semble those of the AH_2-treated samples of Greene *et al.* (1971). Again, nonheme iron may be the lipid oxidation catalyst in this case. When either $NaCO_3$-treated or dark-cutting samples were cooked or when cooked samples were treated with $NaCO_3$, TBA numbers were as high as in untreated controls.

CONCLUSION

General conclusions of the authors are: (1) heme pigments may be more active catalysts when iron is in the 3+ state; (2) nonheme iron may be a more active catalyst in the 2+ state; and (3) sodium chloride appears to be the major factor responsible for cured meat flavor rather than sodium nitrite or the absence of lipid oxidation.

Clearly, a number of factors must be taken into account when studying the cause and prevention of lipid and pigment oxidations in meat. The answer no doubt lies in a combination of these factors as well as others not presented in this paper. Further studies on the role of nonheme iron and the Fe^{2+}/Fe^{3+} ratio might be profitable. These areas have been studied less extensively and may hold a part of the answer.

LITERATURE CITED

Bailey, M. E., Swain, J. W., *Proc. Meat Ind. Res. Conf., Amer. Meat Inst. Found.*, 29 (1973).
Brown, W. D., Harris, L. S., Olcott, H. S., *Arch. Biochem. Biophys.* **101**, 14 (1963).
Cho, I. C., Bratzler, L. J., *J. Food Sci.* **35**, 668 (1970).
Eriksson, C. E., Olsson, P. A., Svensson, S. G., *Lipids* **5**, 365 (1970).
Eriksson, C. E., Olsson, P. A., Svensson, S. G., *J. Amer. Oil Chem. Soc.* **48**, 442 (1971).
Greene, B. E., Hsin, I., Zipser, M. W., *J. Food Sci.* **36**, 940 (1971).
Haurowitz, F., Schwerin, P., Yenson, M. M., *J. Biol. Chem.* **140**, 353 (1941).
Hirano, Y., Olcott, H. S., *J. Amer. Oil Chem. Soc.* **48**, 523 (1971).
Hustad, G. O., Cerveny, J. G., Trenk, H., Deibel, R. H., Kautter, D. A., Fazio, T., Johnston, R. W., Kolari, O. E., *Appl. Microbiol.* **26**, 22 (1973).
Koizumi, C., Nonaka, J., Brown, W. D., *J. Food Sci.* **38**, 813 (1973).
Liu, H., *J. Food Sci.* **35**, 590 (1970a).
Liu, H., *J. Food Sci.* **35**, 593 (1970b).
Liu, H., Watts, B. M., *J. Food Sci.* **35**, 596 (1970).
MacNeil, J. H., Mast, M. G., *Food Prod. Develop.* 7(2), 36 (1973).
Saleh, B., Watts, B. M., *J. Food Sci.* **33**, 353 (1968).
Sato, K., Hegarty, G. R., *J. Food Sci.* **36**, 1098 (1971).
Siegal, S., "Nonparametric Statistics for the Behavioral Sciences," McGraw-Hill, New York, N. Y., 1956.
Simon, S., Ellis, D. E., MacDonald, B. D., Miller, D. G., Waldman, R. C., Westerberg, D. O., *J. Food Sci.* **38**, 919 (1973).
Smith, G. J., Dunkley, W. L., *Arch. Biochem. Biophys.* **98**, 46 (1962).
Steel, R. G. D., Torrie, J. H., "Principles and Procedures of Statistics," McGraw-Hill, New York, N.Y., 1960.
Stewart, M. R., Hutchins, B. K., Zipser, M. W., Watts, B. M., *J. Food Sci.* **30**, 487 (1965a).
Stewart, M. R., Zipser, M. W., Watts, B. M., *J. Food Sci.* **30**, 464 (1965b).
Tarladgis, B. G., Watts, B. M., Younathan, M. T., Dugan, L., Jr., *J. Amer. Oil Chem. Soc.* **37**, 44 (1960).
Wasserman, A. E., Talley, F., *J. Food Sci.* **37**, 536 (1972).
Watts, B. M., *Advan. Food Res.* **5**, 1 (1954).
Watts, B. M., Kendrick, J., Zipser, M. W., Hutchins, B., Saleh, B., *J. Food Sci.* **31**, 855 (1966).
Younathan, M. T., Watts, B. M., *Food Res.* **24**, 728 (1959).
Younathan, M. T., Watts, B. M., *Food Res.* **25**, 538 (1960).
Zipser, M. W., Watts, B. M., *Food Technol.* **16**, 102 (1962).

Received for review June 20, 1974. Accepted December 5, 1974. Presented at the Symposium on Effects of Oxidized Lipids on Food Proteins and Flavor, Division of Agricultural and Food Chemistry, 167th National Meeting of the American Chemical Society, Los Angeles, Calif., April 1974.

Effect of Lipid Antioxidants on the Stability of Meat during Storage

Robert C. Benedict,* Elizabeth D. Strange, and Clifton E. Swift

Deteriorative changes in meat may occur from heme and lipid oxidations, producing alterations in color, flavor, and odor. Samples of ground beef with either low levels (*ca.* 3%) or high levels (*ca.* 10%) of polyunsaturation in the added fat were examined for storage produced changes. High polyunsaturation levels increased meat deterioration. The antioxidant effectiveness of five additives (0.005% level) derived from natural sources (α-tocopherol, ascorbic acid, 1-ascorbyl stearate, citric acid, and ascorbic acid with sodium bicarbonate) was examined during 10 days storage. Samples were adjudged to be commercially unacceptable after 1–4 days storage but monitoring was continued to determine differences in the additive's antioxidant action. Ascorbic acid exerted a definite prooxidant action. The other additives showed only a slight effect in decreasing the rate of lipid and heme oxidations compared to untreated samples. A hypothesis of coupled heme–lipid oxidation is presented.

