

S U M M A R I E S  
o f  
P A S T A N D F U T U R E  
R E S E A R C H

Submitted

By

Think Tank II Participants

Think Tank on Beef Flavor

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## Current Research Activities on Rancidity

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Research activities in our laboratory have included three separate but interrelated areas. Initially, we investigated the effects of vitamin E feeding on the resistance of turkey meat to rancidity. Secondly, we have developed a new method for the direct high performance liquid chromatography (HPLC) determination of free malondialdehyde in meat products. Thirdly, we have begun to develop methods for and determine levels of cholesterol oxidation products in various types of foods.

Many previous studies had indicated that both the lean and fat portions of the animal's carcass may be stabilized against rancidity by vitamin E supplementation. Our study determined that warmed over flavor could be lessened to some extent by vitamin E feeding. However, it is not totally effective. Further, we determined that vitamin E is quite effective at reducing the rancidity found in fresh turkey meat which is stored for up to three weeks at 3°C.

Most investigators have used the TBA method. Although this is a good general method for rancidity, values for TBA are often quoted in terms of micrograms of malondialdehyde per gram of tissue (TBA number). Numerous studies have demonstrated that TBA suffers from a number of chemical interferences. Therefore it would be useful to develop a method for direct determination of free malondialdehyde. In our studies, we used HPLC on TSK G1000 PW column with a mobile phase of .1 molar  $\text{Na}_3\text{PO}_4$ , pH 8.0 buffer at a flow rate of .6 ml per minute. The eluent was monitored at 267 nm. Values from meat samples were compared to the TBA "determination" of malondialdehyde. The results indicated that the TBA result inflates the actual malonaldehyde content of tissue by from two to threefold or higher in some cases. Since malondialdehyde is rated as a putative carcinogen, it was important to have an accurate method. It is obvious that the TBA value is not a good method for direct malonaldehyde determination. In spite of this fact, numerous authors have reported levels of malonaldehyde using the TBA procedure. These data now must be interpreted with caution.

The cholesterol content in meat products has long been a controversial factor. Earlier feeding studies in animals indicated that cholesterol was atherogenic. However, numerous recent findings have leveled serious criticisms at these studies because the cholesterol which had been used is now known to have contained cholesterol oxidation products and that these products, not native cholesterol, were the actual cause of atherosclerosis in these studies. Obviously, cholesterol should come under reexamination as far as its atherogenic properties are concerned. The practical importance of this to the meat industry is obvious. However, before we can claim that cholesterol is not atherogenic as it occurs in meat products, it is very definitely a requirement to determine whether or not oxidation products of cholesterol occur in such products.

The two analytical methods that we are currently working on for cholesterol oxides are GLC and HPLC. GLC, using a capillary column, has been useful in both pure systems and in the actual analysis of foods. Using this system, some cholesterol oxides have been found in certain health food preparations (dried brain and liver) and some fast-foods fried in lard- and tallow-containing oils. We are also working on HPLC methodology. Here, separation and quantification has been accomplished using a normal phase column of microporosil and UV detection. We have also studied the possibility of derivatization of cholesterol oxides with p-nitrobenzoyl chloride. This would greatly increase the sensitivity of our assay. However, derivatization makes necessary the use of reverse phase HPLC. These studies are currently ongoing.

Statement for Think Tank on Flavor Problems in Beef  
by  
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This statement summarizes a concept explained in full in a manuscript by Lozano and Cassens which is scheduled for publication in J. Food Science. The title is "Influence of an extract of heart on stability of color and development of rancidity during storage of sliced bologna".

In cured beef products the pigment, even though relatively stable, is susceptible to fading, and oxidation of lipids also occurs resulting in objectional flavor. It is well established that reducing conditions retard color fading and lipid oxidation. Therefore, ascorbic acid is widely used in the industry not only to accelerate curing but also to protect flavor and color of the retail product. The concept we have under investigation is to utilize an extract of cardiac muscle as a possible means to stabilize color and to slow development of rancidity. The rationale is based on the fact that cardiac muscle has an inherent reducing system in the high content of mitochondria plus an elevated heme protein level mainly in the high content of cytochrome-c. It is hoped that this concept will be of interest to participants in the "Think Tank on Flavor Problems in Beef".

