Lipid Autoxidation in Freeze-Dried Meats

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The rapid absorption of oxygen and appearance of off-odors in freeze-dried meats indicate that oxidative deterioration occurs. However, no peroxides were found and changes in spectral absorption of the fat, thiobarbituric acid reactive substances, and reducing capacity of the dried meats were small and could not be correlated with the absorption of oxygen. Of the several objective methods studied, oxygen absorption was the only one that could be used to indicate oxidation. Freeze-dried beef and chicken have been fractionated into their lipid and nonlipid components. The absorption of oxygen by the isolated fractions has been compared to that of the whole tissues. Autoxidation of freeze-dried meats takes place in two stages. The protein-bound lipids autoxidize first, without an induction period, and their initial rate of autoxidation decreases with time. After a period of lower oxygen absorption, the free glyceride fat begins to autoxidize in the autocatalytic manner characteristic of autoxidation in isolated glyceride fats.

Autoxidation of lipid is a serious cause of deterioration in freeze-dried meat. Lea (1943) recognized two types of defects in stored freeze-dried meats: a tallowy or oily flavor due to fat oxidation; and a mealy defect ascribed to deterioration of nonfat components.

In studies with freeze-dried beef, Tappel (1956) and El-Gharbawi and Dugan (1965) found that protein-bound lipids autoxidized readily, whereas the free glyceride fat was very stable. In addition, Tappel (1956) reported that protein could account for 50 to 100% of the total oxygen absorbed by freeze-dried beef. However, Lea *et al.* (1958) observed that the residual protein of defatted herring meal did not oxidize readily.

These observations suggested that valuable information might be obtained by investigating the autoxidation of protein and lipid fractions isolated from the same samples of freeze-dried meats.

Some of the procedures commonly employed to study autoxidation in foods, however, are not applicable to dehydrated products. Insignificant increases in peroxides have been reported in freeze-dried beef (Tappel, 1956) and salmon (Martinez and Labuza, 1968) that had absorbed substantial amounts of oxygen. Although Pazlar *et al.* (1967) have stated that measurements of peroxides are satisfactory to follow the initial stages of autoxidation, and Kopecky (1968) has reported some correlation between peroxide content and thiobarbituric acid (TBA) value in freeze-dried pork, these authors did not investigate absorption of oxygen and the true degree of autoxidation of their samples was unknown.

Steinberg (1959) reported an increase in reducing substances in stored freeze-dried beef. Chapman and McFarlane (1945) also observed the accumulation of reducing substances in milk powders and isolated milk proteins stored in air. The nature of these reducing compounds is unknown but they are generally considered to indicate protein deterioration.

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EXPERIMENTAL

Materials. COMMERCIAL PRODUCTS. Initial studies were performed on samples of freeze-dried meats supplied by the Armed Forces Food & Container Institute [presently Food Laboratory, U.S. Army Natick (Mass.) Laboratories]. These materials had been sealed in cans in an inert atmosphere and stored at room temperature for up to 2 years prior to examination. The history, treatment, and processing conditions of these samples were unknown to us.

LABORATORY SAMPLES. Six-month-old chickens obtained from the Poultry Husbandry Department of the University of Minnesota were killed, dressed, and kept at 3° C overnight. The breast and thigh muscles were freed of bone, skin, connective tissue, and fat deposits. The meat was cooked in an autoclave at 121° C for 12 min and immediately cooled in a refrigerator.

Longissimus dorsi muscle (rib eye) from beef slaughtered 24 hr earlier was freed of adhering fat deposits and connective tissue and chilled thoroughly to 3° C.

The cooked chicken or raw beef tissues were diced into 1 cm cubes and spread evenly on stainless steel screens, supported approximately 1.5 cm above the bottom of metal canisters, 4 cm deep × 9.5 cm diameter (containers for 100-ft spools of 35 mm film) with tightly fitting covers. A thermocouple was inserted at the center of one of the cubes of meat and the samples were frozen at −20° C overnight. The frozen samples in their open canisters were placed on shelves in the 12 in. diameter bulk drying drum of a freeze-drier (Model 10-100, The Virtis Co., Gardiner, N.Y.). The canister covers were suspended above the containers from metal rods that passed through the lucite drum cover through vacuum tight rubber bushings. This permitted closing the canisters at the end of the drying period before the vacuum was released and the drum opened.

