Measuring Oxidative Stability of Beef Muscles Obtained from Animals Supplemented with Vitamin E Using Conventional and Derivative Spectrophotometry

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ABSTRACT: The measurement of oxidative stability of beef muscle, from animals fed either control or supplemented vitamin E, during refrigerated storage using both conventional and derivative spectrophotometry was examined. Lipid oxidation measured over a 7-d period using both methods was lower in all muscles from animals fed the higher level of supplementation. Area under the curve of MDA-TBA values obtained by derivative analysis were 68 to 80% lower in all muscles than the corresponding TBARS values obtained using conventional spectrophotometry. Overall, both methods resulted in similar patterns of oxidation. It was concluded that both methods were equally as effective in measuring the extent of lipid oxidation in beef muscle during refrigerated display.

Key words: oxidative stability, conventional and derivative spectrophotometry

Introduction

SENSORY PROPERTIES OF MEAT HAVE AN important influence on the purchasing behavior of consumers (Dirinck and others 1996). Consequently, the prevention of lipid oxidation, which can lead to a dramatic reduction in sensory appeal, is of major concern to the meat industry. It is now well established that α-tocopherol (vitamin E) acts as a powerful lipid-soluble antioxidant in cell membranes (Morrissey and others 1994), hence, one of the main purposes of dietary supplementation of animal diets with vitamin E is to delay lipid oxidation in muscle foods (Liu and others 1995). The improvement in oxidative stability of beef muscle from animals fed supplemented vitamin E has been widely reported (Mitsumoto and others 1993, Liu and others 1994 and 1996, Lanari and others 1994 and 1995, Sanders and others 1997). Traditionally, studies investigating the extent of lipid oxidation in food systems have relied on the classical 2-thiobarbituric acid (TBA) test, with single wavelength spectrophotometry, to quantify the amount of malonaldehyde (MDA) at an absorption maximum between 532 and 538 nm (Sinnhuber and others 1958, Tarladgis and others 1960, Espinosa-Mansilla and others 1993). It is now widely accepted that the TBA test is intrinsically nonspecific for MDA. Nonlipid-derived materials, as well as fatty peroxide-derived peroxidation products other than MDA, are TBA-positive (Janero 1990) and can yield a TBA-test product which is spectrophotometrically, chromatographically, and structurally indistinguishable from genuine 1:2 MDA-TBA adduct (Buttkus and Bose 1971, Kosugi and others 1987).

To eliminate or reduce interference, other workers have adopted the use of derivative analysis for resolving spectra, sharpening peaks, and eliminating unwanted background interference (Sánchez and others 199, Vianna-Soares and Martins 1995, Hargis and others 1996). Wen and others (1997) measured the oxidative stability of gluteo biceps obtained from pigs fed a-tocopherol acetate (30, 200, or 1000 mg/kg diet) by both conventional and derivative spectrophotometry. They concluded that the use of spectrophotometry in the derivative mode resulted in elimination of interfering compounds to a large degree, thus giving a more accurate representation of the degree of lipid oxidation in porcine muscle. A similar improvement in results has also been reported for broiler muscle (O'Neill and others 1999). These authors reported that MDA-TBA values were lower than TBARS values during storage. To date, however, the use of derivative spectrophotometry in measurement of the oxidative stability of beef muscle remains uninvestigated.

The aim of the present study was to compare the use of conventional (single wavelength) and first derivative spectrophotometry in measuring the extent of lipid oxidation in raw beef muscle, from animals fed control or supplemental levels of vitamin E, during refrigerated storage at 4 °C under fluorescent light.

Materials and Methods

Reagents

All chemicals used were "AnalaR" grade obtained from BDH Chemicals Ltd. (Poole, Dorset, England) and Rathburn Chemicals Ltd. (Walkerburn, Scotland). α-Tocopherol acetate used in the diets was obtained from Roche Products Ltd. (Welwyn Garden City, Hertfordshire, England). 1,1,3,3-Tetraethoxypropane was purchased from Sigma Chemical Co. Ltd. (Poole, Dorset, England).

Animals and diets

Friesian × Black Hereford steers were divided into two groups. Group 1 (n = 4) was overwintered indoors and fed a basal concentrate diet containing 20 mg α-tocopherol acetate/head/day for 135 d (E20) prior to slaughter. Group 2 (n = 4), also overwintered indoors, was fed a supplemented concentrate diet containing 3000 mg α-tocopherol acetate/head/day (E3000) for the same period of time. Both groups received silage and water freely. Following slaughter, carcasses were centrally split and chilled at 2 °C for 14 d.

