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A Rapid Method to Evaluate Oxidation Capacity of Fresh Beef

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Summary

A method to determine the capacity of beef to oxidize was developed by spectrophotometrically measuring the formation of conjugated dienes after inducing oxidation. The assay was tested by comparing the oxidation capacity and oxidation products (2-thiobabutaric acid reactive substances) of beef stored in a cooler with various packaging types. There was an inverse relationship between oxidation capacity and oxidation products. As oxidation increases, the oxidation capacity of samples declines. This method of measuring oxidation capacity provides useful information without having to wait for oxidation to occur.

Introduction

Oxidation is the primary cause of color and flavor deterioration in beef during storage. This reduction in color stability reduces shelf life in the retail case. Lipid oxidation is influenced by total fat content, especially polyunsaturated fatty acids (PUFA), oxygen exposure, and the presence or absence of antioxidants. The most common method of evaluating oxidation in meat is measurement of 2-thiobarbituric acid reactive substances (TBARS) or malonaldehyde, an intermediate byproduct in lipid oxidation. Unfortunately, this method quantifies oxidation after it happens or after beef oxidizes. The first objective of the current study was to develop a rapid and easy method to predict total oxidation potential of fresh beef before oxidation occurs naturally. The method was based on the spectrophotometric measurement of conjugated diene hydroperoxide production from PUFA in beef by *in vitro* induction of lipid oxidation with copper. The second objective of the study was to assess results from the new method against oxidation changes measured by TBARS for ground, intact and vacuum-packaged beef during cooler storage.

Procedure

Experiment 1

Nine different solvents (n-Propanol, Hexane, Dimethyl sulfoxide, Ethanol, Methanol, Chloroform, 2-Propanol, Tween 20 and Triton X 100) at different concentrations were tested for lipid solubility and interference with absorbance at 234 nm in phosphate-buffered saline (PBS), pH 7.4 with 0.15 M NaCl. The highest fat solubility and least interference with absorbance at 234 nm in PBS (pH 7.4) were observed for 20% 2-propanol; therefore, it was selected as the solvent for the meat extraction.

Samples from three different top blade (m. *Infraspinatus)* muscles of beef were evaluated. A powdered sample (1 g) was dissolved in 10 mL of 20% 2-propanol in 0.1 N PBS, vortexed for 1 minute and centrifuged at 2000 × g for 5 minutes at 39°F. Then, 1 mL of the supernatant was dissolved in 9 mL of 20% 2-propanol in 0.1 N PBS. Absorbances of the sample were taken at 234 nm, and the spectrophotometer was set to zero using the initial reading of each sample. Oxidation of samples was continuously measured spectrophotometrically every 2 minutes by monitoring conjugated diene formation catalyzed by addition of 50 µL of 0.005 M $CuSO_4$ at 99°F. The developed method was validated by monitoring *in vitro* oxidation of different concentrations of commercially available PUFA (0.2, 0.4, 0.6 and 0.8 g of linoleic acid/L). Each sample was tested in triplicate.

Experiment 2

Three beef eye-of-round steaks (m. *Semitendinosus*) were purchased from a fresh beef retail market in Lincoln, Neb. Each steak was cut into three equal-weight pieces. Each piece was randomly assigned to one of three treatments (retail overwrap as ground or whole; vacuum-packaged as a whole piece). All of the treated samples were stored in the cold at 32 $\pm$ 36°F for 21 days. A 10 g sample of each piece was removed on day 0, 3, 7, 14 and 21 of storage and tested for conjugated diene formation using the assay developed in experiment 1 and TBARS using the 2-thiobarbuteric acid reactive substance assay.

An analysis of variance (ANOVA) using the GLIMMIX procedure of SAS (version 9.1, Cary, N.C., 2002) was used to analyze the data. Significant means of each treatment indicated by ANOVA were separated using LSMEANS, DIFF and LINES functions while simple effects of interactions were evaluated by using the LSMEANS, SLICE and SLICEDIFF functions at $P \leq 0.05$ significance. Correlation between conjugated diene formation and TBARS values of beef stored at cooler were analyzed by PROC CORR and PROC REG functions of SAS.

