α-Tocopherol Contents and Lipid Oxidation in Pork Muscle and Adipose Tissue during Storage

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In an on-farm study 1220 pigs were fed diets containing either a basal (40 mg/kg of feed) or supplemented (200 mg/kg of feed) level of α-DL-tocopheryl acetate. The changes in α-tocopherol levels and lipid oxidation in longissimus dorsi muscle (mld) and adipose (backfat) tissue were studied after slaughtering during 14 days of storage of the chops at 4 °C. The α-tocopherol levels were higher in the muscle and adipose tissue from the supplemented group. In muscle tissue from the basal feeding group, there was a marked rise in the amounts of thiobarbituric acid reactive substances (TBARS) by 7 days storage. In muscle from the supplemented group the development of the TBARS remained low during storage. The α-tocopherol levels of muscle tissue did not change during storage. In adipose tissue the α-tocopherol concentration decreased and lipid oxidation occurred.

Keywords: *Tocopherol; pork tissue; storage stability*

INTRODUCTION

Lipid oxidation is a major cause of quality deterioration in meat products. The initial oxidative mechanism of membrane deterioration and the consequences, such as the development of rancidity, the deterioration of texture, color, and nutritional value, and the introduction of health hazard, have been reviewed (Pearson et al., 1983; Ladikos and Lougovois, 1990; Stanley, 1991). α-Tocopherol acts as a free radical scavenger and protects against lipid peroxidation (Tappel, 1962). The antioxidative activity is closely related to dietary vitamin E supplementation (Bieri and Anderson, 1960) and the α-tocopherol content of the tissue (Kornbrust and Mavis, 1980). A higher content of α-tocopherol improves the color (Faustman et al., 1989; Arnold et al., 1993; Asghar et al., 1991), delays the formation of carbonyl compounds (Asghar et al., 1991; Monahan et al., 1990a,b, 1992; Buckley and Connolly, 1980), and decreases the drip loss (Asghar et al., 1991) of meat. The α-tocopherol content is the determining factor for lipid stability of pork muscle tissue (Pfalzgraf et al., 1995a). Therefore, the analysis of α-tocopherol could provide a means to examine the quality of muscle tissues. This research studied the stability of α-tocopherol in relation to lipid oxidation during the storage of meat to determine whether the α-tocopherol concentration in stored meat reflects the present state of oxidation or the original levels of α-tocopherol resulting from the feeding. Lipid oxidation was investigated via the analysis of thiobarbituric acid reactive substances (TBARS).

MATERIALS AND METHODS

Reagents. All reagents were of analytical grade. Methanol was distilled before use. Ethanol, toluene, ethyl acetate, potassium hydroxide, trichloroacetic acid, ascorbic acid, and 2-thiobarbituric acid were purchased from Merck (Darmstadt, Germany). *n*-Hexane was from Promochem (Wesel, Germany); 2,6-di-*tert*-butyl-*p*-kresol (BHT) from Fluka (Buchs, Switzerland), and DL-α-tocopherol from Sigma (Deisenhofen, Germany).

Animals, Feeding, and Sampling. A total of 1220 pigs (barrows and gilts) in the Federal German hybrid breeding program (BHZP), weighing approximately 30 kg each, were randomly divided into two groups. The pigs were housed at a commercial farm belonging to the Erzeugergemeinschaft Osnabrück (EGO). One group of 545 pigs was fed a control diet containing a basal level of 40 mg of α-DL-tocopheryl acetate/kg of feed, and the other group of 675 pigs was fed a diet supplemented with 200 mg of α-DL-tocopheryl acetate/kg of feed. The feed was based on a barley, triticale, soybean meal, and lard mixture. The composition of the feed, especially the vitamin contents during the feeding, was checked by different laboratories.

The pigs were slaughtered at an average weight of 95 kg at the EGO slaughterhouse. Samples for tocopherol and lipid oxidation analysis were taken randomly from 38 animals in the control group and from 35 animals in the supplemented group. The chops from these animals were divided into six slices. One slice of each chop was vacuum packed and stored at −24 °C until analysis. The other slices were packed in commercial displays under an 80% O_2, 20% CO_2 atmosphere and stored at 4 °C. After 2, 5, 7, 9, and 14 days of storage, the sample that consisted of one slice of each chop was vacuum packed and stored at −24 °C. Analyses of all samples were performed randomly within 3 months.

