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Oxidative Deterioration in Meat Fats and Stabilization by the use of Antioxidants

by P. A. T. Swoboda and C. H. Lea

Low Temperature Station for Research in Biochemistry and Biophysics,
University of Cambridge and Department of Scientific and Industrial Research.

After water, fat is the next most abundant constituent of meat. When the boneless meat from a whole carcase is considered pork may have five times, mutton about three to four times and beef two to three times as much fat as protein. Meat therefore contributes a large proportion of the total fat intake of the diet. The other major sources of lipid in the diet are edible fats and oils. The production of lard and other edible animal fats represents one of the most important by-products of the meat packing industry. Thus investigations into the causes and methods of prevention of the development of rancidity in fat must be an important aspect of meat research. This deterioration of fat can occur (a) by hydrolysis due to the action of tissue lipases, (b) by the action of micro-organisms, and (c) by atmospheric oxidation.

The action of tissue lipases.

The hydrolytic action of tissue lipases, which sets in after death, is not very rapid except in metabolically active organs and, at ordinary temperatures it usually tends to be overshadowed by the action of lipolytic enzymes produced by bacteria. However tissue lipases show considerable resistance to freezing and remain active at temperatures as low as -25°C where microbial growth is arrested. They can therefore produce slow changes in the fat of cold-stored meat and poultry. However the higher fatty acids containing fourteen carbon atoms or more, which constitute almost exclusively the fatty acids of the majority of natural oils and fats, are virtually odourless and tasteless, and their slight liberation does not have any considerable deleterious effect on palatability. Indeed the fat of long cured hams, for example, often contains a considerable amount of free fatty acids.

Microbial Spoilage.

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Microbial Spoilage.

Many micro-organisms in the presence of water and nutrients are able to attack fat, resulting in either hydrolysis, or oxidation or both. Although fat

can be used as the main source of carbon, carbohydrate or protein form a more favourable substrate for most micro-organisms. When moulds or slime bacteria grow on chilled meat, a pronounced hydrolysis of fat usually occurs with the liberation of free fatty acids, but the unpleasant tainted flavours probably arise in large part from the action of the organism on the proteins of the connective tissue.

Microbial growth on fatty tissues, can however seriously impair the stability of the subsequently rendered fat towards atmospheric oxidation. Thus beef adipose tissue, which after three days storage in a moist atmosphere had developed a tainted odour was found to give on rendering, a fat which contained 2.6% free fatty acid and which had an induction period before the onset of oxidative rancidity of only 3 to 15 hours at 70°C. In contrast, the fat extracted from the tissues of the freshly killed animal had an induction period of 150 hours whilst the content of free fatty acid was 0.1%.

Oxidative Rancidity.

The most important type of deterioration which affects fats is that caused by the atmospheric oxidation of the unsaturated fatty acids. The primary products of oxidation of mono-ethenoid and nonconjugated poly-ethenoid acids are unsaturated hydroperoxides, which are formed by a free radical chain reaction. The autocatalytic nature of the oxidation results from the decomposition of the first formed hydroperoxides into free radicals which initiate further reaction chains. Thus the course of the reaction is characterised by a period of limited oxygen uptake, the induction period, which is followed by a phase of rapid oxidation. Traces of pro-oxidant and antioxidant substances exert an enormous influence on the duration of the induction period.

The hydroperoxides are only intermediates in the oxidation process. They do not in themselves contribute to rancidity, but react further, by fission, dehydration, polymerization or further oxidation, to give a wide range of degradation products, including volatile aldehydes, ketones and acids which are largely responsible for the objectionable odours and flavours. Other changes associated with free radical oxidation also take place simultaneously with the oxidation of unsaturated fatty acids. Carotenoid pigments such as those of butter and beef fat are bleached, and the vitamins E, A, C and possibly D and K

may be destroyed. In meat, oxidation of the fat resulting in rancidity, and oxidation of the haem pigments resulting in discoloration are closely related and one can accelerate the other. A yellow or orange discoloration can develop in the fat itself when large amounts of the more highly unsaturated fatty acids are present, or when the fat oxidises in the presence of nitrogenus substances.

The main factors which influence the susceptibility of a fat to oxidation can be considered under four headings:-

(1) The composition of the fat as regards the degree of unsaturation of its constituent fatty acids.

(2) The degree of access or exclusion of oxygen.