Results

Experiment 1

Of all solvents tested, 20% 2-proponol had the highest fat solubility and least interference with the absorbance at 234 nm in PBS, pH 7.4, and therefore was selected as the solvent for the meat extraction (data not shown). The time course for oxidation of beef muscle extract showed three consecutive phases, a lag phase (up to 2 minutes), during which diene absorbance increased slowly, a propagation phase (up to 6 minutes), during which dienes absorbance increased

rapidly, and, finally, a plateau or decomposition phase (Figure 1). All the replicates of each muscle showed similar magnitudes of absorbance throughout the diene formation. The assay was validated by monitoring *in vitro* oxidation of different concentrations of PUFA (0.2, 0.4, 0.6 and 0.8 g of linoleic acid/L). The pattern of diene formation increased with the increased concentration of linoleic acid (Figure 2). The new technique revealed that total time required to predict oxidation potential was 20 minutes, since there was no significant difference in absorbance beyond 20 minutes ($P = 0.28$). Therefore, absorbance taken at 20 minutes after incubation at 99°F with $CuSO_4$ was considered the maximum production of conjugated dienes in a beef sample, and that amount was used as the dependent variable to compare treatments in consecutive experiment 2.

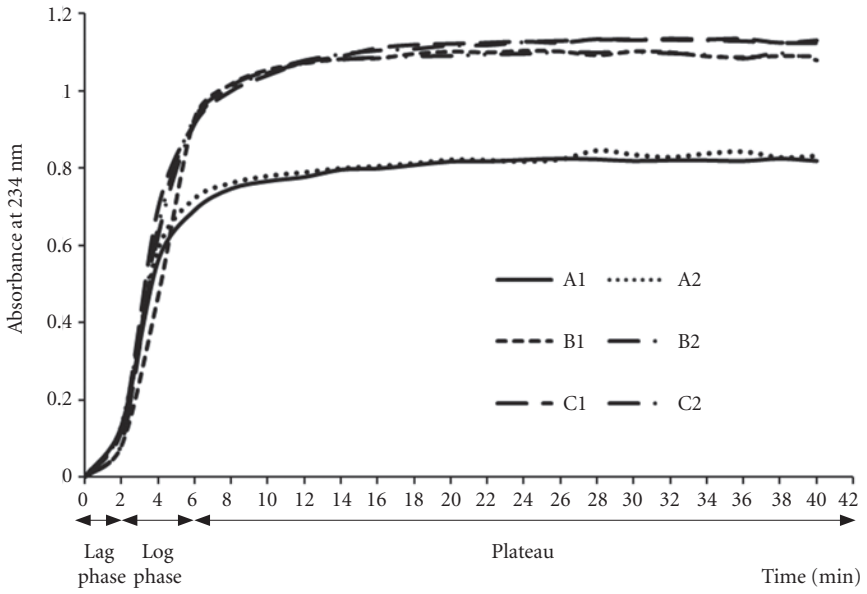

Figure 1. Continuous production of conjugated dienes of three (A, B and C) top blades (m. *Infraspinatus*) of beef in duplicates.

