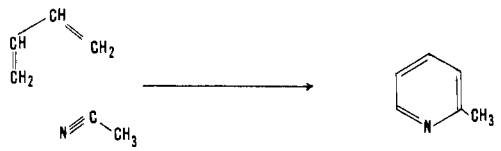
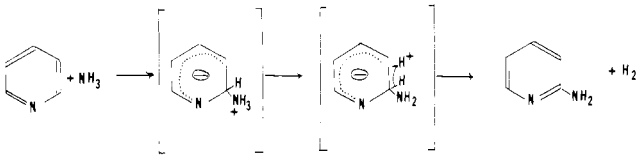


butadiene and butyronitrile would produce 2-propylpyridine.

The formation of 2-aminopyridine differs from that of the alkylpyridines in that pyridine itself is one of the reactants. 2-Aminopyridine is formed by amination of pyridine in a Chichibabin-like reaction. Bergstrom (1937) predicted that ammonia or free aminopyridine could replace the monometal salt used in the Chichibabin reaction as an acceptor of the hydride ion which is liberated from the intermediate formed in the reaction. In the reaction proposed here, the proton liberated after addition of ammonia to the pyridine ring serves as the acceptor for the hydride ion in the final step of the reaction to form diatomic hydrogen and 2-aminopyridine.



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Formation of Monocarbonyl Compounds in Chicken Tissue

Kurt E. Moerck[1] and Hershell R. Ball, Jr.

Broiler adipose and thigh muscle tissue samples were aseptically obtained, ground, and 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 and identified. Acetone was the only monocarbonyl present in fresh tissue samples. There was an increase in the concentration of all classes of aliphatic monocarbonyl compounds in stored tissue samples. More oxidation occurred in stored ground thigh muscle than in stored ground adipose tissue samples. 2-Pentanone was the only methyl ketone formed during storage. C_2–C_{10} alkanals were present in all stored samples. Hexanal and acetaldehyde were present in the highest concentrations. C_6–C_{11} 2-alkenals were isolated from ground thigh muscle samples and C_7–C_{10} 2-alkenals were identified in adipose tissue samples. 2-Nonenal was the predominant 2-alkenal in all samples. 2,4-Alkadienals were composed primarily of heptadienal, nonadienal, and decadienal, with decadienal being the predominant 2,4-alkadienal in all samples.

Lipid oxidation is a major cause of deterioration in the quality of stored poultry and poultry products. This oxidative process involves the reaction of unsaturated fatty acids with molecular oxygen to yield hydroperoxides, which in turn decompose to yield flavorable compounds (Holman, 1954; Lundberg and Jarvi, 1968; Labuza, 1971; Forss, 1972). Many of these flavor and odor compounds are aliphatic monocarbonyl compounds, namely methyl ketones, alkanals, 2-alkenals, and 2,4-alkadienals (Ellis et al., 1961; Gaddis et al., 1961; Badings, 1970). Hoffmann (1961), Smouse and Chang (1967), and others have demonstrated that flavor defects which occur because of autoxidative deterioration are many times due to monocarbonyl compounds which are formed from polyenoic fatty acids. These secondary products are responsible for a wide range of oxidized flavors and odors in chicken tissues (Dimick and MacNeil, 1970; MacNeil and Dimick, 1970; Dimick et al., 1972; Golovkin and Galkin, 1975). Aliphatic monocarbonyl compounds are important flavor and odor sources in poultry and other lipid-containing foods because of their low flavor threshold values (Forss et al., 1962; Meijboom, 1964; Badings, 1970; Siek et al., 1971).

The purpose of this investigation was to isolate and identify the aliphatic monocarbonyl compounds formed

Department of Food Science, North Carolina State University, Raleigh, North Carolina 27650.
[1]Present address: Basic Research, Frito-Lay Research Department, Frito-Lay, Inc., Irving, TX 75061.

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in chicken tissue during storage at both refrigeration and room temperatures.

