Evaluation of 2-Thiobarbituric Acid Reactive Substances (TBRS) in Relation to Warmed-Over Flavor (WOF) Development in Cooked Chicken

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The 2-thiobarbituric acid reactive substances in cooked chicken were evaluated in relation to development of warmed-over flavor. TBA numbers were determined by the distillation method and were shown to be related to absorbance of the TBA complex at 532 nm. TBA numbers were related to sensory scores for warmed-over flavor for cooked chicken white and dark meat with an "r" value of −0.87. Absorbance at 532 nm, which correponds to the malonaldehyde–TBA complex, accounted for an average of 93.3 and 83.0% of the total TBA-reactive substances in the distillates from all cooked chicken white meat samples at 0 and 3 days storage, respectively. Corresponding values in cooked chicken dark meat were 98.5 and 94.0%. Results indicated that the major TBA-reactive substances in the distillate of cooked chicken is malonaldehyde and that its level is indicative of warmed-over flavor.

Malonaldehyde (MA) is found in meat products containing oxidizing unsaturated fatty acids and, in the presence of water, exists mainly as the nonvolatile, bound enolate anion (Kwon and Watts, 1964; Kwon et al., 1965). The reaction of MA with 2-thiobarbituric acid (TBA) has been widely use to quantitate rancidity in foods, since first being related to oxidative changes by Kohn and Liversedge (1944). In fact, the TBA test has become the most widely used method for assessing the extent of oxidative deterioration in muscle foods (Tarladgis et al., 1960; Gray, 1978; Melton, 1983). According to Sinnhuber and Yu (1958) and Tarladgis et al. (1960) the principal reactant is MA, a water soluble substance formed and released upon heating the sample in an acid medium. However, Tarladgis et al. (1964) have demonstrated that MA can also be measured without the acid treatment. Patton and Kurtz (1951) showed that extremely low levels (1 ppm) of malonaldehyde give a distinctly positive TBA test on measuring at 532 nm. They, therefore, postulated that MA results from the oxidation of unsaturated fatty acids as demonstrated by analysis of the red color obtained from oxidized milk fat.

The red pigment obtained in the reaction occurs as a consequence of the condensation of 2 mol of TBA with 1 mol of MA (Sinnhuber and Yu, 1958). The intensity of color is a measure of MA concentration (Tarladgis et al., 1960, 1964) and has been correlated organoleptically with rancidity (Zipser et al., 1964) and with warmed-over flavor (Igene and Pearson, 1979; Igene et al., 1979a). The relationship between MA and rancid odors has been a matter of controversy. Results reported by Tarladgis et al. (1960) and Patton and Kurtz (1951) showed that although MA levels are closely related to the TBA color reaction, it contributes only a part of the total color complex. In addition, other lipid oxidation products may give rise to the color reaction with TBA (Gray, 1978; Melton, 1983). Other workers have shown that other lipid oxidation products, such as alka-2,4-dienals, also react with TBA to form a red complex with the same absorption maximum (532 nm) as the MA–TBA complex (Jacobson et al., 1964; Marcuse and Johansson, 1973). On the other hand, Yamauchi (1972a) working with the distillate from cooked rancid pork suggested that TBRS other than MA decompose under the conditions of the TBA test to produce MA, which then reacts with TBA.

Addition of antioxidants or chelators to muscle foods often significantly retards the extent of lipid oxidation as indicated by lower levels of TBRS or TBA numbers (Igene and Pearson, 1979; Igene et al., 1979a; Pearson et al., 1983; Melton, 1983). The relationship between MA, other carbonyls, and the odors in distillates from pure fatty acids and from meat products, therefore, needs to be investigated thoroughly to assess the utility of the TBA test for estimating rancidity in muscle foods. Thus, the present study was undertaken to determine the levels of TBA-reactive substances (TBRS) including malonaldehyde and to determine their relationship to sensorial perception of warmed-over flavor (WOF) in chicken white and dark meat in the presence or absence of antioxidants chelators.