Three batches of beef bologna containing 0, 5.43 and 10.86% of a heart extract were prepared commercially, packaged in a specially made nylon-surlyn film with relatively high oxygen permeability and studied during a 60-day period. The product containing a greater amount of extract had a more intense, pink color than product with less or no extract. The rate of change of the color and lipid oxidation pattern were found to be the same for the three batches during a 60-day storage period. The positive influence of the extract on color was attributed to the extra amount of pigment provided in the extract, rather than to a protective reducing mechanism.

Further investigation of the composition and properties of the extract is required.

## SUMMARY OF SOME RECENT AND PROPOSED STUDIES ON WARMED - OVER FLAVOR IN MEAT

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The primary objective of the more recent research has been to examine factors responsible for variability in the rates of oxidation of lipids and myoglobin in raw beef. It had been observed by workers in our laboratory that some samples appeared to be very stable to one or the other or both oxidations. If the factor or factors responsible for the observed stability were known, it might be possible to induce or enhance these factors. Some of the factors examined include the selenium glutathione peroxidase (SeGSHpx) system and type of apparatus used for grinding meat.

The SeGSHpx system is believed to serve as an antioxidant in living animals. It has been shown to decompose lipid hydroperoxides (initial products of unsaturated fatty acid oxidation) to non-reactive products, thus breaking the chain reaction of fatty acid autoxidation. The SeGSHpx enzyme was found to be present in post-rigor beef muscle, and some activity was measureable within the pH range for post-rigor beef. Further testing is needed to determine whether all of the components of the SeGSHpx system are present in a quantity and proportion sufficient to allow the system to operate in post-rigor muscle.

The availability of metal ions appeared to be the primary factor responsible for differences in storage stability of raw beef ground in different apparatus. The use of grinder plates composed of a comparatively soft metal resulted in beef patties that developed higher TBA numbers than patties ground with plates of a harder metal which contained slightly less iron.

If studies on the variability of oxidation rates were to be continued, further investigation would include an examination of the activities of metmyoglobin reductase and the glutathione transferases and a comparison of the effects of self-defrosting versus non self-defrosting freezers on the rates of lipid and myoglobin oxidations in stored meat. Metmyoglobin reductase is an enzyme discovered recently to reduce the brown metmyoglobin pigment to the red myoglobin in some living animals. The glutathione reductases are a group of enzymes, some of which had been confused with SeGSHpx originally. Some of these transferases may function in a manner similar to SeGSHpx and may, therefore, have antioxidant activity also. Comments from consumers on an apparent decrease in keeping time of frozen meats in recent years prompted the consideration of a study comparing freezers.

Possibilities for future studies at Oregon State University include an examination of the effect of final internal temperature (IT) on flavor changes in stored cooked meat. It is anticipated that different flavor notes will be perceived at the various IT increments, and that there may be an IT at which warmed-over flavor in cooked meats is least perceptible. A centroid model will be used in the surface response analysis of these data.

## LIPID OXIDATION IN COOKED MEATS

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When uncured precooked meat is stored it develops a characteristic objectionable stale rancid odor and flavor. This phenomenon occurs in a relatively short period of time and is particularly noticeable when refrigerated precooked meat is reheated. This problem has considerable significance because of the increase of fast food service facilities and convenience foods requiring use of precooked or partially cooked meat and meat products. Since beef is one of the primary sources used, finding a solution to this oxidation problem, commonly referred to as warmed-over-flavor, would significantly increase the acceptance of beef.

The primary component of meat that is prone to oxidation is the lipid fraction. Within the lipid fraction, unsaturated fatty acids, particularly those in phospholipids and proteolipids, are most easily oxidized. This process is also referred to as atmospheric oxidation because of the involvement of oxygen, particularly singlet oxygen.

