# VALIDITY OF THE 2-THIOBARBITURIC ACID (TBA) TEST FOR THE EVALUATION OF OXIDATIVE RANCIDITY IN CURED MEAT PRODUCTS

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## INTRODUCTION

The role of sodium nitrite in preservation of meat and development of specific "cured flavour" dates back to antiquity. More specifically, strong antioxidative effect of nitrite has been shown to be responsible for the elimination of "warmed-over" flavour in cured meat products and in retarding the development of oxidative rancidity in processed meats.

The extent of rancidity development in meats and other fatty foods is commonly monitored by the 2-thiobarbituric acid (TBA) test, using the distillation method of Tarladgis et al. (1960). The distilled malonaldehyde (Figure 1) is reacted with the 2-thiobarbituric acid reagent and the absorption intensity of the coloured chromogen produced is measured at 532 nm.

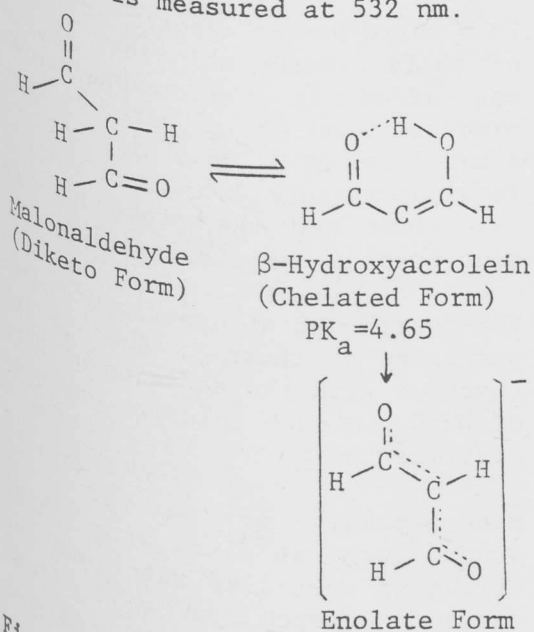


Figure 1. Tautomeric forms of malonaldehyde.

The complete structure of the crystalline adduct from the reaction of the 2-thiobarbituric acid (TBA) and malonaldehyde has recently been determined (Nair and Turner, 1984). It was concluded that while two spectrally equivalent tautomeric structures were present (Figure 2), variations in the concentrations of solution and the presence of trace contaminants may cause prototropic shifts to favour equilibrating structures similar to those given but bearing 3 hydroxyl and 2 amide hydrogens.

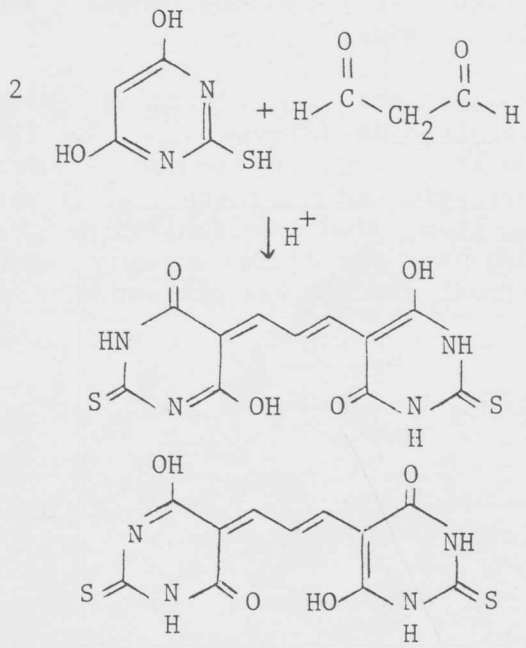

**Figure 2.** Tautomeric structures of 2:1 adduct of TBA and malonaldehyde.

For nitrite-cured meat products, the TBA test has been modified by Zipser and Watts (1962). These authors added sulfanilamide to cured meats prior to distillation in order to inhibit the reaction of malonaldehyde with the residual nitrite. A diazonium salt was produced and thus, they concluded that malonaldehyde present may be determined accurately (Figure 3). Recently Shahidi et al. (1985) reported that in the absence of residual nitrite, addition of sulfanilamide brings about its own

