LIPID OXIDATION AND THE FLAVOUR OF MEAT PRODUCTS

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INTRODUCTION

Our interest in meat flavour stemmed from our work on the development of nitrite-free meat-curing systems. As a curing agent nitrite performs multiple functions -

1. It produces the cooked cured-meat pigment, dinitrosyl ferrohemchrome. This pigment has also been synthesised from beef red blood cells (Shahidi et al. 1985).

2. It gives oxidative stability to meat by preventing lipid oxidation. This effect is complex but is believed to be associated with the appearance of the cured-meat flavour and the prevention of the objectionable warmed-over flavour. However, nitrite is not unique in imparting oxidative stability to meat, as will be shown later.

3. It acts as an antimicrobial agent, and we have relied on it to prevent the outgrowth of C. botulinum in cured meats. However, here too nitrite is not unique (Wood et al. 1986).

Raw meat has little odour and only a blood-like taste. On cooking, the characteristic cooked-meat flavour develops, in part by the formation of a spectrum of carbonyl compounds arising presumably by lipid oxidation. The nature of this spectrum varies with the lipid composition of the original meat - beef, pork, lamb, and poultry. However, this is only part of the story. Hornstein and Crowe (1960 and 1963) have already indicated that aqueous extracts of beef, pork, and lamb had similar aromas when heated. However, heating and fat yielded aromas which were species-characteristic. More recently Mottram et al. (1982) and Mottram and Edwards (1983) confirmed the importance of fat in giving meats their species-characteristic flavour, and that this was largely derived from the more highly unsaturated structural phospholipids rather than the triglycerides. Willemot et al. (1985) reached a similar conclusion. Further oxidation quickly leads to the development of the so-called warmed-over flavour.

There are, therefore, two distinct aspects to meat flavour. There are the flavour volatiles which arise, on heating, in the aqueous phase. There is an abundance of small molecules, including amino acids and carbohydrates, which can interact to give a distinctive aroma. Of these the sulphur-containing compounds may be of particular importance. The other aspect, as already mentioned, is related to lipid oxidation which gives rise to a rich spectrum of carbonyl compounds. These carbonyls are straight-chain although branched chain carbonyls can also be detected, albeit in smaller amounts. These probably arise from the degradation of amino acids, as pointed out by Cross and Ziegler (1965). Together these two aspects produced the overall sensation of meat flavour, of which cured-meat flavour is of special and perhaps quite fundamental interest.

In this paper we do not intend to present a review of the literature, which is vast. An extensive review has only recently appeared (Shahidi et al. 1986a). Other reviews may be of assistance - Bailey and Swain (1973); Chang and Peterson (1977); MacLeod and Seyyedain-Ardebili (1981); Gray et al. (1981); Moody (1983); Gray and Pearson (1984). We plan to deal with concepts giving the "state of the art" as we understand it. We also wish to point to some hazards of working in this difficult field, and to mention some new techniques which have recently become available for separating and identifying the flavour volatiles present in the food matrix. We shall put some stress on lipid oxidation in meat and its effect on meat flavour, but shall also consider the problem as a whole.

CHEMICAL NATURE OF MEAT FLAVOUR VOLATILES

Amongst the volatile flavour components of meat from beef, pork, mutton, and chicken, 995 compounds have so far been identified (Shahidi et al. 1986a). Of the above four sources of meat, the largest number of volatiles have been found in beef, namely 681 as compared to pork with 314. This may be due to the fact that more work has been done on beef than on the meat from other species. In cured pork only 135 compounds have so far been identified in the volatiles, and for this there may indeed be a good reason. Many classes of organic compounds are represented in the meat volatiles such as aliphatic and aromatic hydrocarbons, aldehydes, ketones, alcohols, phenols, carboxylic acids, lactones, furans, pyridines, pyrazines, amines, pyrroles, oxazoles, thiazoles, mercaptans, thiophenes, ethers, and several halogenated compounds. Beilstein is well represented here!

