Influence of Microorganisms on the Carbonyl Compounds of Chicken Tissue

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Broiler adipose and thigh muscle tissue samples were inoculated with bacteria and yeasts, then stored for 3 days at 22 °C or 7 days at 4 °C. Hexane-extracted carbonyl compounds were converted to their 2,4-dinitrophenylhydrazone derivatives, separated, and identified. *Pseudomonas fluorescens* and *Candida lipolytica* produced acetone in both tissue samples. *Rhodotorula aurantiaca* produced acetone in ground thigh muscle. *Pseudomonas fragi* and *Pseudomonas aeruginosa* produced acetone and trace amounts of 2-butanone, 2-hexanone, and 2-octanone in all tissue samples. Five *Pseudomonas* species, *Achromobacter lipolytica*, and *C. lipolytica* decreased the concentration of at least one class of aldehyde in all samples and decreased peroxide levels in stored and rancid adipose tissue. *Trichosporon pullulans* and *R. aurantiaca* decreased the concentration of aldehydes in samples stored for 3 days at 22 °C. Dicarbonyl compounds were removed by *R. aurantiaca* and *A. lipolytica*. A *Micrococcus* sp. increased peroxides and aldehydes in stored samples and also rancid adipose tissue.

Carbonyls are very potent flavor compounds that are formed primarily by the oxidation of unsaturated fatty acids. Carbonylic compounds that are formed include methyl ketones, alkanals, 2-alkenals, and 2,4-alkadienals. These aliphatic monocarbonyls have been associated with "off-flavors" or rancidity as well as many desirable flavors and odors in raw and cooked chicken (Pippen et al., 1958; Lineweaver and Pippen, 1961; Dimick and MacNeil, 1970; MacNeil and Dimick, 1970; Wilson and Katz, 1972). The low flavor threshold values of aliphatic monocarbonyl compounds make them potent flavor and odor sources in trace amounts. Because the characteristic flavors and odors of many of these compounds is concentration-dependent (Hornstein, 1967), microbial induced alteration of aliphatic monocarbonyl concentrations could alter the flavor of raw and cooked, stored chicken. It has been demonstrated that many microorganisms such as *Pseudomonas*, *Achromobacter*, and *Micrococcus* and various yeasts and molds can alter the concentrations of both carbonylic compounds and peroxides in lipid-containing foods (Smith and Alford, 1968, 1969; Alford et al., 1971; Bothast et al., 1973).

The objective of this research was to investigate the influence of various microorganisms on the carbonyl compounds of chicken tissue.

EXPERIMENTAL SECTION

Microorganisms. *Pseudomonas fragi*, *Pseudomonas syncyanea*, *Pseudomonas aeruginosa*, and *Pseudomonas fairmontensis* cultures isolated from spoiled chicken were obtained from Dr. Nelson A. Cox (Animal Products Utilization and Marketing Research Laboratory, USDA, Athens, GA). *Achromobacter lipolytica*, *Micrococcus* sp., and *Pseudomonas fluorescens* were obtained from stock cultures in the Department of Food Science, North Carolina State University. *Candida* (*Saccharomyces*) *lipolytica* (ATCC 8661), *Trichosporon pullulans* (ATCC 9331), and *Rhodotorula aurantiaca* (ATCC 9536) cultures were obtained from American Type Culture Collection (Rockville, MD). Bacteria were maintained on Difco heart infusion agar and yeasts were maintained on malt-extract agar.

Preparation of Inocula. Bacteria were grown static in nutrient broth for 48 h at 22 °C. Yeasts were grown in nutrient broth fortified with 1% yeast extract (pH 5.5) with agitation for 48 h at 22 °C. Cells were harvested by centrifugation at 4000*g* for 20 min at 0 °C and washed twice in sterile physiological saline. Cells were then diluted and resuspended to give a final concentration of approximately 1×10^6 cells/mL of the saline solution.

Treatment of Samples. Eight- to nine-week-old broiler chickens were purchased from a local poultry processing plant. All chickens were obtained within 12 h of slaughter and were packed in ice. Slices of thigh muscle tissue (5–10 g) were asceptically removed and washed four times in 150-mL portions of sterile physiological saline solution. The thigh muscle was immediately transferred to a cool, sterile Waring Blendor and ground for about 30 s. One hundred gram samples were placed in sterile beakers, inoculated with the appropriate microorganism to give a final concentration of approximately 1×10^4/g and covered with aluminum foil. Both inoculated samples and uninoculated controls were held for 3 days at 22 °C or 7 days at 4 °C.

