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Improved sensitivity for determining thiobarbituric acid reactive substances in ground beef☆

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ABSTRACT

To create expected differences in oxidation ground beef samples from grass-fed and grain-fed animals were utilized in six differing percentages with 4 different packaging types. Percentages of grass-fed and grain-fed ground beef (GB) consisted of 100% grain fed GB; 80% grain-fed: 20% grass-fed GB; 60% grain-fed: 40% grass-fed GB; 40% grain-fed: 60% grass-fed GB; 20% grain-fed: 80% grass-fed GB; and 100% grass-fed GB. Packaging treatments included: high oxygen (HO; 80% O_2: 20% CO_2), low oxygen (LO; 65% N_2: 35% CO_2), carbon monoxide (CO; 65% N_2: 34.6% CO_2: 0.4% CO), and overwrap (OV; polyvinyl chloride film wrapped over a styrofoam tray). The modified TBARS method showed greater sensitivity and increased differences between treatments with less variability. The original extraction method showed fewer differences between treatments with greater variability. Data suggest that the modified method of TBARS determination could provide researchers with a better assay to find differences while decreasing the amount of labor.

1. Introduction

The Thiobarbituric Acid Reactive Substances (TBARS) assay has been used for years to identify lipid oxidation in meat samples. The procedure can become labor and time intensive for researchers performing the methods. The procedure can take many hours if there are many samples to analyze.

In an effort to minimize labor and time intensiveness, the objective was to modify a method that was still accurate. Measurements of absorbencies among TBARS assays vary depending upon the method used. Different wavelengths have been used in assays; Chae, Keeton, and Smith (2004) used 530 nm, Buege and Aust (1978) used 532 nm and Jimenez-Villarreal, Pohlman, Johnson, Brown, and Baublits (2003) used 533 nm. After some preliminary research, it was found that absorbances could be read at 540 nm, with some loss in absorbancy, but was justified due to the same approximate loss across concentrations. Therefore, it was determined that the use of an incubator/shaker for incubation of the samples for the reaction and use of a plate reader with only a 540 nm filter could be implemented.

The number of assays available to researchers for TBARS is unknown, but many have inherent problems. Different problems can be, but not limited to: extraction methods, labor intensity, amount and types of chemicals needed and time intensity. Depending on the assay examined, the incubation period can be as long as 18 h (Daniel, Dikeman, Arnett, & Hunt, 2009) or as short as 20 min (Jimenez-Villarreal et al., 2003). Obtaining an assay that can be less time intensive, requires less reagents and offer high precision and sensitivity is a high priority.

The meat sources utilized in this study were ground beef from grain-fed animals and grass-fed animals. Different titrations as well as different packaging types were used to allow for wide variation among the samples. The use of these samples allowed for samples with high amounts of oxidation to be compared to samples with low amounts. This will allow for a greater comparison of methods.

Therefore, the following experiment was designed with the following objectives: 1) modify the TBARS procedure to be less time and labor intensive; 2) modification of the TBARS procedure with the same amount or better sensitivity and precision than the current procedure being used in our lab.

2. Materials and methods

2.1. Materials and solutions

Chemicals used were trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), propyl gallate (PG), and ethylenediaminetetraacetic acid

☆ Practical Application: This modified method decreases the amount of labor and time compared to the older method that was used. The new modified method also showed increased sensitivity which will help food and meat scientist in the determination of product oxidation.

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(EDTA) and tetraethoxypropane (TEP). All reagents were of analytical grade. Ground beef was used as the meat source for the analysis. All analyses were performed on fresh, uncooked meat samples. Solutions were prepared according to Wang, Page, Dessal, Bovell-Benjamin, and Phillips (2002) with 7.5% TCA (w/v), 0.1% EDTA (w/v) and 0.1% PG (w/v); reaction solution was 80 mM TBA. All were mixed with double distilled water to dilute and mix solutions.

2.2. Malonaldehyde stock solution

Malonaldehyde stock solutions were made from modified procedures of Shlafer and Shepard (1984), and Wang et al. (2002). A stock solution was made by adding a sufficient quantity of TEP to double distilled water to obtain 1 mM in an aqueous solution, and was stored at 4 °C in the absence of light. On the day of analysis, stock solution was diluted to 80 nM/mL to make standards.

