The combination of both processes, however, still further reduced the loss of the carotenoids. Lycopene and β-carotene behaved similarly during storage.

It is evident from Table I that there was no difference in the development of browning nor in the lycopene and β-carotene contents between the two moisture levels tested.

The data obtained in the course of this investigation indicate that a combination of blanching and sulfurization would improve greatly the storage quality of Qamareddeen. At the sulfurization level of 4000 p.p.m. of sulfur dioxide, the taste of sulfur was detectable, when the pulp was consumed without reconstitution. In this respect, the 2000 p.p.m. sulfur dioxide level proved more desirable. On the other hand, drying the pulp to a 10% instead of a 25% moisture content produced slight off-flavor and did not show any superiority in storage quality.

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RADIATION PRESERVATION OF FOODS

Carbonyl Compounds of Irradiated Meats

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In irradiated beef, pork, and chicken, iso-octane–soluble carbonyls come from the lipide and aqueous-soluble carbonyls come from the protein. Irradiation of beef, pork, chicken, and beef liver gives rise to a wide variety of long-chain aldehydes and ketones. They appear to be derived from plasmalogens and other lipides, but the precursor-product relationship appears complex. Carbonyls decrease during storage and cooking.

CARBONYL COMPOUNDS produced by irradiation of meat are important in determining the characteristic odor and flavor of irradiated meat (*1, 8*). The slower rate of gastrointestinal absorption of irradiated lard may be explained by the presence of carbonyls, which delay gastrointestinal absorption and inhibit pancreatic lipase (*10*). The two main groups of precursors for the formation of carbonyls are the lipides and the proteins and amino acids. The mechanism for the production of carbonyls from lipide precursors by aerobic irradiation is well known. Scission products including carbonyl compounds result from lipide peroxidation. This mechanism is based upon that of the autoxidation of lipides, and carbonyls have been shown to result from aerobic irradiation of meat fats and pure lipides (*2, 13*).

In our previous research (*5*), beef and pork irradiated in the presence of oxygen developed relatively high lipide peroxides and iso-octane–soluble carbonyl compounds. In contrast, beef and pork irradiated under anaerobic conditions of pure nitrogen did not produce large amounts. Thus, irradiation in the presence of oxygen initiates free-radical lipide peroxidation, which at the dose level proposed for pasteurization or sterilization gives unacceptable levels of oxidative fat rancidity. This is the main reason why meat should be irradiated under anaerobic conditions. Batzer *et al.* (*1*) likewise studied carbonyl compounds produced during aerobic irradiation of beef and pork fat. They found a greater amount of water-soluble carbonyl produced than lipide-soluble carbonyl. The mechanism by which small amounts of carbonyl are produced when lipides are irradiated under anaerobic conditions has not been defined. Carbonyls may arise from the protein and amino acid portion of meat by several mechanisms (*3, 4*).

This paper reports research defining the relative amount of carbonyl compounds derived from the lipide and protein portions of meat and the composition of iso-octane–soluble carbonyl compounds produced by anaerobic irradiation of beef, pork, chicken, and beef liver.

Experimental

Meat Samples and Irradiation. All the meats used as samples were purchased in large quantities from local sources and finely ground and mixed to prepare representative samples. The various meats and meat components were packed in No. 2 enameled cans, and evacuated and gassed four times with pure nitrogen. Transportation for irradiation was at dry ice temperature. Irradiation was at 20° C. with 0.6- to 2-M.e.v. γ-radiation from spent fuel rods at the Materials Testing Reactor, Arco, Idaho. Subsequently, all samples were held at −20° C. prior to analysis.