Adipose tissue was carefully removed from chicken carcasses and rinsed four times as previously described. Samples (10–20 g) were transferred to a cool, sterile Waring Blendor. Two parts of sterile distilled water to one part tissue were added, and the mixture was blended for 3 min. Approximately 100-g samples were inoculated with the test microorganism to give about 1×10^4 cells/g of sample and these, along with uninoculated controls, were stored for 3 days at 22 °C or 7 days at 4 °C.

Peroxide values and carbonyl concentrations were increased in some adipose tissue samples by exposure to ultraviolet light (UV). Approximately 100 g of ground adipose tissue were spread in thin layers in large petri dishes and subjected to UV light for approximately 24 h at 22 °C. After UV treatment, samples were inoculated with the test microorganisms as previously described. Both the inoculated samples and uninoculated controls were held for 3 days at 22 °C.

Enumeration of Aerobic Microorganisms. The total number of aerobic organisms in inoculated and uninoculated samples was determined by the pour plate method at 0 time and after storage. Initial dilution of the tissue slurry was made by blending 11 g of sample with 99 mL of sterile water. The desired serial dilutions were made and duplicate samples were plated on nutrient agar (Difco). The plates were incubated 48 h at 32 °C and all visible colonies were counted with the aid of a Quebec colony counter.

Determination of Lipase Activity. Microbial lipase activity was determined using methods similar to those described by Alford and Elliott (1960). Microorganisms were grown in the appropriate media as previously described. Cells were removed by centrifugation, and the

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Table I. Concentrations of Carbonyl Compounds Isolated from Inoculated and Uninoculated Ground Thigh Muscle after 3 Days Storage at 22 °C and after 7 Days at 4 °C

	μmol/10 g of extract[a]											
	total carbonyls		mono-carbonyls		methyl ketones		alkanals		2-enals		2,4-dienals	
samples	22 °C	4 °C	22 °C	4 °C	22 °C	4 °C	22 °C	4 °C	22 °C	4 °C	22 °C	4 °C
control (0 time)	44.6	44.6	7.2	7.2	6.9	6.9	0.1	0.1	*b*	*b*	*b*	*b*
control (3 days)	57.4	54.2	20.5	16.4	7.6	7.3	5.9	4.0	4.4	3.3	2.5	1.7
P. fluorescens	57.8	55.0	24.6	19.9	15.8	14.6	1.8	0.4	4.3	3.3	2.1	1.8
control (0 time)	48.3	48.3	8.2	8.2	7.8	7.8	*b*	*b*	0.1	0.1	*b*	*b*
control (3 days)	60.1	58.2	22.8	19.1	8.4	8.1	7.1	5.6	4.0	3.6	2.3	1.5
P. fragi	59.6	55.2	21.1	20.3	17.6	16.8	3.2	2.4	*b*	*b*	*b*	*b*
control (0 time)	43.8	43.8	7.4	7.3	6.9	6.9	*b*	*b*	0.1	0.1	*b*	*b*
control (3 days)	59.4	56.2	23.7	20.1	7.5	7.5	7.7	6.5	4.6	4.3	3.1	1.6
P. aeruginosa	58.6	54.7	19.0	17.9	15.6	13.8	2.1	1.7	*b*	0.1	*b*	*b*
control (0 time)	42.7	43.8	6.8	6.8	6.5	6.5	*b*	*b*	*b*	*b*	*b*	*b*
control (3 days)	58.7	57.8	21.3	18.9	6.7	6.4	6.2	6.0	4.8	3.6	2.7	2.1
P. syncyanea	56.4	54.8	15.6	14.8	6.3	6.6	1.1	2.1	4.9	3.3	2.5	2.2
control (0 time)	48.6	48.6	8.1	8.1	7.8	7.8	*b*	*b*	*b*	*b*	*b*	*b*
control (3 days)	62.9	60.4	23.8	20.3	8.4	7.7	6.4	5.8	5.0	4.3	2.9	2.1
P. fairmontensis	59.2	57.7	16.8	15.8	8.6	7.7	0.1	0.5	5.1	4.3	2.9	2.0
control (0 time)	47.7	47.7	7.2	7.1	6.8	6.7	*b*	*b*	*b*	*b*	*b*	*b*
control (3 days)	57.5	56.3	19.8	23.7	6.9	6.6	6.7	5.4	3.7	3.8	2.1	1.7
Micrococcus sp.	86.8	56.9	33.3	24.0	7.0	6.8	11.3	5.2	8.1	3.6	5.8	1.8
control (0 time)	45.8	45.8	8.1	8.1	7.8	7.8	*b*	*b*	0.1	0.1	*b*	*b*
control (3 days)	60.1	58.3	19.9	18.1	7.7	7.8	5.7	5.0	3.6	3.6	2.1	1.5
A. lipolytica	41.7	40.6	14.0	12.7	7.8	7.5	*b*	*b*	3.7	3.4	2.2	1.5
control (0 time)	47.2	47.2	8.4	8.4	7.9	7.9	*b*	*b*	*b*	*b*	*b*	*b*
control (3 days)	58.9	58.6	20.9	16.5	8.4	7.9	6.4	5.4	3.2	2.8	2.0	1.3
T. pullulans	54.7	51.8	12.7	10.8	8.1	8.0	3.1	1.7	*b*	*b*	*b*	*b*
control (0 time)	46.5	46.5	7.0	7.0	6.8	6.8	0.1	0.1	*b*	*b*	*b*	*b*
control (3 days)	59.3	56.4	20.6	17.9	7.4	6.8	6.8	6.1	3.6	3.0	2.0	1.7
R. aurantiaca	43.8	48.7	24.4	21.9	23.6	20.8	0.6	1.3	*b*	*b*	*b*	*b*
control (0 time)	42.9	42.9	7.2	7.2	6.8	6.8	*b*	*b*	*b*	*b*	*b*	*b*
control (3 days)	57.2	59.6	22.9	21.8	7.7	7.6	6.4	6.1	4.9	4.8	3.0	2.5
C. lipolytica	58.1	57.8	19.3	17.3	15.4	14.8	2.9	0.9	0.1	0.2	*b*	*b*