(3) Physical factors such as heat and light which influence the rate of oxidation.

(4) The balance of pro- and antioxidant substances in the system.

Fatty acid composition. Polyunsaturated fatty acids oxidize many times faster than do the mono-ethenoid and saturated acids; in fact so much faster that they are important factors in deterioration even when present only in quite minor proportions. The introduction of each additional double bond into the molecule, after the second, rather more than doubles the maximum rate of absorption of oxygen. Conversely, a partial selective hydrogenation, which considerably decreased the linolenic and arachidonic content of edible beef fat whilst only raising the melting point by about 2°C, has been found to result in greatly improved stability to oxidation.

The enormous variation in susceptibility towards oxidative rancidity shown by natural fats from different sources can thus be partly accounted for by difference in the proportion and degree of unsaturation of the constituent fatty acids. The fats of pork and poultry, for example, are more easily oxidized than those of beef or lamb; the linoleic content of the fat of beef or lamb is usually only of the order of 1 to 4% of the total glycerides, whereas with pork it ranges from 7 to 10% and with poultry from 18 to 31%.

Whilst animals can synthesise saturated fatty acids and oleic acid from carbohydrates, the fatty acids containing two or more double bonds are derived solely from the polyunsaturated acids in the diet. Many species, including pigs and poultry, tend to deposit the fatty acids of the diet in their body

(and egg) fats. Thus the linoleic content of hog fat has been varied from 2% on low fat rations to 32% on soybean rations. Similarly the inclusion of 5% linseed oil in the diet of turkeys for eight weeks before slaughter resulted in the deposition of body fat of greatly increased linolenic content which developed 'fishy' off-flavours on roasting.

The degree of species difference between animals fed on grass pasture is of interest. The rabbit and horse lay down in their body fats large amounts of linoleic and linolenic acids derived from the grass and their fats are readily oxidizable. Indeed in the gutted frozen rabbit, the exposed fat surrounding the kidneys and covering the wall of the abdominal cavity will go rancid and develop a yellow colour comparatively rapidly even when stored at -10°C. Ruminants such as cattle and sheep however, hydrogenate in the rumen much of the polyunsaturated fatty acids of the grass, and their body and milk fat on the same diet are harder and much more stable towards oxidation than those of non-ruminants.

An increased unsaturation of the body fat, however, need not necessarily lead to greater ease of oxidation, for the diet which provides the unsaturated fat may at the same time provide an increased intake of 'natural' antioxidants, some of which can be absorbed and stored in the fat.

Availability of oxygen. Any type of packaging of a meat product which reduces the contact of the fat with oxygen will retard the development of rancidity. When there is very little free space in a sealed container the oxygen present can become exhausted before the development of rancidity has become serious. Vacuum and inert gas packing is practised with some commodities to protect them from atmospheric oxygen. Even with bacon, storage of the sides at -10°C in an atmosphere of carbon dioxide has been found to delay the onset of rancidity up to 12 months whilst the control samples in air were definitely rancid by 4 months, but this process has not been used commercially.

Whenever a solid fat is stored, rancidity develops first at the exposed surface and penetrates only slowly to the interior. The importance of diffusion of oxygen through the fat is illustrated by the fact that green bacon, after storage for 3 months at -10°C had a peroxide value of about 50 at the exposed surface and only 2 or less at a depth of one centimetre. With a relatively finely divided and porous material such as dehydrated meat, the susceptibility of the bulk of the fat to oxidative rancidity is greatly increased.

When the diffusion of oxygen is not limiting, the rate of peroxide formation in a pure olefin has been observed to be independent of the oxygen concentration at high oxygen pressures: thus the oxidation of ethyl linoleate at 25°C only became dependent on oxygen concentration at oxygen pressures below twenty millimetres of mercury. With fats and fatty foods the dependence of oxidation rate on the partial pressure of oxygen may be much greater.

Effect of heat and light. Refrigeration is now generally employed where possible in order to retard both microbial spoilage and the development of oxidative rancidity. In general the rate of oxidation of a pure dry oil or fat is approximately doubled by a 10°C rise in temperature. In the presence of light or a metal catalyst the coefficient may be much smaller.

