+					+
Ethyl acetate		+				+[c]	+	+	+		+[c]		+
Methyl propionate						+[c]							
Methyl mercaptan	+[c]						+		+	+			+
Dimethyl sulfide		+				+[c]		+	+				+
Dimethyl disulfide	+[c]			+[c]		+[c]	+	+	+	+		+	+
Methyl isopropyl sulfide											+[c]		
Methyl thiolacetate						+[c]							+
2-Propanethiol											+[c]		
Xylene							+						+
Ethyl benzene							+						
Unidentified (molecular wt, 102)				+[c]							+[c]		

[a] *n*-Pentane, *n*-hexane, *n*-heptane, *n*-octane, 1-heptene, acetone, benzene, and toluene were present in the head space of radiation-sterilized chicken (1.5 Mrad) after incubation (see Table 4).

[b] Numbers indicate culture number; letters indicate group.

[c] Also detected in the head space volatiles of cultures grown on TSY agar.

tanone, ethyl propionate, and methyl thiolacetate. However, sensory evaluations of combinations of these compounds will be necessary to confirm their relationship to the odor of typical spoilage aroma.

It is also apparent that considerable research remains to be accomplished in relating the components of spoilage aroma to their causative microorganisms. The fact that human sensory evaluation can be considerably more sensitive for the detection of many compounds than current analytical instruments makes correlation difficult. Moreover, this study did not attempt to explore the effect of the growth of mixed cultures on subsequent end products.

By comparing those compounds identified after anaerobic storage of unirradiated muscle with those identified after aerobic storage of unirradiated muscle, it is obvious that anaerobiosis prevented the development of a number of compounds associated with the aerobically developed spoilage aroma. The observation also provides a rationale in substantiating the observation of Van den Berg et al. (37) that the storage life of fresh poultry at refrigerated temperatures could be doubled under aseptic and anaerobic conditions.

The hydrocarbons produced by irradiation most likely originated from lipid compounds, benzene and toluene from phenylalanine, and dimethyl disulfide directly from cystine or methionine (22). Ethanol and methanol were not detected in irradiated samples in this study, although Merritt (21) reported a small amount of ethanol present in chicken receiving a dose of 0.5 Mrad. Ethanol and methanol were both found in aerobically and anerobically stored unirradiated samples, indicating the source of these two compounds was microorganisms that were eliminated by irradiation. The organisms more sensitive to irradiation, *Pseudomonas* and *Flavobacterium*, produced these alcohols in pure-culture growth studies on chicken, whereas *Moraxella*, the more radioresistant organism that predominated on irradiated chicken after storage, did not.

The compounds *n*-heptane, *n*-octane, 1-heptene, acetone, benzene, and toluene were found on samples spoiled aerobically at both 2 and 10°C and on stored samples subjected to sterilizing and pasteurizing doses or irradiation. The origin of these compounds in irradiated samples has been discussed above, but in the unirradiated samples it is possible that they were produced by aerobically growing microorganisms. Toluene was also found in unirradiated,

anaerobically stored samples, and acetone was found in all samples, including fresh chicken breasts analyzed prior to storage.

Dimethyl disulfide was present both in spoiled unirradiated chicken and immediately after irradiation. This leads one to speculate as to its contribution to the aroma of both the irradiation odor of chicken and the normal spoilage odor, since these aromas are dissimilar. The influence of dimethyl disulfide in both aromas may be dependent upon its concentration. At lower concentrations it will not be as objectionable, and its influence may be modified by other compounds.

In pure culture studies of isolates from cod and haddock inoculated onto sterile cod muscle, Herbert et al. (10) reported that the aroma from pseudomonad cultures produced either sweet-fruity or characteristic hydrogen sulfide-sulfury odors and that where fruitiness was detected no sulfides were identified. Miller et al. (24) reported that a *P. fragi*, isolate 18, inoculated onto sterile fish muscle produced both esters and sulfur compounds (dimethyl sulfide, ethyl acetate, and dimethyl disulfide) even though a fruity odor predominated. In the present study, culture 15 of group E, *P. fluorescens* (culture 60), *Pseudomonas* isolate 48, and *Moraxella*-oxidative (isolate 18) produced several sulfur compounds in addition to ester compounds. The known *P. fragi* culture and *P. fragi* culture 37 smelled predominantly fruity, and both produced at least one sulfur compound in addition to methyl and ethyl acetate.

Hydrogen sulfide and methyl mercaptan were produced by the known *P. putrefaciens* culture and isolates designated as *P. putrefaciens* (isolate 36) and *Pseudomonas* sp. (isolate 15). The production of hydrogen sulfide by *P. putrefaciens* strains has been well established (10, 16, 18, 20), and Lea et al. (17) reported that *P. putrefaciens* when grown on chicken breast muscle produced small quantities of hydrogen sulfide at 1°C. Herbert et al. (10) found *P. fragi* strains to be hydrogen sulfide producers, as did Nicol et al. (27) for *Pseudomonas mephitica* isolated from beef.