Generally, the oxidation of lipids in foods is undesired as it may lead to alterations in flavor, odor, and color. The reactivity of fats and other lipids with oxidants within a biological system can vary markedly from the reactivity of extracted and purified lipids. Such factors as the degree of lipid unsaturation, the content of prooxidants and antioxidants, and the cellular and tissue structure all contribute to these differences in oxidation rate (Ledward and MacFarlane, 1971; Love and Pearson, 1971; Kwoh, 1971; Sato and Herring, 1973). With fresh meats, deteriorative changes resulting from oxidation of lipids have not been a major problem to date. Most spoilage in fresh meat occurs as a result of bacterial action, producing an acid odor and flavor and a brown color. The aerobic bacteria on the meat surface and the endogenous reductants within the tissue act to diminish the oxygen available for tissue lipid oxidations (DeVore and Solberg, 1974). In addition, beef fat is not highly polyunsaturated, with beef adipose tissue lipid containing only about 2.5% polyunsaturates (Swern, 1964). In the more unsaturated pork fat (10–12% polyunsaturates, Swern, 1964), the lipid oxidation is limited somewhat by the lower levels of the prooxidant heme pigments, myoglobin and hemoglobin. Fresh pork, however, does have a limited shelf and storage life, even in frozen storage, because of the development of oxidative rancidity and off-flavors.

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118.

Recent developments in meat production, processing, and marketing have altered some of these protective factors; such methods may alter meat and lipid stability. With centralized packaging of meat, the desired reduction in bacterial growth and extension in storage time will increase the tendency for lipid oxidations, as has been observed previously in pasteurized milk. In addition, the development of new hybrid meat-vegetable food products or commercial production of beef with an increased content of polyunsaturated fat (Cook *et al.*, 1970; Dinius *et al.*, 1974) may introduce new problems in food stability from oxidative rancidity. A knowledge of potential stability problems along with possible methods for their control is essential if such products are to be produced.

Color is a prime judgment quality with meat products. The bright red color from oxymyoglobin on the fresh meat surface normally indicates that, at most, limited spoilage has occurred. The alteration from the bright red color to the brown color of metmyoglobin was believed to be produced by an oxidation of the heme iron to the ferric state (Fox, 1966). Although heme oxidations normally result from bacterial action, other causes are possible. Factors such as hydrolysis of meat lipids by added lipases (Govendarajan *et al.*, 1973), presence of prooxidant metal ions (Swern, 1964), and peroxidation of tissue lipids (Greene, 1969) have been implicated in heme oxidations. Whether the lipid peroxides catalyze oxidation of the heme, or whether oxidation of the heme precedes and causes lipid oxidation, or even if cooxidation of the pigments and lipids occurs has not been established with certainty (Greene *et al.*, 1971). There is a natural reducing capacity in fresh meat from endogenous reductants such as nicotinamide adenine dinucleotide reduced (NADH), intermediates in electron transport, and various enzymes and substrates which act to decrease metmyoglobin formation (Saleh and Watts, 1968). As the meat tissue ages, there is a decline in this reducing capacity from the inactivation of these reductants and/or an increase in deteriorative factors. Fresh meat lipids do not normally contain as high a level of natural antioxidants as vegetable oils (Swern, 1964). As a result, animal lipids show less stability to oxidation than vegetable lipids with the same degree of unsaturation. The addition of phenolic antioxidants, such as BHA or PG, has been found to be quite effective in preventing both lipid oxidations in meat fat and metmyoglobin formation (Greene, 1969).

Grinding has several adverse effects on normal meat. In addition to increasing the surface area exposed to air and to bacterial contamination, grinding also increases the loss of intracellular reductants, such as NADH (Ledward and MacFarlane, 1971), and increases the association of the meat proteins with the intracellular enzymes and lipids (Love and Pearson, 1971). The intracellular lipids contain a higher percentage of phospholipids and polyunsaturated fatty acids than depot fat and are more susceptible to oxidation (Govendarajan *et al.*, 1973). With a ground meat product under nonoptimum storage and temperature conditions, very rapid changes in meat color are possible. At a temperature of 25° with a freshly prepared, high quality ground meat sample, the color changed from bright red to brownish red within 15 min and to a complete brown within 120 min. Such behavior could not be attributed to bacterial action (Strange *et al.*, 1974b). Similarly, the TBA (thiobarbituric acid) values of ground meat samples frequently are two-three times the values of unground meat samples under identical conditions, reflecting an increased lipid oxidation (Strange *et al.*, 1974b).

Since consumer acceptability of fresh meat products requires a satisfactory appearance for purchase and for use, the biochemical bases for such deteriorative changes and their control are of importance. In these experiments we have examined the effect produced by differences in fat composition on the color and the stability of meat samples. We used both the relatively saturated fat of beef adipose tissue and the relatively unsaturated fat of pork adipose tissue for the two types of animal fat, and we ground these samples both to produce a more homogeneous sample and to increase the possibility of color deterioration and lipid oxidation. In each experiment, all samples were prepared to the same percentage of fat and heme pigment content with the same lean meat, and were handled under identical storage conditions. A temperature of −1.1° was maintained to decrease interference by bacterial action on the effect of the fat composition. In addition, five antioxidant additives were examined for their effect in preventing deterioration. These additives included two lipid-soluble antioxidants (α-tocopherol and 1-ascorbyl stearate), two water-soluble antioxidants (ascorbic acid and ascorbic acid–sodium bicarbonate), and citric acid. Citric acid is not considered to be a true antioxidant, but is believed to enhance natural antioxidant activity by metal ion deactivation (Swern, 1964).

EXPERIMENTAL SECTION

Materials. Lean beef (top round) was trimmed free of intramuscular fat and ground two times through a 0.25-in. plate. The beef fat and pork fat (both adipose tissue trimmings) were ground separately two times through a 0.25-in. plate. Ground meat mixtures of 25% final fat content were prepared by addition of the ground lean beef to either the ground beef fat or pork fat with thorough mixing. The antioxidants to be tested were dissolved in either 95% ethanol or distilled water (1% w/v) and were added to the ground meat at a concentration of 0.005% by weight, with the exception of the ascorbic acid–sodium bicarbonate mixture, where each was added separately at a concentration of 0.0025%. Appropriate amounts of the two solvents were added to all samples including controls. The concentration of 0.005% antioxidant was chosen to examine the beneficial action of these low levels in supplementing the natural antioxidant activity in the animal fats. When calculated in reference to the total fat, such levels are approximately 0.018%. The ground meat was divided into packets of known weight, wrapped in commercial PVC stretch film (MC-FMC Corporation), and stored at −1.1° in a commercial meat display cooler until assayed.