EXPERIMENTAL SECTION

Source of Meat. The chicken meat samples used in this study were taken from Rhode Island Red cocks raised at the Michigan State University poultry farm and dressed at the poultry processing laboratory. Following electrical stunning and killing by exsanguination, portions of dark (leg) and white (breast) meat were excised from the carcasses without prior scalding and packaged immediately. Thereafter, the meat samples were stored for 24 h, at 4 °C, after which they were frozen and stored at −18 °C until used for the study.

Experimental Treatments. Packages of dark and white meat were thawed overnight at 4 °C in preparation for analysis. Thereafter all visible fat was removed, and the meat cut into pieces, ground once through a $^3/_8$-in. plate, mixed thoroughly, and then reground through a $^3/_{16}$-in. plate. Three experimental treatments were designed to study the development of warmed-over flavor in the dark and white meat: (1) dark meat or white meat plus distilled deionized water (1:1); (2) dark meat or white meat in combination with ethylenediaminetetraacetic acid (EDTA); (3) dark meat or white meat in combination with sodium tripolyphosphate (TP). Each experimental treatment consisted of 50 g of meat that was mixed with 50 mL of distilled deionized H_2O in which the additives were dissolved. The final level of additives in the exper-

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Table I. Mean TBA Numbers and Sensory Scores in Cooked Chicken White Meat and Dark Meat with and without Chelators and/or Antioxidants (±SD)[a]

	0 day storage		3 days storage 4 °C	
treatments	TBA no.[b]	taste panel[c] scores	TBA no.[b]	taste panel[c] score
1 chicken white meat, no chelator/antioxidant	1.25 ± 0.04	3.50 ± 1.12	2.07 ± 0.07	2.33 ± 1.11
2 chicken white meat + 2% EDTA	0.66 ± 0.01	4.0 ± 1.10	0.54 ± 0.10	4.00 ± 0.91
3 chicken white meat + 0.5% TP	0.76 ± 0.02	4.10 ± 0.66	0.66 ± 0.07	4.08 ± 0.76
4 chicken dark meat, no chelator/antioxidant	1.42 ± 0.03	3.37 ± 1.22	2.41 ± 0.26	2.42 ± 0.95
5 chicken dark meat + 2% EDTA	0.74 ± 0.03	4.25 ± 0.66	0.70 ± 0.07	3.33 ± 1.11
6 chicken dark meat + 0.5% TP	1.00 ± 0.22	4.5 ± 1.00	1.29 ± 0.03	3.25 ± 1.0

[a] Experimental treatments were replicated twice. [b] TBA numbers are mg of malonaldehyde per 1000 g of meat. [c] Taste panel scores are means for 10 trained panelists based on the following scoring system: 1 = very pronounced WOF; 2 = pronounced WOF; 3 = moderate WOF; 4 = slight WOF; 5 = no WOF.

imental treatments consisted of 2% EDTA or 0.5% TP.

Cooking and TBA Analysis. The treated samples were placed in unsealed retortable pouches, heated in a boiling water bath to an internal temperature of 80 °C, then cooled, and thoroughly mixed. At 0 day and again after 3 days storage at 4 °C, the treated samples were assessed by the 2-thiobarbituric acid (TBA) test for malonaldehyde with a water–TBA reagent blank (Tarladgis et al., 1960). Results are expressed as mg of malonaldehyde/kg of tissue (TBA numbers). To determine the nature of the TBRS in the distillates, the TBA–MA complex obtained by the TBA test (Tarladgis et al., 1960) was analyzed with a recording Beckman spectrophotometer (Model 24). The absorption spectrum of the TBRS complex was scanned and recorded from 700 to 400 nm. Peak areas of MA and TBRS were calculated quantitatively as the product of peak height × width at half height with shoulders not being included. Results were expressed as a percentage of total area.