[a] Mean values of two trials. [b] None detected.

supernatant fraction was passed through a Seitz filter. Ten milliliters of the supernatant fluid was added to a flask containing 20 mL of a vegetable oil emulsion of 4% oil, 0.04% $CaCl_2$, and 0.01% formaldehyde. Lipase activity was determined at pH 7.0. The reaction mixture was incubated for 8 h at 22 °C using agitation. After incubation, the mixture was adjusted to pH 2.5 using 25% H_2SO_4 (v/v). Ten milliliters of ethyl alcohol was added and the mixture was shaken. Free fatty acids (FFA) were extracted twice with 25-mL portions of petroleum ether. Extracts were titrated with 0.02 N alcoholic NaOH using phenolphthalein as the indicator. The difference between NaOH used for titrating extracts of the test solution and that of a blank from a boiled supernatant solution was used to determine the amount of FFA released.

Analysis of Monocarbonyl Compounds as Their 2,4-Dinitrophenylhydrazone (DNPH) Derivatives. Hexane-extracted carbonyl compounds from uninoculated (control) and inoculated samples were derivatized, isolated, and identified as their 2,4-dinitrophenylhydrazone (DNPH) derivatives according to procedures similar to those reported by Schwartz and Parks (1961), Schwartz et al. (1968), and Moerck (1977).

RESULTS AND DISCUSSION

Acetone was the only monocarbonyl present in fresh tissue samples. There was an increase in all classes of aliphatic monocarbonyl compounds in all stored, uninoculated samples (Tables I and II). These increases in carbonyl concentrations can be attributed to the autoxidation of unsaturated fatty acids.

2-Pentanone was the only methyl ketone formed in stored, uninoculated samples. C_2 to C_{10} alkanals were present in all uninoculated, stored samples with hexanal and acetaldehyde being the predominant alkanals. There was also an increase in the concentration of 2-alkenals in all uninoculated samples (Tables I and II). C_6 to C_{11} 2-alkenals were identified in ground thigh muscle samples. C_7 to C_{10} 2-alkenals were present in stored adipose tissue samples. 2-Nonenal was the predominant 2-alkenal in all samples. 2,4-Alkadienals were composed primarily of heptadienal, nonadienal, and decadienal. Only 2,4-decadienal was detected in adipose tissue stored for 7 days at 4 °C.