Most evidence indicates that the mechanism of oxidation of fatty acids involves 1) Initiation of free radical formation during a relatively slow induction period, 2) Propagation of the free radical by combination with singlet oxygen to form peroxide free-radicals and hydroperoxides leading to compounds with obnoxious (rancid) odor and flavor characteristics and 3) Termination of the oxidation chain reaction when the free radicals are deactivated or destroyed.

The secondary decomposition products of fatty acids include alcohols, aldehydes, ketones, acids, lactones and unsaturated hydrocarbons which are susceptible to further oxidation. It is very difficult to isolate and identify intermediate compounds formed during oxidation. From a flavor standpoint, aldehydes present problems because some of them (2,4-decadienal) have flavor thresholds in low ppb concentrations.

There is considerable experimental evidence that warmed-over-flavor results from oxidation of muscle lipids, particularly in cooked meats. Even in very lean products, oxidation can occur rapidly. It is not necessary that meat have a high fat content to be susceptible to oxidation. It has been shown that the complex phospholipid fraction containing a relatively high proportion of readily oxidizable polyunsaturated fatty acids is susceptible to rancidity development.

The primary catalysts of lipid oxidation in meat are heme (hemoglobin, myoglobin and cytochromes) and non-heme iron in both oxidized and reduced forms. Other metals involved generally influencing oxidation are cobalt, copper, manganese and nickel.

The control of lipid oxidation is very important for the food industry. Conventional antioxidants are approved for use in only a limited number of products. These include fresh pork sausage, brown and serve sausage, Italian sausage products, pre-grilled beef patties, fresh sausage made from beef or beef and pork, dry sausage, dried meats and rendered fats. Antioxidants approved include BHA, BHT, Propyl galate, and TBHQ. Also, glycine, resin guaiac and tocopherols are approved for use in rendered fats.

There are many substances which have been shown to inhibit oxidation in meats and meat products. Of primary importance to the meat industry is nitrite which is very effective, particularly in combination with ascorbates and vacuum packaging. Heated vegetable protein products such as soy flour, textured vegetable protein and cottonseed flour inhibit oxidation. Shelf-stable retorted canned meats have inhibitory substances as do browning reaction products obtained from the interaction of sugars and amino acids. Metal chelators are important in stabilizing food products. These include EDTA, tripolyphosphates, and citric acid.

Packaging is important in preserving food products, including meat. Packaging materials with high oxygen barrier when used with vacuum packaging or controlled atmosphere to exclude oxygen are very important in inhibiting oxidation and prolonging product shelf life. Recent advances in material development have been very instrumental in increasing the types of meats and meat products available to the consumer.

## RESEARCH IN "MEAT FLAVOR" AT RUTGERS UNIVERSITY

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Meat flavor, particularly that of beef, is undoubtedly one of the most desired flavors by the food industry. At the same time, it is also one of the most difficult flavors to study. Our laboratory has been engaged in the isolation, fractionation and identification of beef flavor for eighteen years. We have produced several prominent Ph.D. food scientists through this investigation. However, we have not yet completely solved the mystery of beef flavor, even though we believe we are very close to the summit.

Many novel compounds were identified in meat flavor by our laboratories. 2,5-Dimethyl-1,3,4-trithiolane and 2,4,5-trimethyl-3-oxazoline were reported as early as 1968 in the flavor of boiled beef. They have since been studied extensively by other researchers for their use or their homologous series as flavor compounds.

Recently we identified a total of 44 nitrogen-containing heterocyclic compounds in the volatiles of roasted beef. They consisted of 15 thiazoles, 1 thiazoline, 6 oxazoles, 11 pyrazines, 6 pyrroles, 2 piperidines and 3 pyridines.