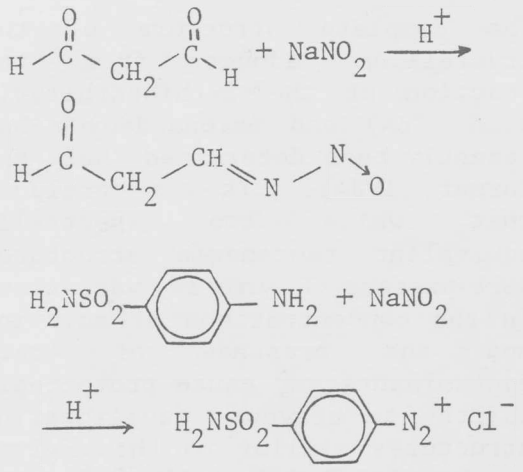

**Figure 3.** Reactions of sodium nitrite with malonaldehyde and sulfanilamide.

complications by reacting with malonaldehyde (Figure 4). The TBA values so obtained were underestimated and therefore, it was suggested that sulfanilamide be added to cured meats only when residual nitrite was present.

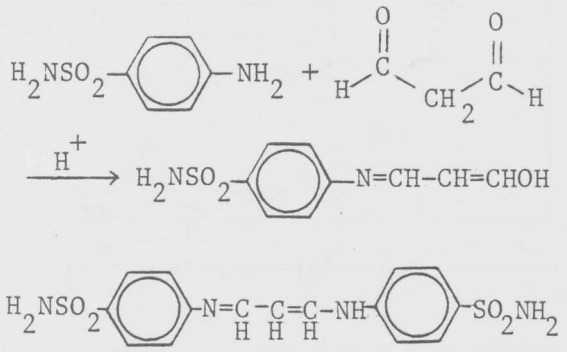

**Figure 4.** Reaction of sulfanilamide with malonaldehyde.

In this paper, interaction of sodium nitrite and sulfanilamide with malonaldehyde will be examined in order to study the validity of the 2-thiobarbituric acid test in cured meats.

## MATERIALS AND METHODS

### Materials

All chemicals used in this study were reagent-grade commercial products and were purchased from Fisher, Sigma or Aldrich Chemical Companies. They were used without any further purification.

Fresh loin pork, obtained from Newfoundland Farm Products, was trimmed of all of its surface fat and was ground twice using a 0.79 cm and then a 0.48 cm plate.

### Methods

The distillation method of Tarladgis et al. (1960) as modified by Shahidi et al. (1987a) or by Zipser and Watts (1962) was used in this study.

### Model Systems

A stock solution of malonaldehyde precursor namely 1,1,3,3-tetramethoxypropane (TMP) at a concentration of 0.220 mg/ml was used. Between 0 and 1 ml of this solution was added to enough distilled water in a 500 ml round bottom flask to make a total volume of 97.5 ml. To this solution 2.5 ml of 4N HCl was added and the mixture was distilled to collect 50 ml of distillate. An aliquot of the distillate was reacted with TBA reagent and the absorbance of the coloured complex so obtained was read at 532 nm.

In a second set of experiments 5 mg of $NaNO_2$ was added to each of the above solutions and the procedure was continued as given above.

In a third set of experiments, 2 ml of sulfanilamide solution (10 mg) was added to the malonaldehyde precursor and enough water to make a total volume of 98.5 ml solution. To this mixture 1.5 ml of 4N HCl was added and the procedure was continued as described earlier.

The fourth set of experiments were performed as those in the 3rd set together with the addition of 5 mg of $NaNO_2$ to each solution prior to distillation.