An exception to the usual rule of higher temperatures leading to reduced storage life has been observed with certain complex foods such as milk powder and dried herring, and this effect may possibly arise in certain meat products. It appears to be due mainly to the production at the higher temperature of substances which inhibit the oxidation of fat, particularly products of Maillard-type browning reactions between amino acids and sugars, and liberated sulphydryl groups of proteins.

At very high temperatures, such as those used in cooking, the oxidation of fat is greatly accelerated, although the increase may not always be reflected by increased peroxide values owing to the lability of peroxides to heat. The effect of cooking on the balance of 'natural' pro- and antioxidant factors in a meat product is so drastic that no prediction may safely be made as to the comparative susceptibilities to rancidity of the cooked and uncooked fat. One of the desirable characteristics of a chemical antioxidant which is added to fat before its use in the manufacture of a food is that part at least of its activity should 'carry through' to the final product.

Light accelerates not only the development of rancidity but also the discoloration of haem pigments in meat products. Illumination of fats not only causes more rapid oxidation during the period of exposure to light, but also an increased rate of peroxide formation after the light is removed. Autoxidation of fat is most powerfully accelerated by ultraviolet light and by the blue end of the spectrum up to a wavelength of about 450 mμ. Even the comparative yellow

light of a tungsten filament lamp is not entirely without effect, and modern fluorescent lighting is considerably more active. Ultraviolet light which has been used to retard the growth of micro-organisms on meat in cold storage, unfortunately tends also to accelerate the development of oxidative rancidity. This has been observed for example, with skinned pork carcasses. The range of intensity of light encountered in handling and marketing of food can be very great, ranging from direct sunlight to artificial light thousands of times weaker. Exposure of fat to direct sunlight or skylight is to be particularly avoided because of its high intensity and ultraviolet content.

Pro- and antioxidants. These substances may be natural constituents of the meat fat itself; haem pigments for example are pro-oxidant and tocopherols antioxidants. They may also be introduced into the fat accidentally or intentionally during manufacture; trace metal contamination from machinery and salt used in curing tend to act as pro-oxidants whilst antioxidatns can arise from the addition spices or during smoking. Finally, antioxidant chemicals may be used, in so far as they are permitted by the Preservatives in Food Regulations, as additives specifically to extend the storage life of the product.

Antioxidant Additives. Antioxidant chemicals may be classed under two types depending on their mode of action. The function of a primary antioxidant, such as an ortho or para substituted phenol or aromatic amine, is to react with the free radicals, particularly with the hydroperoxide radical, so as to break the chain reaction of the oxidation. With powerful antioxidants the induction period of the oxidation does not come to an end until the inhibitor has been almost completely destroyed.

At the present time all the primary antioxidants which have received legal approval for addition to human foods are phenols, either polyphenols such as the gallates, 2,4,5-trihydroxybutyrophenone (THBP) or nordihydroguaiaretic acid (NDGA) or ortho and para substituted ('hindred') monophenols such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). The polyphenols usually show higher activity in dry fats but their poorer stability to heat and alkali and their less favourable partition ratio between fat and water often make them less effective than the 'hindered' monophenolic inhibitors in complex foods and in baked or fried products.

Aromatic amine derivatives are widely used as oxidation inhibitors in rubber and petroleum products but have not been utilised to date in human food. Presumably this is in the main because of doubts concerning their non-toxicity and, in part perhaps, because of the tendency which they often show towards discoloration on oxidation. Antioxidants of this type, such as diphenyl paraphenylenediamine (DPPD) and 6-ethyl-2,2,4,-trimethyl-1,2-dihydroquinoline ('Santoquin') have however, been found particularly effective for the stabilization of carotenoids, including vitamin A, and have been employed for this purpose in animal feeds. The use of DPPD in poultry feed was however discontinued in the U.S.A. when the antioxidant was detected in trace amounts in the eggs and carcases of the birds, and BHT is now used instead.

The second class of antioxidant chemicals comprise the synergists or metal deactivators. Substances of this type are usually inactive or only feeble active when used alone, in the absence of primary antioxidants, but function by increasing the activity of the latter or by inhibiting the pro-oxidant activity of trace metals. They include such substances as citric acid, ascorbic acid and other hydroxy acids, phosphoric acid and polyphosphates, certain phospholipid fractions and various amino acids.