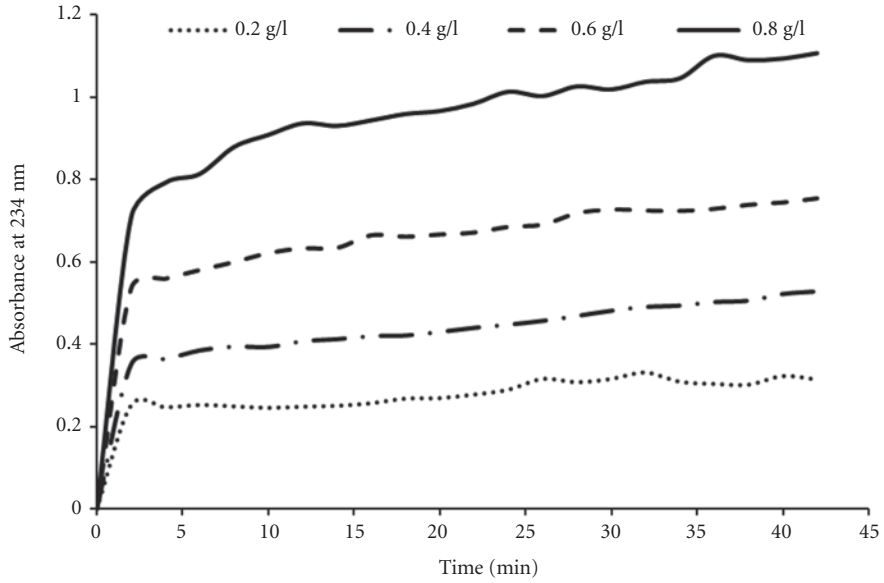

Figure 2. Conjugated diene formation of 0.2, 0.4, 0.6 and 0.8 g of linoleic acid/L.

Experiment 2

There was a significant interaction between sample type and day of storage. Oxidation was greatest with cell membrane destruction (grinding) and least when exposure to oxygen was minimized (vacuum packaging). Therefore, oxidation capacity or conjugated diene formation decreased gradually ($P = < 0.001$) in all treatments during cold storage, indicating that oxidation occurred (Figure 3). The order of magnitude of reduction in oxidation capacity was ground, whole and then vacuum-packaged beef. A high reduction in oxidation potential of ground beef during cold storage was due to maximized exposure of PUFA in cell membranes to proxidants as a consequence of grinding. Therefore, there were significant ($P \leq 0.05$) reductions in oxidation capacity of ground beef at each level of cold storage except storage at days 3 and 7. In whole muscle, oxidation capacity at day 0 was significantly ($P \leq 0.05$) higher than that at days 3, 7, 14 and 21, since PUFA located on the surface of the beef piece more easily reacted with oxygen than did PUFA located inside the beef piece. Vacuum packaging prevented exposure of PUFA to proxidants (oxygen) and therefore oxidation potential of vacuum-packaged beef slowly decreased during cold storage.

An important concept with this new method of measuring oxidation capacity is that an increase in sample oxidation results in a decrease in subsequent oxidation capacity. Thus, the gradual decrease in oxidation capacity for all treatments during cooler storage is indicative that oxidation occurred. The greatest reduction in oxidation capacity, and thus the most extensive oxidation, was observed in ground samples over time, followed by whole muscles that were wrapped in oxygen-permeable film, and then by vacuum-packaged samples.

The TBARS of ground and whole beef increased with time of cold storage ($P \leq 0.05$), whereas TBARS of vacuum-packaged beef did not significantly ($P > 0.05$) change during the