EXPERIMENTAL SECTION

Preparation 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. Using sterilized equipment, multiple slices of thigh muscle (5–10 g) and adipose tissue (10–20 g) were aceptically removed from each of four–six broiler chickens used per experiment. Because microorganisms have been shown to both produce and remove carbonyl compounds (Smith and Alford, 1968, 1969; Alford et al., 1971; Bothast et al., 1973; Moerck, 1977), the initial levels of microorganisms on the tissue samples were lowered by washing four times in 150-mL portions of sterile physiological saline. Thigh muscle or adipose tissue slices were immediately transferred to a cool, sterile Waring Blendor and ground for 45–60 s. One-hundred-gram samples were placed in sterile beakers and covered. Both ground thigh muscle and ground adipose tissue samples were held for 3 days at 22 °C or 7 days at 4 °C.

Enumeration of Aerobic Microorganisms. The total number of aerobic organisms was determined at 0 time and after storage by the pour plate method. 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.

Preparation of DNPH Derivatives. DNPH derivatives used as standards and for calibration purposes were prepared by adding the carbonyl compounds to a solution consisting of 2 g/L of 2,4-dinitrophenylhydrazine in 2 N HCl. DNPH derivatives were recrystallized from hot 95% ethanol to constant melting points. Melting points agreed closely with literature values (Reich and Helfe, 1956; Shriner et al., 1956; Brewster et al., 1960; Owen, 1969). DNPH derivatives were also analyzed using GLC and TLC to assess purity.

Preparation of Reaction Column. A modification of the method of Schwartz and Parks (1961) was used to prepare the 2,4-dinitrophenylhydrazine reaction column. 2,4-Dinitrophenylhydrazine (0.75 g) was dissolved in 0.9 mL of 85% H_3PO_4 by grinding in a mortar. Six milliliters of distilled water was added and the precipitated 2,4-dinitrophenyldrazine was redissolved by grinding. Fifteen grams of analytical grade Celite (Fisher Scientific Co.) was added to this mixture and ground. The analytical grade Celite had been activated for 24 h at 150 °C. The mixture was packed into a 2.5 × 30 cm column. The column was flushed with 50 mL of carbonyl-free benzene, followed by 100 mL of carbonyl-free hexane.

Solvents. All solvents were rendered carbonyl free by passing them through the reaction column and then distilling them using a Vigreux column. Chloroform was washed four times with half its volume of distilled water and dried over anhydrous $CaCl_2$ prior to passage through the column.

Extraction of Carbonyl Compounds. Fifty grams of chicken thigh tissue or 10 g of adipose tissue were homogenized in a cold Waring Blendor with 200 mL of carbonyl-free hexane. The extract was filtered through Whatman No. 1 paper, using a Buchner funnel, and dried with anhydrous sodium sulfate.

Efficiency of the hexane extraction procedure was determined by homogenizing thigh or adipose tissue samples as previously described and then drying under vacuum to determine the hexane-extractable lipid present. The percent hexane-extractable lipid was compared with the percent lipid as determined according to AOAC (1970).

Reaction with 2,4-Dinitrophenylhydrazine. Carbonyl compounds present in the hexane–lipid extract were converted to their DNPH deriatives by passage through the 2,4-dinitrophenylhydrazine reaction column (Schwartz and Parks, 1961). The DNPH derivatives were eluted from the column with 200 mL of carbonyl-free hexane. Total concentration of DNPH derivatives present was determined by reading absorbance against hexane at 340 nm, using a Bausch and Lomb 600 spectrophotometer, and converting to micromoles, using $E = 22\,500\ M^{-1}\ cm^{-1}$ (Jones et al., 1956).

Removal of Lipid and Ketoglyceride Derivatives. The procedures described by Boyd et al. (1965) were followed with minor modifications. The eluate from the reaction column was defatted on 15 g of Celite 545-Sea Sorb 43 (Fisher Scientific Co.) in a 2.5 × 30 cm column, prepared as described by Boyd et al. (1965). Lipid was eluted with 250 mL of carbonyl-free hexane and the adsorbed DNPH derivatives were eluted with 150 mL of chloroform–nitromethane (3:1, v/v). Eluate volume was reduced to approximately 20 mL with a rotary evaporator at room temperature. The remaining solvent was evaporated under a stream of nitrogen gas. The residue was then dissolved in 10 mL of carbonyl-free hexane.