'Natural' antioxidants. In plant tissues there is a wide distribution of such phenolic substances as tocopherols, flavonoids, chlorogenic acid and tannins which could function as primary inhibitors of autoxidation. In addition there are, in both animal and plant tissues, many substances such as phospholipids, organic hydroxy and amino acids, sulphydryl compounds, and ascorbic acid which could act as synergists and metal deactivators.

The chief 'natural' antioxidant present in animal fat is vitamin E, the homologues of tocopherol, which may be present in concentrations of up to about 0.005%, and are wholly derived from the diet. This concentrations is less than optimal for stabilisation against autoxidation. Much larger quantities are usually present in vegetable oils and account in part for the observed differences between vegetable and animal fat in general stability and response to added antioxidants. The possibility exists of increasing the stability of an animal fat by supplementing the tocopherols of the diet, but stabilisation in this way is usually uneconomic because of the low efficiency of absorption from the gut and depostion in the tissues.

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'Natural' antioxidants from vegetable sources may also be added to the animal fat during the process of manufacture. The use of spices for flavouring and preserving foods dates from antiquity and recent work has shown that some of them do have a marked effect in delaying the development of oxidative rancidity in fats. Their relative antioxidant potencies however, vary widely with the type of product to which they are added and the active constituents of the different spices have usually not be identified.

Wood smoke has recently been shown to contain many phenolic compounds, mainly derivatives of catechol and pyrogallol. Tests in a model haematin-catalyzed methyl linoleate emulsion system showed that in smoke deposited in the traditional manner, almost the whole of the antioxidant activity was present in the 'volatile' phenol fraction, containing 1,3-dimethyl pyrogallol, guaiacol, creosol and 2-methoxy-4-ethyl phenol.

Pro-oxidant Catalysts. The haem compounds, haemoglobin, myoglobin and the cytochromes are the predominant catalysts of animal tissue for fat oxidation. This catalysis takes place particularly in heterogeneous systems where both an aqueous and a lipid phase are present. In contrast to the well characterized lipoxidases of vegetable origin, no specific enzymes from animal tissues have been implicated as important factors in the development of rancidity.

The nitric oxide haemoglobin which is formed during the curing of meat is still an effective catalyst. However heating haemoglobin, muscle extract or meat sufficiently to coagulate the haemoglobin and myoglobin reduces their pro-oxidant efficiency. Such heating does not destroy the iron porphyrin, which is the active catalyst, but presumably inactivates it by rendering it insoluble as the protein is denatured. A search for practical inhibitors for meat products which would function by inactivating the haem pro-oxidants has not so far been successful. Instead a combination of BHA or BHT, NDGA and ascorbic acid has been found to give the best protection against the haemoglobin catalysed oxidation of lard, and such combinations have also been shown to be effective in preliminary experiments on pork and salmon adipose tissues.

The presence in fat of trace amounts of metal, of the order of parts per million, can seriously affect the stability of fat towards oxidation. Contamination of a fatty food by copper and iron, both potent pro-oxidants, can easily arise as

a result of corrosion of the manufacturing plant. Metal complexing agents, such as citric acid or the more oil soluble citrate esters, can be used to prevent the pro-oxidant effect of the metal. They also prevent the discoloration which otherwise may arise by interaction of iron with water soluble polyphenolic antioxidants. If the metal contamination is high the use of phenolic antioxidants alone results in very little protection against rancidity and the metal deactivators is more important than the phenolic antioxidant.

Finally comment might be made on the fact that although the curing of meat results in increased resistance to microbial spoilage, it often enhances the susceptibility of the fat to oxidation. This has been attributed to a pro-oxidant effect of the salt which is added during curing. Thus in salted pork, both during salting and storage at -18°C, oxidation has been shown to be directly related to the concentration of salt.

Recent studies at the Low Temperature Research Station.

Following on from this brief review, mention may be made of some relevant investigation which we have recently carried out.

The most commonly used chemical test for the detection and estimation of oxidative rancidity is the determination of the peroxide value of the fat. The usual iodometric procedure is a macro-method which involves titration with thiosulphate of the iodine liberated by the anaerobic reaction of the fat peroxide with iodide under acid conditions. A new modification of the method has been developed so that milligram samples may be assayed. The iodine liberated is now estimated colorimetrically in an acidic aqueous phase which is stabilized against further atmospheric oxidation whilst any coloured lipid material is rejected in a chloroform phase.