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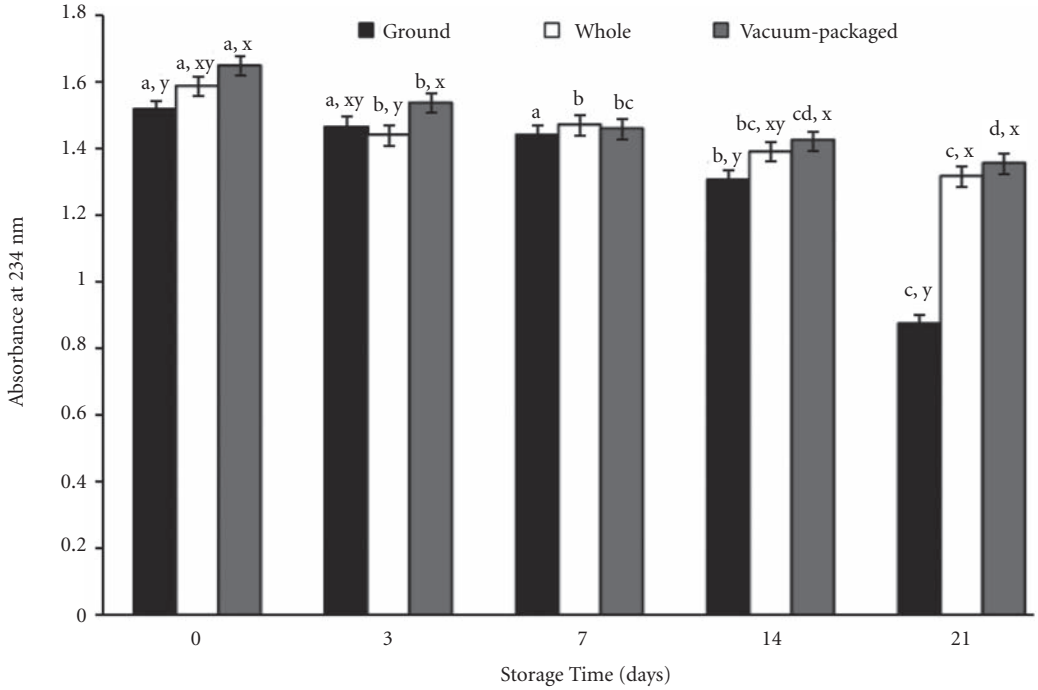
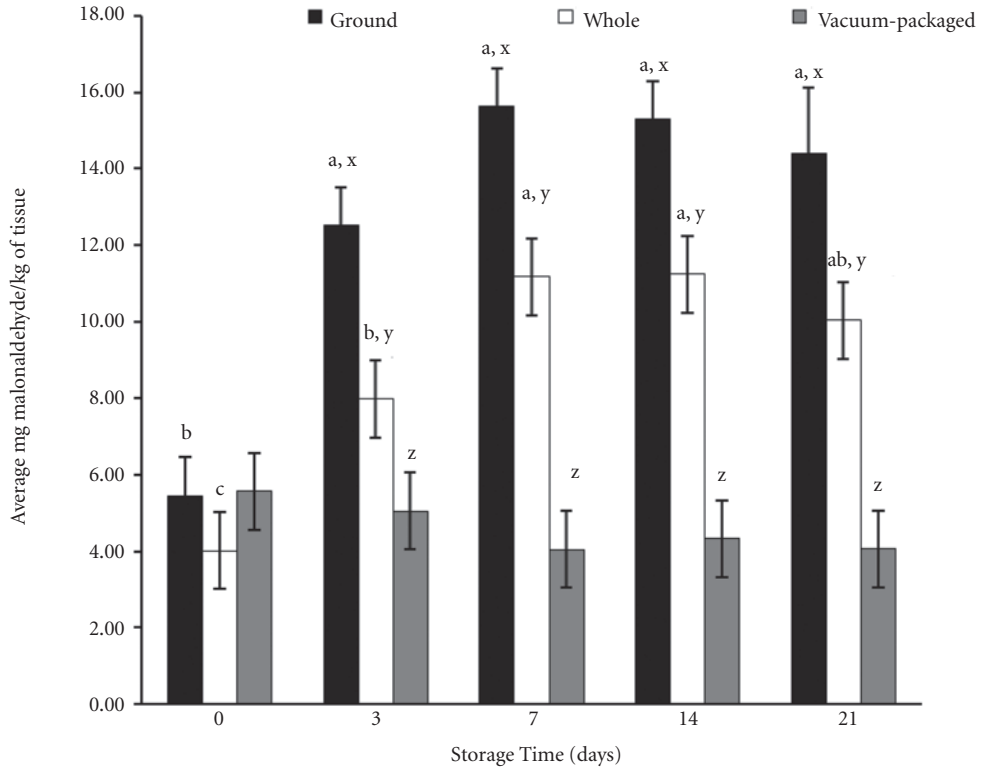


[a-d]Means within type of beef stored with different superscripts are significantly different ($P \leq 0.05$).
[x-y]Means within time point with different superscripts are significantly different ($P \leq 0.05$).

Figure 3. Conjugated diene formation of ground, whole and vacuum-packaged beef eye of round (m. *Semitendinosus*) at cold storage for 0, 3, 7, 14 and 21 days.



[a-b]Means within each type of beef stored with different superscripts are significantly different ($P \leq 0.05$).
[x-z]Means of storage type of beef within each time point with different superscripts are significantly different ($P \leq 0.05$).

Figure 4. Mean TBARS values (mg of Malonaldehyde/kg of tissue) of ground, whole and vacuum-packaged beef eye of round (m. *Semitendinosus*) at cold storage for 0, 3, 7, 14 and 21 days.