Ketoglyceride derivatives were separated from the monocarbonyl derivatives on a 1.2 × 30 cm column containing 15 g of partially deactivated alumina (Fisher Scientific Co.). The monocarbonyls were eluted with 250 mL of carbonyl-free benzene–hexane (1:1, v/v). The solvent was removed as described above and the residue dissolved in a known volume of chloroform. Concentration of monocarbonyl DNPH derivatives was determined spectrophotometrically at 365 nm and converted to micromoles using $E = 22\,500\ M^{-1}\ cm^{-1}$ (Jones et al., 1956).

Separation of Monocarbonyl DNPH Derivatives into Classes. After solvent evaporation, monocarbonyl derivatives were fractionated into classes on a 10 g of Celite 545-Sea Sorb 43 (1:1, w/w) column (Boyd et al., 1965). DNPH derivatives were dissolved in 10 mL of hexane and applied to the column. Separation of DNPH classes was accomplished with the following solvents: 150 mL of 15% chloroform in hexane, 125 mL of 30% chloroform in hexane, 100 mL of 60% chloroform in hexane, 150 mL of 100% chloroform, and 100 mL of 0.5% methanol in chloroform. The eluate was collected in 5-mL aliquots, using a Gilson fraction collector.

Each fraction was evaporated to dryness under nitrogen and dissolved in a known volume of chloroform. Each tube was examined spectrophotometrically to establish the class of monocarbonyl DNPH derivative present. Classes were established and pooled on the basis of the following absorption maxima: methyl ketones, 365 nm; alkanals, 355 nm; 2-alkenals, 373 nm; 2,4-alkadienals, 390 nm. Concentrations were calculated, using the suitable molar extinction coefficient as described by Jones et al. (1956).

Percent recovery and stability of the four DNPH classes were determined by applying a mixture of four representative DNPH derivatives to the columns and separating as previously described (Table I).

Separation and Tentative Identification of Individual Compounds within a Class Using TLC. Ascending TLC on Kieselguhr G (E. Merck) impregnated with Carbowax 400 (Applied Science Laboratories, State College, PA) was used to separate and tentatively identify

Table I. Recovery of 2,4-Dinitrophenylhydrazone Derivatives from Celite 545-Sea Sorb 43 Columns[a]

compd	concn, μM	recov., %
2-pentanone	0.50	97
octanal	0.56	95
2-octenal	0.47	96
2,4-decadienal	0.55	96

[a] Average of three determinations.

the individual components within each class of monocarbonyl compounds (Badings and Wassink, 1963). All chromatoplates were prepared from a slurry of 15 g of Kieselguhr G, 8 g of Carbowax-400, and 30 mL of distilled water. The slurry was applied to 20.3 × 20.3 cm glass plates at a thickness of 0.25 mm. The chromatoplates were air-dried for 30 min and then heat activated for 1 h at 110 °C. The plates were stored over calcium chloride until needed. DNPH classes to be separated were dissolved in hexane, applied to the chromatoplates, and developed with methylcyclohexane. Known DNPH derivatives of the appropriate monocarbonyl class were developed on the same chromatoplates. DNPH derivatives were visualized by spraying with 5% KOH in ethanol. Compounds were tentatively identified by comparing their R_f values and characteristic colors on KOH-treated plates: methyl ketones, brown; alkanals, tan; 2-alkenals, pink; 2,4-alkadienals, red.

GLC Analysis of Individual Compounds within a Class. Each class of DNPH derivatives was analyzed using GLC. A Barber-Colman 5000 gas chromatograph equipped with a 1.83 m × 4 mm silanized glass column, containing 2% OV-1 on Chromosorb W (60–80 mesh, acid washed), and a flame ionization detector was used. The column was operated isothermally for 5 min at 185 °C and then programmed to 285 °C at 3 °C/min. Nitrogen was used as the carrier gas at 40 mL/min. The injector temperature was 290 °C and the detector temperature was 320 °C.

Usually, 1.0-μL samples were injected into the columns with a 10-μL Hamilton syringe. Relative amounts of the DNPH derivatives in the injected samples were determined, using an integrator.

DNPH derivatives used for GLC calibration were prepared as previously described. Derivatives were dissolved in carbonyl-free hexane at 1 mg/mL concentrations, and 1-μL portions were injected into the columns. Chromatographic peak areas were measured, using an integrator.