Taste Panel Evaluation. Sensory evaluation for warmed-over flavor (WOF) was carried out by 10 trained panelists immediately after cooking of the samples (0 day) and again after 3 days storage of the cooked samples at 4 °C. At each setting, all panelists were presented with six different coded samples representing the different treatments. All experimental samples were reheated to 80 °C and served while hot. The panel scoring system was as follows: 1 = very pronounced WOF; 2 = pronounced WOF; 3 = moderate WOF; 4 = slight WOF; 5 = no WOF as previously described by Igene et al. (1979a).

Statistical Analysis. Means, standard errors, and correlation coefficients were calculated between mean values for TBA numbers and panel scores by using methods outlined by Gill (1978).

RESULTS AND DISCUSSION

Relationship between TBA Values and Panel Scores for WOF. Figure 1 presents a plot of TBA numbers against panel scores and demonstrates that the relationship is linear. The correlation coefficient between these two measurements was −0.87, which was statistically significant ($P < 0.01$). This shows that the TBA values were closely related to WOF panel scores and indicates that changes in TBA numbers account for over 75% of the variation in WOF. The regression curve, where $a = 4.734$ and $b = -1.012$, was also statistically significant ($P < 0.01$) and demonstrates that the slope of the curve is significantly different from zero, thus showing that TBA values give a good estimate of WOF.

Changes in TBA Values and Panel Scores for Chicken White Meat. Mean TBA numbers and the corresponding mean taste panel scores for cooked chicken white meat are presented in Table I. For chicken white meat at 0 day, treatment 1, which contained no chelators, had the highest TBA values and the lowest taste panel

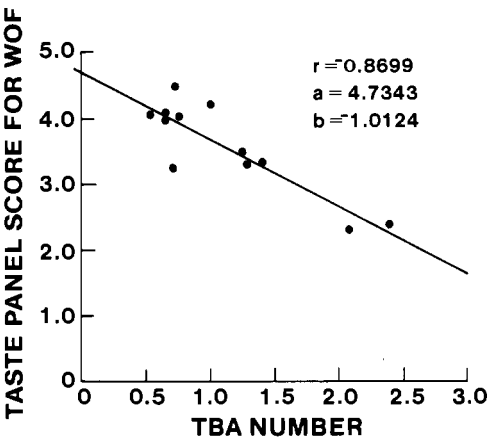

Figure 1. Relationship between panel scores and TBA numbers.

scores. Treatments 2 and 3, which contained EDTA and tripolyphosphate, had about 50% lower TBA values than treatment 1 and were consistently rated higher by the panelists. At 3 days following storage at 4 °C, the TBA values for treatment 1 had approximately doubled with a corresponding further increase in off-flavor development, which was described as "pronounced" WOF by the taste panel. On the other hand, lipid oxidation (TBA numbers) remained low and relatively unchanged in treatments 2 and 3 after 3 days storage, which indicated that EDTA and TP, respectively, were effective in blocking WOF development.

The low TBA values exhibited by treatments 2 and 3 were closely related to the higher taste panel scores, which were described as having "slight" WOF. These results emphasize the importance of using antioxidants to protect cooked meats against off-flavor development, which is in agreement with other studies (Tims and Watts, 1958; Yamauchi, 1972b; Igene et al., 1979a).

Changes in TBA Values and Panel Scores for Chicken Dark Meat. Lipid oxidation in cooked chicken dark meeat is shown in Table I, and closely follows the pattern observed in the case of chicken white meat. However, the magnitude of lipid oxidation, as indicated by higher TBA values and the corresponding lower taste panel scores, was generally greater in the chicken dark meat than in the white meat, irrespective of the treatments and length of storage. The results of this study are supported by previous studies in relation to the behavior of poultry meat in cooked model systems (Igene and Pearson, 1979) and in cooked meat during frozen storage (Igene et al., 1980).