2.3. Standards and standard curve

Standards (0, 2, 4, 6, 8, 10, 20, 30 nM/mL) were made by using 80 nM/mL dilution and diluting each of the standards to their respective concentration. The absorbencies for each dilution were then used to calculate the standard curve for the meat samples. Standards were run on each plate and the standards curve generated was used on the respective samples from each plate. Nanomoles per mL were then converted to mg/kg for comparison of methods.

2.4. Samples and sample preparation

Ground beef was utilized as the source of meat. The ground beef had six different percentages of grain-fed and grass-fed beef. Mixtures consisted of 100% grain fed GB; 80% grain-fed, 20% grass-fed GB; 60% grain-fed, 40% grass-fed GB; 40% grain-fed, 60% grass-fed GB; 20% grain-fed, 80% grass-fed GB; and 100% grass-fed GB. Additionally, four different packaging treatments were utilized. Packaging treatments included: high oxygen (HO; 80% O_2: 20% CO_2), low oxygen (LO; 65% N_2: 35% CO_2), carbon monoxide (CO; 65% N_2: 34.6% CO_2: 0.4% CO), and overwrap (OV; polyvinyl chloride film wrapped over a #2S Styrofoam tray). Modified atmosphere packages were sealed with their respective gases using a Koch ILPRA Model FoodPack (400 V/G; Corso Pavia, Italy). The HO, LO, and CO treatments were packaged in #3 × 1.6 trays and sealed with Cryovac T7225B Laminate (Duncan, SC) film with an oxygen transmission rate of 0.3 cm^3/645 cm^2/d.

Three 454 g ground beef samples for each packaging type by ground beef titration were used. The entire process was then replicated the next day for a total of 6 samples per packaging type by ground beef titration. Independent ground beef batches served as the replicate in the statistical model for each TBARS method. After packaging, samples were placed in dark storage at 2 °C for 5 days. Packages were then placed in a simulated retail display case for 5 days at 2 °C with the illumination intensity of 800 lx at the surface of the package, utilizing Sylvania© Designer Cool White Plus bulbs (F40/DCWP).

Five gram meat samples were placed in 50 mL centrifuge tubes with 15 mL TCA extraction solution. Samples were then homogenized using a Kinematica Polytron blender (Kinematica, Inc., Bohemia, NY, USA) with a variable speed attachment at speed 3.5 for 30 s. Samples were centrifuged at 6000 × *g* for 5 min and then filtered through No. 4 Whatman paper into 16 mL glass tubes.

2.5. New TBARS method

Samples were loaded in 96 well plates with each well having a capacity of 300 μL. Equal amounts of sample (125 μL) and TBA were added to each well. Standards and sample were run on each plate in triplicate. Plates were then incubated for 130 min in a VWR microplate incubator and shaker (VWR International, LLC., West Chester, PA, USA) with the speed of the shaker set at 100 and temperature at 40 °C. After incubation, plates were read at 540 nm using a Thermo Multiscan EX (Thermo Fisher Scientific, Waltham, MA, USA) microplate reader. The average of the three samples was taken and values of the TBARS were calculated using the standard curve regression equation for each plate.

Table 1
Analysis of variance tables for transformed (log_{10}) data for the old (Buege & Aust, 1978) and new TBARS methods.

Old method

analysis of variance

source	DF	Sum of squares	Mean square	F ratio	Prob > F
Model	23	8.997539	0.391197	1.3708	0.1399
Error	118	33.675752	0.285388		
C. Total	141	42.673291			

Effect tests

source	DF	Sum of squares	F ratio	Prob > F
Packaging	3	4.151379	4.8488	0.0032
%Grass-fed GB	5	2.2840309	1.6007	0.1652
Packaging * %Grass-fed GB	15	2.3385707	0.5463	0.9089

New method

analysis of variance

source	DF	Sum of squares	Mean square	F ratio	Prob > F
Model	23	13.579911	0.590431	13.3978	<0.0001
Error	120	5.288317	0.044069		
C. total	143	18.868228			

Effect tests

source	DF	Sum of squares	F ratio	Prob > F
Packaging	3	10.992102	83.1425	<.0001
%Grass-fed GB	5	2.161995	9.8118	<.0001
Packaging * %Grass-fed GB	15	0.425813	0.6442	0.8329