## THE EFFECT OF CATTLE DIET ON BEEF FLAVOR AND CHEMICAL COMPOSITION - Sharon Melton

During the past 6 years research at the University of Tennessee (U.T.) has been involved with the effect of diet on beef flavor and composition. The following abstract is a summary of the results of these studies. Grass-produced beef compared with grain-produced beef has a less desirable flavor (1 to 1-1/2 units on an 8-point scale) due to a higher intensities of sour, milky-oily (rancid) flavors and a lower intensity of a cooked beef fat flavor. This flavor difference is not due to lower fat levels in grass-produced beef but most likely to its fatty acid composition. Grass-produced beef has higher levels of stearic acid, 13-methyl pentadecanoic acid, and omega-3 acids (18:3 $\omega$ 3, 20:3 $\omega$ 3, 20:4 $\omega$ 3, 22:5 $\omega$ 3 and 22:6 $\omega$ 3) and lower levels of monounsaturated (14:1, 16:1, 17:1, 18:1 and 20:1) and, generally, omega-6 acids (18:2 $\omega$ 6, 20:3 $\omega$ 6 and 20:4 $\omega$ 6) than grain-produced beef. The percentage of 18:3 $\omega$ 3 in beef has been negatively correlated ( $P < .001$ ) with its desirable flavor score and cooked beef fat flavor intensity and positively correlated ( $P < .001$ ) with its milky-oily flavor intensity. The correlation coefficient between the acid and each flavor score was approximately 0.5 in each study. Changes found in beef fatty acid composition can be related to fatty acids in the diets and to the rumen microflora present on each diet. Fatty acids of grass diets (Kentucky 31 fescue, orchard grass and clover) studied at U.T. were composed of >40% 18:3 $\omega$ 3 and 20-30% 18:2 $\omega$ 6, but fatty acids of the grain diet (mainly corn) included no 18:3 $\omega$ 3 and >70% 18:2 $\omega$ 6. Results of the U.T. studies showed that when steers were changed from grass to a grain diet, the major changes in beef fatty acid composition, flavor and other components occurred during the first 100 days on grain. Besides changes in fatty acids and flavor, the  $\alpha$ -tocopherol content in beef was found to decrease but contents of  $\delta$ -decalactone,  $\gamma$ -dodecalactone and  $\delta$ -tetradecalactone in cooked ground beef increased. Changes in lactone concentrations could be explained by fatty acid changes in the raw beef. Future research at U.T. will be concerned with the effect of other types of grass diets on beef flavor and composition.

## Recent and Current Research at the US Army Natick

### R&D Laboratories on Warmed Over Flavor

BACKGROUND: During the last ten years, the Food Engineering Laboratory, pursuant to the Department of Defense (DOD) Food Program has undertaken research in restructured meats, both flaked and formed and chunked and formed. Two sensory problems were noted immediately: a vague off-flavor and a discoloration described as gray to green mottling. It was suggested by the writer that this behavior resembled trace oxidation in other comminuted or roasted meats, as described by Greene, Pearson and others. Representative examples of recent FEL research to confirm this are abstracted below:

#### Recent research:

Beef Roast, Chunked and Formed, Frozen: Chunked and formed beef roasts with and without antioxidants and with standard polyethylene packaging or oxygen-impermeable packaging have been stored at 0°F for 9 months. A flavor profile panel found that oxygen-impermeable packaging significantly reduced flavor degradation over standard packaging and that antioxidants helped to retard degradation although not uniformly.

Beef Steaks, Flaked and Formed, Frozen: Flaked and formed beef steaks were prepared with salt, salt and sodium tripolyphosphate (STPP), and salt, sodium tripolyphosphate, butylated hydroxyanisole (BHA) and sodium erythorbate and stored at 0°F. Samples were withdrawn after 3 and 6 months and evaluated by a flavor profile panel. The panel found that samples containing salt only, had the highest degree of flavor degradation, that samples containing salt and STPP had less flavor degradation and that samples containing salt, STPP, BHA, and sodium erythorbate had the least amount of flavor degradation although they had developed a slightly stale beef and oxidized tallow flavor after 6 months.

