Methods for Following Lipid Oxidation in Meats

Betty M. Watts and Barbara Greene
Department of Food and Nutrition
Florida State University
Tallahassee, Florida

Abstract

Advantages and limitations of various tests for lipid oxidation are discussed. Oxygen consumption, either by manometric techniques or by the oxygen electrode, is of limited use in cooked meats. Peroxides increase regularly only in cured meats, where ferric hemes are not present to decompose the peroxides. Malonaldehyde is an extremely useful measure of lipid oxidation in raw, cooked and cured meats, correlating well with rancid odors. In irradiated or freeze dried meats held at room temperature, malonaldehyde and other carbonyls derived from lipid oxidation enter into secondary reactions with amines and cannot therefore be used as a measure of lipid oxidation. In such products, hydrocarbon assay by gas-solid chromatography may prove useful.

Products of the oxidation of unsaturated fatty acids contribute much more than has been realized to off odors even in very lean meats. The lack of valid methods for measuring such oxidation in meats has delayed understanding of its role in flavor deterioration and its control. This paper is a review of work in progress in our laboratories on methods for evaluating lipid oxidation and their application to various meat products.

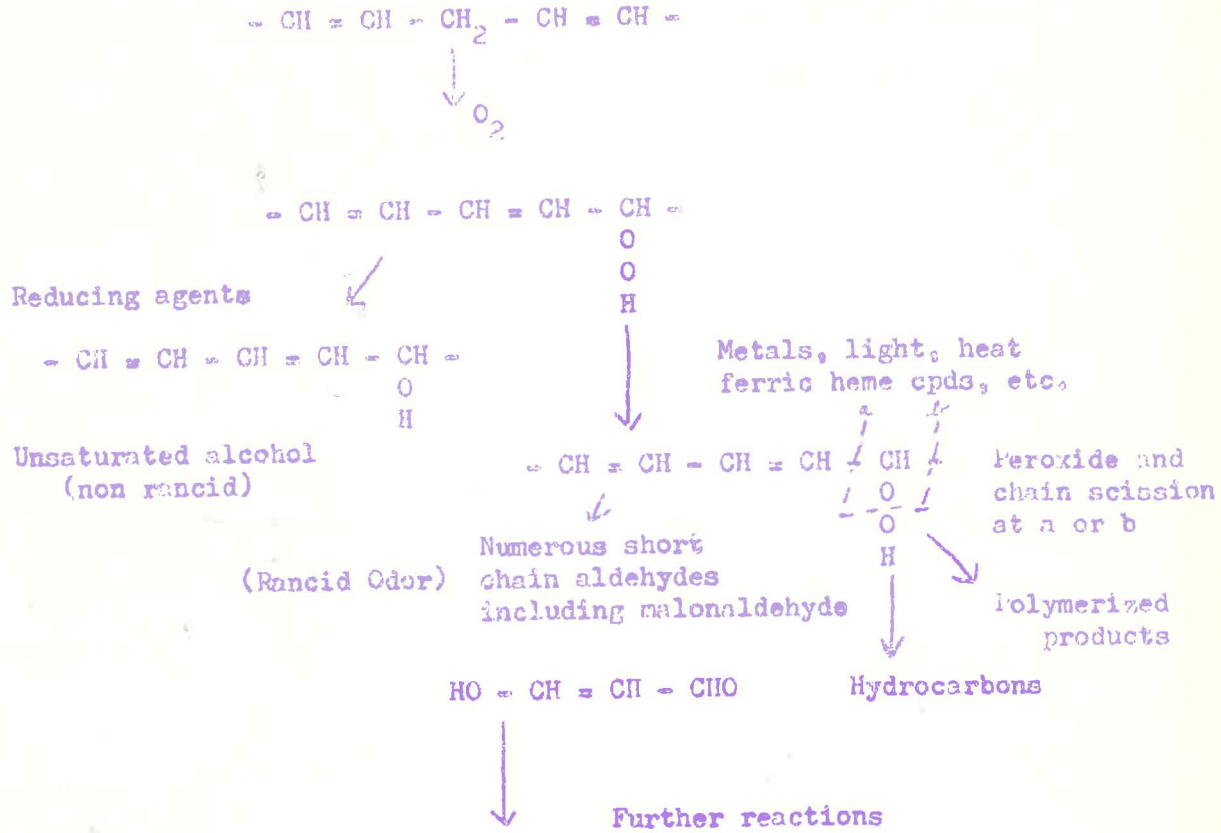
The schematic representation of various stages in the oxidation and decomposition of polyunsaturated fatty acids (PFA) illustrates the kind of analytical procedures which can be used to measure rancidity and indicates also their limitations (Fig. 1).

The overall loss of PFA from a meat product is not a reliable measure of rancidity. A very rancid odor can appear at a time when the loss of PFA is too small to be measurable by GLC techniques.

The uptake of O_2, either by Warburg manometric techniques (Greene and Watts 1966) or by use of a polarographic oxygen electrode has proven useful in the study of antioxidants in cooked meats. The residual oxygen uptake after enzyme destruction by the cooking process is apparently mainly associated with lipid oxidation and can be greatly reduced by suitable antioxidants.

The first relatively stable intermediates in the oxidative process are conjugated hydroperoxides. Before these can be measured they must be quantitatively extracted from meat. They can then be assayed with relative ease, either by a spectrophotometric assay of diene conjugation or by reactions of the peroxide group. Unfortunately, the diene peroxides may

Figure 1-Stages in the Oxidation of Polyunsatured Fatty Acids



show little or no correlation with rancid odor or with other tests for rancidity. Ferric hemes are powerful peroxide decomposers, so that, with the exception of cured meats in which the pigment remains in the non catalytic ferrous state, peroxides do not build up consistently. They may decompose almost as rapidly as they form.

