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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?

Pearson: No, because it is in the food much of the time. There is a difference between an additive and what's there.

Dupuy: What criteria can be used to screen the raw materials you work with to insure that they are not already undergoing oxidative rancidity?

Pearson: Because changes occur so rapidly, you must use fresh meat; not meat from a retail store but freshly slaughtered meat.

Dupuy: If a person tasted a sample with considerable warmed-over flavor first, would his ability to distinguish various levels of warmed-over flavor be diminished on subsequent samples?

Pearson: I would say my abilities would be diminished and I would assume this would be the case for most people. In some studies I was involved with some years ago with irradiated meats, as long as the samples were presented in an order with increasing amounts of irradiation, panels could discriminate very well; but then after being given samples with higher levels of irradiation, couldn't pick out the lower levels.

A. Gardner-Grebe: Dr. Pearson, you mentioned that the off-flavor detected in chicken is due to malondialdehyde. Is this also true for other meats?

Pearson: We haven't looked at this, so we don't really know.

Addis: I would just comment on the fact that the warmed-over flavor problems that we observe are due to more than just malondialdehyde. These compounds also can react with the TBA reagent to give you color. The point I want to make is that I think we need further studies correlating some of the newer techniques with sensory procedures.

Regenstein: Dr. Pearson, your data would suggest that 80% to 90% of TBA is malondialdehyde, while Dr. Addis, your data would indicate a lower figure. Has anyone looked at these other compounds that might be chemically reactive and see what their spectra is? Is it possible that the 532 nm is not a pure malondialdehyde peak.

Addis: We are not necessarily in disagreement. There are other compounds, other than malondialdehyde, that will react with the TBA reagent.

Pearson: What Paul is saying is that these compounds, while not malondialdehyde, may be measuring oxidation and also absorb at 532 nm.

Regenstein: Perhaps it might be advantageous to call it 532 nm-absorbing material rather than malondialdehyde.

J.C. Acton: I have several questions. My first question: Is there any relationship over time between free fatty acids and eventual TBA numbers?

Addis: In relation to oxidative rancidity, free fatty acids don't tell you much. In the fast-food trade, there is good evidence that with frying oils there is extensive breakdown to what some feel are toxic cholesterol oxides. Thus, we should be looking at a measure of oxidative rancidity and not just free fatty acids.

Acton: Is there any kind of relationship or correlation between initial phospholipid content and the potential for rancidity or warmed-over flavor, whether it is measured sensorially or by TBA tests?

Addis: As was discussed this morning, phosphatidyl ethanolamine is important, but as far as relating it to the susceptibility for rancidity, I don't believe it is viable.

Pearson: There is good evidence that the phospholipids are involved and as you get higher levels, you have more problems. But phospholipid levels are quite constant once you take in the residual tissue. They may vary maybe from 0.5% to 1.0% and, as you can see, are relatively low to begin with.

Acton: Is there any relationship between peroxide values and TBA numbers?

Pearson: Recent work (*Arch. Biochem. Biophys.* 237: 314) shows that the initiation step occurs by hydrogen peroxide interacting with myoglobin. Nonheme iron is the catalyst of oxidation in the propagation step.

Acton: Is this just a displacement of iron to the nonheme form?

Pearson: No, it is the hydrogen peroxide species of myoglobin that is the initiator. Then, during the breakdown of myoglobin, the iron released from the porphyrin ring is the propagator.

Acton: We can look at the reactions and see an initiation and very clearly the propagation phase and at some point the end products being formed and determined by TBA. My question is: Isn't peroxide value a fairly good indication of where this thing got started?

Pearson: I can't tell you about initiation, but it is often advantageous to use more than one method to measure oxidation. One method might be to measure unsaturation and then another, the TBA test. Dr. Kanner and also the people in our biochemistry department feel TBA is the best approach for oxidation and subsequent correlation with sensory scores for rancidity. However, there will be situations where you can have a very rancid product and also a very low TBA number; that happens because it's not an end product.

J.T. Keeton: Have there been any studies done on the chilling rate and how this affects oxidation in meat products, particularly after pre-cooking?

E.D. Aberle: At Purdue we were involved with prerigor vs post-rigor grinding and the pH effect on the reaction. We found that below pH 6 the oxidation reaction goes quickly, perhaps due to a pH effect on the catalytic ability of iron to catalyze the reaction.

Addis: I believe you attributed that to the release of nonheme iron from protein, which then allowed it to catalyze the oxidation reaction.

Aberle: The nonheme iron was partially responsible, but in looking at our data closely, there is not enough difference in the nonheme iron content to account for the marked increase in oxidation rate below pH 6.0.

Pearson: In the model system, you only need 1 ppm change in nonheme iron to get a marked increase in oxidation of nonheme iron.

Aberle: The point is that at pH 6.5 we still had 2 to 3 ppm of nonheme iron. It went up 1 ppm as you dropped pH to 5.8. I don't think that is a large enough change in nonheme iron.

Pearson: When your pH dropped, you get the oxidation. Dark-cutting beef doesn't oxidize, but does develop a definite off-flavor. The high pH of dark-cutting beef blocks oxidation.

R.C. Benedict: I would like to comment on the existence of other methods besides TBA that are important. Oxygen uptake, lipid peroxides, determination of aldehydes, pentane and ethane which are saturated gases formed respectively

from omega 6 and omega 3 fatty acids, and reduction in unsaturation. Malonaldehyde can only be formed if you have more than one double bond, so you can have extensive oxidation of oleic acid and detect it with oxygen uptake or with peroxides, but would get a zero TBA value. Consequently, TBA values are a reflection of oxidation and fatty acid makeup.

Addis: Capillary gas liquid chromatography can be used to separate these compounds.

M.E. Bailey: We have just published a procedure which is a modification of a methodology worked out by Dr. Harold Dupuy and the USDA group in New Orleans. The approach, which measures volatiles by quantitative GC mass spectroscopy, provides not only information on the degree of oxidation, but also the mechanism of the degradation of unsaturated fatty acids to yield these volatile compounds. We believe this particular kind of methodology will be extremely

important in solving what type of mechanisms are responsible for oxidative rancidity.

D.T. Bartholomew: What are the times required to do the TBA test and the HPLC procedure?

Addis: For TBA, it will vary from several hours to 24 hours for procedures with an overnight room incubation. With the HPLC approach, it takes about 50 minutes for just the chromatographic separation. Of course, it takes longer when you prepare the sample. The big problem is that you tie up the HPLC for 50 minutes just for one run.

M.C. Hunt: Are there possible differences in malondialdehyde from different suppliers?

Addis: I think it is useful from time to time to look at the purity of malondialdehyde that you would hydrolyze from tetrahydroxy propane by looking at its extinction coefficient to insure there aren't some contaminants present.