### Beef, Veal, Pork and Lamb Steaks, Flaked and Formed, Frozen:

Evaluation of flaked and formed samples stored at 0°F for 9 months shows that oxygen-impermeable packaging preserved the sensory qualities of the beef, veal and lamb steaks, but made little difference in the pork steaks.

### Implications of research for the DOD Food Programs.

DOD procures and distributes restructured meat to its troops in dining facilities via a frozen storage system. With this system, color changes present no particular problems to the consumer. Flavor degradation, on the other hand, is of primary concern to DOD. It has been concluded that, although it is expensive, oxygen-impermeable packaging is probably the best temporary solution to the warmed over flavor problem in restructured meat. Storage stability requirements in general are for one year at 0°F. There is no question, however, that warmed over flavor in meats presents a procurement problem to DOD, particularly for pre-cooked, chunked and formed meats.

DOD is therefore, also strongly interested in amendments to current regulations which prevent the use of antioxidant and chelating additives in fresh and frozen meats, and is willing to participate in needed research and applications.

### Future Research.

DOD is currently concerned with flavor degradation in combat rations as well. Research continues on a rationale for appropriate antioxidants for whole tissue foods, in which there are membranes and micellar materials of high lipid surface to volume ratio. Research also is in progress and will be reported on oxidation detection methods other than TBA and PV. Chief of these is vapor phase polyamide fluorescence. Other methods are saline extract fluorescence (altered myosin) and chloroform-methanol extract (altered phospholipids) fluorescence. Overlap with, and discrimination of these methods from, the similar sugar-amine browning fluorescence will be discussed.

## Summary of Work on Warmed-Over Flavor (WOF)

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### SUMMARY

Our work has focused upon the mechanism responsible for development of WOF and some methods for preventing its development. Our review on the subject indicated that myoglobin was responsible for WOF (Pearson et al., 1977). On attempting to produce WOF by using either metmyoglobin or <sup>non</sup>heme iron in a model meat system, however, Love and Pearson (1974) found that <sup>non</sup>heme iron was indeed the catalyst for WOF development, which verified the earlier report by Sato and Hegarty (1971). Later Igene et al. (1979) demonstrated that on heating of meat pigment extracts the amount of <sup>non</sup>heme iron increases due to the breakdown of meat pigments. We (Chen et al., 1983a) have since obtained evidence that the porphyrin ring breaks down on heating, which increases the amount of nonheme iron. The heme iron increases during heating, with slow heating being more effective than rapid heating, which may explain why earlier workers (Sato and Hegarty, 1971; Love and Pearson, 1974) failed to obtain an increase in oxidation on using metmyoglobin in model systems.

Work in our laboratory clearly showed that phospholipids play a more important role in WOF than the triglycerides (Wilson et al., 1976; Igene and Pearson, 1979). The polyunsaturated fatty acids (PUFAs) are involved in the oxidative deterioration with phosphatidyl ethanolamine (PE) being the chief phospholipid degraded during development of WOF (Igene and Pearson, 1979; Igene et al., 1981).

Research has demonstrated that nitrite protects against development of WOF (Zipser et al., 1964; Bailey and Swain, 1973; Fooladi et al., 1979; Igene et al., 1979). Chen et al. (1983b) have obtained evidence that nitrite stabilizes the porphyrin ring, thus protecting against release of nonheme iron. The metal chelator EDTA has also been shown to inhibit development of WOF (Igene and Pearson, 1979). There is also evidence that over-heating of meat produces antioxidative activity and inhibits WOF (Sato et al., 1973). Other antioxidants may also be used to inhibit the development of WOF in cooked meats (Chen et al., 1983).

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Current and Future Research at Texas A&M University on Oxidative  
Rancidity Development in Meat and Meat Products

(Prepared by K.S. Rhee)

I. Previous and On-going Research

Area 1: Use of natural food ingredients to retard oxidative rancidity in meat and meat products.