Sample Treatment Prior to Analysis. For analysis of tocopherol levels and TBARS, pieces of about 2 g of longissimus dorsi muscle (mld) and outer backfat tissue were cut separately from the frozen chop slices. Seven to ten pieces were pooled and homogenized with a Moulinette. Four pooled samples of identical feeding groups and storage times were obtained from each tissue. Each pooled sample was analyzed twice.

Tocopherol Analysis. For tocopherol analysis we used a saponification, single-step extraction method with HPLC/fluorescence determination (Pfalzgraf et al., 1995b). Two grams of muscle or 1 g of adipose homogenate was weighed into amber 50 mL laboratory bottles (Schott no. 9071980) followed by the addition of 0.5 g of ascorbic acid and either 5 mL of potassium hydroxide solution (375 g of KOH dissolved in 750 mL of H_2O and 450 mL of methanol) for muscle or 1.5 mL of KOH solution for adipose tissue. The bottles were flushed with 100 mL of nitrogen and capped with PTFE sealed screw caps (Schott no. 9209032). The samples were saponified at 80 °C for 40 min and shaken manually every 10 min. After cooling, 20 mL of H_2O/ethanol (4:1 by volume for muscle or 1:1 by volume for adipose tissue) and 10 mL of *n*-hexane/

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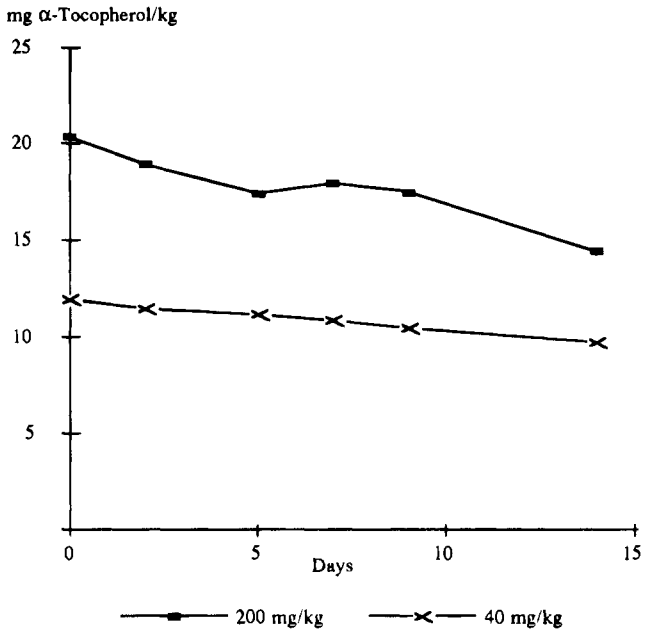

Figure 1. α-Tocopherol content (mg/kg) during storage of adipose tissue (backfat) from pigs fed either 200 or 40 mg of vitamin E/kg.

toluene (1:1 by volume) containing 0.01% BHT were added. The mixture was vigorously shaken (10 min, 250 strokes/min) and centrifuged (10 min, 2500*g*). Twenty microliters of the upper layer was injected into the HPLC.

Measurement of TBARS. Ten grams of tissue was homogenized with 20 mL of 10% (w/v) trichloroacetic acid using an Ultra-Turrax (90 s, 20 000 rpm). The homogenate was centrifuged and the supernatant decanted through a paper filter (Schleicher & Schüll no. 311643, Dassel, Germany). Two milliliters of the filtrate was mixed with 2 mL of the TBA reagent (300 mg of 2-thiobarbituric acid/100 mL of H_2O). The mixture was heated in a water bath for 20 min to 97 °C. After the mixture had cooled to ambient temperature, the extinction was measured at 531 nm. TBARS were exhibited as milli extinction units per gram of tissue (mE/g).

RESULTS

α-Tocopherol Contents. α-Tocopherol was the only isomer in muscle and adipose tissues. The mean α-tocopherol content of adipose tissue was 20.3 mg/kg of tissue for animals fed the vitamin E-supplemented diet and 12.0 ± 0.4 mg/kg for animals in the control group. Linear decreases to 14.4 ± 0.6 and 9.7 ± 0.9 mg/kg occurred during the 14 days of storage at 4 °C. Figure 1 displays the α-tocopherol contents of the adipose tissue, and changes can be seen. The correlation coefficient between storage time and α-tocopherol content of adipose tissue was −0.95 for the supplemented group and −0.99 for the basal group.