Mass Spectrometry of DNPH Derivatives. Identities of DNPH derivatives were confirmed by comparing their mass spectra with those of authentic standards. A Finnigan 3300 mass spectrometer (Research Triangle Institute, Research Triangle Park, NC) was operated with an ionizing voltage of 70 eV. Samples were introduced into the mass spectrometer using direct probe or a combination of GC–MS. The GC column operating conditions previously described were used. The top of each gas chromatographic peak was scanned as it was eluted from the column. Samples for direct probe insertion were eluted from TLC scrapings from plates prepared as previously described. The DNPH derivatives were eluted with carbonyl-free methylene chloride or carbonyl-free hexane.

RESULTS AND DISCUSSION

The lipid content of adipose tissue and ground thigh muscle was approximately 71.7 and 5.8%, respectively. When hexane was used, approximately 9% less lipid material was extracted.

The levels of aerobic microorganisms present after storage of ground thigh muscle and adipose tissue samples were relatively low, indicating that the washing procedure was effective in reducing the initial bacterial population. Initial numbers were 10 and 18 per gram of adipose tissue and thigh muscle, respectively. Final numbers for adipose tissue were 45 and 47 per gram after 3 days at 22 °C and 7 days at 4 °C, respectively. Final numbers for thigh muscle were 1300 and 62 per gram after 3 days at 22 °C and 7 days at 4 °C, respectively.

Except for trace quantities of alkanals and 2-alkenals detected in some samples, the only monocarbonyl compound present in both fresh adipose tissue and fresh ground thigh muscle samples was acetone. Trace levels of 2-alkenals and alkanals found in some of the samples could possibly have been formed during extraction.

There was a substantial increase in the concentration of total carbonyl, total monocarbonyl, and all classes of aliphatic monocarbonyls in stored adipose tissue and ground thigh muscle samples (Table II). The total carbonyl fraction includes ketoglycerides, all monocarbonyls, and dicarbonyls, while total monocarbonyls include alkanals, 2-alkenals, 2,4-alkadienals, and methyl ketones. It is noticeable that more oxidation occurred in stored ground thigh muscle samples than in stored adipose tissue samples. Similar results were obtained when ground thigh muscle and adipose tissue samples were stored at the higher temperature (Table II).

2-Pentanone appears to be the only methyl ketone formed in ground thigh muscle and adipose tissue samples during storage. Adipose tissue samples stored for 7 days at 4 °C did not have any detectable levels of 2-pentanone. Methyl ketones apparently are not typical end-products of lipid oxidation in chicken and do not appear to be involved in the oxidative flavor deterioration in raw, stored chicken.

There was a substantial increase in the concentration of alkanals in all stored tissue samples (Table II). Ground thigh muscle, stored for 3 days at 22 °C, had the highest concentration of alkanals. Adipose tissue and ground thigh muscle samples stored for 3 days at 22 °C, also had higher concentrations of alkanals than did samples stored for 7 days at 4 °C.

Gas chromatograms typical of the DNPH derivatives of alkanals isolated from stored ground thigh muscle and adipose tissue samples are shown in Figures 1 and 2.

Table II. Concentrations of Carbonyl Compounds Isolated from Ground Thigh Muscle and Adipose Tissue[a]

	μmol/10 g of extract					
samples[b]	total carbonyls	total monocarbonyls	methyl ketones	alkanals	2-enals	2,4-dienals
GTM (0 time)	45.8 ± 1.9	7.6 ± 0.5	7.2 ± 0.5			
GTM (7 days, 4 °C)	57.6 ± 1.5	19.3 ± 1.8	7.4 ± 0.5	5.6 ± 0.5	3.7 ± 0.4	1.8 ± 0.3
GTM (3 days, 22 °C)	59.1 ± 1.2	21.6 ± 1.2	7.7 ± 0.4	6.5 ± 0.4	4.2 ± 0.6	2.5 ± 0.4
GAT (0 time)	64.6 ± 3.6	5.7 ± 0.5	5.4 ± 0.4			
GAT (7 days, 4 °C)	68.9 ± 3.7	13.1 ± 1.4	5.7 ± 0.3	3.0 ± 0.4	2.7 ± 0.4	1.2 ± 0.2
GAT (3 days, 22 °C)	70.5 ± 3.2	13.4 ± 1.2	5.7 ± 0.4	3.4 ± 0.5	3.1 ± 0.5	1.6 ± 0.3

[a] Each value is the mean and standard deviation of 20 trials. [b] GTM, ground thigh muscle; GAT, ground adipose tissue.