On the day of collection, the muscles rhomboideus (RB), semimembranosus (SM), and semitendinosus (ST) were removed and duplicate muscle cores (2.5 × 5 × 4 cm, 50 g approx.) were vacuum-packaged, using a Webomatic type D463 (Werner Bonk, Bochum, Germany) vacuum packer, and stored at –20 °C for vitamin E analysis. The vacuum packaging material consisted of Cryovac® film (W. R.

Grace Europe Inc., Lausanne, Switzerland) characterized as 45 $cm^3/m^2/24$ h at STP.

For analysis of oxidative stability, duplicate muscle samples were sliced into 2.5-cm-thick samples, and 2.5 × 10 × 6 cm portions were cut from these and placed on polystyrene/EVOH/polyethylene trays. Samples were subsequently overwrapped with oxygen-permeable (permeability to 0_2, 6000-8000 $mL/m^2/24$ h at STP) polyvinylchloride film (Wrap Film Systems Ltd., Telford, Shropshire, England) for aerobic packaging. Samples were then stored in a retail display cabinet at 4 °C under fluorescent lighting (616 Lux).

Determination of a-tocopherol

α-Tocopherol was extracted from muscle following the method of Buttriss and Diplock (1984) and determined by reverse-phase HPLC as described by Sheehy and others (1991).

Oxidative stability of raw beef

The oxidative stability of raw beef during refrigerated display at 4 °C was determined on days 1, 3, 5, and 7 following the 2-thiobarbituric acid distillation method of Tarladgis and others (1960) as modified by Ke and others (1977). TBARS were expressed as mg malonaldehyde/kg muscle. After conventional TBARS measurement, first derivative spectral analysis was carried out as outlined by Wen and others (1997).

Preparation of standard curves for first derivative spectrophotometry

1, 1, 3, 3-Tetraethoxypropane (TEP) (0.066 g) was weighed into a 1-L volumetric flask and diluted to volume with distilled water to produce a 3 10^{-4} M stock solution. This solution was refrigerated (4 °C). A 3 10^{-5} M working solution was then prepared by diluting 10 mL of the stock solution to 100 mL with distilled water. Aliquots of 0, 0.33, 0.66, 1.0, 1.33, 1.67, and 2.0 mL of working solution were accurately pipetted into 10-mL volumetric flasks and distilled water was added to volume. Five mL was pipetted into screw-capped test tubes containing 5 mL of TBA reagent (0.02 M TBA in 10 % glacial acetic acid) and the tubes were placed in boiling water for 15 min and subsequently cooled to room temperature. The absorbances of the standards were determined by wavelength scan between 480 and 580 nm, and first derivative analysis was carried out. A calibration curve was constructed by plotting the distance between peak and trough of the first derivative spectrum against the concentration of each of the working standard solutions. The concentration of MDA-TBA in samples was determined by using slope and intercept data of the computed least-squares fit of the calibration curve and expressed as mg of MDA-TBA/kg meat after accounting for dilution factors and the average percentage recovery of MDA.

Table 1-Mean (s.e.) α-tocopherol concentration (mg/kg) of muscle from crossbred cattle fed control (E20; n = 4) and supplemented (E3000; n = 4) α-tocopherol acetate.

	Muscle					
	SM		ST		RB	
Diet	Mean	s.e.	Mean	s.e.	Mean	s.e.
E20	1.4[a]	0.40	1.5[a]	0.31	1.7[a]	0.18
E3000	3.9[b]	0.49	3.9[b]	0.35	4.8[b]	0.43

[a,b]Means, within a column, without a common subscript differ significantly (P<0.05).

Recovery of malonaldehyde in distillates

The recovery of malonaldehyde from the distillation procedure was determined as follows. Ten mL of TEP solution (3 10^{-5} M) was transferred to a 500 mL distillation flask with distilled water (90 mL), 10% aqueous disodium EDTA (1 mL) and propyl gallate (about 0.5 g). The pH was adjusted to 1.5 using 4 N HCL (2 mL); 0.1 mL silicone antifoaming agent (BDH Chemicals Ltd.) was added and the solution subsequently analyzed following the conventional TBARS method of Tarladgis and others (1960) as modified by Ke and others (1977). A second 10-mL aliquot of the TEP solution (3 10^{-5} M) was diluted to 50 mL with distilled water and 5 mL of this solution was also mixed with 5 mL TBA reagent in a glass-stoppered tube and placed in a boiling water bath for 35 min. The tubes were cooled in cold water for 10 min and the absorbances of the distilled and undistilled samples were read at 538 nm against the reagent blank. The percentage recovery of MDA during distillation was calculated as follows: Absorbance of distilled TEP solution 100, divided by absorbance of undistilled TEP solution. The average recovery was 89.5%. Values from both the conventional TBARS method and the first derivative method were then adjusted for recovery.