The antioxidants selected for use as possible additives in meat products were those which occur naturally. Ascorbic acid, sodium bicarbonate, α-tocopherol, and citric acid (all reagent grade quality) were purchased from commercial sources. L-Ascorbyl stearate (LAS) was prepared by the method of Swern *et al.* (1943) and was recrystallized two times before use. Although other ascorbyl esters such as ascorbyl 2-sulfate have been isolated from natural sources, LAS is not known to occur naturally. It is synthesized from and is metabolized to l-ascorbic and stearic acids. Sodium bicarbonate and ascorbic acid were added separately since we had determined that application of the ascorbic acid–sodium bicarbonate mixture to unground meat either to the surface or within a packet within the meat package increased the storage life of beef by the production of a bacteriostatic carbon dioxide gas (Benedict *et al.*, 1974).

Methods. The color of the ground meat samples was monitored daily by the method of Strange *et al.* (1974a). The difference in per cent reflectance of the meat samples at 630 and 580 nm decreases linearly with the increase in metmyoglobin formation. A Beckman DB-G recording spectrophotometer equipped with reflectance attachment was employed with a magnesium carbonate reflectance standard also wrapped with the plastic meat wrap.

The pH of the ground meat was determined with a combination pH electrode probe. The pH of an aqueous meat filtrate (10 g of ground meat blended with 40 g of distilled water for 2 min at high speed in a laboratory

blendor) was also measured. The redox potential of both the ground meat and the aqueous filtrate was determined by use of a platinum electrode and silver|silver chloride half-cell. Redox measurements were standardized against a ferrous–ferric solution of known potential (Light, 1972). In addition, the employment of resazurin, a redox indicator dye, as a colorimetric test of redox changes was also examined. One milliliter of standard resazurin solution was added to 2.0 ml of the above described meat filtrate. The color of the solutions was noted at 2-hr intervals. A 10-ml aliquot of the meat filtrate was freeze-dried and stored at −20° for subsequent chromatographic analysis.

Lipids of the ground meat were extracted by triturating with 5 vol of chloroform–methanol (2:1) three times and combining. Excess solvent was removed under reduced pressure and samples were stored under nitrogen in chloroform–methanol solvent at −20° until examination. Lipids were examined for fatty acid content as methyl esters on glc by the method of Herb *et al.* (1960). Peroxide values on the extracted lipids were determined by the AOAC method (AOAC, 1970).

A protein free extract was prepared from the ground meat sample by filtration following a 10% trichloroacetic acid precipitation. This filtrate was used for determination of the thiobarbituric acid value by the method of Witte *et al.* (1970) and for determination of the free tyrosine value by the method of Pearson (1968).

Bacteria counts of the meat samples were monitored by shaking and dilution and plating on standard nutrient agar. Plates were incubated at 20° for 48 hr. Colonies were selected and examined by staining and microscopic techniques. Bacterial counts were expressed as logarithms of colonies per gram of meat sample and were determined in triplicate.

Chromatographic separation of the meat filtrate components was conducted on Sephadex G-75 and G-15 gels; elution patterns were monitored by absorption at 245 nm which measured most eluted solutes. Organoleptic analysis was conducted on both uncooked (odor only) and cooked samples (flavor and odor) of the ground meat. Samples were cooked individually for 45 sec in a microwave oven.

RESULTS AND DISCUSSION

Although there is no accepted procedure for measuring the rate of color change in ground meat products, values obtained by reflectance spectrophotometry of the meat products can provide an indication of the myoglobin changes resulting from heme oxidation at the meat surface, and can be followed with time as a measure of color deterioration (Strange *et al.*, 1974a). As the heme iron becomes oxidized, the per cent reflectance (R) at 630 nm contributed by the oxymyoglobin form decreases, and the per cent reflectance at 580 nm contributed by the metmyoglobin increases. With increasing storage of the meat, the difference ($\Delta\%R$ = $\%R$ at 630 nm − $\%R$ at 580 nm) decreases and approaches zero, and even negative values. With continued storage and increased bacterial growth, a reduction of the metmyoglobin form may occur because of exogenous bacterial reductants. Such meat, however, would not be offered for sale. With fresh ground beef, a $\Delta\%R$ value greater than 25 indicates a desirable red color.

Figure 1 indicates the behavior under optimum storage conditions of −1.1° of a normal beef sample ground with beef fat (A) and normal beef ground with pork fat (B). Curve A represents the normal commercial ground beef, and it maintained the desired red color ($\Delta\%R$ greater than 25) for approximately 4 days. Replacement of the beef fat with pork fat led to a more rapid loss in desired color, such that the desired red color was gone in 3 days. Continued storage resulted in a continued heme pigment alteration. Some measure of this alteration can be calculated by noting the time at which the $\Delta\%R$ crosses the lines equivalent to values of 20 or 10. On this figure the times are 5.5 and 7.8 days for the beef with the added beef fat (A) and 3.5 and 5.5 days for the beef with the added pork fat (B). These pairs of values are both below the generally accepted $\Delta\%R$ of 25 for desirable color, but provide a measure of the effectiveness of additives in retarding or accelerating further heme oxidations and color changes.

