Revisiting the thiobarbituric acid reactive substances (TBARS) assay to measure antioxidant activity in a lipid system

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Introduction

- The TBARS assay is one of few methods used to measure the effectiveness of an antioxidant in a lipid system [1].
- Antioxidants (AH) inhibit lipid oxidation and % inhibition can be measured using the TBARS assay [2].
- However, the TBARS assay was reported to be highly variable [3].
- This study investigated the sources of variability including the lipid substrate, different concentrations of Trolox and the order of addition of the reagents.

Methods

- TBARS assay: Linoleic acid (LA) was oxidised for 20 h with Cu²⁺, with or without antioxidant at 37 °C followed by 10 min boiling with thiobarbituric acid (TBA). The pink coloured TBARS adducts were extracted in butanol and measured at 532 nm (Fig-1).
- Different batches of LA were investigated.
- 250, 1000, 2500 and 5000 µM Trolox (AH) were used to inhibit lipid oxidation and the decrease in TBARS formation was measured (Fig-2).
- Different orders of addition of reagents of LA, control solvent/antioxidant and metal ion were conducted.
- Reproducibility was assessed by coefficient of variance (%CV).

Results and Discussion

- Different batches of LA without antioxidant gave different absorbance values (Fig-3).
- Within a batch of LA, different Trolox concentrations gave different %CV, with highest %CV at 1000 µM (Fig-4).
- LA, control solvent/ Trolox and metal ion gave the most reproducible results (Fig-5).

Conclusion

- Variability of the TBARS assay is due to the LA substrate, concentration of Trolox used, and the order of addition of the reagents.
- Further research is being conducted to understand this variability so that the TBARS assay can be used to reliably assess antioxidant activity in a lipid system.

References


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