The samples were freeze-dried at 0.010 to 0.025 Torr for 2 hr longer than required for the thermocouple to indicate that the meat had reached room temperature (24 to 28 hr). At this point the canisters were covered and the vacuum was released with pure nitrogen.

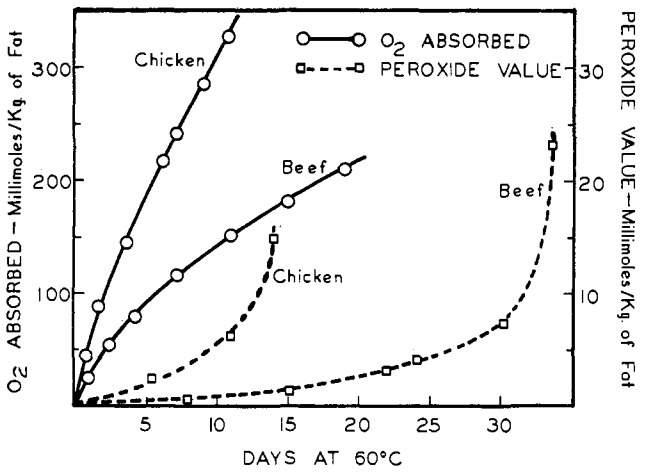

Figure 1. Absorption of oxygen and accumulation of peroxides in freeze-dried beef and chicken

Methods. LIPID EXTRACTION. Each sample of freeze-dried meat was broken into small pieces and thoroughly mixed to give a uniform sample. The material was extracted first with petroleum ether (bp 30–60° C) in a Soxhlet apparatus for 16 hr. The petroleum ether extracted tissue was freed of solvent and further extracted three times with chloroform-methanol (3:1, v/v) in a Waring Blendor at low speed for 2 min, and the three extracts were filtered and combined. Thus, each sample yielded five fractions for study: the original tissue; petroleum ether soluble fat; petroleum ether extracted tissue; chloroform-methanol extract; and the fat-free tissue.

The freeze-dried meats prepared in the laboratory were extracted, and the fractions isolated in a nitrogen atmosphere in a specially constructed cabinet. The cabinet was 2.5 × 4 × 3 ft high and equipped with three pairs of gloved access ports, several observation windows, electrical, air, vacuum, and water services, a small air lock chamber which could be evacuated and which opened both in the room and in the cabinet, and a removable gas tight panel for introduction of larger pieces of equipment.

The cabinet containing the necessary apparatus was alternately filled with carbon dioxide and purified nitrogen through inlets at the bottom and top of the cabinet, respectively, until the atmosphere inside contained not more than 0.1% of oxygen as determined by gas chromatography on 5 Å molecular sieve. Thereafter, the cabinet was kept under a slight positive pressure of nitrogen. The oxygen content of the atmosphere was monitored at intervals during the entire experiment and did not exceed 0.1%.

The tightly covered canisters containing the freeze-dried meat were removed from the nitrogen atmosphere of the freeze-drier and immediately placed in the air lock chamber of the nitrogen cabinet. The chamber was sealed, thoroughly evacuated, and refilled with nitrogen three times, and then the samples were introduced into the cabinet for extraction and fractionation as described.

OXYGEN ABSORPTION MEASUREMENTS. Approximately 1 g of the original tissue, petroleum ether extracted tissue, or fat-free tissue, or an aliquot of the petroleum ether extract or chloroform-methanol extract containing 0.4 to 0.6 g of lipid was introduced into tared 35-ml Warburg flasks, and any solvents present were removed in a stream of nitrogen. In the case of the laboratory freeze-dried meats, this was done in the nitrogen cabinet. The Warburg flasks were quickly weighed, attached to manometers, filled with pure oxygen, and placed in a bath at 60° C, where they were equilibrated for 5 min before absorption of oxygen was measured.