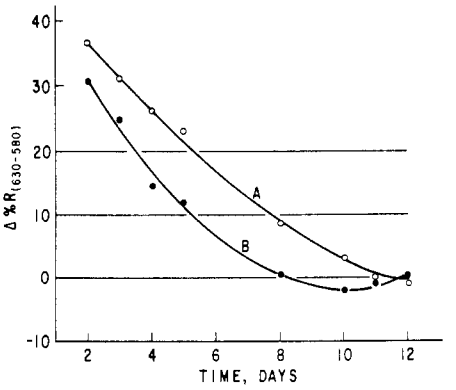

Figure 1. Decrease with time on the oxymyoglobin content of samples of ground beef with different fat added as determined by reflectance spectra difference values at 630 and 580 nm. Samples were stored under optimum commercial conditions at −1.1°. Fat levels were prepared to 25%: (A) (O) ground lean beef with beef fat; (B) (●) ground lean beef with pork fat.

Table I. Mean Time (Days) for Color Reflectance Difference Values of Meat Samples to Reflect Increase Due to Metmyoglobin Formation[a]

	Control	Tocoph.	LAS	Ascorb.	Asc-Bic	Citric
$\Delta\%R = 20$						
Beef fat	4.7	3.5	*4.85*	3.4	2.1	*5.0*
Pork fat	1.75	*2.0*	1.5	0	2.0	*2.15*
$\Delta\%R = 10$						
Beef fat	8.0	6.7	*8.0*	*8.3*	5.0	*8.0*
Pork fat	5.0	*5.5*	4.9	3.25	4.9	*5.7*

[a] Color reflectance difference value ($\Delta\%R$) is the difference in per cent reflectance values of ground meat samples at 580 and 630 nm. Control meat is untreated. Additives at 0.005% concentration are α-tocopherol (Tocoph.), 1-ascorbyl stearate (LAS), ascorbic acid (Ascorb.), ascorbic acid and sodium bicarbonate (Asc-Bic), and citric acid. Samples which had times equal to or exceeding that of the control are in italics.

The rate of metmyoglobin formation under optimum storage conditions shown in Figure 1 could not be demonstrated with all samples. In most experiments the samples containing added pork fat developed metmyoglobin sufficiently by the first spectral assay at 24 hr that the $\Delta\%R$ values were below the acceptable color values of 30 or 25. For comparison of the effectiveness of the additives under the different experiments, data from each sample were plotted separately and were compared for differences occurring from the type of fat or additive. The graphs of $\Delta\%R$ were interpolated to determine the time required in days for the $\Delta\%R$ values to decrease to 20 and 10. The mean time in days required based on the average of three complete runs is shown in Table I and is used as an indication only of the effect on surface color, as these samples were already unacceptable from a consumer standpoint. Two distinctions are apparent. First, the addition of pork fat produced a much more rapid decrease in red color or a more rapid heme oxidation, as reflected by the lower times. Second, the additives were not effective at the concentrations used in preventing the oxidation of the heme iron. The two water-soluble additives containing ascorbic acid promoted metmyoglobin formation with respect to

Table II. Representative Data from Sample of Ground Beef with 25% Added Beef Fat during Storage

Day of storage	Bacteria count (log value)	Filtrate pH	Redox potential, mV	Tyrosine value, mg/g of meat
2	6.00	5.43	+205	0.33
3	6.00	5.60	+253	0.49
5	5.84	5.85	+323	0.50
8	7.08	5.72	+375	0.50
10	7.15	5.65	+203	0.54

the untreated control samples. Of the lipid-soluble additives, α-tocopherol showed a value slightly above control times with the pork fat samples, and 1-ascorbyl stearate appeared about the same as the control samples with both fats. The citric acid additive equaled or surpassed control times with all samples.

Measurement of the bacterial growth of the samples during 10 days storage indicated that no significant differences existed between the untreated and treated samples with the same type of fat. The beef fat samples had an initial mean log value of 5.97 colonies/g, and a mean log value of 8.07 colonies/g after 10 days storage. The beef fat samples which had been treated with additives had mean log values within 0.5 log unit of this value, whereas differences of at least 1 log value would be necessary for significance. The pork fat samples had an initial mean log value of 5.10 colonies/g, and a mean log value of 6.56 colonies/g after 10 days storage. With the exception of the sample treated with ascorbic acid, which had a mean log value of 7.70, the samples containing pork fat and the additives had mean log values within 0.5 log unit of that for the untreated pork fat control. The samples which contained pork fat rather than beef fat had lower bacterial counts in all experiments. Either the initial bacterial loads of the two fats differed or the bacterial growth was hindered. We could not determine with accuracy the initial bacterial loads contributed by the fat alone. Products of high fat content, such as adipose tissue, are difficult to analyze for bacteria by standard methods because of their insolubility in aqueous diluents. Pork fat samples showed a slightly slower increase in counts during storage. Analysis of the bacterial colonies indicated mainly catalase positive, Gram negative bacilli, mainly varieties of *Pseudomonas*, a common meat spoilage organism. The increased metmyoglobin formation reflected in the $\Delta\%R$ values by the pork fat samples definitely could not be attributed to an increased bacterial growth.

The measurements of the aqueous filtrate from the ground meat samples showed variations in values within the same sample with time of storage. Data from one experiment with the untreated beef fat sample are shown in Table II. Both the bacterial content and the amount of free tyrosine in the sample showed increases with the time of storage. This might be expected if the tyrosine is released as a result of bacterial proteolysis (Pearson, 1968). The values for the pH and electrometric redox potential also showed increases to a point in these data, but this trend was not universal in all experiments. Comparison of these data with those from the pork fat samples or the additive samples indicated that the pH and other measurements on the aqueous extract appeared to be affected slightly if at all by the lipid oxidation, but were related more to the bacterial growth. Such behavior might be expected since many of the products resulting from lipid oxidations would be hydrophobic in nature. Malonaldehyde is an exception, being released into the aqueous phase following the splitting of linolenate, and being measured by the TBA test. Metabolites resulting from bacterial activity are generally water soluble and would affect the pH

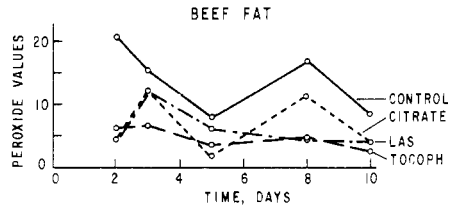

Figure 2. Peroxide values (milliequivalents of peroxide/kilogram of lipids) of lipids extracted from lean beef ground with beef fat (fat content of 25%) untreated or with 0.005% concentration of antioxidant after storage at −1.1°: (O—O) control untreated; (O---O) citrate; (O---O) LAS (1-ascorbyl stearate); (O--O) tocoph (α-tocopherol).