The levels of aerobic microorganisms present after inoculation and storage of ground thigh muscle and adipose tissue samples are presented in Table III. Very low levels of microorganisms were detected in saline-rinsed (uninoculated) adipose tissue and saline-rinsed ground thigh muscle samples after storage. *Micrococcus* sp. did not grow well on tissue samples held at 4 °C, nor did *T. pullulans* and *R. aurantiaca* grow well on adipose tissue samples. In general, microorganisms grew better on ground thigh muscle samples than on adipose tissue. Aerobic microbial counts were also consistently higher in samples stored for 3 days at 22 °C than in samples stored for 7 days at 4 °C.

Four microorganisms, *P. fluorescens*, *P. fragi*, *P. aeruginosa*, and *C. lipolytica*, increased the concentration of methyl ketones in ground thigh muscle and adipose tissue samples (Tables I and II). *R. aurantiaca* increased the methyl ketone concentration in ground thigh muscle

Table II. Concentrations of Carbonyl Compounds Isolated from Inoculated and Uninoculated Adipose Tissue after 3 Days Storage at 22 °C and after 7 Days at 4 °C

	μmol/10 g of extract[a]											
	total carbonyls		mono-carbonyls		methyl ketones		alkanals		2-enals		2,4-dienals	
samples	22 °C	4 °C	22 °C	4 °C	22 °C	4 °C	22 °C	4 °C	22 °C	4 °C	22 °C	4 °C
control (0 time)	58.2	58.2	5.4	5.4	4.8	4.8	0.1	0.1	b	b	b	b
control (3 days)	64.2	62.6	13.6	10.8	5.8	5.0	2.6	1.5	2.7	2.1	1.2	0.8
P. fluorescens	60.2	58.9	14.5	13.1	9.6	9.1	0.2	0.1	2.8	2.0	1.1	0.9
control (0 time)	60.3	60.3	5.6	5.6	5.2	5.2	b	b	b	b	b	b
control (3 days)	64.8	62.2	13.7	11.5	5.4	5.6	3.2	1.5	3.5	3.1	1.2	0.9
P. fragi	62.4	64.6	14.9	13.0	13.6	11.6	0.9	0.5	b	0.1	b	b
control (0 time)	63.4	63.4	6.3	6.3	5.6	5.6	0.1	0.1	0.1	0.1	b	b
control (3 days)	70.1	65.1	16.1	13.9	5.9	5.8	3.7	3.4	4.2	3.6	1.7	1.6
P. aeruginosa	65.8	63.9	17.0	15.6	14.3	13.1	0.8	1.7	b	0.1	b	b
control (0 time)	67.7	67.7	5.1	5.1	4.9	4.9	b	b	0.1	0.1	b	b
control (3 days)	71.6	70.2	15.3	12.6	5.3	5.1	4.0	3.8	3.2	2.6	1.8	1.0
P. syncyanea	71.9	71.8	10.2	11.6	5.2	5.0	1.1	1.4	3.1	2.8	1.9	0.9
control (0 time)	63.1	63.1	6.0	6.0	5.7	5.7	b	b	b	b	b	b
control (3 days)	67.8	67.1	13.1	12.1	5.6	5.6	2.9	2.4	2.1	1.9	1.0	1.1
P. fairmontensis	66.4	66.8	9.3	11.6	5.5	5.8	0.6	1.4	2.0	1.8	1.1	1.3
control (0 time)	68.5	68.5	6.4	6.4	6.1	6.1	b	b	b	b	b	b
control (3 days)	74.8	74.6	14.1	13.9	6.6	6.5	3.1	3.1	2.6	2.7	1.3	1.2
Micrococcus sp.	88.9	75.3	30.1	13.2	7.0	6.4	9.6	3.0	8.0	2.9	4.6	1.2
control (0 time)	61.9	61.9	5.3	5.3	5.3	5.3	b	b	b	b	b	b
control (3 days)	69.9	69.1	13.9	12.5	5.6	5.2	3.6	3.3	3.1	3.0	3.1	1.0
A. lipolytica	43.8	45.8	11.7	9.3	5.3	5.4	0.2	0.4	2.9	0.4	3.1	1.1
control (0 time)	68.6	68.6	4.9	4.9	4.9	4.9	b	b	b	b	b	b
control (3 days)	74.7	72.4	13.0	13.2	5.2	5.5	3.1	3.3	3.0	2.1	1.3	1.5
T. pullulans	74.8	72.6	13.2	13.5	5.1	5.6	3.0	3.1	3.2	2.2	1.3	1.5
control (0 time)	71.3	63.8	5.1	5.1	5.0	5.0	b	b	b	b	b	b
control (3 days)	76.9	70.5	13.9	13.9	5.5	5.7	3.3	3.8	2.6	2.9	1.7	1.4
R. aurantiaca	77.3	69.2	13.2	14.3	5.4	5.7	3.1	3.7	2.7	2.7	1.7	1.5
control (0 time)	62.7	62.7	6.7	6.7	6.5	6.5	b	b	0.1	b	b	b
control (3 days)	70.3	75.2	17.7	16.6	6.5	6.5	4.6	4.1	4.4	3.2	1.8	1.6
C. lipolytica	67.9	72.9	12.9	13.7	9.8	9.6	0.7	1.3	b	0.8	b	b