Statistical analysis

All analyses were performed in duplicate. One-way analysis of variance (ANOVA) and best-fit regression analysis were carried out using SPSS v 6.1 (SPSS Inc., Chicago, USA).

Results & Discussion

α-Tocopherol concentration of muscle

The effect of dietary supplementation on the α-tocopherol concentration of beef SM, ST, and RB can be seen in Table 1. It is evident that the higher level of supplementation (E3000) resulted in a significantly higher α-tocopherol content in all three muscles compared with the corresponding values for the control values. RB had a significantly ($P < 0.05$) higher α-tocopherol content than both SM and ST. No other significant ($P > 0.05$) differences were found.

These results are in agreement with previous studies (Arnold and others 1992, 1993; Mitsumoto and others 1995; Sanders and others 1997) that reported higher α-tocopherol concentrations in muscles from cattle fed supplemental α-tocopherol acetate. There are no clear explanations as to why skeletal muscles differ in α-tocopherol concentration (Liu and others 1995). Porter and Palade (1957) reported differences between muscles that contained different proportions of red and white muscle fibers and showed that red fibers contained more but smaller mitochondria than white fibers. Smaller muscle fibers and reduced mitochondrial sizes should, in theory, provide greater surface areas with increased biological membrane volume, thereby providing more interactive sites and membrane surfaces for α-tocopherol incorporation. Another contributing factor may be the differences in the rate of lipolysis between muscles as reported for chicken thigh (higher) and breast (lower) muscles (De Winne and Dirinck 1996). Factors such as rates of oxidative stress, higher concentrations of phospholipids, polyunsaturated fatty acids and prooxidants (iron in particular) in meat may also account for differences in α-tocopherol levels between different meat types (Wen and others 1997).

Oxidative stability

The time course of oxidative stability of SM, ST, and RB measured using conventional (single wavelength) spectrophotometry can be seen in Fig. 1. TBARS values from SM obtained from cattle fed the higher level of α-tocopherol acetate (E3000) were significantly ($P < 0.05$) lower on days 3 and 5 than in the equivalent muscles obtained from animals fed the control (E20) diet. No significant differ-

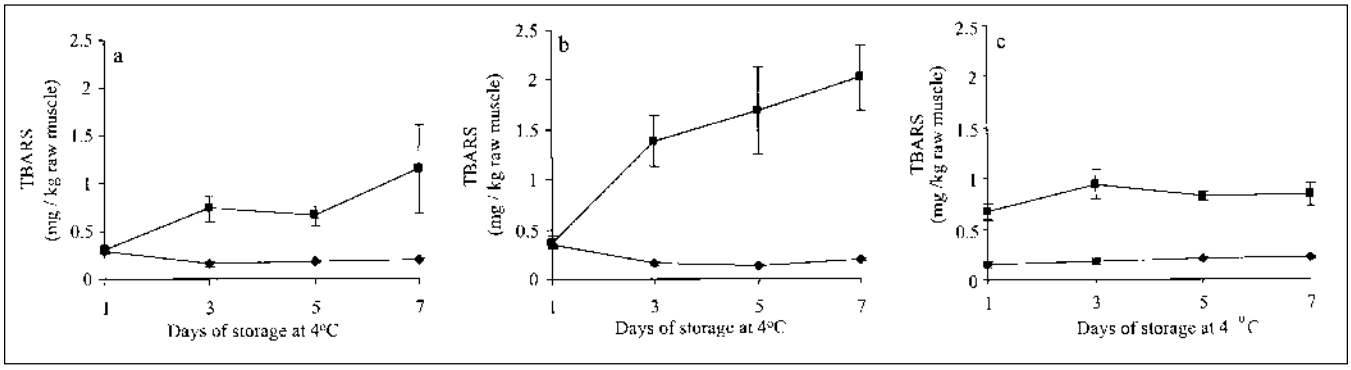

Fig. 1—Effect of α-tocopherol acetate supplementation on lipid oxidation measured using conventional spectrophotometry and expressed as TBARS numbers in (a) semimembranosus, (b) semitendinosus, and (c) rhomboideus: ■ 20 mg/kg, ◆ 3000 mg/kg. Standard error is indicated by bars.

