Malonaldehyde, Lipid Oxidation, and the Thiobarbituric Acid Test

Sir: Marcuse and Johansson (JAOCS 50:387 [1973]) have emphasized that the abundant literature on the thiobarbituric acid (TBA) test for lipid oxidation does not make clear what are optimum reaction conditions for the test and what the test is measuring at its two principal absorption maxima (450 and 532 nm). To clarify this issue, I draw attention to some apparently obscure published findings.

Since the lipid will be exposed to heat, light, oxygen, and possibly trace metals during the test, it is reasonable to assume that the test conditions themselves will contribute, in varying degrees, to the results that are obtained. The presence of cupric ion or simply exposure to air can induce 2-enals to generate absorbance at 532 nm, as well as at 450 nm in the test (Patton and Kurtz, J. Dairy Sci. 38:901 [1955]). Thus, for example, the presence of copper or iron proteinates, such as in blood, meat, tissue, etc., could be expected to influence test results. With respect to decadienal, we observed that the freshly synthesized compound gives no absorbance at 532 nm in the TBA test but readily does so on exposure of the compound to air for 48 hr or when tested in the presence of $CuSO_4$ (40 ppm) (Patton, Barnes, and Evans, JAOCS 36:280 [1959]). Consequently, it appears that there are complex relationships existing among the pigment precursors during the test and that enals and dienals can be precursors of both the 450 and 532 nm absorbances. No doubt other classes of lipid oxidation products, e.g., hydroperoxides, also are involved. The value in the TBA test of absorbance at 450 nm in assessing lipid oxidation may be limited. This absorbance is produced in the reaction by aldehydes in general. Not only do fatty aldehydes yield this absorbance but also glyceraldehyde (Patton, Food Res. 25:554 [1960]), hydroxymethylfurfural (Keeney and Bassette, J. Dairy Sci. 42:945 [1959]) and a variety of aromatic aldehydes (Dox and Plaisance, J. Am. Chem. Soc. 38:2164 [1916]). In fact, Keeney and Bassette (loc. cit.) have suggested that the absorption at 450 nm in the TBA test, when used to measure hydroxymethylfurfural (absorption maximum 443 nm), affords a basis for assessing the extent of the browning reaction. Of course, in many food systems and frying oils, fat oxidation and browning are proceeding simultaneously. A further consideration is that reactive aldehydes released from plasmalogen glycerides during the TBA reaction would be a measure of neither fat oxidation nor browning. Marcuse and Johansson (loc. cit.) express some uncertainty as to the origin and character of the pigment absorbing at 532 nm. It has been shown that this pigment from oxidized lipids involves the reaction of a three carbon compound with the reagent (Bernheim, Bernheim, and Wilbur, J. Biol. Chem. 174:257 [1947]. Patton and Kurtz (J. Dairy Sci. 34:669 [1951]) demonstrated that malonaldehyde, a three carbon compound, gives the characteristic absorbance at 532 nm in the test. Sinnhuber and Yu (Food Res. 23:626 [1958]) established that the pigment so formed involves a 2:1 reaction between TBA and malonaldehyde. Patton, Keeney, and Kurtz (JAOCS 28:391 [1951]) showed that oxidizing milk fat contains malonaldehyde. While compounds other than malonaldehyde may yield the essential three carbon structure during pigment formation, so far as I am aware there is no evidence yet that the pigment absorbing at 532 nm in the TBA test may have a variety of structures. Thus, the test appears to be measuring malonaldehyde and structures capable of yielding a closely related three carbon derivative of it during the test reaction.

The TBA test has proven highly sensitive and useful as a method of monitoring lipid oxidation in many systems and under a wide variety of conditions. In light of the complex factors leading to pigment production in the TBA reaction, test results need to be considered with caution and should be compared with organoleptic evaluation or with findings by other suitable chemical tests.

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