[a] Mean values of two trials. [b] None detected.

Table III. Growth of Microorganisms on Ground Thigh Muscle and Adipose Tissue[a]

	microorganisms/gram[b]			
	thigh muscle		adipose tissue	
inoculum	22 °C, 3 days	4 °C, 7 days	22 °C, 3 days	4 °C, 7 days
fresh (control)	1.3×10^2	6.2×10^1	45	47
P. fragi	6.3×10^8	5.3×10^8	4.9×10^8	6.7×10^6
P. aeruginosa	3.2×10^8	2.5×10^7	3.6×10^7	2.6×10^6
P. fluorescens	6.6×10^8	6.2×10^8	5.4×10^8	5.1×10^6
P. syncyanea	1.2×10^8	2.4×10^7	6.9×10^7	8.3×10^6
P. fairmontensis	5.9×10^8	3.8×10^7	3.2×10^7	3.8×10^6
A. lipolytica	5.3×10^8	2.9×10^7	2.6×10^8	4.4×10^6
Micrococcus sp.	3.4×10^8	7.3×10^3	7.3×10^7	2.6×10^6
C. lipolytica	3.9×10^8	6.2×10^6	3.9×10^8	1.1×10^6
T. pullulans	4.8×10^7	1.4×10^6	1.2×10^5	4.8×10^4
R. aurantiaca	6.7×10^7	3.0×10^6	3.1×10^5	2.9×10^4

[a] Initial counts after washing were 20 and 10 per g of thigh muscle and adipose tissue, respectively. After inoculation, counts ranged from 10^4 to 10^5/g. [b] Duplicate determinations of ten independent samples.

samples, but not in adipose tissue samples (Tables I and II). This can be attributed to the poor growth and consequent low levels of *R. aurantiaca* on adipose tissue (Table III). The only methyl ketone produced by *P. fluorescens*, *R. aurantiaca*, and *C. lipolytica* was acetone, while *P. aeruginosa* and *P. fragi* produced acetone and trace quantities of 2-butanone, 2-hexanone, and 2-octanone.

Acetone production by *P. fragi*, *P. fluorescens*, *P. aeruginosa*, *C. lipolytica*, and *R. aurantiaca* could possibly be due to the decarboxylation of acetoacetate (Wood et al., 1945; Harvey, 1960). The mechanism of bacterial acetoacetic decarboxylase has been shown to occur via formation of a Schiff's base between enzyme and substrate, followed by decarboxylation and hydrolysis (Wood et al., 1945). Production of trace quantities of 2-butanone, 2-hexanone, and 2-octanone by *P. fragi* could be attributed to the oxidation of hydrocarbons. Several researchers have demonstrated the microbial conversion of *n*-alkanes to methyl ketones (Leadbetter and Foster, 1960; Lukins and Foster, 1963; Fredricks, 1967; Klein et al., 1968; Forney and Markovetz, 1970). Transformation of hydrocarbons into methyl ketones appears to involve some type of subterminal methylene-carbon oxidation (Forney and Markovetz,

Table IV. Lipolytic Activity of Microorganisms[a]

microorganism	mL of 0.02 N alc. NaOH[b]
C. lipolytica	47.6
A. lipolytica	42.3
P. fluorescens	37.6
P. fragi	35.9
Micrococcus sp.	30.6
T. pullulans	29.7
P. aeruginosa	24.6
P. syncyanea	21.8
P. fairmontensis	19.7
R. aurantiaca	18.8

[a] Bacteria were grown in nutrient broth for 48 h at 22 °C. Yeast were grown in nutrient broth (pH 5.5) fortified with 1% yeast extract for 48 h at 22 °C. [b] Means of duplicate determinations.