Table III. Concentrations of Individual Monocarbonyl Compounds within Each Class Isolated from Stored Ground Thigh Muscle and Adipose Tissue[a]

compounds[b]	ground thigh muscle		adipose tissue	
	3 days, 22 °C	7 days, 4 °C	3 days, 22 °C	7 days, 4 °C
methyl ketones				
acetone	7.25 ± 0.65	6.85 ± 0.76	5.39 ± 0.68	5.2 ± 0.63
2-pentanone	0.41 ± 0.11	0.25 ± 0.09	0.13 ± 0.02	
alkanals				
acetaldehyde	1.50 ± 0.25	1.03 ± 0.14	0.81 ± 0.05	0.69 ± 0.12
propanal	0.63 ± 0.13	0.54 ± 0.12	0.37 ± 0.04	0.17 ± 0.03
butanal	0.65 ± 0.05	0.68 ± 0.03	0.43 ± 0.05	0.16 ± 0.03
pentanal	0.79 ± 0.10	0.84 ± 0.09	0.35 ± 0.06	0.45 ± 0.14
hexanal	1.57 ± 0.18	1.38 ± 0.12	0.93 ± 0.20	1.01 ± 0.23
heptanal	0.77 ± 0.09	0.61 ± 0.13	0.21 ± 0.03	0.31 ± 0.05
octanal	0.26 ± 0.05	0.22 ± 0.10	0.08 ± 0.01	0.07 ± 0.02
nonanal	0.18 ± 0.04	0.05 ± 0.01	0.12 ± 0.02	0.12 ± 0.04
decanal	0.13 ± 0.04	0.15 ± 0.02	0.07 ± 0.02	0.03 ± 0.01
2-alkenals				
2-hexenal	0.22 ± 0.06	0.18 ± 0.01		
2-heptenal	0.19 ± 0.05	0.25 ± 0.03	0.26 ± 0.02	0.27 ± 0.07
2-octenal	1.24 ± 0.26	0.94 ± 0.09	0.83 ± 0.11	0.56 ± 0.09
2-nonenal	1.68 ± 0.15	1.21 ± 0.13	1.52 ± 0.21	1.05 ± 0.22
2-decenal	0.63 ± 0.13	0.48 ± 0.07	0.60 ± 0.19	0.39 ± 0.16
2-undecenal	0.17 ± 0.10	0.21 ± 0.02		
2,4-alkadienals				
2,4-heptadienal	Tr[c]	Tr	Tr	
2,4-nonadienal	0.61	Tr	Tr	
2,4-decadienal	1.39	Tr	Tr	Tr

[a] Each value a mean and deviation of ten trials. Expressed as μmol/10 g of hexane extract. [b] Fresh samples contained only acetone. [c] TR, trace amounts.

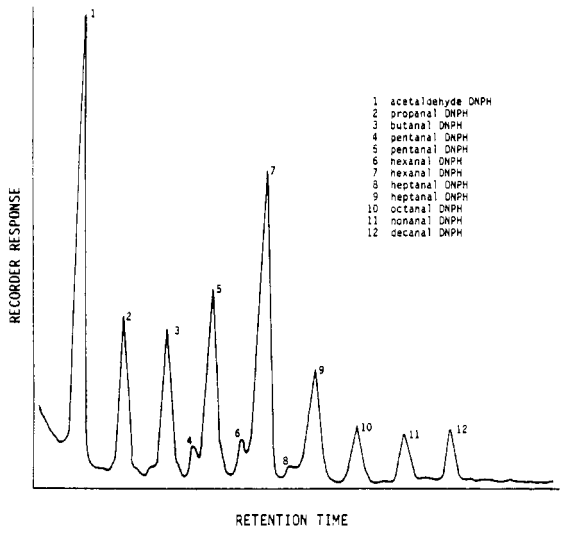

Figure 1. Typical chromatogram of the DNPH derivatives of alkanals isolated from stored thigh muscle.