For measurement of iso-octane– and acid-soluble carbonyls in the first experiment, beef round, boned pork chops, and the muscles of chicken thighs were used. For separation into the com-

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ponent parts, the meats were first freeze-dried, then extracted twice with 10 volumes of butyl alcohol to 1 volume of meat in a blender. After the butyl alcohol phase had been discarded, the remaining butyl alcohol was removed from the protein portion by washing with 10 volumes of ethyl ether per volume of protein. The protein was dried under vacuum at 40° to 50° C. for 2 to 3 hours. This dried meat protein was then rehydrated to the water content of the original ground meat. The lipide portion of meat was separated by rendering another sample of the meat fat at low temperature. Part of these two fractions, the rehydrated protein fraction and the lipide, were made up to the original portions of the ground meat sample.

Carbonyl Measurement and Other Analysis. Iso-octane (2,2,4-trimethylpentane), benzene, and methanol were all of reagent or C.P. grade. Contaminating carbonyl compounds were removed by refluxing the solvent with 2,4-dinitrophenylhydrazine and hydrochloric acid for 30 minutes and distilling. The 2,4-dinitrophenylhydrazine was crystallized twice from carbonyl-free methanol before use in analytical procedures. The iso-octane–soluble carbonyls were determined using the method of Henick, Benca, and Mitchell (*6*) in the first experiment. The acid-soluble carbonyls were determined by the method of Lappin and Clark (*7*). These carbonyls were extracted using a solvent of 2% metaphosphoric acid in saturated sodium chloride solution. The metaphosphoric acid and saturated sodium chloride were used to decrease the solubility of other tissue constituents.

For measurement and identification of iso-octane–soluble carbonyls in the second experiment the following meats were used: beef round steak, boned pork chops, the meat of chicken thighs, beef liver, and white meat albacore tuna. Dry weight was determined by freeze-drying. Lipide was extracted from the ground freeze-dried meat using the Soxhlet apparatus with 1 to 1 volume of methanol-chloroform for 18 hours. This hot extraction with polar solvent assured good extraction of the plasmalogen phospholipides. Total plasmalogens in the methanol-chloroform extract were determined as carbonyl dinitrophenylhydrazones (*11*, *12*). Iodine numbers were determined by the Wijs method.

Four representative 25-gram samples of meat were each extracted with four 20-ml. aliquots of iso-octane, using a mortar and pestle. The combined iso-octane extracts were centrifuged to remove suspended meat particles. Total carbonyl compounds were determined by reacting a suitable aliquot of this extract with 1 ml. of a saturated solution of 2,4-dinitrophenylhydrazine in benzene and 1 ml. of 4.3% trichloroacetic acid in benzene at 50° C. for 30 minutes in glass-stoppered containers. After cooling, the reaction mixture was diluted to about 18 ml. with benzene, 5 ml. of 4% potassium hydroxide in absolute ethyl alcohol were added, and the volume was adjusted to 25 ml. with benzene. The absorbance at 530 mμ at the time of addition of potassium hydroxide was determined by graphic extrapolation of readings obtained with a Beckman Model DU spectrophotometer. After correction for a reagent blank, these readings were converted to molar equivalents of carbonyl groups, using a molar absorptivity value of 8.80×10^3 (*9*).

The remainder of the iso-octane extract was combined with 1/2 volume of methanol saturated with dinitrophenylhydrazine and a few drops of concentrated hydrochloric acid. The mixture was heated at 50° C. for 30 minutes in a closed container, and then evaporated to dryness under reduced pressure at temperatures not exceeding 50° C. The residue was dissolved in a minimum volume of benzene, and suitable aliquots of this solution were extracted twice with 5% aqueous sodium carbonate to remove interfering acidic materials. Samples thus prepared were subjected to chromatographic separation and subsequent spectrophotometric examination (*9*) for determination of the distribution by carbon chain length of the aldehydes and ketones.