STORAGE TESTS. Approximately 30 g of dried meat was placed in a 250-ml beaker and covered loosely with a piece of filter paper. The samples were stored in air in ovens at 40° C or 60° C and examined from time to time for changes in odor, peroxide content, and malonaldehyde content.

ANALYTICAL DETERMINATIONS. Thiobarbituric acid (TBA) reactive substances were measured on the reconstituted freeze-dried meats using the methods of Turner *et al.* (1954), Sinnhuber and Yu (1958), and Tarladgis *et al.* (1960). In a series of experiments, malonaldehyde was estimated by a direct spectrophotometric procedure described by Kwon and Watts (1963).

Peroxide values and spectral absorption were determined on the fat extracted from 3 to 4 g of dried meat with petroleum ether. Each sample was extracted three times with 25-ml portions of petroleum ether for 2 min in a Waring Blendor in an atmosphere of nitrogen. The combined extracts were filtered, dried over a small amount of anhydrous sodium sulfate, and the solvent removed under vacuum in a rotating evaporator. Peroxide values were determined on a portion of the residual fat by the iodometric procedure of Wheeler (1932). Spectral absorbancies at 233 and 268 nm were measured on solutions of the fats in isooctane using a Beckman DU spectrophotometer.

Soluble reducing substances were measured on aqueous extracts obtained by macerating in a Waring Blendor for 2 min 2 g of freeze-dried meat with 200 ml of a solution of 0.5 *M* KCl and 0.03 *M* $NaHCO_3$, followed by centrifugation and filtration.

Free thiol groups were determined on 50 ml of the extract to which were added 70 ml of 4% HCl and 25 ml of 5% potassium iodide. The solution was then titrated with 0.03 *M* potassium iodate, as described by Okuda (1924).

Total reducing substances were measured in 5 ml of the extract by an adaptation (Steinberg, 1959) of the potassium ferricyanide method of Chapman and McFarlane (1945).

RESULTS AND DISCUSSION

Autoxidation Measurements. PEROXIDE VALUE. When duplicate portions of samples of freeze-dried beef and chicken were examined concurrently at 60° C in a Warburg apparatus or in an oven, it was found that peroxides did not accumulate in the petroleum ether extractable fat, although all the samples absorbed oxygen rapidly. Typical results in Figure 1 show that rates of oxygen absorption are initially high and decrease with time. These oxygen absorption curves, without an induction period, differ markedly from those usually associated with autoxidation of common fats. In contrast, peroxide accumulation is typical of autocatalytic fat autoxidation characterized by an induction period during which little peroxides accumulate, followed by a rapidly increasing rate of peroxide formation.

Similar data are given in Table I for different samples of beef and chicken. In this case, aliquots of each material were allowed to absorb oxygen to predetermined levels, then immediately extracted with petroleum ether and the fat analyzed for peroxides and spectral characteristics. Again no peroxides accumulated in the extracted fat until more than 250 mmoles of O_2 per kg of fat has been absorbed by the samples.

ULTRAVIOLET SPECTRAL ABSORPTION. The increased spectral absorbance of autoxidized lipids at 233 and 268 nm has been taken as an indication of autoxidation (Maloney *et al.*, 1966; Thomas *et al.*, 1968; Wills, 1965) even in absence of

Table I. Autoxidation of Cooked Freeze-Dried Beef and Chicken at 60° C

Time Hr	O_2 Absorbed[a] mmoles/kg fat	Peroxide Value[b] mmoles/kg fat	Absorptivity[b] 233 nm	Absorptivity[b] 268 nm
		Beef		
0	0	1.0	0.869	0.008
139	100	1.5	0.909	0.009
307	150	2.3	0.890	0.009
507	200	2.3	0.927	0.008
~883	250	3.2	0.940	0.008
1316	300	62.6	0.850	0.004
		Chicken		
0	0	0.6	...	...
17	50	2.0	0.540	0.009
41	100	2.8	0.547	0.010
66	150	2.7	0.626	0.010
101	200	2.9	0.567	0.009
137	250	3.0	0.597	0.009
355	300	24.1	0.655	0.008