Which of the compounds so far identified in meat volatiles make a contribution to meat flavour? One is tempted to answer - not too many. Thus MacLeod and Ames (1986a) report that 740 compounds have so far been characterised in meat volatiles and state - "Many of these are unimportant". Min et al. (1979) studied the volatile compounds in the neutral fraction of beef and concluded that lactones, substituted aromatics, furans, and sulphur compounds are important contributors to roast beef flavour. Minor et al, (1965) suggested that sulphur-containing compounds were responsible for the "meaty" aroma of cooked chicken muscle, while the "chickeny" aroma could be traced to the high level of H_2S. This perhaps illustrates nicely the importance of concentration in judging the contribution made by individual volatiles to meat aroma. What is meant by a "high level" of H_2S, and at what even higher level would it become highly obnoxious? Chang and Peterson (1977) consider carbonyl compounds, aliphatic and aromatic hydrocarbons, saturated alcohols, esters, and ethers unimportant as contributors to beef aroma. On the other hand, they list lactones, furanoid and hydrofuranoid compounds, and heterocyclic compounds containing sulphur, nitrogen, and oxygen as important contributors.

The literature is full of contradictions and no firm conclusions can be drawn. Perhaps we can say with some degree of confidence that sulphur-containing compounds are of importance in the formation of the "meaty" flavour and that carbonyls play a role in species differentiation. Shahidi et al. (1986a) have reviewed the

Table 1

Odour Characteristics of the Volatile Compounds in Meat

Compound	Odour
1-Hexene	dull, cardboard
Benzene,1,4-dimethyl	fruity, solvent-like, sickly, fatty
n-Hexanal	strong, rancid, unpleasant, green, pungent, sickly
Benzaldehyde	sweet, almond-like, strong, sweet-metallic
2,3-Butanedione	buttery, sickly
δ-Decalactone	creamy, sweet, nut-like
3-Furanthiol,2-methyl	sweet, meaty
Pyrazine, trimethyl	nutty, roasted
Thiazole,2,4-dimethyl	meaty, cocoa-like
3-Thiazoline,2,4,5-trimethyl	meaty, nutty, onion
Mercaptan, methylthioethane	meaty odour
Thiophene,2-carboxaldehyde	spicy, meat, nutty, sharp, sweet
1,4,5-Trithiane,2,4,6-trimethyl	heated meat

literature and list all compounds (about one seventh of the total) in meat volatiles which have so far been assigned odour characteristics. A few examples are given in Table 1. Of the 150 or so compounds listed by Shahidi et al. (1986a) only a few are referred to as meat like, and these are given in Table 1. It is also interesting to note that n-hexanal, which is so prominent a component in the volatiles of meat, is given a set of descriptors which runs from rancid to sickly!

A number of sulphur-containing compounds with meat-like characteristics have been synthesised. These were listed by Shahidi et al. (1986a). Unfortunately, none of these has so far been found in meat volatiles. Chang and Peterson (1977) reported the interesting case of 3,5-dimethyl-1,2,4-trithiolane which was thought to be a major contributor to beef flavour. On synthesis it was, however, found not to be beef-like at all. This can be listed as one of the hazards referred to above. Did the compound isolated from beef volatiles contain a minor impurity with intense beef-like aroma? If so, it may be worthwhile to look for it.

There is some light at the end of the tunnel. MacLeod and Ames (1986a) reported on the isolation and characterisation of 2-methyl-3-(methylthio)furan from beef volatiles. Its odour was described as "meaty, not roasted". On synthesis the compound still had a meaty aroma.

LIPID OXIDATION PRODUCTS

The importance of lipid oxidation in the overall mechanism of meat-flavour production has already been mentioned, and it is the polyunsaturated fatty acid of the phospholipids which are largely involved. The primary products of oxidation are hydroperoxides which are colourless and odourless. However, on decomposition secondary products are produced (Fig.1) which influence the flavour of meat and are felt to be responsible for species differentiation. The spectrum of these secondary products of lipid peroxidation will, of course, depend on the fatty acid composition of the lipids present in the meat.