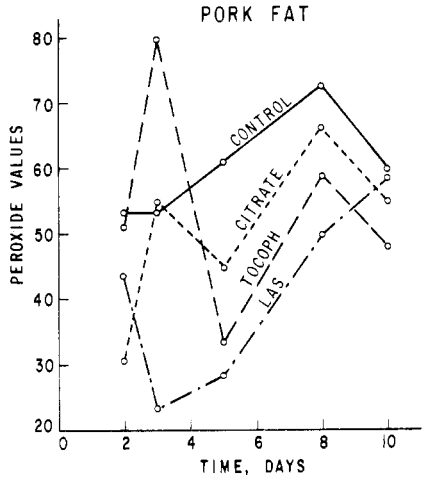

Figure 3. Peroxide values for lipids extracted from lean beef ground with pork fat. For explanation of terms, see legend for Figure 2.

and redox potentials more readily. The decreases noted after 8 and 10 days of storage may be a reflection of alteration of bacterial metabolism.

Resazurin is a redox indicator dye which has been employed for colorimetric determinations of bacterial contamination in meat. High bacterial populations compete for available oxygen and produce a reducing environment, which leads to a decolorization of added Resazurin when the redox potential reaches a value of −80 mV. The time required for the decolorization of the Resazurin solution is generally inverse to the bacterial load, with populations greater than 1×10^9 colonies/g producing a decolorization in 1–2 hr. None of the meat filtrates decolorized Resazurin unless they were incubated for 12 hr at 25°. The individual redox potential values and pH values represent contributions from several different factors and cannot generally be employed alone for indication of bacterial growth (Watts *et al.*, 1966). Such values might be employed for monitoring of quality in conjunction with other data (Strange *et al.*, 1974b).

Analysis of the fatty acid composition of the extracted fat from the control ground meats indicated that the major differences between the fat samples were in the content of polyunsaturates. The beef fat showed values of 47.4% for content of saturated fatty acids (C_{10}–C_{22}), 50.7% monoenoic (C_{15}–C_{20}), and 1.8% polyenoic. The pork fat sample showed values of 40.9% saturated, 49.1% monoenoic, and 10.0% polyenoic. No major alterations in the fatty acid compositions were noted during the storage period.

Oxidation of lipids leads generally to increases in the peroxide values and the TBA values. Peroxide values of the beef fat samples were (Figure 2) much lower than those of the pork fat samples (Figure 3), as might be expected from the differences in unsaturation. A comparison of the effect of the additives, citric acid, α-tocopherol, and

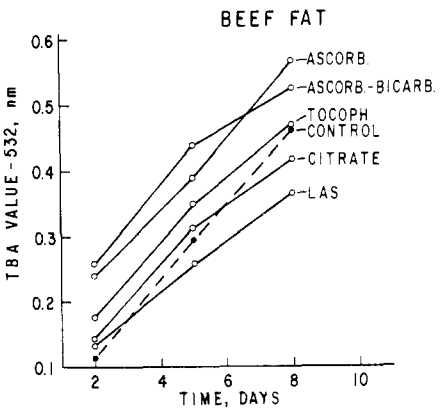

Figure 4. Thiobarbituric acid (TBA) values expressed as optical density values at 532 nm, for trichloroacetic acid extract from lean beef ground with beef fat after storage at −1.1° untreated or with additives: (●--●) untreated control; (ascorb) 0.005% ascorbic acid; (ascorb-bicarb) 0.0025% ascorbic acid and 0.0025% sodium bicarbonate added separately; (tocoph) α-tocopherol; (citrate) citric acid; (LAS) 1-ascorbyl stearate.

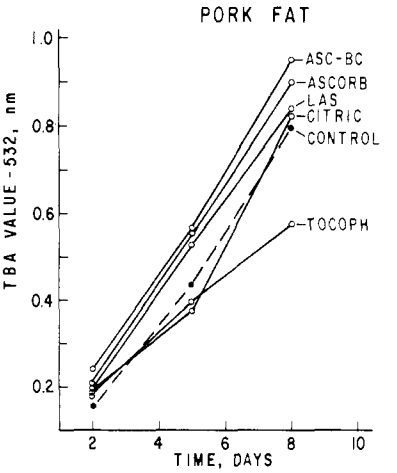

Figure 5. Thiobarbituric acid (TBA) values for lean beef ground with pork fat. See legend to Figure 4 for explanation of terms.

1-ascorbyl stearate, on the peroxide values of beef fat when added to the meat samples showed consistently lower mean peroxide values (Figure 2). The data from the experiments in which ascorbic acid or ascorbic acid with sodium bicarbonate were added are not shown for purposes of clarity in the figure, but reflected consistently higher values than the untreated sample. Somewhat similar results were noted with the pork fat samples (Figure 3). Here also the samples with ascorbic acid additives showed higher peroxide values than untreated samples, but are omitted for clarity. The graphs of peroxide values may differ from those normally observed with pure fats during oxidation, but could reflect both the formation of peroxides and their destruction. Such behavior has been reported by Tappel *et al.* (1961) who noted that *in situ* lipid was more labile to peroxidation than lipid which was not in contact with tissue constituents. Berner *et al.* (1974) found that increased peroxide values in a lipid–hemin emulsion led to a decreased induction period for lipid oxidation, whereas active antioxidants produced an increased induction period. The untreated control samples showed much higher peroxide values at the second day than the samples to which antioxidants had been added. Zero day values were not determined. The destruction of peroxides may occur in a biological system enzymatically from bacterial and tissue catalases or pseudoenzymatically from other metalloproteins.