[a] Oxygen absorbed by whole tissue but expressed in terms of fat contained in the tissue. [b] Measured on fat extracted with petroleum ether.

measurable amounts of peroxides (Privett and Blank, 1962). Table I also shows clearly that no substantial change occurred in absorbance of the fats from freeze-dried beef or chicken that had absorbed up to 300 mmoles of O_2 per kg. Tappel (1956) suggested that initial lipid autoxidation in freeze-dried beef might be restricted to the protein-bound lipids. In our studies, peroxides could not be detected in samples of isolated crude or washed beef-bound lipids that had absorbed up to 80 mmoles of oxygen per kg.

TBA Reactive Substances and Malonaldehyde. The methods of Sinnhuber and Yu (1958), Tarladgis *et al.* (1960), and Turner *et al.* (1954), for the determination of TBA reactive substances gave a pink color absorbing at 530 nm, similar to that obtained with malonaldehyde, but the values consistently lacked reproducibility and did not increase during storage of the freeze-dried meat in air or oxygen at 60° C. Tarladgis (1961) suggested that interference from substances of non-lipid origin might be minimized by performing the analysis under much milder conditions using aqueous thiobarbituric acid at room temperature. Although malonaldehyde bis-(diethylacetal) gave a typical color absorbing at 532 nm with this procedure, only a yellow color absorbing at 453 nm was obtained with freeze-dried meats. Täufel and Zimmermann (1961) observed such a color when they reacted simple saturated and unsaturated aldehydes or several other compounds with TBA under mild conditions, although the pink coloration absorbing at 532 nm was obtained when the reaction was carried out at 100° C.

The direct spectrophotometric determination of malonaldehyde described by Kwon and Watts (1963) also failed to show increasing concentrations of this compound in raw freeze-dried beef stored at 40° C for 4 days, although odor evaluation showed definite deterioration.

Reducing Substances. Total reducing substances measured by potassium ferricyanide increased slightly in samples of the same freeze-dried beef and chicken used to obtain the data in Table I. However, the amounts of reducing compounds in both materials were quite similar, and since chicken absorbed oxygen four to five times more rapidly than beef, the nearly equal amounts of reducing substances in the two products indicate no direct relationship between these compounds and autoxidation.

No thiols were found in these samples and therefore, if the reducing substances indicate protein degradation, it is not accompanied by release of sulfhydryl groups. The reducing