Malonaldehyde is a major product of lipid peroxidation and its concentration in meats is determined by the well-known 2-thiobarbituric acid (TBA) test. It has been suggested that the warmed-over flavour becomes recognisable when the TBA value exceeds 0.5 to 1.0 (Tarladig et al. 1960). Malonaldehyde has very little odour and may, therefore, not contribute much to the flavour of meat. However, it has served as a useful indicator of lipid oxidation.

Hexanal is the dominant breakdown product of lipid peroxidation and has been assigned a variety of odour characteristics, all of them unpleasant. Its effect on meat flavour must be substantial. Shahidi et al. (1987a) found that the hexanal content and TBA numbers were closely correlated, and there was an indication that the two correlated with sensory scores (Table 2). We should like to suggest that the hexanal content after only 2 days of storage may be a more suitable indicator of lipid oxidation than the traditional TBA test.

In our search for a substitute for nitrite, as far as lipid oxidation is concerned, we examined a number of sequestering agents (Shahidi et al. 1986b) and

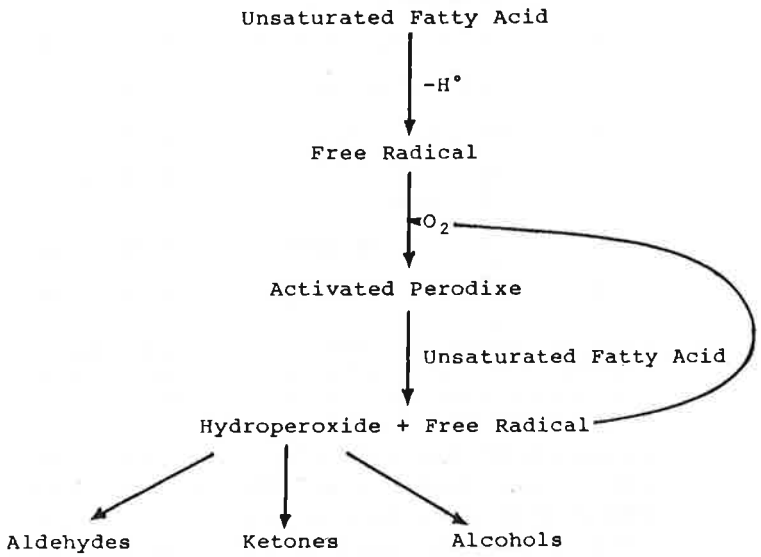

Figure 1. Mechanism of Lipid Oxidation.

Table 2

Hexanal Content, TBA Numbers, and Sensory Scores of Meat Systems[1] (Shahidi et al. 1987a)

Experiment No.	Additives	Hexanal[2]	TBA Number	Average Sensory Score[3]
1	Control[4]	100.0	15.93	2.8
2	(1) + SA (550 ppm)	50.1	8.30	-
3	(1) + STPP (3000 ppm)	38.0	4.79	5.1
4	(2) + STPP (3000 ppm)	4.1	1.32	-
5	(4) + BHA (30 ppm)	1.6	0.22	5.3
6	(4) + TBHQ (30 ppm)	3.0	0.27	5.6
7	(4) + $NaNO_2$ (30 ppm)	2.1	0.56	-
8	(4) + $NaNO_2$ (150 ppm)	2.0	0.43	5.7
9	(2) + $NaNO_2$ (150 ppm)	2.8	0.39	-

[1] Cooked meats were stored at 4°C for 2 days prior to the determination of hexanal content and sensory evaluation. The TBA numbers (mg malonaldehyde equivalent/1000 g meat) were determined after 35 days. The amount of hexanal in the control (corresponding to the area under the curve) is arbitrarily set at 100. The additives were sodium ascorbate, SA; sodium tripolyphosphate, STPP; butylated hydroxyanisole, BHA; and t-butylhydroquinone, TBHQ.

[2] Average of three determinations.

[3] Average of two determinations.

[4] The control as well as all experimental samples, contained 2% NaCl and 1.5% sucrose.