The antioxidants seemed to be more effective in cooked chicken white meat than in cooked chicken dark meat, especially in relation to taste panel scores. This is to be expected since chicken dark meat contains a greater proportion of total lipid and especially of phospholipids than chicken white meat (Peng and Dugan, 1965; Acosta et al.,

Table II. Mean Absorption Spectra of TBA-Reactive Substances in the Distillates of Cooked Chicken Samples as Percent of Total Spectrum[a]

treatments	0 day storage		3 days storage at 4 °C	
	substance(s) absorbing at 532 nm, %	substance(s) absorbing at 450–452 nm, %	substance(s) absorbing at 532 nm, %	substance(s) absorbing at 450–452 nm, %
1 chicken white meat, no chelator/antioxidant	82.5	17.5	84.0	16.0
2 chicken white meat + 2% EDTA	100.0	0	86.0	14.0
3 chicken white meat + 0.5% TP	97.5	2.5	79.0	21.0
av, %	93.3	6.70	83.0	17.0
4 chicken dark meat, no chelator/antioxidant	98.5	1.5	96.0	4.0
5 chicken dark meat + 2% EDTA	97	3.0	88.0	12.0
6 chicken dark meat + 0.5% TP	100	0	98.0	2.0
av, %	98.5	1.5	94.0	6.0

[a] Values are means of two replicate determinations.

1966; Igene et al., 1980) and therefore, possesses greater susceptibility to lipid oxidation (Igene et al., 1979b). Another factor in the greater susceptibility of chicken dark meat to lipid oxidation may be its higher content of nonheme iron, which has been demonstrated to serve as a catalyst of lipid oxidation in cooked meats (Igene et al., 1979a; 1985). Regardless of the cause of oxidation, results of this study are in agreement with our previous work (Igene et al., 1979a,b) which has emphasized that although MA and/or TBRS can be used to follow lipid oxidation in muscle foods, sensory evaluation is also a necessary step in studying warmed-over flavor in meats.

Analysis of the TBA-Reactive Substance in Cooked Meat Distillates. In analyzing the TBRS in oxidizing cooked chicken products spectral measurements of the visible wavelength (400–700 nm) were made to determine the absorbance of TBRS in the chromogen following determination of the TBA numbers at 532 nm wavelength (Tarladgis et al., 1960). The mean absorption spectra of TBRS in the distillates of cooked chicken samples are presented in Table II. In cooked chicken white meat, which did not contain antioxidants or chelators (treatment 1), the mean spectral concentration of substances measured at 532 nm and at 450–452 nm had changed only slightly. The higher TBA numbers observed for the meat stored for 3 days (Table I) was verified by the characteristic spectral curves (Figure 2).

When EDTA was added, as in treatment 2 at 0 day, all of the TBA-reactive substances were accounted for by absorption measured at 532 nm (100%). Following the 3 day storage period at 4 °C for the cooked meat, the TBRS absorbing at 532 nm had decreased in concentration to 86%, which is verified by the lower TBA number of 0.54 as shown in Table I. When 0.5% tripolyphosphate was used, the TBRS were 98% at 532 nm at 0 day and 79% after 3 days storage (Table II). The reduction in the concentration of TBRS at 532 nm after 3 days storage is reflected by lowering of the TBA number from 0.76 at 0 day to 0.66 after 3 days storage (Table I).

As shown in Table II the chicken dark meat behaved in a similar pattern to the chicken white meat in regard to the TBRS. However, the chicken dark meat treatments exhibited higher concentrations of TBRS measured at 532 nm than chicken white meat treatments, both at 0 day and at 3 days storage. This is illustrated by the characteristic absorption spectra presented in Figure 2. Although the absorption spectra for chicken white meat are not presented, they are similar to those shown in Figure 2. The higher concentrations of TBRS observed for chicken dark meat is in good agreement with the corresponding TBA values for these treatments (Table I).