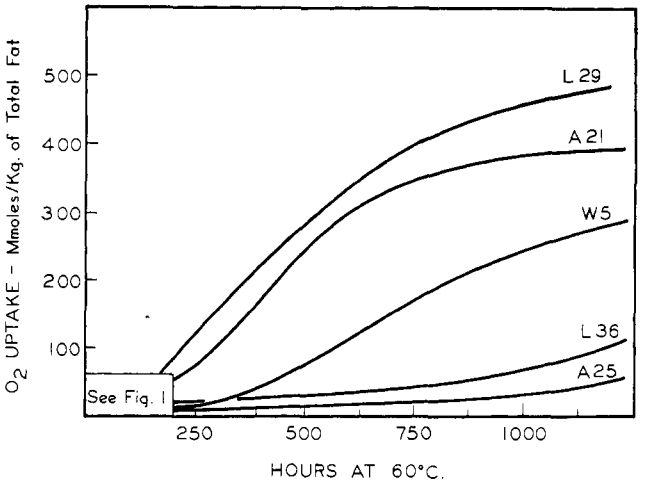

Figure 3. Overall autoxidation of freeze-dried beef

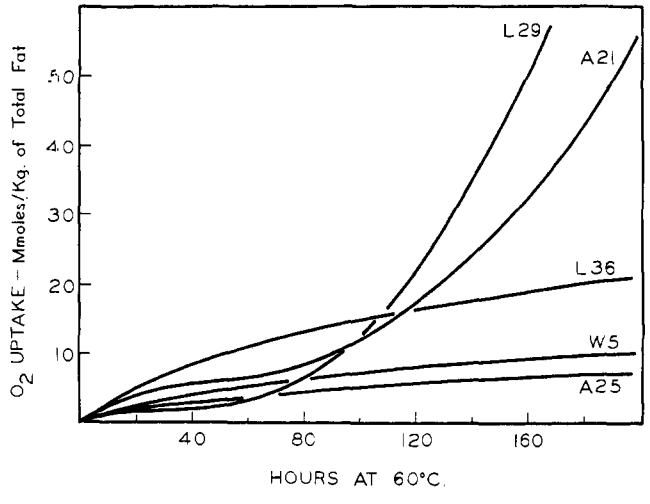

Figure 2. Initial oxygen absorption by freeze-dried beef

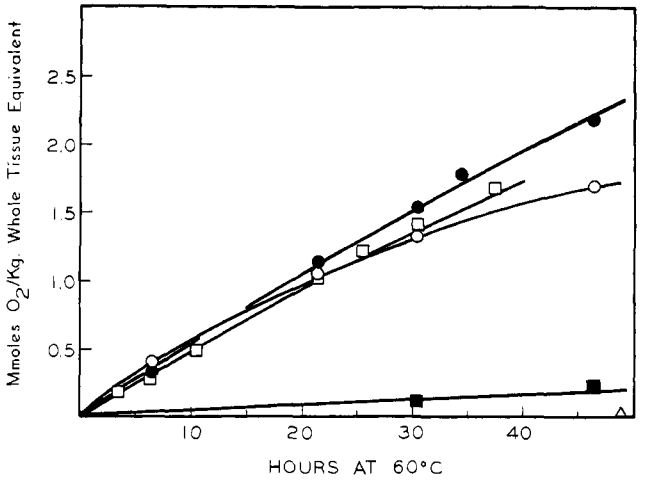

Figure 4. Autoxidation of fractions from commercial freeze-dried beef A25. Whole tissue ○; petroleum ether extracted tissue ●; bound lipids □; protein ■; free fat △

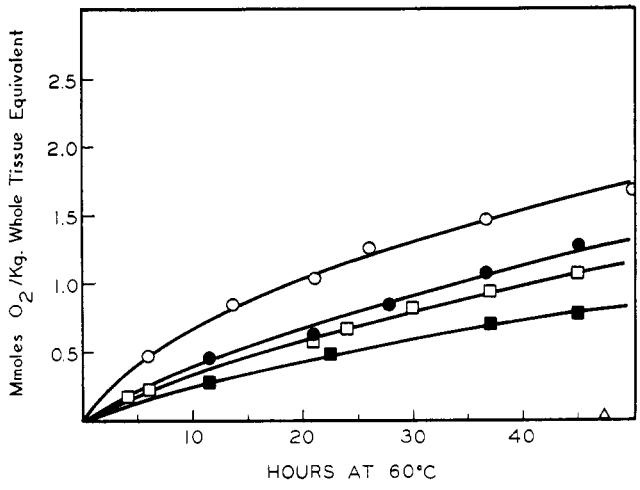

Figure 5. Autoxidation of fractions from commercial freeze-dried beef L36. Whole tissue ○; petroleum ether extracted tissue ●; bound lipids □; protein ■; free fat △

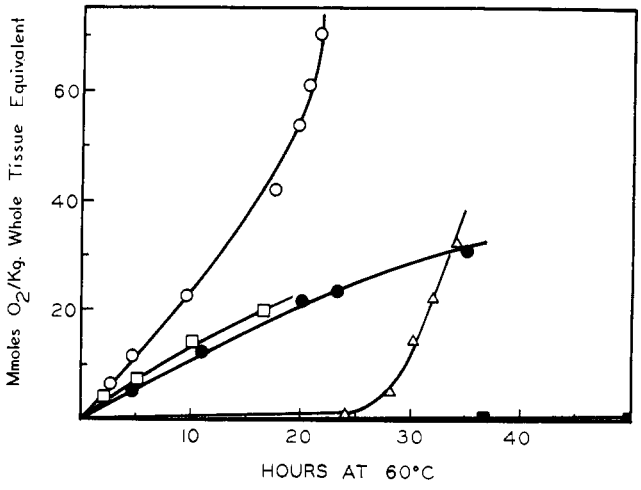

Figure 7. Autoxidation of fractions from freshly prepared freeze-dried chicken. Whole tissue ○; petroleum ether extracted tissue ●; bound lipids □; protein ■; free fat △