It is mainly the carbonyl products resulting from peroxide decomposition and chain scission that are responsible for the off odors characteristic of rancid products. General tests for carbonyl products, although useful with pure fats, have not been successfully applied to meats, since carbonyls can arise from many other sources in addition to oxidizing fats. However, the three C fragment malonaldehyde (β hydroxy acrolein) appears to arise in meats only as a result of lipid oxidation. It can be detected in small concentrations either by its well known reaction with thiobarbituric acid or by its differential spectrum in acid versus base (Kwon and Watts, 1963). It has proven highly useful as an indicator of lipid oxidation and flavor deterioration in refrigerated meats although it does not itself contribute significantly to the off odor (Watts, 1961).

However, malonaldehyde is a highly reactive species. Both the enolic carbon atom of the α, β unsaturated system (Crawford et al, 1966) and presumably also the aldehyde group can combine with amine groups of amino acids and proteins as well as other food constituents (Kwon and Watts, 1964; Kwon et al, 1965). The extent to which the malonaldehyde is recoverable from such products under testing conditions may be expected to vary with the type of bonding and the degree of polymerization. Other carbonyl products, responsible for off odors, would also be expected to react with amine groups in meat in Maillard-type reactions.

Where such secondary reactions of the carbonyls resulting from lipid oxidation become significant, as for example in heat or irradiation sterilized meats or in dehydrated meats, all of which can be held at room temperature for extended periods, the only end products of lipid oxidation which could be expected to accumulate indefinitely are the hydrocarbons. Assay of hydrocarbons resulting from lipid oxidation by gas-solid chromatography is still at a very early stage, but offers possibilities (List et al, 1965).

It is also possible that at least some fraction of the lipid hydroperoxides may be reduced to the corresponding alcohol rather than decomposing to give rancid end products. It has been suggested that some antioxidants, such as selenium and sulfur amino acids, may owe their antioxidant activity to ability to reduce peroxides (Hamilton and Tappel, 1963). There is little direct evidence for this, but simplified procedures for the preparation of purified hydroperoxides (Kokatnur et al, 1965) will make possible more complete studies on the fate of such peroxides in the presence of either antioxidants or lipid oxidation catalysts. Mr. Hirano, in our laboratory, is investigating this problem.

With this brief view of the theoretical possibilities for following lipid oxidation in meats, let us now turn to some actual observations we have made on lipid oxidation in various kinds of preserved meats.

Cooked meats have been rather thoroughly explored in recent years. The cooking itself denatures the globin and converts the heme to brown ferric pigments which act as lipid oxidation catalysts and peroxide decomposers. Rancidity, as followed by malonaldehyde tests, takes place at all exposed surfaces. The malonaldehyde follows a typical pattern, increasing for

several days in the refrigerator and then generally leveling off at a maximum value usually within the limits 5-20 mg malonaldehyde/1000 g meat. Malonaldehyde values correlate well with organoleptic evaluations. Peroxide values, as might be expected, are low and irregular.

Raw meats have been much less thoroughly studied but here again malonaldehyde values seem to be the only established method for following lipid oxidation. The extent of such oxidation in stored ground meats correlates significantly with the conversion of pigments to the ferric form. This in turn is correlated with enzymatic reducing activity in the meat. Malonaldehyde values usually increase more slowly in raw as compared to cooked meats and tend to level off at lower values. In a few samples, the malonaldehyde number never exceeded one, even after 7 days storage, but the usual range was 3 to 15 (Hutchins et al, 1966 unpublished). Rancid odors, correlating with the malonaldehyde values, are commonly encountered and highly objectionable in refrigerated ground meats (Greene, 1966). They can be prevented with butylated hydroxy anisole (BHA), a food antioxidant.

Table 1

TBA numbers and odor scores for raw beef stored at 0°C for 6 days

Sample	TBA number	*Odor Score
Raw ground beef	4.3	3.2
Raw ground beef + 0.01% BHA	0.5	5.1

*difference significant at 0.01%
(6 = no off odor; 1 = very strong off odor)

With cured meats, the pigment is not a lipid oxidation catalyst and rancid odors do not develop rapidly in refrigerated cured meats as they do in raw and cooked meats. However, if cured meats are held in a freezer for some months, salt catalyzed oxidations occur. Both peroxides and malonaldehyde builds up and both correlate with rancid odor. Table 2 (Zipser et al, 1964) shows the ratio of peroxide number to malonaldehyde number in cooked versus cured meats, both stored in the freezer for some months.

Table 2

Ratio of peroxide number to TBA number in cured vs uncured frozen ground pork.

Sample Treatment	No. of Samples	Peroxide : TBA Range	Peroxide : TBA Average
Uncured	25	0.52-2.8	1.5
Cured	42	5.8-27	11.4

In irradiated meats stored at room temperature, malonaldehyde, whether added as such or produced as a result of lipid oxidation, disappears during storage (Table 3). We know of no good way to follow lipid oxidation in such products.

Table 3

Retention of added malonaldehyde in stored cooked irradiated beef

Days in sealed can	Malonaldehyde (mg/1000 g meat) Unirradiated (freezer)	Irradiated (room temp)
0	44.4	37.2
41	39.3	12.7
104	41.8	6.5

Freeze dried meats represent a similar problem. Mr. Seo, in our laboratory, is attempting to work out an analysis for hydrocarbons in head space gases which may shed some light on lipid oxidation in such products.

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