Sensory Evaluation Techniques to Assess Oxidative Rancidity

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Introduction

I would like to introduce my co-workers for this session. Al Pearson, from Michigan State University, is a well-known researcher in the area of warmed-over flavor, including the sensory aspects; Brad Berry, who is also doing quite a bit of work in this area. I would like to begin our comments by saying that I am convinced that the area of warmed-over flavor (WOF) and sensory evaluation is an important one that needs more research, especially the relationship between chemical measures of rancidity and sensory measures. Many studies have been done correlating the TBA tests; for example, with sensory evaluation of WOF or other types of rancidity. However, there is need for further work in this area for the following reasons:

1. Much of the work has been done by people like ourselves, meat scientists, and I do think the time has come that we call upon the expertise of those people who are specifically educated in sensory evaluation. People who have Ph.D. degrees in that particular specialty can help us because they have developed new and more sophisticated statistical designs for the evaluation of sensory variation by taste panels. I think we are not doing as good a job as we can with respect to the utilization of our colleagues in the sensory area.
2. A very large number of new techniques for determining rancidity by chemical procedures have been developed in the last two or three years, many of which have not yet been correlated to sensory evaluation. I think all of us will agree that before we do too much work with these newer techniques, we have to show that they do predict what the consumer perceives as a WOF problem.
3. A lot of confusion surrounds the TBA tests, whether it is the traditional Tarladgis method or the later-developed Witte procedure. The results for these methods are often expressed in micrograms of malonaldehyde (or malondialdehyde) per gram of tissue. The fact is that this test was never really meant to just simply determine malonaldehyde (MA). It is a misuse of the test if we say we are reporting MA because what we are determining is a broader test of oxidative rancidity. I will show you some data later which has been confirmed by other researchers, where the TBA test greatly overestimated the amount of MA in tissue.
4. In recent years, there has been a lot of research which indicates that MA itself is an active, mutagenic compound and possibly is carcinogenic. For that reason alone, it is important that we not express TBA data in terms of MA if the TBA tests overestimate the amount of MA present. It is much better to quote our data in terms of TBA-reactive substances (TBARS) to get away from automatically overestimating the MA present.

Warmed-Over Flavor

I would like to make a few comments about WOF. First of all, the endpoint temperature of cooking is important and as we go up in temperature we have a tendency to produce more and more WOF, up to the point of retort temperature. At sterilization temperatures, there is a greatly diminished WOF development, possibly due to the development of browning-type reaction compounds or reductions. WOF varies with species and also, within a species, from one muscle to another, mostly with the red/white fiber ratio.

With respect to taste panel testing, there is a great deal of person-to-person variation. Dr. Pearson and I have discovered that our wives cannot detect WOF. I am extremely sensitive to it, having studied it for a long period of time. In doing taste panels, it is absolutely critical that you test whether or not the people on the panel can determine or detect this flavor defect and screen out those who cannot. It might also be helpful to try to get people to detect differing levels of WOF by mixing freshly cooked meat with that which has had warmed-over flavor at different levels to see if the different levels can be detected by your panel. I'm sure you all are aware of the fact that it is the phospholipids that are primarily involved in WOF development. The triglycerides

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can also be involved but seem to be a little less important.

When serving a sample to a taste panel, it is important to serve the sample hot as it is more difficult (if not in some cases impossible) to detect WOF in cold meat. Although I do think I can probably detect some more severe cases of it in cold samples, it is important for a panel to serve it warm.

I would like to just mention a few studies that illustrate how the taste panel work has been done. We obviously do not have time to cover everything, but I will talk about one of Dr. Pearson's papers. A paper by Fooladi et al. (1979) looked at WOF in beef, pork and chicken in the absence of and presence of nitrite. TBA values and sensory evaluations of warmed-over flavor were determined. Sensory evaluation was done at zero hours and at 48 hours storage at 4° C and they used the three-member trained taste panel. The scoring system ranged from 1 to 5 with 1 representing very pronounced WOF and 5 representing no WOF. I would like to discuss the correlations between the sensory and chemical or TBA scores. For chicken, they found on 32 samples a correlation of −.71, significant at the one percent level; for pork, −.57, also at the one percent level; for beef −.36 correlation, significant at the five percent level. The first two numbers have appeared to give us a pretty good correlation, but with the latter one we are dealing with a pretty low degree of correlation.