1970). Hydrocarbons have been shown to be formed by the autoxidation of unsaturated fatty acids (Evans, 1961; Horvat et al., 1964; Forss and Holloway, 1967).

Smith and Alford (1968) and Alford et al. (1971) reported large increases in the concentration of methyl ketones when *P. fragi* and *C. lipolytica* were grown on lard. These researchers postulated that these and possibly other lipolytic bacteria, yeasts, and molds produce methyl ketones by decarboxylation of β-keto acids formed during the β oxidation of lipase liberated fatty acids. All of the microorganisms in the present study were shown to be active lipase producers (Table IV). If these bacteria and yeasts produced methyl ketones by an aborted β-oxidative system, a variety of odd-chained methyl ketones of intermediate chain lengths (C_5–C_{15}) should be present in the tissue samples (Patton, 1950; Darty and Kinsella, 1973). Except for acetone and pentanone, odd-chained methyl ketones were not detected in any samples, indicating that decarboxylation of β-keto acids, which is known to produce methyl ketones in fungal cells, did not occur.

The *Micrococcus* sp. increased the concentration of aldehydes in ground thigh muscle and adipose tissue samples stored for 3 days at 22 °C (Tables I and II) and also in rancid adipose tissue samples (Table V). This culture also increased the concentration of peroxides in both fresh (Table VI) and rancid adipose tissue samples (Table V). The concentrations of individual alkanals and 2-alkenals isolated from *Micrococcus* sp. inoculated ground thigh muscle is shown in Tables VII and VIII. All of the alkanals and 2-alkenals detected in the samples inoculated with *Micrococcus* sp. were also present in the uninoculated samples but in lower concentrations.

The increase in the concentration of aldehydes and the acceleration of peroxide formation suggests the possibility of lipoxidase-like activity, Smith and Alford (1969) reported that *Micrococcus freudenreichii* produced a large increase in the concentration of saturated and unsaturated aldehydes and observed an acceleration in peroxide formation. These researchers postulated the presence of lipoxidase in this microorganism. Several other reports contain evidence of lipoxidase in *Aspergillus*, *Penicillium*, *Rhizopus*, *Pseudomonas*, *Achromobacter*, and *Pediococcus* (Mookherjee, 1951; Fukuba, 1952; Shimahara, 1966; Bothast et al., 1973).

Nine out of the ten microorganisms tested decreased or completely removed at least one class of aldehydes that were present in stored, uninoculated ground thigh muscle and adipose tissue samples. *P. fluorescens*, *P. syncyanea*, *P. fairmontensis*, and *A. lipolytica* decreased the concentration of alkanals in all tissue samples (Tables I and II). *P. aeruginosa*, *P. fragi*, and *C. lipolytica* decreased or completely removed saturated and unsaturated aldehydes that were present in uninoculated tissue samples (Tables I and II). *T. pullulans* and *R. aurantiaca* decreased or completely removed both saturated and unsaturated aldehydes when grown on ground thigh muscle, but did not decrease the concentration of these carbonyls when grown on adipose tissue samples, presumably because of their poor growth on adipose tissue (Table III). The *Micrococcus* sp. increased the concentration of alkanals, 2-alkenals, and 2,4-alkadienals in ground thigh muscle and adipose tissue samples stored for 3 days at 22 °C, but not in samples stored for 7 days at 4 °C (Tables I and II) because there was little growth of the *Micrococcus* sp. at 4 °C in either muscle or adipose tissue (Table III).

Removal of alkanals by 9 out of 10 microorganisms tested is contrary to results obtained by Smith and Alford (1969). These researchers had reported that 28 strains of microorganisms, including *P. fragi* and *C. lipolytica*, had little or no effect on the alkanal fraction of fresh lard. Instead, these workers found that *P. fragi* and *C. lipolytica*