In the supplemented group the α-tocopherol content of muscle tissue was also higher than in the control group. The mean values in muscle tissue were 4.1 ± 0.2 mg/kg (supplemented group) and 2.5 ± 0.3 mg/kg (control group). These values did not change during the storage period, as shown in Figure 2.

Formation of TBARS. For the first 2 days after slaughter, the amounts of TBARS in muscle tissue were the same in both feeding groups. After the 7 days of storage, TBARS increased rapidly in the tissues from the basal group. Figure 3 indicates that in muscle samples from the vitamin E-supplemented group, the formation of TBARS was suppressed compared to the basal group.

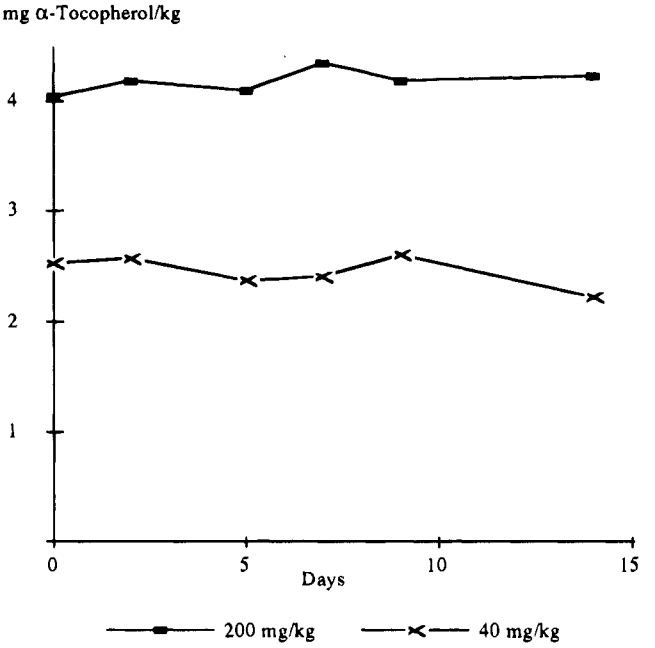

Figure 2. α-Tocopherol content (mg/kg) during storage of muscle (longissimus dorsi) tissue from pigs fed either 200 or 40 mg of vitamin E/kg.

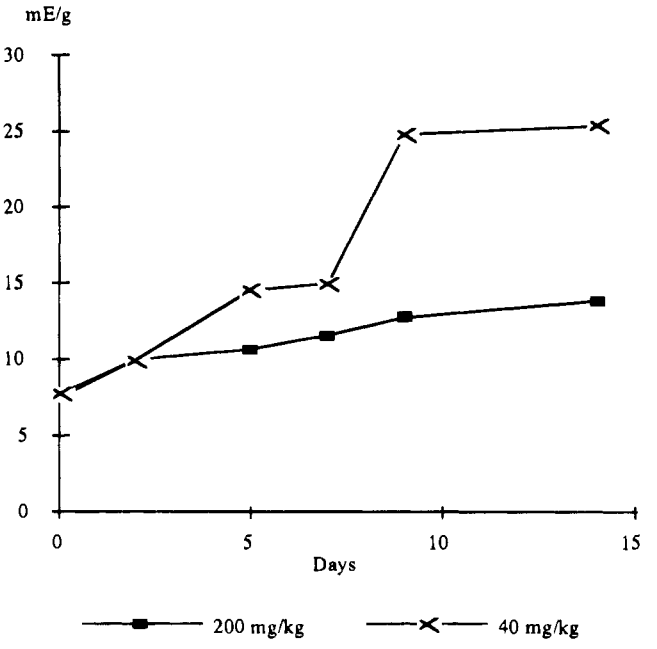

Figure 3. Development of TBARS during storage of muscle (longissimus dorsi) tissue from pigs fed either 200 or 40 mg of vitamin E/kg.

The development of TBARS in adipose tissue was also influenced by the vitamin E intake of the animals. From the date of slaughter to the end of the storage, the amounts of TBARS were higher ($P > 0.05$) in the basal group than in the supplemented group (Figure 4). In samples from both feeding groups the amounts of TBARS linearly increased by about 35% during storage. In adipose tissue the coefficient of correlation between storage time and TBARS was 0.96 for the basal and 0.98 for the supplemented group.