Whereas the peroxide value indicates the potential for possible lipid oxidations, the TBA test has been employed as a measurement of oxidative rancidity development. The two values may show independence based upon the lipid composition (Dahle *et al.*, 1962). In these experiments, the lower peroxide values produced in the samples by certain additives did not relate to the TBA values observed (Figures 4 and 5). Again, the ascorbic acid additives produced values greater than those in the untreated samples with both types of fat. The other additives did not produce any marked differences from the values of the untreated controls. The rate of development of TBA reactive material is more rapid with the pork fat samples, as would be expected from the higher level of polyunsaturation present. These samples were below commercial quality after 1 or 2 days, as judged by color, and the development of flavor defects from oxidative rancidity followed the color deterioration closely. Storage stability when applied to meat products implies the maintenance of desirable color and appearance and the lack of development of off-flavors and odors during the storage period. This period includes the display and purchase as well as the subsequent home storage. For the purposes of these experiments, the organoleptic evaluation was limited to those factors that the average consumer would consider in their evaluation of acceptability. Color and odor can be determined on the uncooked sample by the consumer and consequently contribute more to the overall consumer evaluation than does flavor which requires a cooked sample. This overall evaluation differs therefore from panel organoleptic analysis where individual factors are considered. The main flavor defect noted was oxidative rancidity. A TBA value of 0.20 in these samples appears to be related to the threshold level of oxidative rancidity, which was evident as a metallic or oily flavor. All samples were judged as definitely rancid by the fifth day of storage. The ascorbic acid treated samples, which were found to produce higher peroxide values and TBA values than the untreated or other samples, also developed an overt oxidative rancidity before the other samples. The ascorbic acid–sodium bicarbonate treated pork fat sample was definitely rancid by the second day of storage, followed closely by the beef fat sample so treated, and subsequently by the ascorbic acid treated pork fat and the other pork fat samples. Other workers have reported a relationship of TBA values to the development of off-flavors (Sato and Herring, 1973). In these experiments with ground meat, the TBA values also bore a relationship to the metmyoglobin formation. The mean time for the sample to reach the $\Delta\%R$ value of 20 corresponded closely to the time at which the TBA value reached 0.28. At this time, the samples were unacceptable for color as well as flavor. This relationship may not hold for unground meat samples.

Although distinct changes in the heme of the myoglobin were produced during storage by the effect of oxidation, there did not appear to be changes in the protein of the myoglobin or corresponding changes in the other proteins as revealed by gel filtration. Such changes might be expected, based on the findings of Taborsky (1973) with model systems, who noted protein oxidations in the presence of ferrous iron and oxygen, both of which are present in these samples. However, the oxidative modifications in the proteins in Taborsky's experiments were dependent upon the composition of the media and required sensitive instrumentation for detection. Gel filtration of the soluble sarcoplasmic proteins on Sephadex gels showed similar elution patterns at the beginning and end of 10 days storage. Myoglobin showed a difference in color and a slight decrease in quantity, approximately 5%, and differences were also found in the quantity of a low molecular weight (under 1000) nitrogenous material. This substance eluted last and had spectral characteristics similar to those of inosinic acid. These data agree with the findings of Petropa-

kis (1971) and Rampton *et al.* (1965) who found no alteration of soluble proteins content but an increase in the content of low molecular weight nonprotein nitrogenous compounds during aging of meat.

Oxidation of the thiol groups on the proteins to disulfides or other sulfur compounds might be expected from the increased content of peroxides (Little and O'Brien, 1968). However, attempts to measure the free sulfhydryl content of the soluble proteins with Ellman's reagent [5,5′-dithiobis(2-nitrobenzoic acid)] met with negative results because of large interference at the assay wavelength (412 nm) by the Soret band of the hemoproteins. The stromal proteins and the myofibrillar proteins showed essentially the same solubility characteristics after storage as at the beginning.

Addition of the natural additives in the concentrations employed did not extend the storage stability of the ground meat product sufficiently to make a significant improvement, particularly when the more polyunsaturated pork fat was used. The subsequent behavior of the samples following initiation of lipid oxidation gives some indication of a possible secondary method in biological systems for lipoxidative changes.

Alternative Mechanism of Coupled Heme–Lipid Oxidation. The literature on meat lipid oxidations frequently contains examples of results that could not be explained on the basis of the accepted theory of free-radical initiation and propagation for lipid oxidation. These mechanisms are believed to be responsible, at least in part, for the lipid oxidations in cooked meats which result in the familiar "warmed over flavor" (Sato *et al.*, 1973; Sato and Herring, 1973). However, in uncooked meat, alternative mechanisms which involve enzymatic reactions are possible. Coupled reactions are common in biological enzymatic reactions, necessitating a strict configuration on the enzyme and substrates. A coupled reaction with the heme protein–lipid oxidation would necessitate an aqueous–lipid interface. Such an interface requirement was reported by Banks (1944) and more recently by Haurowitz *et al.* (1973) for lipid oxidations by heme compounds in model systems. The presence of water was required for significant oxidation of the lipid to occur. Misra and Fridovich (1972) presented evidence for a coupled oxidation of heme proteins and epinephrine in aqueous solution, producing adrenochrome and the methemoprotein. In their postulated reaction (eq 1), the binding of oxygen to the hemopro-

$$Fe^{2+} + O_2 \rightleftharpoons [Fe^{3+}\text{–}O_2^{-}]^{2+} \longrightarrow Fe^{3+} + [O_2^{-}] \quad (1)$$

teins in the oxygenation step involves a charge migration from the heme to the oxygen, such that the structure becomes a superoxoferriheme. This portion of the reaction is reversible with low oxygen tension. The dissociation of the superoxoferriheme to yield $[O_2^-]$, the superoxide radical, and the oxidized heme would be expected to be slow because of the electrostatic attraction between the oppositely charged groups. Resonance Raman spectroscopy supports the classification of the iron in oxyhemoglobin as a low-spin Fe(III) rather than a low-spin Fe(II) structure (Yamamoto *et al.*, 1973; Spiro and Strekas, 1974).