Table 3

Effect of Combinations of Antioxidants with Sodium Ascorbate and Sodium Tripolyphosphate or Ethylenediaminetetraacetic Acid on the TBA Values of Cooked Pork during Storage at 4°C

Experiment No.	Additives[1]	Storage time (days)			
		1	7	21	35
1	Control	3.17	6.63	10.0	13.05
2	STPP (3000 ppm)	0.22	0.36	1.13	1.86
3	(2) + SA (550 ppm)	0.17	0.16	0.31	0.27
4	(3) + BHA (30 ppm)	0.16	0.14	0.20	0.20
5	(3) + TBHQ (30 ppm)	0.19	0.16	0.18	0.18
6	Na_2EDTA (500 ppm)	0.18	0.17	0.25	0.29
7	(6) + SA (550 ppm)	0.19	0.21	0.23	0.23
8	(7) BHA (30 ppm)	0.19	0.21	0.23	0.23
9	(7) + TBHQ (30 ppm)	0.21	0.18	0.22	0.22

[1] The additives were STPP, sodium tripolyphosphate; SA, sodium ascorbate; BHA, butylated hydroxyanisole; TBHQ, t-butylhydroquinone; NA_2EDTA, disodium salt of ethylenediaminetetraacetic acid.

antioxidants (Shahidi et al. 1987b). Some very effective additives were found. Thus, EDTA kept the TBA number down to less than one over a 5-week storage period. Two common antioxidants, BHA and TBHQ, at 30 ppm were particularly effective, certainly as good as 150 ppm of nitrite.

Surprisingly, the cured-meat pigment, dinitrosyl ferrohemochrome, has a modest antioxidant effect.

Combinations of antioxidants and chelators were also tested and the results are shown in Table 3 as adapted for Shahidi et al. (1987c). A combination of such commonly used food additives as sodium ascorbate and sodium tripolyphosphate gives an excellent result, which is somewhat better in the presence of 30 ppm of BHA of TBHQ.

Nitrite is not unique in preventing lipid oxidation in cooked meats. Even the flavour acceptability of nitrite-cured meats can be duplicated by a judicious choice of additives referred to above. The results, which to be sure are preliminary, are given by Yun et al. (1987). Nitrite is unique in producing the cured-meat colour. It is also unique in that it, by itself, can produce a cured-meat product having all the proper attributes - colour, oxidative stability and flavour, and microbial stability. To replace it, a system of "curing" agents is needed.

VOLATILES FROM UNCURED AND CURED MEATS

More than 20 years have passed since Cross and Ziegler (1965) published their paper call "A Comparison of the Volatile Fractions from Cured and Uncured Meat". This seminal paper did not receive the attention it deserved. Gas-chromatographic analysis of the volatiles showed