### Meat Systems

Ground pork was mixed with 20% by weight of distilled water and was cooked, as such, to an internal temperature of 75±1°C for nearly 45 minutes.

To 10 g of the cooked meat, different amounts of malonaldehyde (up to 0.5 ml of 0.2124 mg/ml solution) was added and the mixture was distilled off as described previously (Shahidi et al., 1987a). The absorbance units were corrected for that due to the meat itself.

In another set of experiments, ground meat was mixed with 20% water and 150 ppm of sodium nitrite and was then cooked as described above. The distillation procedure was followed without the addition of sulfanilamide.

## RESULTS AND DISCUSSION

Table 1 summarizes the absorbance units of the thiobarbituric acid - malonaldehyde (TBA-MA) complex at 532 nm for model systems. Results indicate that addition of 5 mg or more of sodium nitrite to malonaldehyde precursor eliminated the complex formation between TBA reagent and malonaldehyde. This indicates that malonaldehyde had reacted about entirely with the added sodium nitrite. Addition of sulfanilamide (SA) to the above system, prior to distillation, eliminated the reaction between nitrite and MA almost completely and the absorbance units, although lower, were close to those of the control.

Addition of sulfanilamide to the malonaldehyde precursor had its own implications. Prior to the addition of TBA reagent, a bright fluorescent yellow colour was observed, thus, indicating the formation of condensation products of an amino-iminopropene structure. Addition of TBA reagent to this mixture, unlike when nitrite was present, resulted in colour development. Therefore, it is conceivable that either the reaction between SA and MA was incomplete or that the product so obtained was unstable and in the presence of TBA reagent gave rise to the formation of TBA-MA complex. At any rate, results so obtained indicate depressed absorbances of the complex as compared to those of

**Table 1.** Effect of Nitrite and Sulfanilamide (SA) on the Absorbance of 2-thiobarbituric acid - malonaldehyde (TBA-MA) complex at 532 nm.

| Malonaldehyde (MA) | Malonaldehyde + Additives[a] | | | |
|---|---|---|---|---|
| Weight, mg | No Additive | $NaNO_2$ | SA | $NaNO_2$ + SA |
| 0.044 | 0.62 | 0.00 | 0.58 | 0.57 |
| 0.088 | 1.23 | 0.00 | 1.00 | 1.07 |
| 0.132 | 1.87 | 0.01 | 1.50 | 1.77 |
| 0.176 | 2.38 | 0.01 | 2.00 | 2.09 |
| 0.220 | 2.98 | 0.01 | 2.60 | 2.73 |

[a]Sodium nitrite and sulfanilamide were added to meat at 5 and 10 mg levels, respectively.

the controls. Similar results were noted when sulfanilamide was added to meat samples which did not contain nitrite, as reported previously (Shahidi et al., 1985) and as shown in Figure 5. In the latter case, a contribution from the reaction of the other TBA reactive substances such as 2,4-alkadienals with the TBA reagent to the TBA-MA absorption at 532 nm may be expected (Kosugi et al., 1988).