Evidence for the involvement of superoxide in the oxidation of epinephrine to adrenochrome was demonstrated by Misra and Fridovich (1972) by the effectiveness of the enzymes superoxide dismutase and catalase in reducing the extent of oxidation. Superoxide radical is quite reactive, and would react with water to form molecular oxygen, plus peroxide and hydroxide ions. Many aerobic cells contain the enzyme superoxide dismutase which reduces the potentially harmful effect of superoxide on cellular systems. Participation of the superoxide radical in the aerobic reduction of ferricytochrome *c* in the presence of ferrous iron, a high pH, and high oxygen tension in a model system could not be supported nor eliminated when examined by Zipper *et al.* (1972). However, with the hemoproteins, the presence of hydrophobic regions in the immediate area of the heme might allow an introduction of a nonpolar molecule as a lipid to approach the heme. Alteration of the dielectric constant surrounding the superoxoferriheme could permit dissociation, as well as a coupled oxidation. If such reactions were to occur, however, the interfacial tension would be expected to have a large effect on the rate of oxidation, as reported by Haurowitz *et al.* (1973). The effect of various metal ions in increasing the rate of meat lipid oxidation (Ellis *et al.*, 1968) might be related to their effect on the interfacial tension, as well as the effect on membrane structure and enzyme activity. Two of the additives examined, citric acid and 1-ascorbyl stearate, might be expected to show some effect on the interfacial tension. Citric acid, along with certain other acid salts such as oxalate, polyphosphate, and EDTA, act as scavengers of divalent metals from the aqueous phase, and alter the lipid–aqueous interfacial tension. The molecule, 1-ascorbyl stearate, is amphipathic with limited solubilities in both aqueous and nonaqueous phases. As such, it would be expected to concentrate in the interface, similar to phospholipids, where it could exert its antioxidant activity. Both citric acid and 1-ascorbyl stearate showed a limited effect in decreasing lipid and heme oxidations.

The lipid-soluble antioxidant, α-tocopherol, is believed to protect lipids from oxidation through formation of a semiquinoid structure. The phenolic antioxidants, PG, BHT, and BHA, also act in this manner quite effectively (Greene, 1969). In our experiments, α-tocopherol showed a limited effect of antioxidant action, possibly as a result of the low levels used.

With all fats, the oxygen tension will be a factor in the rate of oxidation since oxygen is one of the reactants. In biological systems, the oxygen tension will be modified by the solubility in the aqueous phase and by the content of biological oxidants and reductants. These also can be modified by enzymatic action. In addition to superoxide dismutase and catalase, certain animal tissues contain peroxidases such as glutathione peroxidase. With low levels of substrate, catalase can also act as a peroxidase, as can other heme compounds, and some nonheme iron compounds. Nonheme iron components with unknown catalytic effects which are present in most cells are transferrin, ferritin, iron–sulfur proteins, and other enzymatically active compounds from the respiratory chain (Liu and Watts, 1970; Kwoh, 1971). Oxidation–reduction interactions between the various iron-containing compounds can also occur within the cells as with the cytochromes. As the meat ages, the activity of the enzymes and the reductants decreases, and their protective action toward oxidations diminishes, allowing an increased probability for oxidations. This behavior is true of ascorbic acid, which is a naturally occurring reductant in fresh meat. Ascorbic acid is believed to act as a free-radical acceptor, a hydrogen donor, and an enzyme activator. It may or may not have some chelation ability with copper. In our experiments, the addition of ascorbic acid, alone or with bicarbonate, was completely ineffective in preventing oxidation of the heme and lipids, and appeared to exert a prooxidation effect. Sodium bicarbonate addition may have decreased the effectiveness of ascorbic acid as a reductant further. Ascorbic acid has been reported to inhibit the action of catalase (Orr, 1967), and may also act on other heme and nonheme iron compounds. Consequently, the addition of ascorbic acid to ground meat samples for the purpose of extending the stability cannot be recommended.

LITERATURE CITED

Association of Official Analytical Chemists, "Official Methods of Analysis," 11th ed, 1970, p 445, No. 28.023 and 28.024.
Banks, A., *J. Soc. Chem. Ind. London* **63**, 8 (1944).
Benedict, R. C., Strange, E. D., Swift, C. E., Abstracts of the