compounds could be reductones formed by the carbonyl-amine reactions responsible for nonenzymic browning, although Tappel (1956) concluded that this reaction could not be responsible for the oxygen absorption of freeze-dried beef and Venolia and Tappel (1958) were unable to demonstrate it in an aqueous lipid-protein model system. However, results obtained by Andrews *et al.* (1965) indicated that carbonyls derived from autoxidized linoleate were responsible for protein deterioration in dry methyl linoleate-gelatin and methyl linoleate-insulin systems exposed to air at 50° C. The shape of the protein denaturation-time curve, however, showed a definite induction period (Koch, 1962) and was quite different from the oxygen absorption curves observed in our study (Figure 1).

The failure of peroxides, spectral absorption, TBA reactive substances, and malonaldehyde to increase in samples of freeze-dried meats that had absorbed substantial amounts of oxygen or had been stored in air at elevated temperature until organoleptic odor deterioration was evident, and the lack of correlation between reducing substances and oxygen uptake indicate that these analytical procedures are not suitable to detect and follow autoxidative deterioration in freeze-dried meats. These methods measure accumulated autoxidation products, and the types and amounts of these compounds vary widely with the materials and conditions studied. Absorption of oxygen must be considered a more basic indication of autoxidative deterioration, and this measurement was used in all subsequent studies.

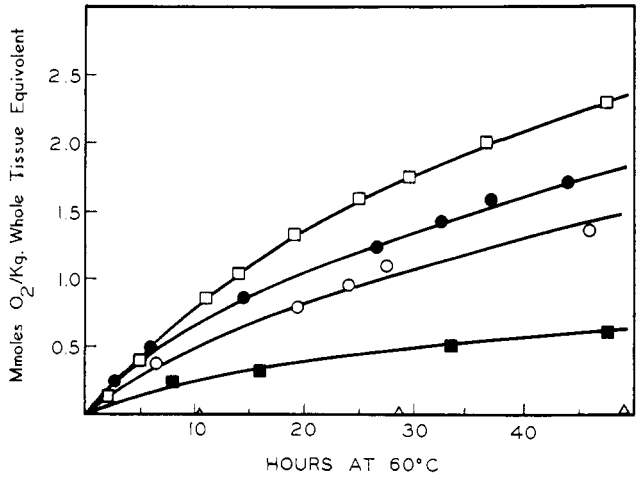

Figure 6. Autoxidation of fractions from freshly prepared freeze-dried beef. Whole tissue ○; petroleum ether extracted tissue ●; bound lipids □; protein ■; free fat △

Autoxidation of Freeze-Dried Meats. AUTOXIDATION OF WHOLE SAMPLES. Figure 2 shows oxygen absorption curves obtained with five representative samples of commercially prepared freeze-dried beef. None of these samples displayed the induction period commonly associated with autoxidation of fats. Instead, oxygen was absorbed immediately by all the samples at a rate which was greatest at the beginning of the experiment and which decreased gradually with time.

Upon further incubation, two of these samples (L29 and A21) showed a second active period of oxygen absorption during which uptake of oxygen increased rapidly, and this portion of the curves resembled typical fat autoxidation. After a prolonged time, the other samples also entered into an accelerated oxygen absorption phase, as is shown in Figure 3. In this figure, samples W5, A21, and L29 show evidence of approaching their maximum level of oxygen absorption. The second phase of oxygen absorption began for samples A25 and L36 after 1000 hr.