Table V. Effect of Microorganisms on Peroxides and Monocarbonyls in Rancid (UV Treated) Adipose Tissue[a]

	peroxide value[b]		monocarbonyls[c]			
samples[d]	start	end (3 days at 22 °C)	methyl ketones	alkanals	2-enals	2,4-dienals
control	51.6	56.5	7.6	12.6	6.7	4.4
P. fluorescens	51.6	33.7	10.6	*e*	6.2	4.4
control	47.2	46.8	6.9	11.7	6.5	3.8
P. fragi	47.2	10.8	14.6	2.1	*e*	*e*
control	53.4	52.8	5.9	10.6	5.9	4.0
P. aeruginosa	53.4	8.3	16.7	2.7	1.8	*e*
control	58.2	60.3	6.3	9.8	6.1	3.2
P. syncyanea	58.2	39.8	6.2	1.2	6.2	3.0
control	49.8	47.6	5.7	11.6	4.9	3.6
P. fairmontensis	49.8	26.7	6.0	0.8	5.1	3.6
control	51.6	52.7	7.0	12.5	4.3	3.1
Micrococcus sp.	51.6	68.6	7.4	18.6	8.7	5.2
control	60.3	59.1	6.3	8.9	4.0	2.9
A. lipolytica	60.3	32.6	6.2	0.5	4.2	2.8
control	53.4	56.5	5.3	13.7	6.4	3.4
C. lipolytica	53.4	25.7	14.6	7.2	2.1	*e*

[a] Mean values of two trials. [b] Milliequivalents/kilogram of fat. [c] Micromoles/10 g of extract. [d] *R. aurantiaca* and *T. pullulans* had no apparent effect on moncarbonyls or peroxides. [e] None detected.

Table VI. Effect of Microorganisms on Peroxides in Adipose Tissue Stored for 3 Days at 22 °C[a]

samples	microorgan./g	peroxide value, meguiv/kg of fat
control	2.1×10^1	12.3
P. fragi	3.4×10^8	*b*
P. aeruginosa	4.9×10^7	*b*
P. fluorescens	1.3×10^8	2.6
P. syncyanea	2.8×10^7	5.2
P. fairmontensis	3.6×10^7	6.4
A. lipolytica	9.8×10^7	2.6
Micrococcus sp.	8.2×10^7	28.4
C. lipolytica	5.1×10^8	*b*
T. pullulans	3.3×10^5	12.9
R. aurantiaca	4.3×10^5	11.8

[a] Average of duplicate determinations of two independent samples. [b] None detected.

increased the level of this class of carbonyls. However, Bothast et al. (1973) later reported that *P. fluorescens* decreased the concentration of saturated aldehydes in ground porcine muscle by 39%.

The ability of most of the microorganisms tested to decrease or completely remove at least one class of aldehyde conceivably explains why Dimick and MacNeil (1970), Harris and Lindsay (1972), Dimick et al. (1972), and others found either low levels or no saturated or unsaturated aldehydes in stored chicken tissue samples. Since these researchers did not inhibit microbial growth, it seems feasible that microorganisms which were present had removed any aldehydes formed during autoxidation.

Removal of saturated and unsaturated aldehydes by the microorganisms investigated can be construed as evidence for dehydrogenase or reductase activity. Keenan et al. (1967) demonstrated that a number of *Pseudomonas* species were capable of reducing acetaldehyde, propanal, and butanal to the corresponding alcohols. An alternate metabolic consequence can lead to the oxidation of aliphatic aldehydes to the corresponding acids, as has been reported in yeasts (Seegmiller, 1953). The ability of some of the microorganisms examined to selectively attack and utilize only saturated aldehydes, while others removed both saturated and unsaturated aldehydes, suggests differences in enzyme specificity or enzyme populations.

In order to investigate the possibility of the presence of reductase activity, *P. fragi* and *P. fluorescens* cultures were grown for 48 h in nutrient broth with 60 ppm butanal added. Alcohols present in the nutrient broth were analyzed using procedures similar to those described by Keenan et al. (1967). Both microorganisms were shown to convert butanal to the corresponding alcohol, indicating the presence of reductase activity.

Except for *A. lipolytica*, none of the microorganisms investigated completely removed all of the alkanals that were present in uninoculated samples. Pentanal, hexanal, and heptanal were detected in most tissue samples (Table VII). Keenan et al. (1967) noted that the extent of aldehyde reduction by a variety of *Pseudomonas* species was dependent on the substrate. *P. fragi*, for example, reduced acetaldehyde and propanal to their corresponding alcohols more readily than it reduced butanal. However, there could also be a concentration effect involved, since pentanal, hexanal, and heptanal were present in relatively high concentrations.