167th National Meeting of the American Chemical Society, Los Angeles, Calif., March–April, 1974.
Berner, D. L., Conte, J. A., Jacobson, G. A., *J. Amer. Oil Chem. Soc.* **51**, 292 (1974).
Cook, L. J., Scott, T. W., Ferguson, K. A., McDonald, I. W., *Nature (London)* **228**, 178 (1970).
Dahle, L. K., Hill, E. G., Holman, R. T., *Arch. Biochem. Biophys.* **98**, 253 (1962).
DeVore, D. P., Solberg, M., *J. Food Sci.* **39**, 22 (1974).
Dinius, D. A., Oltjen, R. R., Satter, L. D., *J. Anim. Sci.* **38**, 887 (1974).
Ellis, R., Currie, G. T., Thornton, F. E., Bollinger, N. C., Gaddis, A. M., *J. Food Sci.* **33**, 555 (1968).
Fox, J. B., Jr., *J. Agr. Food Chem.* **14**, 207 (1966).
Govendarajan, S., Hultin, H. O., Kotula, A. W., 33rd Annual Meeting of the IFT, 1973, Abstract 332.
Greene, B. E., *J. Food Sci.* **34**, 110 (1969).
Greene, B. E., Hsin, I. M., Zipser, M. W., *J. Food Sci.* **36**, 940 (1971).
Haurowitz, F., Groh, M., Gansinger, G., *J. Biol. Chem.* **248**, 3810 (1973).
Herb, S. E., Magidman, P., Riemenschneider, R. W., *J. Amer. Oil Chem. Soc.* **37**, 127 (1960).
Kwoh, T. L., *J. Amer. Oil Chem. Soc.* **48**, 550 (1971).
Ledward, D. A., MacFarlane, J. J., *J. Food Sci.* **36**, 987 (1971).
Light, T. S., *Anal. Chem.* **44**, 1038 (1972).
Little, C., O'Brien, P. J., *Biochem. J.* **106**, 419 (1968).
Liu, H., Watts, B. M., *J. Food Sci.* **35**, 596 (1970).
Love, J. D., Pearson, A. M., *J. Amer. Oil Chem. Soc.* **48**, 547 (1971).
Misra, H. P., Fridovich, I., *J. Biol. Chem.* **247**, 6960 (1972).
Orr, C. W. M., *Biochemistry* **6**, 2995 (1967).
Pearson, D., *J. Sci. Food Agr.* **19**, 366 (1968).
Petropakis, H. J., Ph.D. Dissertation, Oregon State University, 1971, *Diss. Abstr.* **32**, 1010B (1971).
Rampton, J. H., Anglemier, A. F., Montgomery, M. W., *J. Food Sci.* **30**, 636 (1965).
Saleh, B., Watts, B. M., *J. Food Sci.* **33**, 353 (1968).
Sato, K., Hegarty, G. R., Herring, H. K., *J. Food Sci.* **38**, 398 (1973).
Sato, K., Herring, H., *Food Prod. Develop.* 7(9), 78 (1973).
Spiro, T. G., Strekas, T. C., *J. Amer. Chem. Soc.* **96**, 338 (1974).
Strange, E. D., Benedict, R. C., Gugger, R. E., Metzger, V. G., Swift, C. E., *J. Food Sci.* **39**, 988 (1974a).
Strange, E. D., Benedict, R. C., Swift, C. E., Abstracts of the 34th Annual Meeting of the IFT, 1974b.
Swern, D., Ed., "Bailey's Industrial Oil and Fat Products," 3rd ed, Interscience, New York, N.Y., 1964, pp 186–195.
Swern, D., Stirton, A. J., Turer, J., Wells, P. A., *Oil Soap* **20**, 224 (1943).
Taborsky, G., *Biochemistry* **12**, 1341 (1973).
Tappel, A. I., Brown, W. D., Zalkin, H., Maier, V. P., *J. Amer. Oil Chem. Soc.* **38**, 5 (1961).
Watts, B. M., Kendrick, J., Zipser, M. W., Hutchins, B., Saleh, B., *J. Food Sci.* **31**, 855 (1966).
Witte, V. C., Krause, G. F., Bailey, M. E., *J. Food Sci.* **35**, 582 (1970).
Yamamoto, T., Palmer, G., Gill, D., Salmeen, I. T., Rimai, L., *J. Biol. Chem.* **248**, 5211 (1973).
Zipper, H., Person, P., O'Connell, D. J., *Biochemistry* **11**, 4686 (1972).

Received for review June 11, 1974. Accepted September 24, 1974. Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned. Presented at the Symposium on Effects of Oxidized Lipids on Food Proteins and Flavor, 167th National Meeting of the American Chemical Society, Los Angeles, Calif., March 31–April 4, 1974.

Dietary Induction of Hepatic Microsomal Enzymes by Thermally Oxidized Fats

Ana Maria G. Andia and Joseph C. Street*

Functional changes associated with the hepatomegaly commonly observed upon feeding thermally oxidized (TO) fats were investigated. Rats were fed purified diets in which the fat consisted of fresh corn oil, TO oil, or the proportional amount of non-urea-adduct-forming material (NUAF) from TO oil plus fresh oil. Increases in relative liver weights and the concentrations of microsomal protein and endogenous malondialdehyde were observed when TO oil or NUAF plus fresh oil were fed rather than pure fresh oil with two types of dietary protein, casein and soy. Both the basal and DDT-induced mixed function oxidase activities were higher in animals fed TO oil and NUAF than in those given fresh oil. The TO oil also increased cytochrome P-450 and the activity of *S*-adenosylmethionine:phosphatidylethanolamine methyltransferase whereas the NUAF did not. Oxidized fat thus appears to stimulate SER proliferation and induce a complex of microsomal enzymes.

Certain natural food constituents such as fats are subject to changes during storage, processing, or cooking. Most cooking oils marketed today are rich in polyunsaturated fats which are highly susceptible to peroxidation. Thus, the heating of such cooking oils at high temperatures and in the presence of oxygen, as in deep fat frying, results in their oxidative deterioration. For example, Steibert and Koj (1973) found that, in an industrial scale deep fat frying of meat using rapeseed oil, the resulting fried meat products contained 0.63–1.1% of the nonvolatile oxidation products of the fatty acids taken up from the heated cooking oil. Similarly, Kurkela and Karjalainen (1973) have observed that French fried potatoes absorb some of the cooking fat including its high molecular weight secondary oxidation products. Peroxidation reactions *in vivo*, as well as the accumulation of peroxidation products, like polymerized fatty acids, produce varying degrees of cellular damage (Tappel, 1973).

The prolonged laboratory heating of fats in the presence of air, to simulate the conditions of deep fat frying, has been reported to cause appetite and growth depression, diarrhea, histological changes in various tissues, kidney and liver enlargement, and even death in some cases when fed to experimental animals (Binet and Wellers, 1966; Nolen *et al.*, 1967; Ohfuji and Kaneda, 1973; Kajimoto and Yoshida, 1973). Liver enlargement is frequently accompanied by an increased activity of microsomal enzymes resulting from proliferation of the smooth endoplasmic reticulum (SER). Most toxicological investigations on the effects of thermally oxidized fats have been quite general with little consideration of events at the molecular level. In this present study, the functional changes associated with the hepatomegaly commonly observed upon feeding a thermally oxidized fat and the non-urea-adduct-forming fatty acids contained therein were investigated. The investigation specifically considered the possi-

Department of Chemistry and Biochemistry, Utah State University, Logan, Utah 84322.