Table I. Components Carbonyls of Beef, Pork, and Chicken Meat

Meat	Radiation, Rad × 10^{-6}	Mmoles/Kg. Unfractionated meat	Recombined protein and lipide	Lipide	Protein
		Iso-octane–Soluble Carbonyls			
Beef, 7% lipide	0	0.49	1.47	4.07	0.0
	1.9	1.33	1.70	11.48	0.0
	7.5	1.97	2.09	14.96	0.0
Pork, 16% lipide.	0	0.81	2.50	5.51	0.03
	1.9	2.71	5.94	8.96	0.04
	7.5	3.06	6.17	18.77	0.08
Chicken, 7% lipide	0	0.23	0.17	1.36	0.02
	1.9	0.31	0.86	4.28	0.02
	7.5	0.96	1.37	10.55	0.06
		Acid-Soluble Carbonyls			
Beef	0	0.9	0.6	0.0	0.2
	1.9	1.3	1.9	0.0	1.8
	7.5	2.1	2.3	0.0	2.1
Pork	0	0.5	0.8	0.02	1.4
	1.9	0.5	1.6	0.02	1.9
	7.5	1.4	2.4	0.02	4.2
Chicken	0	0.5	1.5	0.001	1.3
	1.9	0.7	2.1	0.001	1.4
	7.5	1.3	2.5	0.001	2.0

Table II. Analysis of Meats

Meat	G./100 G. Wet Wt. Dry weight	Lipide	Iodine No. of Lipide	Total Plasmalogens, Mmoles Carbonyl/ Kg. Wet Wt.
Beef	32	11.8	45	0.85
Pork	44	27.5	62	1.12
Chicken	33	9.0	64	0.76
Beef liver	27	8.5	72	...
Tuna	29	2.5	85	0.50

Results and Discussion

Carbonyls from Lipide and Protein. The concentrations of acid-soluble and iso-octane–soluble carbonyls are shown in Table I. Both the iso-octane–soluble and the acid-soluble carbonyl concentrations increased with increasing radiation dosage. The concentration of iso-octane–soluble carbonyls in the unfractionated meats was approximately the same as that of acid-soluble carbonyls.

Meats were fractionated into protein and lipide to determine the amounts and solubility characteristics of carbonyls produced from these two meat fractions. The simplest relation which could exist in meat would be a noninteraction of lipide and protein. If this were the case, carbonyls from the lipide would be derived only from free radical lipides produced by a direct radiation effect. Carbonyls from protein would be produced mainly by its reactions with the primary irradiation products of water, the hydroxyl, and hydrogen radicals. The experimental data show that this interpretation ap-

plies to a limited extent. Irradiated beef had more iso-octane–soluble carbonyls on the basis of its lipide content than did the extracted lipide. This suggests that some of the carbonyls of beef fat are due to the direct reaction of ionizing radiations and some are due to free radicals from the aqueous protein part. Unfractionated beef developed amounts of acid-soluble carbonyls similar to those from plain beef protein and recombined beef protein plus lipide. Unfractionated pork and chicken and the extracted lipide had similar amounts of iso-octane–soluble carbonyls on a lipide basis. Unfractionated pork and chicken had less acid-soluble carbonyls than their protein fractions, showing that other components must interact with the protein in unfractionated meats. Irradiation of recombined beef lipide plus protein and unfractionated beef produced similar amounts of iso-octane– and acid-soluble carbonyls. The agreement between unfractionated pork and chicken and their corresponding recombined lipide and protein was not so good.

The presence of acid-soluble carbonyls in the protein fraction and the corresponding absence of detectable iso-octane –soluble carbonyls suggest that water-soluble carbonyls of low molecular weight are formed from the protein portion during irradiation. The presence of iso-octane–soluble carbonyls in the irradiated lipide fraction and the corresponding absence of detectable quantities of acid-soluble carbonyls suggest that the carbonyls derived from lipide are lipide–soluble carbonyls of higher molecular weight. The selectivity of these two methods of analysis for carbonyls is useful in defining the amount of carbonyls arising from protein and the amount from lipides.

Quantitative and Qualitative Analysis of Carbonyls. Table II gives the analysis of the meats used in the second experimental study. The dry weight, lipide, and iodine numbers characterize these meats as average examples of their class. Plasmalogens are determined on the chloroform-methanol extract. This hot polar solvent assures good extraction of the plasmalogen phospholipides. Plasmalogens react faster than free aldehydes with aldehyde reagents. This analytical method of determining total plasmalogens is based on those of Rapport and Alonzo (*11*) and Wittenberg, Korey, and Swenson (*12*).