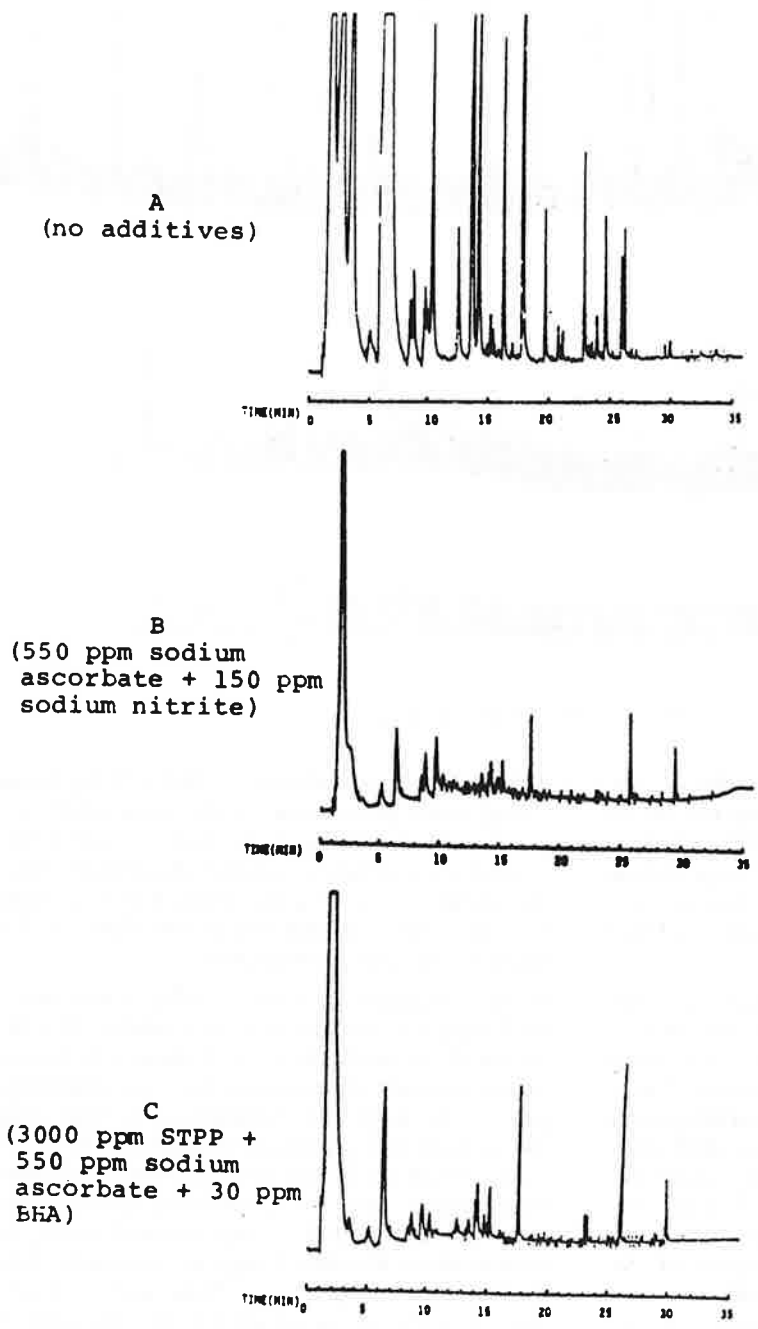

Figure 2. Gas Chromatograms of Volatile Components of Cooked Pork.

that cooked uncured and cured ham were qualitatively similar but quantitatively very different. The volatiles of uncured ham contained relatively large amounts of n-hexanal and n-pentanal. These almost disappeared in the cured product. In fact, when the volatiles from cured and uncured ham were passed through a solution of 2,4-dinitro-phenylhydrazine, both effluent streams had the characteristic ham odour. The volatiles from cured and uncured chicken and beef, on passage through 2,4-dinitrophenyl-hydrazine, also had an aroma similar to that of cured ham.

Cross and Ziegler postulated that the higher aldehydes are lipid oxidation products, the formation of which is almost eliminated by the presence of nitrite. It is on the basis of this work that we proposed that any other agent, or combination of agents, that prevented lipid oxidation would do the same thing, and this we amply confirmed in the last few years. The chromatogram of the volatiles of uncured pork (Fig.2A) was almost "decimated" in the presence of nitrite (Fig.2B) or one of our better systems for preventing lipid oxidation, e.g., sodium tripolyphosphate, sodium ascorbate, and the antioxidants BHA (Fig.2C) (Yun 1984). This was confirmed by organoleptic evaluation. The panelist did not distinguish between nitrite-cured pork samples and those treated with our systems, as already mentioned above.

Perhaps the reason for the slow acceptance of the work of Cross and Ziegler is that they proposed a rather unusual concept. The cured-meat flavour is arrived at by subtraction, by stripping away the rich overlay of carbonyls. They concluded -

"We consider that cured ham flavour represents the basic meat flavour derived from precursors other than triglycerides, and that the different aromas of the various types of cooked meat depend on the spectra of carbonyl compounds, derived by oxidation of fat".

Work since that time, including our own, has lent support to this concept.

OLD HAZARDS AND NEW TECHNIQUES

Beware of artifacts! Flavour components are present at the parts per million (10^6) and parts per billion (109) level. Buttery et al. (1984) reported that the odour threshold of bis-(2-methyl-3-furyl)disulphide (not isolated from meat) is 2 parts per 1014 parts of water, which must be a record, or close to it. As wisely pointed out by MacLeod and Ames (1986a).

"It follows therefore that only minute traces of these compounds need be present for them to be aroma effective, creasing enormous analytical difficulties for their detection".