I think this points out one of the problems; the variations from one species to another. Throughout all the literature that I have reviewed, there is the underlying problem of our correlation not always being as high as we would like. Obviously, we would like to have the ideal correlation of near perfection, which may not ever be developed. But I think one of the points I'm stressing today is that we have many new chemical methods that are being developed now. I think it is time that we look at these methods with respect to their relationship to each other and also their relationship to the sensory evaluation.

Another study that came from the University of Minnesota was published in the *Journal of Food Processing and Preservation*, Vol 1, 1977. In this study, they tried to explain why, in retorted turkey, you get the inhibition of WOF. They oven-cooked and retorted turkey and subjected these samples to taste panel analysis and also tried to characterize the chemical compounds that might be responsible for inhibition of WOF (at retort temperatures). They used a screened taste panel of eight people. Their evaluations were based on a score of zero = no warmed-over flavor and five = very strong WOF. Results showed that WOF was inhibited at retort temperatures but could develop after cooking rather quickly at the lower oven-cooking temperature. They ascribed the inhibition of WOF to the development of reducing compounds or reductones that were the result of the browning reaction, which agrees with the work of Sato et al. (1973). Einerson and Reneccius (1977), however, were quite critical of the TBA tests, listing a number of interferences that they were able to note.

These and other criticisms of the TBA test led us (Csallany et al., 1984) to develop a method for the direct determination of free MA in meat. It has long been suggested that many compounds interfere with the TBA tests which, as I stated earlier, tend to give you an inflated value for MA. It has not been possible to quantify this degree of inflation of MA by TBA because we didn't have a method for determining free malonaldehyde directly. This is what we set out to do. After about three years of work, we believe we finally have a method to do so. The importance of this method stems in part from the hypothesized toxicity of MA. We used high-performance liquid chromatography (HPLC) with a size exclusion column (TSK 6 1000 pw) and a mobile phase of .1 M sodium phosphate at pH 8. The eluent is monitored at 267 nanometers. The properties of these compounds vary quite a bit. For example, the inner ester is a more volatile compound than the enolate anion. Therefore, depending on the pH of the system, MA can adopt a number of different chemical forms and these must be considered when you're trying to determine it or study its properties.

Table 1. Comparison of HPLC and TBA[a]

	Method		
Sample (N)	*HPLC[b]*	*TBA[b]*	*TBA/HPLC*
Beef (9)			
Mean	0.14	0.44	4.6
S.D.	0.085	0.19	3.0
Pork (9)			
Mean	0.11	0.39	5.3
S.D.	0.06	0.12	2.8

[a]Adapted from Csallany et al. (1984). Anal. Biochem. 142, 277-283.
[b]μg MA/g wet tissue.

One of the things about MA that was found by Pryor and his coworkers here at LSU was that you can generate MA by the heat or acid used in the TBA test from prostaglandin-type endocyclic precursors tending to overestimate the actual amount of MA in the tissue. Our sensitivity is one nanogram per injection, indicating a high degree of sensitivity. It takes about 50 minutes for a run to elute MA, and this is the major disadvantage of this procedure.

In Table 1, some of the data we've collected studying beef, pork and chicken are presented. The ratio of the TBA-determined MA to the HPLC-determined MA shows that at the minimum we have an amplification of two-fold of the MA present by the TBA test and at the worst we find an amplification of 9.2. A great need exists to study the correlation, if any, of free MA by HPLC to sensory score and to other types of rancidity measurements.

Our conclusions from this work were that the TBA method overestimates MA, that we can quantify free MA, and we obviously need further development to relate this to sensory values and to all the other new MA and TBA procedures that are being developed. These comments should not suggest that the TBA method is not useful. Rather, they suggest that some improvements are possible, newer methods should be tested for their degree of correlation to older methods and to sensory scores, and we should be suspicious of data reporting MA content if the TBA test was used.