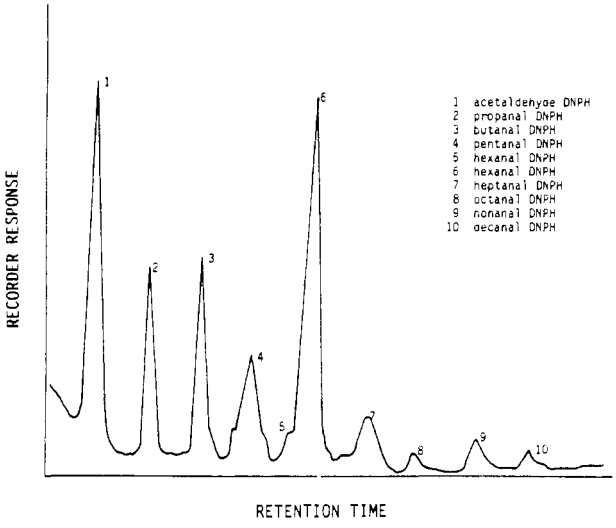

Figure 2. Typical chromatogram of the DNPH derivatives of alkanals isolated from stored adipose tissue.

Double peaks were obtained with a few DNPH derivatives. The double peaks are evidently due to isomeric DNPH derivatives.

GC–MS indicated that acetaldehyde, propanal, butanal, pentanal, hexanal, heptanal, octanal, nonanal, and decanal were present in all samples. Hexanal and acetaldehyde were present in the highest concentrations for both ground thigh muscle and adipose tissue samples (Table III).

Storage conditions influenced the concentrations of individual alkanals in ground thigh muscle and adipose tissue samples. Adipose tissue samples stored for 7 days at 4 °C, for example, had higher concentrations of pentanal, hexanal, and heptanal, but had lower concentrations of acetaldehyde, propanal, butanal, octanal, and decanal than samples held for 3 days at 22 °C (Table III). Ground thigh muscle samples held for 3 days at 22 °C had slightly lower concentrations of butanal, pentanal, and decanal but had higher concentrations of the remaining alkanals.

There was also an increase in the concentration of 2-alkenals in all samples during the storage periods (Table II). Ground thigh muscle held for 3 days at 22 °C had the highest concentration of 2-alkenals. Adipose tissue stored for 7 days at 4 °C had the lowest concentration of these carbonyls.

GC–MS indicated that 2-hexenal, 2-heptenal, 2-nonenal, 2-decenal, and 2-undecenal were present in ground thigh samples. 2-Heptenal, 2-octenal, 2-nonenal, and 2-decenal were isolated from adipose tissue. 2-Hexenal and 2-undecenal, which were isolated from ground thigh muscle, were not detected in stored adipose tissue samples. 2-Nonenal was the predominant 2-alkenal in all stored samples.

Individual 2-alkenals isolated from samples stored for 3 days at 22 °C and those isolated from samples stored for 7 days at 4 °C were not quantitatively similar (Table III). Ground thigh muscle stored for 3 days at 22 °C had higher

concentrations of 2-hexenal, 2-octenal, 2-nonenal, and 2-decenal and lower concentrations of 2-heptenal and 2-undecenal than did samples stored for 7 days at 4 °C. Concentrations of individual 2-alkenals isolated from adipose tissue stored for 3 days at 22 °C were consistently higher than those held for 7 days at 4 °C.

The 2,4-alkadienal class was composed primarily of heptadienal, nonadienal, and decadienal. Ground thigh muscle stored for 3 days at 22 °C had the highest concentration of these unsaturated aldehydes. 2,4-Decadienal and 2,4-nonadienal were present in the highest concentrations (Table III). Trace amounts of 2,4-heptadienal were also found. In ground thigh muscle stored for 7 days at 4 °C and adipose tissue samples held for 3 days at 22 °C, only trace amounts of these unsaturated aldehydes were detected. Only 2,4-decadienal was isolated in adipose samples stored for 7 days at 4 °C.