One of these difficulties is the presence of artifacts, substances which can be detected in the blank, that is in a run done with the same equipment, water and organic solvents, but in the absence of meat. MacLeod and Ames (1986b) using an adsorption/thermal desorption (ATD) technique found 26 such compounds, 3 of which were even characterised as meaty or meat-like!

In our laboratories (LJR) we also used the ATD technique and our blank contained a great number of contaminants (Fig.3A). The source of the contaminants was not the nitrogen nor the absorbent (Tenax TA). They

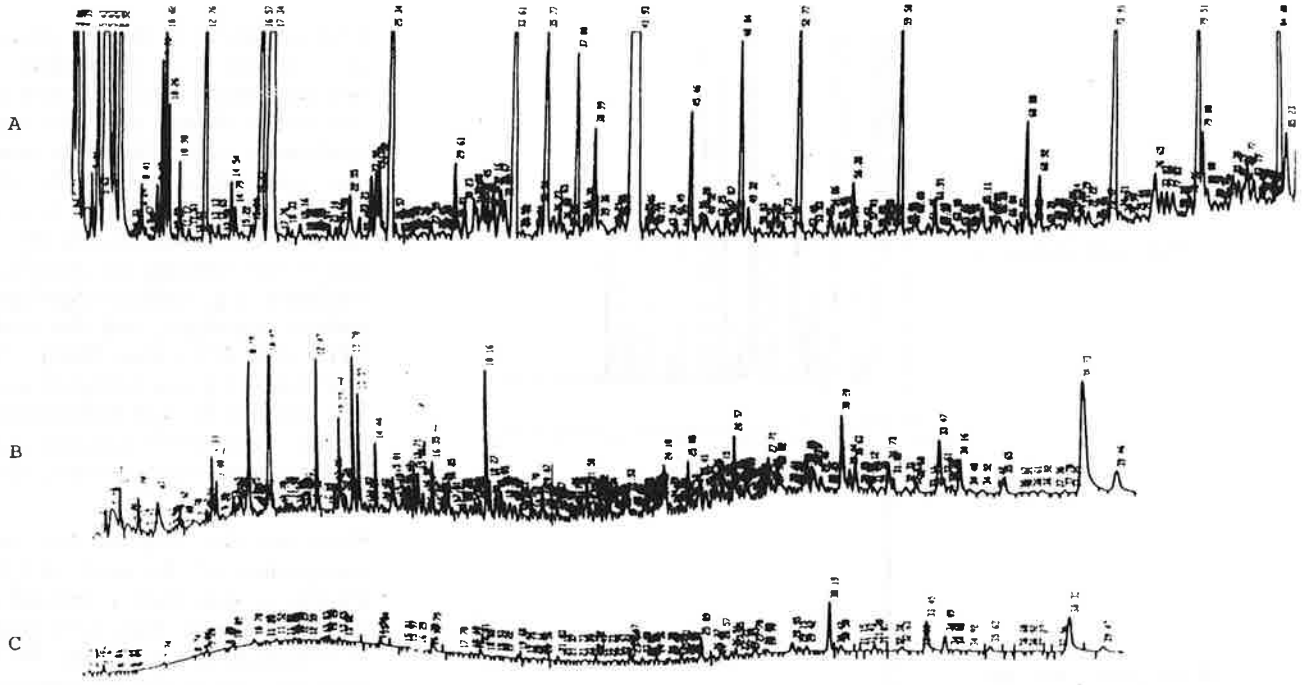

Figure 3. Volatile Compounds in Blanks.

could have come from the grease used to lubricate the stirrer or from water. Even the glassware cannot be excluded. Pankow et al. (1988) used the ATD technique with Tenax in the analysis of trace organic compounds in water. Because of the sensitive of the technique, they were able to detect contaminants even highly purified water.