Additional Comments

A.M. Pearson

Our primary interest in warmed-over flavor goes back to about 1965 when I visited Armour and Co. in Chicago. At that time, Sato and Hegarty were working on warmed-over flavor.

Harold Herring was also involved with them. They convinced me that warmed-over flavor was a real problem and that it was very important in processed meats, particularly in precooked items. They also demonstrated that warmed-over flavor can develop in fresh meats in a very short period of time if you grind it, spread it out in thin layers and expose it to air. Jane Davis Love was the first graduate student we had working in this area. Since that time we have been involved almost continually in warmed-over flavor research. Many people have laughed at us and said we were off-base, but it has become more and more evident that warmed-over flavor is very important, especially as more and more meat products are pre-cooked. This is true in airline foods and in other precooked meat items. It has become even more important in producing restructured products, where it has now created a major problem.

In studying warmed-over flavor, it is very important that one have a trained panel, which must be a selected panel. One person sitting here in this room was very quickly eliminated from our panels, because he preferred warmed-over flavor. I am sure that there are others who are the same way, i.e., they prefer warmed-over flavor, and have become used to it. Perhaps we could reach the stage where that is what consumers want. I hope not, but nevertheless there are people who prefer warmed-over flavor. With that in mind, we must select our panelists. Then we have to train them. Training is difficult, but one must first select a sample with warmed-over flavor and train panelists to recognize it. Then one must screen out those panelists who are not able to detect warmed-over flavor. The general public certainly objects to warmed-over flavor, as evidenced by the term itself. This is a housewife's term. Dr. Margaret Younathan, who spoke this morning, was one of the first scientists to use the term. I don't know how many of you recognize that she was the Tims of Tims and Watts (1958). When she was married she became Margaret Younathan. They recognized the problem and gave it the name "warmed-over flavor."

Another point I wish to make is that one cannot say that they are measuring warmed-over flavor by running TBA numbers unless they can show that there is a significant correlation coefficient between TBA values and panel scores. TBA values alone do not mean anything. Just because you have a high TBA number does not mean that the meat is oxidized. First, I want to point out that malonaldehyde is not a final oxidation product, which is one of the unfortunate things about it.

Two TBA molecules react with 1 mole of malondialdehyde in the presence of heat and water to produce the TBA chromagen. The TBA chromagen which is formed is measured at 532 nm and is a pink or a red pigment. This reaction is the basis of the TBA test. There has been a lot of work on this, with many saying that malonaldehyde is not a good measure of warmed-over flavor. However, it is the best one available at the present time. Dr. Aust, who works in our biochemistry department and is one of the foremost lipid oxidation authorities in the United States, uses the TBA test to measure oxidation.

Dr. Younathan pointed out this morning that there are three steps in oxidation. They are initiation, propagation and termination. We have done a lot of work showing that nonheme iron is the active catalyst of lipid oxidation, but this occurs only during the propagation step. In visiting with Dr. Herring, who used to be in the Armour group and has worked extensively in this area, he indicated that there is convincing evidence to support the fact that nonheme iron is the active catalyst of the propagation step. However, it is not the initiator. I think that's an important thing to remember, i.e., that it is not the initiator, but it plays a part and catalyzes the reaction at the propagation step. Dr. Herring pointed out, and our work has verified this fact, that adding EDTA to the system inhibits lipid oxidation. This means that chelating of the metal ions stops oxidation. Our earlier work has shown that when you heat meat, you break down some of the myoglobin and release nonheme iron from the porphyrin ring, which will then catalyze the oxidation reaction.

We also have a paper that was published recently in the *Journal of Agric. and Food Chemistry*, March/April issue, Vol. 33, page 364, 1985. In it, we examined the TBA-reactive substances in relation to warmed-over flavor in chicken meat. First, we determined that taste panel scores and TBA numbers were closely related, with a correlation coefficient of −.87, which is a good correlation with sensory evaluation. We also showed the relationship between chicken dark and light meat and the scan between 400 and 700 nm. We measured it first while the chicken was still warm and then after heating it again following 3 days storage at 4° C. EDTA prevents this reaction since it is a metal chelator. As little as 1 ppm of nonheme iron can catalyze lipid oxidation and materially increase TBA numbers. Other work from our laboratory has shown that heating breaks down the porphyrin ring of myoglobin and releases nonheme iron, which then catalyzes lipid oxidation. This study shows that somewhere between 82% and 98% of the TBA-reactive substances was due to malondialdehyde at 0 days storage, while about 17% was due to TBA-reactive material absorbing at 450-452 nm. After three days storage of chicken light meat, malondialdehyde accounts for somewhere between 84% and 98% of the TBA-reactive substances. In other words, from 2% to 16% of the TBA-reactive materials absorb at 450-452 nm. Thus, we have good proof that the nonheme iron is the main problem, and that the TBA-reactive substances are mainly accounted for in cooked chicken by malondialdehyde.