The overall autoxidation of these materials, therefore, appears to take place in two stages. An initially rapid but small absorption of oxygen is followed by a period of oxidation at a low rate. At some later time a second phase of oxygen absorption occurs, and the shape of the curve of this stage indicates that it is of an autocatalytic nature, characteristic of the usual autoxidative deterioration of common fats. The intermediate period between these two active phases of oxygen absorption may vary considerably in duration, and the rate of oxygen absorption probably depends largely on the amount and oxidizability of the compounds responsible for the other stages.

AUTOXIDATION OF FRACTIONS. Figures 4, 5, and 6 show oxygen absorption by fractions obtained from three samples of freeze-dried beef. Figure 7 shows similar data for freeze-dried chicken. In these figures, autoxidation is expressed as the amount of oxygen absorbed by the amount of each particular component present in 1 kg of whole tissue. This permits an estimate of the extent to which the individual components contribute to the autoxidation of the whole tissue. Also, it allows the detection of interaction between the various

components and of differences in their rates of autoxidation when they are isolated or in combination.

In all beef samples the triglyceride fat removed from the meat by extraction with petroleum ether was remarkably resistant to autoxidation and did not begin to absorb appreciable amounts of oxygen for at least 700 hr.

The material remaining after extraction with petroleum ether and the isolated bound lipids absorbed oxygen at nearly the same rate. The same was true for corresponding fractions from freeze-dried chicken (Figure 7), although the rates and extents of oxygen absorption in these materials were approximately 20 times greater than for beef. These similarities suggest strongly that the autoxidation of the petroleum ether extracted tissues was due exclusively to the bound lipids they still contained. The isolated fat-free proteins absorbed small amounts of oxygen, also, but in all cases autoxidation of the petroleum ether extracted tissue was considerably less than the sum of the oxygen absorbed by its two components. Apparently autoxidation of one or both of these constituents was repressed when they were combined. Tsen and Tappel (1958, 1960) have shown that sulfhydryl groups are mostly responsible for autoxidation in dry proteins, and these polar groups are most likely to be protected by lipids bound to the protein.

The lipids of chicken autoxidized much more rapidly than those of beef, but chicken protein absorbed oxygen to about the same extent as beef protein, and its contribution to autoxidation of chicken tissue was insignificant. The fact that in chicken as well as in beef the autoxidations of petroleum ether extracted tissue and of bound lipids were nearly equal is a strong indication that autoxidation of bound lipid was not inhibited by the protein.

Autoxidation of whole freeze-dried beef varied from sample to sample. In two cases (Figures 4 and 6) it was less than that of the petroleum ether extracted tissue, whereas in Figure 5 it was considerably higher. These differences may indicate interaction between the various components but they could also be due to the removal or destruction of pro- or antioxidants from the whole tissue during lipid extraction. The shape of the oxidation curves, however, suggests that initial autoxidation of intact freeze-dried beef was probably due largely, if not exclusively, to the bound lipids.

Figure 7 shows that isolated chicken-free fat was much more susceptible to autoxidation than beef fat, and it began absorbing oxygen rapidly after only 25 hr at 60° C. This is probably due, to a large extent, to the much higher content of polyunsaturated fatty acids in chicken fat (Chang and Watts, 1952). In this sample of freeze-dried chicken, the relatively rapid initial autoxidation of the whole tissue, and the increasing rate of oxygen absorption, indicate that the free fat autoxidized concurrently with the bound lipids. The already low stability of the free fat was probably further decreased by the prooxidant effect of the autoxidizing bound lipids.

These studies indicate that autoxidation of freeze-dried meat takes place in two stages. The protein-bound lipids autoxidize first without an induction period, and their initial rate of autoxidation decreases with time. After a period of lower oxygen absorption the free fat begins to autoxidize in the autocatalytic manner characteristic of autoxidation in glyceride fats.

The rate of oxygen absorption during the two active phases of autoxidation and during the intermediate period, as well as the length of the intermediate period, undoubtedly depend basically on the fatty acid composition and physical state of the lipids, and on the presence or absence of pro- and antioxidants. These, in turn, are greatly influenced by the history of the tissue, its nature, source, and treatment beore it is examined.

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