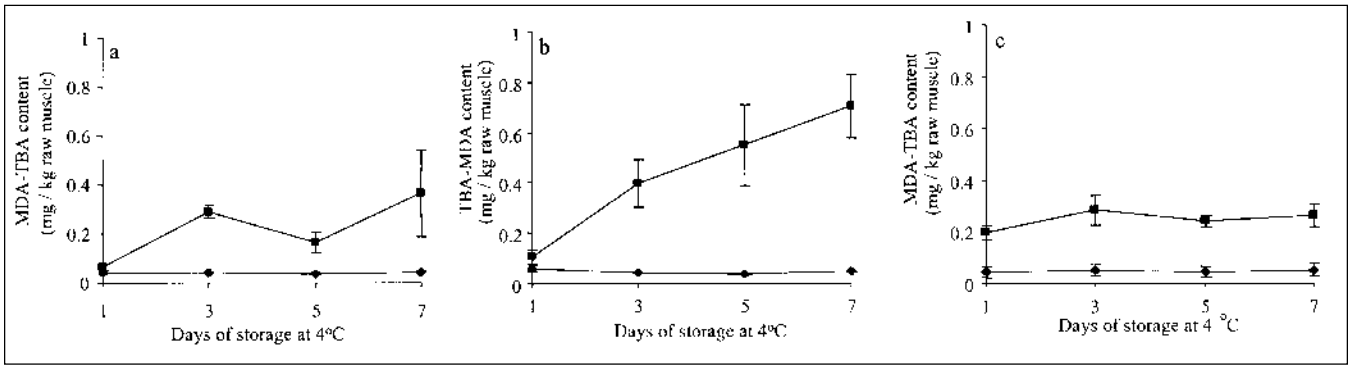

Fig. 2—Effect of α-tocopherol acetate supplementation on lipid oxidation measured using first derivative spectrophotometry and expressed as MDA-TBA values in (a) semimembranosus, (b) semitendinosus, and (c) rhomboideus: ■ 20 mg/kg, ◆ 3000 mg/kg. Standard error is indicated by bars.

ence was found on day 7, possibly due to the large standard error in control samples on this day. ST treatment and control samples were significantly different on days 3, 5, and 7, while RB treatment and controls were significantly different on all days. The ability of vitamin E to delay lipid oxidation in beef muscle during refrigerated display has been shown in several previous studies (Faustman and others 1989; Mitsumoto and others 1991, 1993; Arnold and others 1992, 1993; Liu and others 1996).

Results of lipid oxidation measurement using first derivative spectrophotometry are shown in Fig. 2. MDA-TBA values from SM obtained from cattle fed the higher level of α-tocopherol acetate (E3000) were significantly ($P < 0.05$) lower on days 3 and 5 than in the equivalent muscles obtained from animals fed the control (E20) diet. No significant difference was found on day 7, again, possibly due to the large standard error in control samples on this day. ST treatment and control samples were significantly different on days 3, 5, and 7, while RB treatment and controls were significantly different on all days. It is apparent from a comparison of Figures 1 and 2 that although MDA-TBA values in each muscle during refrigerated storage were lower than their corresponding TBARS numbers, values from both methods followed the same pattern of increase. In order to compare results further, the areas under the TBARS and MDA-TBA curves were determined and compared. MDA-TBA values were 85, 70, and 76% lower than their corresponding TBARS numbers in SM, ST, and RB treatments, and 69, 78, and 70% lower in controls. Similar findings were reported by Wen and others (1997). These authors also calculated the area under the curves to compare MDA-TBA and TBARS values and observed that MDA-TBA values in pork muscle were 59-69% lower than their corresponding TBARS values. O'Neill and others (1999) on the other hand observed that no MDA-TBA complex was detected in raw chicken patties stored for up to 10 d, indicating that non-MDA compounds contributed significantly to TBARS values in those samples during this period.

In conclusion, when measured using conventional (zero-order) spectrophotometry, appreciable amounts of lipid oxidation products (TBARS) were observed during refrigerated storage of beef muscle from animals fed the control diet. When the first derivative method was applied, a much lower MDA-TBA content was observed in samples from both control and supplemented animals. However, the overall pattern of lipid oxidation was similar for both methods. Therefore, both methods appeared to be equally as effective in reflecting the extent of lipid oxidation in beef muscle.

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