Steam distillation at atmospheric or reduced pressure has been the most common method for the isolation of volatiles from meat. These volatiles must be subjected to further concentration steps by solvent extraction. Large amounts of meat, as much as one to several kilograms, need to be used because of the trace levels at which some of the aroma volatiles are present. This necessitates the use of large volumes of water and solvent. The volatiles in the solvent extract must be concentrated, and this brings about a simultaneous concentration of the contaminants. On using a modest 120 mL of water containing 6 g of salt and extracting with ether-hexane, the gas chromatogram shown in Fig.3B was obtained for the concentrated extract. For transferring the solvent a syringe was used in which the needle was attached by a plastic adaptor. Was this partly to blame?

When using freshly purchased solvents of high purity and a proper syringe, our results were better. Even in this case diethyl ether must be freshly distilled in an all-glass apparatus. In this case, the ether-hexane extract gave the chromatogram shown in Fig.3C. Small peaks can be seen, but the situation is tolerable. When one finds bromotrichloromethane in meat volatiles, as has been reported in the literature, it is time to become suspicious.

Now to consider some techniques which have only recently become available. To remove the volatiles from the meat matrix harsh conditions have been used such as steam distillation, or flushing with nitrogen for 24 hours at 9°C (MacLeod and Ames 1986b). What harm are we doing to the constituents of the meat volatiles some of which we would judge to be highly sensitive by a mere examination of their structure? Aside from this, some of the apparatus used for the isolation of meat volatiles has become rather complex (Chang and Peterson 1977), and there is a hazard in complexity.

In the extraction of meat volatiles a new and exciting technique has recently become available. It is the use of supercritical fluids. It is a well known phenomenon that above a certain temperature, the critical temperature, a gas can no longer be compressed to form a liquid no matter how high a pressure is applied. These so-called supercritical fluids are good solvents and have in fact been widely used in specialised applications. The solubility of material in supercritical fluids, the most commonly used to date being carbon dioxide, depends on temperature and pressure. Both can be varied readily. Temperatures are generally low and pressures high, in the order of 5000 lb per square inch (35 MPa). Isolation of the dissolved material can be readily achieved by reducing the pressure or, alternatively, fractionation can be carried out by reducing the pressure in stages. It may be interesting to note that General Foods is building a $60 million plant using supercritical CO_2 in the first instance, offers a new dimension in dealing with the flavour of meat. Partial decomposition of the volatiles already recognised may be avoided, and their levels quantified. It is almost certain that many compounds never seen before because of their sensitivity may thus be revealed. Some of them may be of critical importance in the spectrum of compounds responsible for meat flavour.

The advantages of supercritical-fluid extraction have been utilised by Hawthorne et al. (1987). Unfortunately, only an abstract of this paper is so far available. The authors point out that the solvent power of supercritical

CO_2 can surpass that of the more usual solvents and that temperatures can be kept lo, about 4°C. The supercritical CO_2 stream can be passed directly into a capillary gas chromatograph giving a new analytical coupling - SFE-GC. The loss of extracted flavour volatiles should be minimal.

The proceedings of two very useful symposia have recently been published. The first is called "Supercritical Fluid Extraction and Chromatography" and the editors are Charpentier and Sevenants (1988). The second is the proceedings of the 5th Weurman Flavour Research Symposium edited by Martents et al. (1987).

MacLeod and Ames (1986b) pointed out that the best opportunity for the more detailed analysis of flavour volatiles lay in the use of the excellent fused-silica capillary columns for gas chromatography which had recently become available, in conjunction with mass spectrometry. Certainly the usefulness of CG-MS in this difficult field cannot be underestimated. However, Change and Peterson (1977) made the point that not all the components of the flavour volatiles could be eluted from the GC column, and some may have been decomposed by heat. They suggested the use of high-pressure liquid chromatography (HPLC) where the conditions are milder, and which would be particularly suitable for the fractionation of the less volatile components of meat flavour. When using HPLC identification by MS becomes more difficult. Nevertheless, we feel that this is a technique which is worthy of further consideration.

Infrared spectroscopy is an excellent means for identifying function groups as well as for fingerprinting the molecules as a whole. However, relatively large samples are needed, about 100 g and perhaps 1-10 g with improved instruments. Recently Fourier transform infrared spectrometers (FT-IR) have become available. By using multiple scanning with an FT-IR spectrometer a large increase in sensitivity can be achieved. A spectrum for less than 1 ng of non-volatile polymeric film has been reported. With this level of sensitivity we can consider using FT-IR in conjunction with the excellent method of separation new available - GC and HPLC.

