# A COMPARISON OF TWO THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS) METHODS APPLIED TO AGED BEEF EVALUATION

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## I. INTRODUCTION

The rate of lipid oxidation in meat and processed meat products is often evaluated using a thiobarbituric acid reactive substance (TBARS) assay. In practice, this assay has also been used to provide insight into eating quality and shelf-life characteristics of beef. Furthermore, TBARS limits for consumer acceptance of a fresh beef product have been defined. Campo et al. [1], for example, reports levels ≥ 2.28 as indicative of unacceptability; McKenna et al. [2] instead considered levels > 1.00 as unacceptable; and Hughes et al. [3] found TBARS levels up to 2.60 as still acceptable to consumers. Each of these studies, however, used a unique TBARS method and this suggests that protocol selection can influence interpretation of lipid oxidation in food using this approach – an observation supported by past research [4]. Consequently, the direct comparisons between TBARS values derived using different methods merits caution, unless their comparability is validated. This study investigated two different 'in-house' methods of TBARS determination for unprocessed, aged beef samples to evaluate their potential for comparison.

## II. MATERIALS AND METHODS

From the boning room of an Australian abattoir, a total of 40 beef *M. longissimus lumborum* (LL) were selected at random. These were divided into eight equal portions, which were vacuum-packaged and randomly assigned to one of 72 temperature-time combinations (TTC; $n$ = 4 per TTC) – albeit balanced by portion location. These included four temperature settings (3 °C, 5 °C, 7 °C and control) and six time intervals (T1-T5 and control) applied so that TTC had constant temperature throughout a time interval, and only one variation, at most, in temperature was permitted within their assigned total time period. The control TTC refers to a total of 32 LL portions, held in duplicate temperature control units for 14 d at approximately 1 °C to replicate commercial practice. At the completion of their TTC, sample sections were removed and frozen at – 25 °C to be analysed for TBARS concentration. These then acted as the source for samples tested herein, using each of the following methods:

**Method 1**: Adapted from Hopkins et al. [5], approximately 100 mg samples were homogenised, using micro-tube pestles, with 500.0 µL radioimmunoprecipitation assay (RIPA) buffer (no. 10010263, RIPA buffer concentrate, Cayman Chemicals™ Ltd., Michigan, USA). These were centrifuged and the supernatant absorbance measured at 532 nm, using a benchtop spectrophotometer and in accordance to OXI-tek TBARS Assay Kit Technical Bulletin (no. ALX-850-287-KI01, Enzo® Life Sciences Inc., New York, USA).

**Method 2**: The Witte et al. [6] protocol was used, wherein approximately 10 g of samples were homogenised with 30 mL of chilled extracting buffer that contained 20% trichloroacetic acid and 2 M phosphoric acid. Further 30 mL chilled water was added, homogenised for 15 seconds and the contents were filtered through Whatman No. 1 filter paper. Two mL of filtrate (in duplicate) was mixed with 2 mL of 2-thiobarbituric acid (5 millimolar). Sample solutions were held overnight, under darkness and at room temperature, before absorbance were measured at 532 nm using a benchtop spectrophotometer.

For both methods, the averages of technical duplicates are given and expressed results as mg malondialdehyde (MDA) per kg LL (Table 1). Data were evaluated in Genstat (18th Edition, VSN International Ltd., www.vsni.co.uk) using a linear mixed model with method fitted as a fixed effect; and LL, portion, TTC and their interactions fitted as random effects. Level of significance was set at $P < 0.05$.

Table 1 Summary data for beef samples categorised by method used for TBARS determination

| | *n* | Mean | Range | Median | SD |
|---|---|---|---|---|---|
| TBARS Method 1 | 314 | 1.10 | 0.02 - 2.55 | 1.13 | 0.50 |
| TBARS Method 2 | 314 | 1.44 | 0.04 - 10.72 | 0.59 | 2.00 |

## III. RESULTS AND DISCUSSION

Figure 1 shows the mean TBARS content was highest (P<0.05) when samples were analysed using Method 2. This outcome could have resulted from sample preparation and storage, the use of different extraction reagents, time of incubation and/or mixing procedural differences – which have previously been shown to prompt disparity between TBARS methods [4, 6]. If we consider the beef tested, which include samples held within TTC of 7 °C and up to 12 d, it would be reasonable to expect high levels of lipid oxidation and this to be reflected in the TBARS values (Table 1). It is therefore interesting that only Method 2 reported TBARS values > 2.55 – and yet, this is not to say there was not a relative distribution within the range observed with Method 1 (Table 1). Kerth and Rowe [4] made a similar observation when comparing an 'old' and 'new' method, in that both methods demonstrated a decline in TBARS as percentage grass-fed mince increased within high-oxygen modified atmospheric packaging – but, a more severe relative change was evident for the 'old' method. That said, we can still see that if inappropriately compared to the existing limits for consumer acceptability, there are important implications on the accuracy of any interpretation. This could be overcome with the development of consumer benchmarks based on other TBARS methods or the potentially more practical option of understanding the relationship (conversion) between TBARS method data.

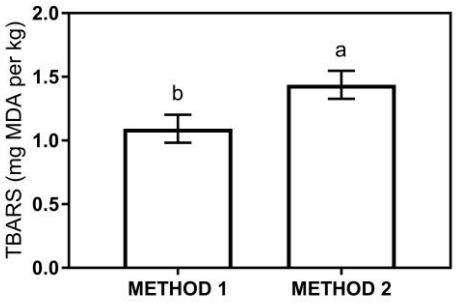


Figure 1. Predicted mean TBARS content (± standard error) for aged beef evaluated using each of two TBARS assay methods

## IV. CONCLUSION

These findings show that there is a need to consider TBARS method selection to avoid misinterpretation.


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