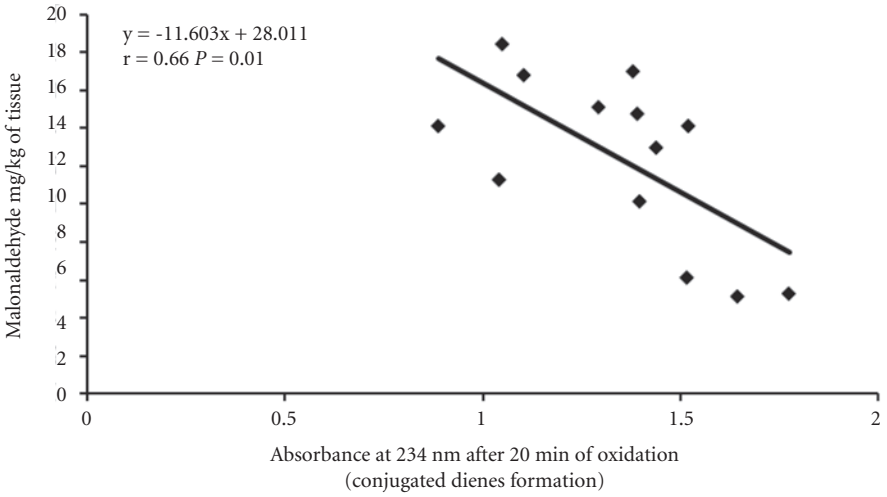


Figure 5. Relationship between TBARS production to conjugated dienes formation during 0, 3, 7, 14 and 21 days of cold storage of ground beef eye of round (m. *Semitendinosus*).

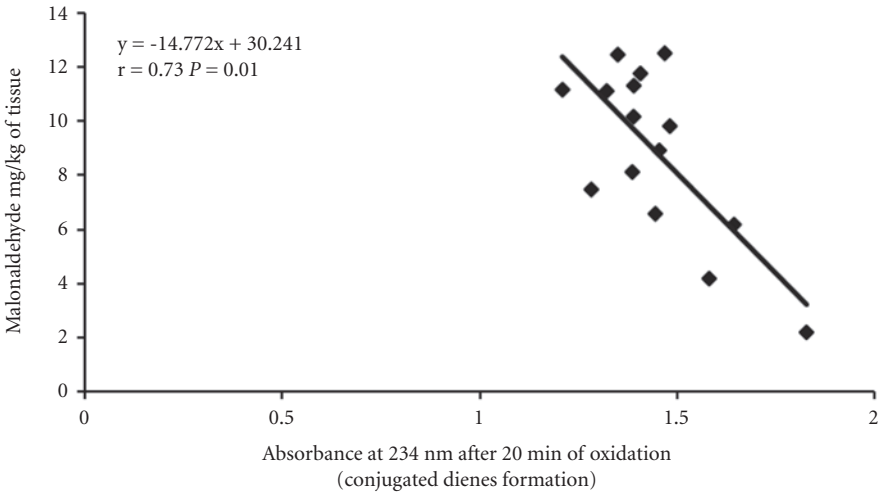


Figure 6. Relationship between TBARS production to conjugated dienes formation during 0, 3, 7, 14 and 21 days of cold storage for whole piece beef eye of round (m. *Semitendinosus*).

storage period (Figure 4). The TBARS values after storage were highest for ground beef and lowest for vacuum-packaged beef ($P \leq 0.05$). Therefore, there were significant negative linear relationships between oxidation capacity and TBARS in ground ($P = 0.014$) and whole ($P = 0.002$) beef during cold storage (Figures 5 and 6, respectively). The correlation coefficient (r) between conjugated dienes formed at 20 minutes and the TBARS of ground and whole pieces of beef eye of round during cold storage for 0, 3, 7, 14 and 21 days were 0.62 and 0.70, respectively. However, we were unable to see any significant linear correlation between conjugated diene formation and TBARS of vacuum-packaged beef stored at cold storage at different days.

Therefore, the new technique reveals that the oxidation capacity of beef decreases during cold storage and the reduction in oxidation capacity is concomitant with an increase in TBARS. Thus, this method of measuring oxidation capacity provides useful information without having to wait for oxidation to occur.

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