Model systems came into some disrepute this morning as well as our work on nonheme iron, but I want to defend them. We have used not only model systems but also meat systems, and find essentially the same thing in meat systems as we find in model systems. One exception was in the study with Steve Aust, where we used a model meat system and a meat pigment extract system. Results did not completely agree. There were some differences in the way some of the additives reacted in those two systems. The paper is in *Food Chemistry* and has just been published.

In closing, I want to say that Joseph Kanner from Israel visited us about a month and a half ago. I think Joseph Kanner is one of the foremost lipid oxidation researchers in the world today. He has shown that the actual propagation step of oxidation in meat takes place because of H_2O_2 reacting with myoglobin. That paper is found in the *Archives of Biochem. and Biophysics*; I think it's the March or April issue of 1985. Anyone interested in oxidation should certainly read Joseph Kanner's paper because he is far ahead of everyone else in regard to lipid oxidation of meat products.

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Discussion

A.W. Kotula: In view of the survey information presented this morning questioning the importance of warmed-over flavor plus the limitations on research funds, how important is warmed-over flavor in cooked products vs rancidity in stored frozen products?

Addis: Both are important. The classical triglyceride oxidation problem causes real problems in Lake Superior lake trout and salmon in several months if the fish are not properly packaged. In the case of warmed-over flavor, it is a tougher problem to solve. Packaging will help, but it is not the total solution. There are some spices that will help and that are acceptable to consumers.

Pearson: As Paul has pointed out, these are two aspects here. In the case of rancidity during frozen storage, it is a mishandling problem. With proper packaging and handling, beef can be stored for a year, pork for six months and chicken and fish for three to six months. With warmed-over flavor, you're talking about something that is much easier to work on from the standpoint of trying to produce it. As far as the meat industry is concerned, warmed-over flavor is the bigger of the two problems and is a difficult one to solve.

H.P. Dupuy: How many members should be on a panel for detecting rancidity?

Addis: In my reviews I've seen as few as three to as many as thirty. Probably the rigorous approaches haven't been taken in this area using statistics to determine the appropriate number of panelists.

B.W. Berry: In dealing with a problem like rancidity, considerable screening of prospective panelists is required due to their variability in detecting rancidity. Some individuals, to a certain degree, prefer a rancid flavor at a low intensity and those people should be screened out or at least identified in the selection process. Once a panel has been assembled that is capable of detecting rancidity at a uniform degree of precision, one has to be careful in how the panel's data is extrapolated to a broad population of consumers, who vary greatly in their abilities to detect rancidity or even know what it is.

J.L. Secrist: We have found at the U.S. Army Natick Labs that it is not a good idea to do your basic work with a large panel. To test out your methodology for correlation purposes, five to six members are sufficient.

T.D. Bidner: How do you set up a 1 to 5 scale for detecting rancidity by smell or taste?

Pearson: We use a scale where 1 = very pronounced warmed-over flavor; 2 = pronounced warmed-over flavor, 3 = moderate warmed-over flavor; 4 = slight warmed-over flavor and 5 = no warmed-over flavor. That is just an arbitrary scale and any other is just as good.

J.M. Regenstein: Has anyone tried to add graded levels of malondialdehyde to meats to ascertain the perception of that compound and at the same time calibrate and train panelists to be more objective?

Pearson: There would be a hard time getting human clearance if it is a carcinogen, as has been claimed.

Regenstein: But aren't you giving panelists the same thing when you give them samples that are rancid or have warmed-over flavor?