If aldehydes in tissue samples are reduced to their corresponding alcohols, there could conceivably be a significant alteration in the flavor of chicken tissue during storage. In addition to lesser volatility, alcohols possess conspicuously different flavor properties. While saturated aldehydes have been described as having a wide range of offensive odors and flavors (green, sharp, oily, fatty, tallowy), saturated aliphatic alcohols are relatively flavorless (Forss, 1972). Unsaturated aliphatic alcohols have been shown to have unique flavors, many of which have been described as perfume-like (Bedoukian, 1971), whereas unsaturated aliphatic aldehydes have been reported to contribute oxidized "fatty" or "deep-fried" flavors to poultry (Hoffman, 1962; Dimick and MacNeil, 1970). Reductase activity could also provide a source of alcohol for ester formation by the microorganisms investigated. *P. fragi* has been shown to produce fruity odors in a variety of foods and the development of this fruity aroma has been attributed to the formation of esters.

It is possible that the microorganisms tested decreased carbonyl concentrations by disrupting the normal sequence of autoxidation. Hydroperoxides that are formed during autoxidation may have been decomposed to compounds other than carbonyls, or the formation of hydroperoxides may have been inhibited. Table VI illustrates that 7 out of 10 microorganisms tested decreased the concentration of peroxides in adipose tissue stored for 3 days at 22 °C. The microorganisms also removed peroxides and aldehydes from adipose tissue that had been subjected to UV radiation to increase peroxide and carbonyl concentrations (Table V). Apparently, these microorganisms are capable of not only removing aldehydes, but can also decompose hydroperoxides. Smith and Alford (1968, 1969) noted that 25 out of 28 species of bacteria, molds, and yeasts, including *P. fragi*, *C. lipolytica*, and a *Pseudomonas* sp., decomposed peroxides in fresh and rancid lard.

Even though dicarbonyl compounds were not isolated and identified, it was evident that some of the microorganisms tested removed dicarbonyl compounds. After growth of *R. aurantiaca* and *A. lipolytica*, the typical dicarbonyl band was no longer visible on the magnesia

Table VII. Concentrations of Alkanals Isolated from Uninoculated and Inoculated Ground Thigh Muscle after 3 Days Storage at 22 °C[a]

compd	control, 3 days, 22 °C	microorganism[b]			
		P. fluorescens	*P. fragi*	*P. aeruginosa*	*Micrococcus* sp.
acetaldehyde	1.65	*d*	Tr[c]	Tr	2.06
propanal	0.53	*d*	*d*	*d*	0.78
butanal	0.70	*d*	0.21	*d*	1.14
pentanal	0.85	0.42	0.74	0.76	1.38
hexanal	1.67	0.95	1.13	0.85	3.05
heptanal	0.72	0.32	0.33	0.41	1.05
octanal	0.21	*d*	Tr	Tr	0.76
nonanal	0.23	*d*	*d*	*d*	0.54
decanal	0.09	*d*	*d*	*d*	0.29

[a] Average of three trials. Expressed as μmol/10 g of extract. [b] *P. fairmontensis, R. aurantiaca, and P. syncyanea* removed all alkanals. [c] Trace amounts. [d] None detected.

Table VIII. Concentrations of 2-Alkenals Isolated from Ground Thigh Muscle Inoculated with *Micrococcus* sp. and Uninoculated Ground Thigh Muscle after 3 Days Storage at 22 °C[a]

compd	control	*Micrococcus* sp.
2-hexenal	0.18	0.91
2-heptenal	0.21	0.83
2-octenal	1.05	1.87
2-nonenal	1.53	2.66
2-decenal	0.71	1.01
2-undecenal	0.11	0.15

[a] Average of three trials. Expressed in μmol/10 g of extract.

columns. This was also reflected in the decrease in total carbonyl concentration, which includes dicarbonyl compounds (Tables I and II). In an earlier study, Moerck and Ball (1974) noted that microorganisms appeared to be removing dicarbonyl compounds in mechanically deboned chicken meat. The progress of oxidation of tissue lipids is frequently followed by reacting thiobarbituric acid (TBA) with dicarbonyl compounds, especially malonaldehyde. It seems feasible that removal of these dicarbonyls could influence TBA determinations. Moerck and Ball (1974) noted that TBA values were greater when microbial growth was inhibited.

It is apparent that microbially induced alteration of monocarbonyl and peroxide concentrations could cause a wide range of flavor changes in raw and cooked, stored chicken. Since the ultimate flavor contribution of monocarbonyl compounds is concentration dependent, changes in the concentration of these compounds could have a significant effect on the quality of stored chicken. It is conceivable that the microflora of chicken or microbial enzymes could be manipulated to bring about desirable changes in flavor.

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