Almost all of the unsaturated and saturated monocarbonyl compounds identified in ground thigh muscle and adipose tissue samples can be attributed to the autoxidation of C_{18}–C_{20} polyunsaturated fatty acids, namely linoleic, linolenic, and arachidonic acids (Patton et al., 1959; Badings, 1960 and 1970; Dimick and MacNeil, 1970; Forss, 1972). Moerck and Ball (1974) also noted that considerable autoxidation occurred in hexenoic, pentenoic, and tetrenoic fatty acids of the phospholipid fraction when mechanically deboned chicken meat was stored for 15 days at 4 °C. Relatively little research has been conducted on carbonyl compounds formed during the oxidation of highly unsaturated fatty acids. Fisher and Wishner (1968) did study the oxidation of methyl ercosapentenoate and methyl docosahexenoate and found that the carbonyls produced were in agreement with the accepted mechanism of olefinic autoxidation. In all cases, the major products were propanal, 2-pentenal, 2-hexenal, and 2-heptenal.

Ellis et al. (1961) demonstrated the role of fatty acids in the production of carbonyl compounds. These researchers found that unsaturated C_{18} fatty acids produced alkanals, 2-alkenals, and 2,4-alkadienals. Gaddis et al. (1961) examined the volatile monocarbonyls of mildly oxidized esters of linoleic and linolenic acids and was able to isolate alkanals having from three to eleven carbons and alkadienals having from seven to twelve carbons. Van der Poel (1961) oxidized linoleic acid at 37 °C and demonstrated that hexanal, 2-octenal, and 2,4-decadienal were the primary aldehydes formed. These monocarbonyl compounds along with acetaldehyde and 2-nonenal were also the primary aldehydes present in stored ground thigh muscle and adipose tissue samples.

The occurrence of 2,4-decadienal can be explained by the oxidation of linoleic acid via the formation of the 9-hydroperoxide and also from the 11-hydroperoxide of arachidonic acid (Badings, 1960). The precursors of hexanal are presumably the 13-hydroperoxide of linoleic acid and the 15-hydroperoxide of arachidonic acid. 2-Octenal has been shown to be derived from the decomposition of the 11-hydroperoxide of linoleic acid and the decomposition of the 13-hydroperoxide of arachidonic acid (Badings, 1960). It is interesting to note that partially autoxidized samples of arachidonic acid have a flavor resembling chicken (Harkes and Begemann, 1974).

Some monocarbonyl compounds isolated from ground thigh muscle and adipose tissue cannot be formed by simple breakdown of oleic, linoleic, and arachidonic hydroperoxides. Heptanal, 2-nonenal, and 2,4-nondienal, for example, which were present in most adipose and ground thigh muscle samples may have been derived from more complex hydroperoxide reactions, by stepwise oxidation of higher homologues, or by oxidation of other polyunsaturated fatty acids (Keppler et al., 1967; Forss, 1972). Badings (1970), for example, exposed linoleic, linolenic, and arachidonic acids to air and isolated the volatile components by high volume degassing. Most of the monocarbonyl compounds isolated could be predicted by autoxidation mechanisms. Badings (1970) postulated that the presence of additional compounds could be explained either by further oxidation or by intramolecular rearrangements.

It is noteworthy that only *trans*-2-alkenals and *trans*-2,4-alkadienals appeared to be present in the stored, ground thigh muscle and adipose tissue samples. Conversion of the native cis unsaturated fatty acids to the trans configuration may have occurred during autoxidation. Cis isomers of carbonyl compounds have also been shown to isomerize into more stable compounds, e.g., *cis*-3-hexenal to *trans*-2-hexenal (Forss, 1972). Badings (1970) also noted that only *trans*-2-alkenals were isolated from cold, stored butter and postulated that isomerism had occurred. For example, autoxidation of oleic acid was expected to yield *cis*-2-undecenal, but the trans isomer was isolated.

Because of the low flavor threshold values of saturated and unsaturated aldehydes, it is suspected that these compounds contribute to the ultimate flavor of stored chicken.

Supplementary Material Available: Table IV presents a summary of the molecular ions and m/e peaks of interest obtained from the mass spectra of the DNPH derivatives isolated (1 page). Ordering information is given on any current masthead.