The total amounts of iso-octane–soluble carbonyl compounds found in several meats before and after irradiation are cited in Table III. In general, exposure to radiation led to substantial increases in carbonyl compounds. Tuna proved to be an exception, displaying a significant decrease in carbonyl content with increasing radiation dose.

The joint application of chromatographic and spectrophotometric measurements provided a mole-fraction analysis of the carbonyl compounds extracted from the tissues. The mole fractions were converted to micromoles of carbonyl compounds by use of the data of Table III. The quantitative results of these analyses are summarized in Table IV. For convenience in presentation, the compounds detected have been grouped according to carbon chain lengths. Table V summarizes the individual compounds recognized by discrete zones in the chromatographic analyses.

In interpreting the joint chromatographic-spectrophotometric studies for Tables IV and V, it has been assumed that all the compounds studied were *n*-aliphatic monocarbonyl compounds. The presence of an alkene bond in a carbon chain would produce the chromatographic behavior of the corresponding saturated compound with a chain length shorter by one carbon. The presence of the alkene bond would be detected only if its proximity to the carbonyl group induced an alteration

Table III. Total Fat-Soluble Carbonyl Compounds

(Mmoles per kilogram)

Meat	Radiation Dose, Rad × 10^{-6} 0	4.6	9.3
Beef	0.30	0.74	1.01
Pork	0.50	1.34	2.80
Chicken	0.16	0.27	0.39
Beef liver	0.03	0.10	0.19
Tuna	0.12	0.06	0.03

Table IV. Distribution by Carbon Chain Length of Fat-Soluble Carbonyl Compounds of Irradiated Meats

Meat	Dose, Rad × 10^{-6}	Carbonyl Compounds, Mmole/Kg. Fresh Tissue: Above 10 carbons	6–10 carbons	4–6 carbons	Below 4 carbons
Beef	0	0.16	0.05	0.06	0.03
	4.6	0.29	0.24	0.13	0.06
	9.3	0.16	0.18	0.52	0.15
Pork	0	0.17	0.18	0.09	0.07
	4.6	0.57	0.35	0.35	0.08
	9.3	0.70	0.23	1.03	0.85
Chicken	0	0.012	0.072	0.058	0.019
	4.6	0.046	0.084	0.105	0.035
	9.3	0.045	0.074	0.213	0.065
Beef liver	0	0.0018	0.0082	0.0061	0.0140
	4.6	0.011	0.027	0.044	0.018
	9.3	0.042	0.086	0.054	0.010

Table V. Qualitative Nature of Carbonyl Compounds of Irradiated Meats

Meat	Dose, Rad × 10^{-6}	Predominant Compounds	Minor Compounds
Beef	0	Long-chain[a] aldehydes[b]	
	4.6	Long-chain aldehydes and ketones	8–9 C ketones 8–9 C aldehydes 5 C aldehydes 4, 3 C aldehydes
	9.3	Long-chain aldehydes, ketones 4–5 C ketone[c] 5 C aldehyde[c]	3, 4–5, 8–9 C ketones
Pork	0	Long-chain aldehydes, ketones	
	4.6	Long-chain aldehydes, ketones	6, 9 C aldehydes
	9.3	Long-chain aldehydes, ketones 4, 5 C aldehydes 3, 4 C ketones	6, 9 C aldehydes
Chicken	0	6, 9 C aldehydes 3, 4, 6, 7 C ketones	Long-chain aldehydes
	4.6	Long-chain aldehydes, ketones 6 C aldehyde	4, 5 C aldehydes 7, 9 C ketones
	9.3	5 C aldehyde[d] Long-chain aldehydes, ketones	3, 4, 6, 9 C aldehydes 3, 6–7, 9 C ketones
Beef liver	0	3, 7 C aldehydes 3, 7 C ketones	4, 5, 8 C aldehydes 3, 8 C ketones
	4.6	Long-chain aldehydes 7 C ketones	4, 5, 6, 8 C aldehydes 3, 4 C ketones
	9.3	Long-chain aldehydes 8 C aldehyde	4, 5 C aldehydes 4, 7 C ketones

[a] Chain length greater than 10 carbon atoms.
[b] Represented over 50% of total.
[c] 4–5 C aldehyde + ketone represented about 50% of total.
[d] Represented 44% of total.