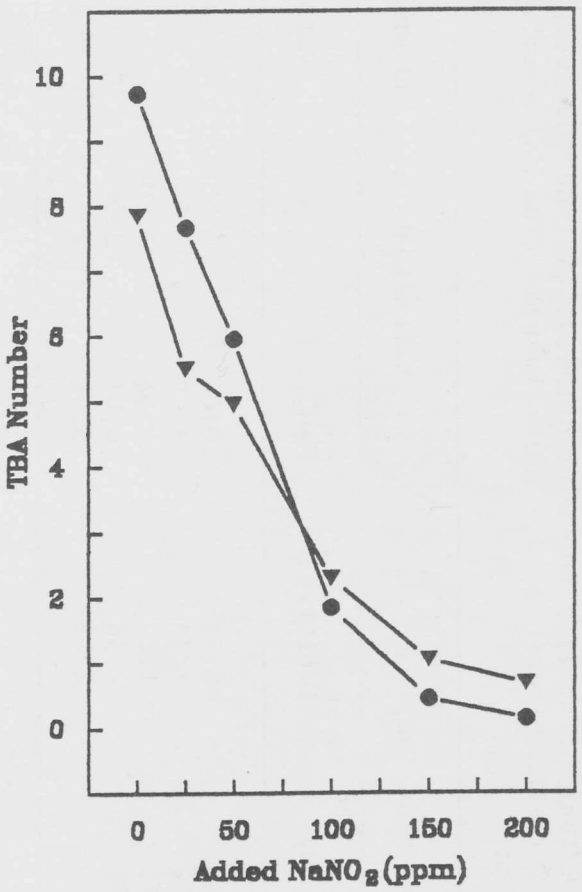


Figure 5. TBA numbers of nitrite-cured meats with, ●——● ; and without, ▼——▼ ; sulfanilamide addition.

In order to convert the absorbance units to TBA numbers, generally malonaldehyde or one of its precursors such as TMP is added to meat and the absorbance units due to the added MA is related to the original amount of malonaldehyde. Absorbance increase versus the concentration of added MA to meat is given in Figure 6. From this figure, a value of 8.1 may be calculated for the conversion of the absorbance units to TBA numbers (mg malonaldehyde equivalents per kg sample). This value was significantly larger (10.8) when meat cured with 150 ppm of sodium nitrite was used. This again indicates that part of the added malonaldehyde was reacted with the residual nitrite (about 40-50 ppm) present. When sulfanilamide was present, the conversion value was reduced to about 8.8.

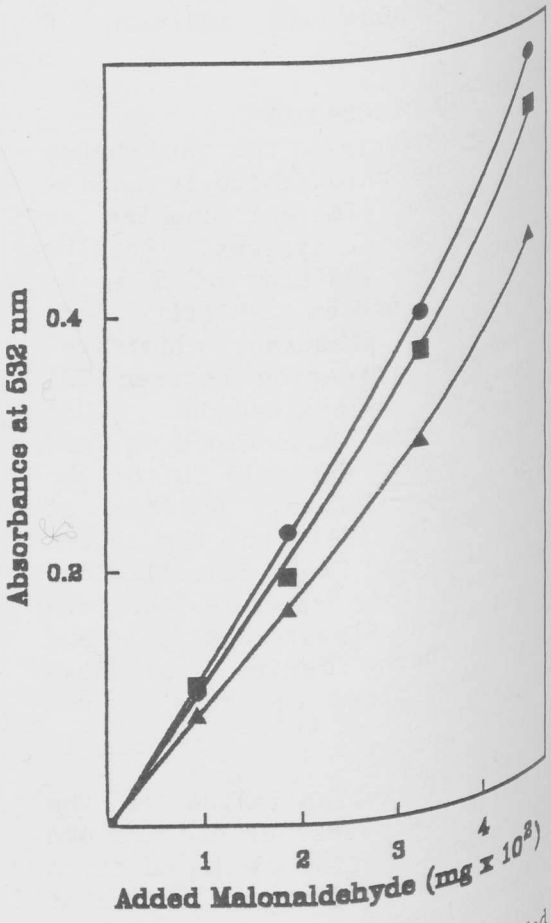


Figure 6. Effect of added malonaldehyde on the absorbance of TBA-MA complex at 532 nm. Cooked-uncured meat, ●——● ; nitrite-cured meat, ▲——▲ ; and nitrite-cured meat with added sulfanilamide, ■——■ .

## CONCLUSIONS

This study together with our previous findings (Shahidi et al., 1985) indicate that TBA methodology is of limited usefulness in determination of the extent of

rancidity development in cured meats. Therefore, as we have suggested elsewhere (Shahidi et al., 1987), the content of hexanal, a predominant volatile of cooked meats, may be used as an indicator of quality for the evaluation of oxidative rancidity in cured meat products.

**ACKNOWLEDGEMENTS**

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