- . Antioxidant activity of aqueous and alcoholic extracts of various oilseed food ingredients in model lipid peroxidation systems of meat.
- . Antioxidant activity increase in heating oilseed food ingredients with glucose.
- . Retardation by oilseed ingredients of oxidative rancidity development in precooked beef patties.
- . Use of oilseed products as an ingredient of gravy or sauce for precooked meat products to retard oxidative rancidity development.
- . Retardation by oilseed ingredients of lipid oxidation and discoloration in salt-containing raw meat products.

Key Findings from Area 1:

1. Various oilseed food ingredients can reduce or retard both heme iron- and nonheme iron-catalyzed lipid oxidation.
2. Nonenzymatic browning between oilseed protein ingredients and a reducing sugar can enhance the antioxidant value of oilseed ingredients.
3. Oilseed ingredients can retard rancidity development (resulting from lipid oxidation) in precooked meat products when incorporated, before cooking, in the products at a low level (e.g. 3.3% as a dry ingredient).
4. Oilseed ingredients can be incorporated in gravy or sauce for precooked meat products to retard rancidity development.
5. Certain oilseed ingredients may retard salt-catalyzed lipid oxidation in fresh (uncooked) meat products.

Area 2: Effect of reduction or replacement of sodium chloride on rancidity development in raw (non-inoculated or inoculated) and cooked meat products.

Key Findings from Area 2:

Among the three chloride salts tested, i.e., NaCl, KCl, and MgCl<sub>2</sub>, NaCl increased lipid oxidation the most. Replacement of NaCl with KCl may be most effective for decreasing rancidity in processed meat products.

Area 3: Studies are being conducted to: (1) determine if an enzyme-catalyzed lipid oxidation system exists in microsomal fractions of beef skeletal muscle; (2) determine its operational requirements; and (3) evaluate the magnitude of its activity for microsomes isolated from the muscle samples that have been stored for varying lengths of time after removal of muscle from the carcass.

## II. Future Research

- . Retardation of oxidative rancidity development, during processing, storage and reheating, in precooked/convenience meat products.
- . Control of off-flavor development in restructured meat products.
- . Microsomal enzymic lipid peroxidation in bovine, porcine and ovine skeletal muscles as affected by muscle fiber differences and postmortem carcass treatment.
- . Relative importance of enzymic vs. nonenzymic lipid peroxidation for oxidative rancidity development in fresh (uncooked) meat and meat products.

## CURRENT AND FUTURE RESEARCH

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After 25 years of flavor research by the group called Food Quality Research Unit, emphasis has been changed to studies of plant constituents which modify insect behavior with flavor research reduced to a minor topic, and the name of the group has been changed to Biocommunication Chemistry Research Unit. Instrumentation and techniques, such as gas chromatography, mass spectrometry, nuclear magnetic resonance, etc., applied to flavor chemistry are now being applied to other topics such as insect attractants and detection of estrus in dairy cattle. Studies of the identities of volatile odor compounds associated with the major crops (corn, rice, oats, wheat, alfalfa, etc.) and the effect of these volatiles on the various insect pests are being continued with improvements in both the techniques of chemical isolation and identification and insect testing. Another area of investigation is that of corn gluten hydrolyzate volatiles used as fruitfly attractants. These volatiles are products of protein and carbohydrate fragments reacting to form flavor compounds, compounds of interest to insects and humans.

Warmed-over flavor problems are probably due to compounds formed during storage and warming because freshly cooked beef is one of the universally desirable foods. If priorities are established in the USDA, Agricultural Research Service program, the Biocommunication Chemistry Research Unit at the Western Regional Research Center, Albany, California, can work on the warmed-over beef flavor problem on the molecular basis, using similar techniques as used in the above hydrolyzed protein problem. The goal would be to find what compounds contribute to this off-flavor. Knowledge of the identities of the compounds responsible for the warmed-over flavor would give scientific basis for the development of methods of preventing their formation or suppressing their effects.