CONCLUDING REMARKS

In the world of food flavour, meat perhaps offers the most complex case. In the aqueous phase we have the low-molecular-weight water-soluble materials which are considered as meat flavour precursors. These include free amino acids and carbohydrates. The high-molecular-weight proteins (in muscle fibrils and sarcoplasmic fluid do not contribute to meat flavour in a direct sense. Fats, and particularly the structural fats of the cell membrane, produce on cooking and oxidation a rich assortment of aldehydes and ketones which no doubt contribute to the overall sensation of meat flavour, particularly in the differentiation of meat from different

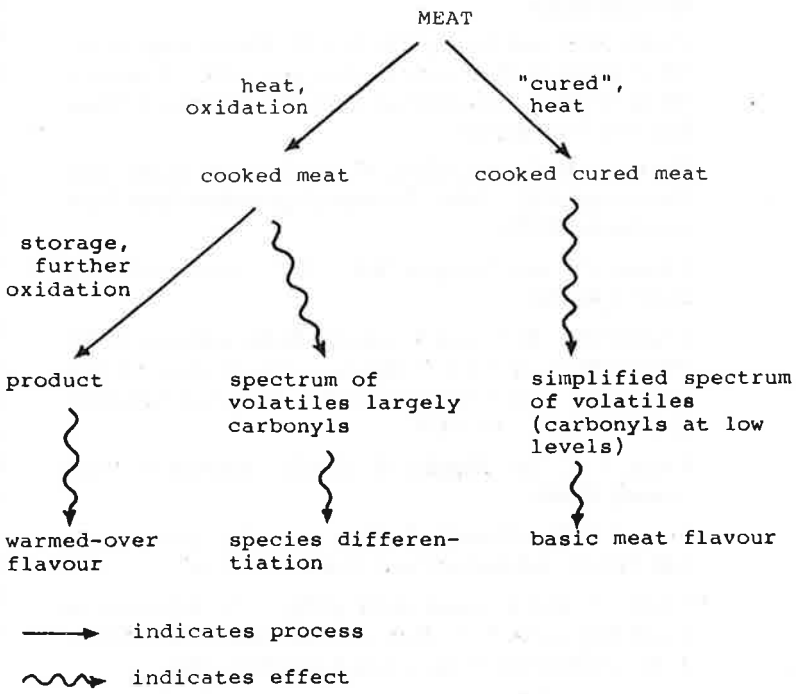

Figure 4. Approach to the Mechanism of Meat Flavour Formation.

species. These differences which relate to species are another complicating factor in considering meat flavour as a whole. On further oxidation, as already mentioned, we get the warmed-over flavour, also very characteristic of meat.

In Fig.4 we attempt to present a unifying theory, simplistic as it is, of the origin of the basic flavour of meat, species differentiation, and warmed-over flavour. Nature is not likely to turn out to be this simple. However, the scheme is in keeping with most of the facts as we know them, and is perhaps a reasonable place to start.

Remarkable progress has been made in meat-flavour research in the last 20 years. A large number of compounds have been identified, 995 to date. Is it worth while to continue to search for new compounds? On balance we would say yes. It is possible that compounds are present in very low concentrations but are organoleptically extremely potent, and hence are major contributors to meat flavour. The literature gives some support for this view.

The other question is, at what point in the scheme given in Fig.4 should we start? We suggest that a study of the basic flavour of meat would be the logical point. It is no longer necessary to disrupt the meat structure by separating the fat from the lean in order to study each part separately. This has been done in the past. It is much easier to use nitrite or one of the effective antioxidant systems referred to above. Another good reason for starting with the basic meat flavour, if it should indeed turn out to be so, is that its GC spectrum is greatly simplified. This does not mean that the problem is an easy one. However, its solution would make a fundamental contribution which should lead to the unravelling of the overall problem of meat flavour.

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