However peroxides are not themselves responsible for the 'off' flavours of rancid fat and it has long been believed that a major contribution to these 'off' flavours is made by volatile aldehydes and ketones produced by degradation of the peroxides. A method has therefore been worked out by which these compounds can be quantitatively isolated from a fat or oil and then estimated by an established colorimetric procedure involving the preparation of the 2,4-dinitro-phenylhydrazone derivatives. The technique employed involves distillation in a simple type of pot still, of the volatiles from the fat, which is heated at 50°C

under a moderate vacuum of 0.01 mm Hg, and their condensation on the surface of a cold finger which is cooled either by solid carbon dioxide-ethanol or by liquid nitrogen. In model systems quantities of 2 to 4 μM/g. of aldehyde were added to an oil previously stripped of its volatiles and good recoveries obtained with aldehydes ranging in molecular weight from hexanal (C_6) to myristic aldehyde (C_{14}). The C_4 compounds, butyraldehyde and croton aldehyde, failed to condense when solid carbon dioxide-ethanol was used as coolant, but half to two-thirds of these substances was recoverable with liquid nitrogen. When applied to autoxidized fats the standard half hour period of distillation resulted in the condensation of 3% of the total carbonyls of lard, 2.5% of those of cod liver oil and 0.5 to 0.7% of those of cotton seed oil. These results show that only a small proportion of the carbonylic substances formed during the oxidation of a fat are volatile, but this volatile fraction is probably of great importance in determining odour and flavour.

The above studies indicated a need for data on the effects of low concentrations of the volatile carbonylic oxidation products on palatability. The threshold concentration at which the flavour of each aldehyde could just be detected has therefore been determined. The results showed that in water the flavour threshold decreased from C_3 to C_{12} and then rose again sharply for C_{14}; the most potent members of the series (C_8 to C_{12}) were all detectable at weight concentrations of 10^{-8} to 10^{-9}. In groundnut oil or in paraffin the aldehydes C_3 to C_{12} all had flavour thresholds in the region of 10^{-6} to 10^{-7} whilst that for C_{14} aldehydes was greater than 10^{-4}.

A number of naturally occurring phenolic compounds have been tested for antioxidant activity in accelerated stability tests. Gossypetin and quercetagetin, two hexahydroxyflavones of rather rare occurrence in plant tissues, have been found to be potent antioxidants for methyl linoleate at 50°C. Certain polyphenolic constituents of tea, L-epigallocatechin gallate and L-epicatechin gallate as well as D-catechin itself have about the same activity as propyl gallate in methyl esters of lard fatty acids; the degree of protection in a light-accelerated system at 20°C was less than in a heat-accelerated system at 90°C. A number of commercial crude tannin extracts were however relatively ineffective. The antioxidant activities of the seven known tocopherols (vitamin E) have also been

compared at 50°C in linoleate. Both the degree of nuclear methylation and the position of the methyl groups influence activity; the tendency appears to be for effectiveness to decrease in the order monomethyl > dimethyl > trimethyl, and 8-methyl > 7-methyl > 5-methyl.

One of the disadvatanges of BHA and BHT is their complete loss by distillation during, for example, the steam deodorization of lard to which they have been added. It was thought that the methylene linked bisphenol in which two molecules of the 'hindered' monophenol are linked through a methylene group might prove less volatile. On testing, their antioxidant activity in crude methyl linoleate, on a weight basis, has been found to be approximately the same as those of the parent monophenol.

Some of the oxidative changes which can occur in a complex fat containing food are illustrated by a recent study of deterioration of fish meal during storage. This product, which is important as an animal feeding stuff, contains a considerable quantity of highly unsaturated fat. A very rapid oxidation of the fish mea stored in air, particularly during the first few weeks was observed. However these was no accumulation of peroxides during storage at 37°, and only a slight one at 25°C. At these relatively high storage temperatures oxidative changes were actually less marked than at 10°C, probably owing to a more rapid build up of oxidation inhibitors produced by Maillard-type reactions. These reactions occur to a greater degree at a higher moisture content and oxidative changes were indeed found to be slower at a moisture content of 11.1% than at 6.2%. Changes observed in the oil extracted from stored meal were a darkening in colour, reduction in iodine value and polymerization. No changes occurred when the fish meal was stored under nitrogen. BHT when added together with citric acid to the eal before storage, considerably retarded oxidation of the oil.

The work described in this paper was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

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