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Role of Citric Acid in the After-Cooking Darkening of γ-Irradiated Potato Tubers

Paul Thomas,*[1] Sieghard Adam, and J. Friedrich Diehl

With the aim of exploring the reasons for radiation-induced after-cooking darkening of potato tubers, organic acids from a naturally darkening ("Irmgard") and a nondarkening cultivar ("Hansa") were purified by ion-exchange chromatography and quantified by gas–liquid chromatography of the trimethylsilyl derivatives. Citric, malic, and pyroglutamic acids were the main components, citric acid forming 70–80% of the total acids. Major differences in citric and malic acid content were observed between the darkening and nondarkening cultivars. A significant decrease in citric acid content accompanied by increases in malic and pyroglutamic acids were noted in irradiated tubers during storage. The induction of after-cooking darkening in irradiated potatoes is attributed to decreased citric acid levels and enhanced polyphenols in the tuber tissues, both changes favoring the formation of iron–phenolic complexes responsible for the discoloration.

The bluish-grey discoloration usually referred to as "after-cooking darkening" appearing in certain potato cultivars shortly after cooking is attributed to the interaction of iron with chlorogenic and/or caffeic acids (Juul, 1949; Kiermeir and Rickerl, 1955a). The darkening is generally more intense at the stem end than at the bud end of the tuber. Various agronomic and climatic factors (Baerug and Enge, 1974; Smith et al., 1942), as well as the composition of the tubers, especially the content of iron, orthodiphenols, organic acids, and pH, are known to influence the darkening tendency (Bate-Smith et al., 1958; Hughes and Swain, 1962a,b; Heisler et al., 1963, 1964; Hunter et al., 1957; Kiermeir and Rickerl 1955b; Smith, 1959; Wurster and Smith, 1963, 1965; Vertregt, 1968).

Among the various factors known to affect the after-cooking darkening, the role of citric acid, because of its known ability to chelate iron, has been investigated by many workers. Juul (1949) showed that the discoloration was influenced by citric acid, which he attributed to the pH effect. Mulder (1949) and Bate-Smith et al. (1958) recognized the citric acid action as a chelating effect and stated that the distribution of blackening in individual cooked tubers is governed mainly by the competition between chlorogenic and citric acid for iron. Hughes and Swain (1962a) studied the distribution of the after-cooking darkening within an individual tuber and found correlation of blackening with the ratio of citric to chlorogenic acid. A correlation between citric acid content and after-cooking darkening was reported by Heisler et al. (1963). Based on in vitro experiments on the effect of citric, orthophosphoric, and malic acids on the color of various phenol–iron complexes, Hughes and Swain (1962b) concluded that citric acid was the most important of these factors in reducing the intensity of color of the chlorogenic acid–iron complex.

In an earlier study, it was observed that γ irradiation at sprout-inhibiting dose levels (10 krad) induces after-cooking darkening in several Indian potato cultivars which normally do not show this phenomenon (Thomas and Joshi, 1977; Thomas, 1977). Since irradiated tubers stored at 15 °C showed higher tissue pH (near neutral) in comparison to unirradiated tubers stored at 2 °C, it was postulated that the radiation-induced darkening might be due to a reduction in the organic acid level, especially citric acid.

The studies reported here were undertaken to gain more information on the effect of radiation on the organic acid content of potatoes and its relation to after-cooking darkening.

MATERIALS AND METHODS

Chemicals. Chemicals and reagents were obtained as follows: All organic acids and pyroglutamic acid (PCA) were from Sigma Chemical Co., St. Louis, MO. Dowex-1×8, Dowex-50 W×8, *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), and trimethylchlorosilane (TMCS) were from Serva, Heidelberg, FRG, and pyridine was from Merck, Schuchart, Darmstadt, FRG.

Potatoes. Two potato cultivars, one showing natural after-cooking darkening ("Irmgard") and the other with no darkening ("Hansa") were obtained from Versuchsfeld für Sortenprüfung, Rheinstetten, FRG. Tubers were about

Bundesforschungsanstalt fur Ernahrung (Federal Research Center for Nutrition), Engesserstrasse 20, 7500 Karlsruhe, Federal Republic of Germany.
[1]Present address: Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Bombay-400 085, India.

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