Miller et al. (25) used peptone iron agar to detect hydrogen sulfide production by *P. putrefaciens*. In the current study, hydrogen sulfide was detected for two of the three isolates by both gas chromatographic-mass spectrometric analysis and by triple sugar iron agar. However, for culture 15, hydrogen sulfide was detected by gas chromatographic-mass spectrometric analysis but not by triple sugar iron agar. This observation supports the position of Padron and Dockstader (28) that triple sugar iron agar does not reveal hydrogen sulfide production by organisms producing it in relatively small quantities.

Methyl mercaptan may have originated from the decomposition of methionine. Segal and Starkey (31) reported that a *P. fluorescens* strain first deaminated methionine, followed by demethiolation with the production of methyl mercaptan. Further transformation would occur upon oxidation to dimethyl disulfide. Miller et al. (25, 26) identified both methyl mercaptan and dimethyl disulfide when cultures of *P. putrefaciens*, *Achromobacter*, *P. fluorescens*, and *P. perolens* were grown in a sterile homogenate of sterile fish muscle. Miller et al. (24) also reported identification of dimethyl disulfide from the volatiles of *P. fragi* when grown in fish homogenate, but methyl mercaptan was absent. In this study dimethyl disulfide accompanied methyl mercaptan in the head space of four cultures. In instances where dimethyl disulfide but not methyl mercaptan was present, it is likely that all of the methyl mercaptan was oxidized to the former (16).

Dimethyl sulfide was produced by group E, cultures 37 (*P. fragi*) and 15, *P. fluorescens* (isolate 60), and the known *P. fragi*. Herbert et al. (10) reported dimethyl sulfide production by *Pseudomonas* species from methionine, and Miller et al. (24) reported dimethyl sulfide production by *P. fragi* strain 18 when grown on sterile fish muscle. The disulfides are less volatile than the sulfides but have a more offensive odor (16).

Herbert et al. (10) observed that *Pseudomonas* species of the *P. fragi* type produced fruity odors when grown on sterile cod muscle and that this odor was probably due to the presence of esters. In this study ethyl acetate was identified with group E, cultures 37 (*P. fragi*) and 15, *P. fluorescens* (isolate 60), group D (culture 48), *Moraxella*-oxidative (isolate 18), and the known *P. fragi* culture. Of these cultures only *P. fragi* and culture 37 were found to produce ethanol in addition to the ester. These two organisms were also the only cultures among the 12 to produce methyl acetate together with methanol. However, *P. fluorescens* culture 60 did produce the ester, methyl propionate, in addition to methyl thiolacetate. Reddy et al. (29, 30) and Miller (24) found that *P. fragi* isolated from fruity-flavored cottage cheese and pasteurized milk, when grown in sterile homogenized milk at 21 and 7°C or in fish until a fruity aroma developed, produced ethyl acetate, ethyl butyrate, and ethyl hexanoate. The addition of either 0.1 or 0.2% ethyl alcohol or butyric acid to the substrate markedly enhanced ester production.

Although both the known strain of *P. fragi* used in the present study and *P. fragi* culture 37 produced methanol and methyl acetate in addition to ethyl acetate and ethanol, no other esters or alcohols were detected in samples containing these two isolates.

It cannot be assumed that isolates capable of producing pronounced spoilage odors on one substrate will do so on others. An isolate from spoiled haddock, which was always accompanied by a strong spoilage aroma on TSY agar, failed to produce a strong off-odor on chicken breast. Herbert et al. (10) reported that when *Moraxella*-like strains were inoculated onto sterile scampi tails they produced strong spoilage odors but that these strong odors were not present after growth on sterile cod muscle.

It appears that a relatively broad spectrum of microorganisms isolated from commerically processed chicken breasts at spoilage are capable of producing compounds associated with the spoilage aroma of poultry stored at refrigeration temperatures. Whereas *Pseudomonas* species have traditionally been the organisms found in the greatest abundance on unirradiated chicken spoiled at chill temperatures and appear to play a major role in the production of the typical spoilage odor, the data from this and other studies demonstrate that other taxa contain organisms that can contribute to spoilage odor. It should be noted that approximately 40% of the *Pseudomonas* isolates in groups C and E did not produce strong offensive odors on sterile chicken breasts. Herbert et al. (10) reported that spoilage odor-producing organisms on spoiled cod and haddock never accounted for more than 10 to 20% of the total flora. McMeekin (19) found that, whereas most strains isolated from spoiled chicken were capable of producing a spoilage odor, there were stains within each *Pseudomonas* group that did not. The contribution that these non-odor-producing organisms make to the spoilage aroma bouquet remains to be elucidated.

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