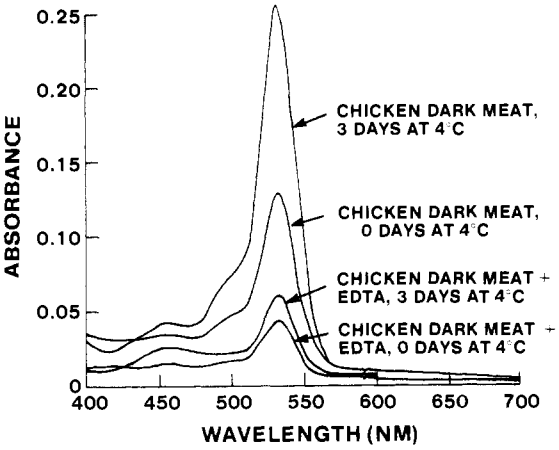

Figure 2. Characteristic absorption spectra for cooked chicken dark meat distillates. Similar plots were obtained by using other antioxidants and/or chelators.

There is a concensus in the literature that MA gives a distinctly positive TBA test on measuring at 532 nm (Patton and Kurtz, 1951; Sinnhuber et al., 1958; Yamauchi, 1972a; Gray, 1978; Melton, 1983). In effect, the distillation method extracts MA, which is considered substantive TBRS and is measured at 532–535 nm (Tarladgis et al., 1960, 1964; Yamauchi, 1972a).

The results obtained in this study demonstrate that the major TBRS in the distillate of cooked chicken probably is MA, although other TBRS may give similar absorption peaks at 532 nm. However, it is clear that most of the TBRS are found at 532 nm. For instance, the average contribution of MA to all TBRS in cooked chicken white meat at 0 day was 93.3%, which decreased to 83.0% after 3 days storage at 4 °C. On the other hand, in cooked chicken dark meat the corresponding levels of MA were 98.5% and 94.0% at 0 and 3 days storage, respectively. Results of this study are, therefore, supportive of the work by Yamauchi (1972a) who reported that MA comprised 99.2% of the TBRS in cooked rancid pork. On the basis of spectral measurements, the present study suggests that the reaction of MA with TBA reagent is largely responsible for the pigment measured at 532 nm, and that MA levels probably reflect the degree of rancid flavor development in cooked chicken meat which was indicated in this study by lower panel scores.

The relationship between TBA numbers and the development of undesirable flavor characteristics in muscle foods is certainly not an easy one. Apart from the red

pigment, which is usually measured at 532 nm during the TBA test, other pigments have been observed to form, notably a yellow pigment, which has a maximum absorbance at 450 nm. A low level concentration (2–18%) of this pigment was observed in most of the treatments of this study after 3 days storage. The absorption peak at 450–452 nm appeared to have increased after 3 days storage at 4 °C, with a consequent lowering of the level of absorbance at 532 nm, which in some cases indeed reflects lowered TBA numbers (Table I).

MA as a secondary product of lipid oxidation does not always result in increased TBA numbers in stored muscle foods. In fact, TBA values have been observed to decline during storage of cooked meat and fishery product (Tarladgis and Watts, 1960) and also during the frozen storage of raw and cooked meat (Benedict et al., 1975; Igene et al., 1979b). The decreasing levels of TBA numbers in stored muscle foods are due to the reaction of malonaldehyde with proteins according to Buttkus (1967). Marcuse and Johansson (1973) have associated the absorbance of the TBA chromogen at 450 nm as indicative of rancidity as is the value at 532 nm. Of course the latter is generally taken as the characteristic TBA–malonaldehyde complex, and the results of the current study (Tables I and II) seem to confirm the relevance of the absorbance at 532 nm. Since the use of TBA test has often been improperly employed and as MA may be involved in other physicochemical interactions, in evaluation of rancidity in foods the test should be accompanied frequently by corresponding sensory evaluations with a trained sensory panel.

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