DISCUSSION

The effect of a high vitamin E intake on the lipid stability of adipose tissue has been studied less inten-

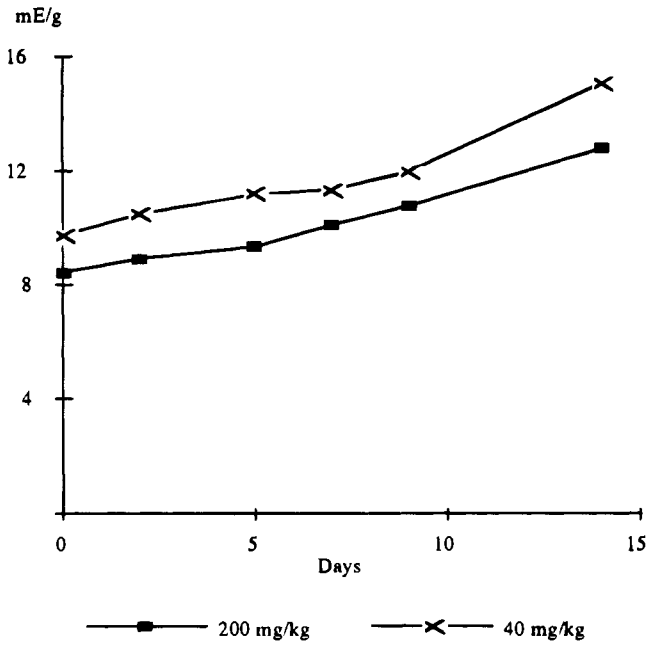

Figure 4. Development of TBARS during storage of adipose tissue (backfat) from pigs fed either 200 or 40 mg of vitamin E/kg.

sively than the effect on muscle tissue. In adipose tissue the TBARS failed to indicate lipid oxidation, but peroxide formation was inversely correlated to vitamin E intake (Grau and Fleischmann, 1966). We observed higher amounts of TBARS in the basal group. In contrast to our findings in muscle tissue, the increase in oxidation products in adipose tissue was the same in both groups. However, oxidation was retarded for about 5 days by supplementation due to the lower level of TBARS immediately post-mortem. In adipose tissue an antioxidant effect was accompanied by a decrease in α-tocopherol. The α-tocopherol loss was inversely proportional to the increase in TBARS. In adipose tissue α-tocopherol content was thus influenced by the oxidation state of the tissue and was not merely a function of vitamin E intake.

In muscle tissue the level of TBARS immediately postmortem was not affected by α-tocopherol supplementation (Asghar et al., 1992; Monahan et al., 1990a,b, 1992), although in rats the rate of in vivo lipid oxidation depends on the α-tocopherol content of the diet (Dillard et al., 1978; Lee and Csallany, 1994). However, in contrast to the rats none of the pigs was fed a vitamin E-deficient diet. Obviously the basal levels of 20–40 mg of vitamin E/kg of feed were sufficient to enable the endogenous antioxidant systems (Chan and Decker, 1994) to control the in vivo lipid oxidation. Depending on storage conditions and diets, the differences in lipid stability reported in the literature have occurred after 1–9 days. In the present study, as shown in Figure 3, TBARS increased rapidly in the basal group after 7 days. The results confirm that high levels of vitamin E in animal rations delays lipid oxidation in muscle and adipose tissue during storage not only in a laboratory setting (where a few animals are fed under optimal conditions) but also in the large-scale setting of a commercial pig farm as well.

α-Tocopherol acts as an antioxidant in tissue. During storage, however, no change in α-tocopherol content was seen in muscle tissue from the animals in either group. In biological membranes ascorbate regenerates oxidized tocopherol (Tappel, 1962). The antioxidant synergism of α-tocopherol and ascorbate is not fully understood. Dmitriev et al. (1994) suggested that α-tocopherol acts as a radical scavenger but is also able to restore oxidized lipids by reducing lipid peroxyl radicals to their primary structure. The resulting oxidation product of α-tocopherol is also regenerated by ascorbate. Regeneration of α-tocopherol apparently occurred during the 14-day post-mortem storage period in this study. The α-tocopherol content of the chops stored for 14 days still reflected the amounts at slaughter. We conclude that the α-tocopherol content of muscle tissue depends on feeding conditions and particularly on vitamin E intake and absorption. Analysis of α-tocopherol appears to be a suitable method of monitoring compliance with requirements for meat with high lipid stability.

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