Table VI. Iso-octane–Soluble Carbonyls of Stored Irradiated Meat

Meat	Dose, Rad × 10^{-6}	Storage Temp., ° F.	Carbonyls, Mmoles/Kg. Initial	1 month	2 months	4 months
Beef	0	−6	0.54	0.78	0.57	0.71
	2.3	−6	2.15	1.1	1.0	1.1
	2.3	69	2.15	1.1	0.92	0.91
	2.3	94	2.15	0.97	0.88	0.74
Pork	0	−6	...	1.1	1.4	1.5
	2.3	−6	2.1	2.1	1.9	1.7
	2.3	69	2.1	1.7	1.3	1.4
	2.3	94	2.1	1.7	1.0	1.1

in the spectrum of the dinitrophenylhydrazone. Spectral shifts typical of such configurations were not detected in the present studies.

For simplicity, ketones were assumed to be of the *n*-alkan-2-one family. This assumption concerning the position of the carbonyl group could lead to errors only when dealing with chains of nine carbon atoms or more—for example, *n*-undecan-6-one would be indistinguishable from *n*-decan-2-one in the present studies.

The data of Tables IV and V indicate the wide variety of carbonyl compounds occurring in irradiated meats. Profound qualitative and quantitative differences occur both between tissues and within a tissue with varying doses of radiation. Unfortunately, tuna extracts contained unidentified materials which interfered with the chromatographic analyses, and the qualitative nature of the effects of irradiation on tuna could not be determined.

The following generalizations are suggested by the present data. Irradiation at 4.6 × 10^6 rad produces predominantly aldehydes and ketones of chain length greater than 10 carbon atoms. The chromatographic analyses clearly indicated that this group of compounds was largely of chain length 14 or greater. Hence, it seems likely that these compounds are derivatives of the unsaturated ether moiety of plasmalogens. Already apparent after doses of 4.6 × 10^6 rad, and becoming predominant at doses of 9.3 × 10^6 rad, are shorter chain aldehydes and ketones which are probably produced by ·OH oxidation of unsaturated fatty acids.

A comparison of the chemical characteristics of the meats (Table II) and carbonyls (Tables III and IV) reveals that no simple correlation exists between the amount of unsaturated fatty acid present in the tissues as determined by iodine number and the amount of shorter chain carbonyl compounds that result from irradiation. Although there is no obvious correlation between the amount of long-chain carbonyl compounds produced by irradiation and the amount of plasmalogen originally present, it appears likely that much of the long-chain aldehydes comes from this source. Plasmalogens constitute the largest source from which long-chain aldehydes could be easily produced by irradiation. Many factors in the meats, physical and/or chemical, profoundly affect the response of lipides to ionizing radiation.

Loss of Carbonyls. In a third experimental study the loss of carbonyls was measured during storage and cooking. Table VI shows the carbonyl content of irradiated beef and pork as a function of storage temperature and time. There was about 50% loss during 4 months' storage at temperatures commonly found in storage of canned goods. A likely mechanism for this storage loss is the active carbonyl-amine condensation reaction. Another possibility is a coupled oxidation or reduction of the carbonyls. In these uncooked meats the reactions could be enzymic. Alcohol dehydrogenase could catalyze reduction of the carbonyls to alcohols and aldehyde dehydrogenase could catalyze oxidation of the carbonyls to acids. Upon cooking of beef which was only exposed to storage at 6° F. there was about 50% loss of carbonyls from both the 2.3 × 10^{-6} rad irradiated and nonirradiated samples.

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