EFFECT OF DIETARY VITAMIN E SUPPLEMENTATION AND PACKAGING METHODS ON OXIDATIVE STABILITY IN RAW PORK MEAT

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Background:
Lipid oxidation is the major cause of quality deterioration in meats and meat products, leading to undesirable colour and flavour changes that can limit their shelf-life (Pearson et al., 1983). These reactions are initiated in the celular and subcelular membranes because of their high content in polyunsaturated fatty acids (Monahan et al., 1993). Autoxidation process may also produce several compounds that exhibit toxicological effects (Draper et al., 1990). In this sense, cholesterol oxidation products (COPS) have been related with several diseases, such as carcinogenesis, mutagenesis, atherogenesis, antiestrogen activity and alteration of sterol metabolism (García-Crusset et al., 2001; Schroepfer, 2000). Preventing lipid oxidation during storage and retail display is therefore essential in order to keep meat safety and sensory quality of meats and meat products. Increasing α-tocopherol levels in muscle by dietary vitamin E supplementation has been widely considered as an excellent approach to improving oxidative stability in raw meat (Mitsumoto et al., 1993; O`Sullivan et al., 1997). Endogenous vitamin E is located into biological membranes (Stillwell et al., 1996) where have been shown to act as a powerful free radical-scavenger (Decker, 1998). In addition, oxidative rate is affected by external conditions, such storage temperature, illuminating conditions and contact with atmospheric gases (Kim et al., 1993; McCluskey et al., 1997). Consequently the combined application of vacuum packaging and chilling temperatures could be considered as complementary methods for reduction of oxidative reactions (Formanek et al., 1998; Guerrero Legarreta et al., 1998).

Key-words: lipid oxidation, α-tocopherol, cholesterol, cops, pork meat, vacuum packaging

Objective:
The objective of this work was to study the effect of dietary supplementation at different levels of α-tocopherol and packaging methods on lipid and cholesterol oxidation in raw pork during refrigerated storage time.

Methods:
Source of meat: *Longissimus dorsi* were obtained from pigs (n=24) fed with different levels of α-tocopheryl acetate (VE): control group (C) (10 mg VE/kg feed), low-level supplemented group (100 ppm) and high-level supplemented group (200 ppm). Animal were slaughtered with 90-95 kg weight. Carcasses were previously selected according to pH and CE measurements, rejecting PSE and DFD meats (Bañón et al., 1996).
Packaging and storage conditions: Loin pork was sliced into chops of 1 cm of thickness and divides in two batches according to packaging method. Aerobic (AP): chops were placed on polystyrene trays over-wrapped with an oxygen-permeable PVC wrap (6000-8000 cm^3 0_2 / m^2/ 24h); Vacuum (VP): samples were full vacuum packed in low oxygen permeable bags (BB4L Cryovac, 8-12cm^3 O_2 /m^2/24h). All packed meat samples were held under retail display conditions at 4ºC under fluorescent lighting (616 Lux) for 7 and 21 days respectively.
Chemical analysis:
α-Tocopherol and cholesterol determination: 1 g of meat was subjected to direct saponification with 7.3 ml of saponification solution (11,5 % potassium hydroxide in a mixture of absolute ethanol:water 55:45 v/v) and 250 mg of ascorbic acid. Reaction occurred on nitrogen atmosphere during 15 minutes at 85ºC. Unsaponified fraction was chilled at room temperature and extracted with isooctane. 20 µl of organic phase were injected into HPLC system equipped with Waters Resolve 5 µm spherical silica column (3.9 x 150 mm), and a mixture of isooctane:tetrahidrofuran as mobile phase. Identifications of α-tocopherol and cholesterol were carried out by a Fluorescence and Light Scattering detectors respectively. Calibration curves were made for compounds quantification. Meat samples were analysed in triplicate at day 0.
Lipid oxidation: Oxidative stability in pork chops was assessed by measuring thiobarbituric acid reacting substances (TBARS) using the method described by Botsoglou et al. (1994)(Botsoglou et al., 1994). Results were expresed as mg malonaldehyde (MDA)/Kg muscle. Lipid oxidation was evaluated in duplicate at day 1, 3, 5 and 7 for AP and at 1, 3, 7, 11, 14, 18 and 21-day for VP samples.
Cholesterol oxidation products (COPS): Total lipids were extracted according to the Folch procedure (Folch et al., 1957). Lipid extract was purified in silice column (Varian Bond Elut) and after was eluted serially with hexane-ethyl acetate of 9:1 (v/v) and 8:2 (v/v) respectively. Finally, the COPS fraction were extracted by eluting with acetone. The dry residue was derivatizated by reaction with BSTFA. Oxysterols (7-β-hidroxicholesterol, α-epoxicholesterol, triol, 25-hidroxicholesterol and 7-ketocholesterol) were detected and quantified by using a GC, with capillary column (HP-5), equipped with a FID detector. The COPS levels were measured in triplicate at days 1, 7 (AP) and 21 (VP).

Results and discussion:
α-Tocopherol concentration in muscle: Endogenous addition of vitamin E significantly increased ($P<0.05$) the α-tocopherol levels quantified in fresh meat (day 0). Vitamin E concentration in 100 ppm samples (4.01 mg/kg) were significantly lower than those obtained for 200 ppm (4.93 mg/kg) chops, and those levels were 2.3 and 2.8 fold higher than the obtained for C meat. Our results are in good agreement with those published by (Asghar et al., 1991; O`Sullivan et al., 1997).
Lipid oxidation (TBARS): Dietary vitamin E level, packaging condition and their interaction significantly ($P<0.05$) affected lipid oxidation rate in raw meat. However no differences were found over display time (Table 1). After 3 days of refrigerated storage, AP control samples showed higher ($P<0.05$) TBARS numbers than supplemented chops, regardless of the dose. However, no significant differences were noticeable among 100 or 200 ppm vitamin E supplemented samples (Figure 1). These results are similar to those published by several authors (Jensen et al., 1998; Lauridsen et al., 2000; Monahan et al., 1990). By comparison with AP samples, lipid oxidation values significantly decrease ($P<0.05$) under vacuum conditions at day 3 and 7, and similar TBARS numbers were found for different dietary treatments (Figure 2). TBARS values and α-tocopherol tissues levels showed a negative and significant correlation coefficient ($r=-0.209$; $P<0.001$).
Cholesterol and COPS: Cholesterol concentration quantified in fresh meat (day 0) was 61.4 mg/100 g muscle. This results are consistent with those obtained by Arneth et al. (1995). Cholesterol levels did not modify the rate of production of total COPS. Moreover, no significant ($P>0.1$) differences were found for total COPS levels among packaging conditions, neither dietary vitamin E levels (Galvin et al., 2000) (Table 1). Within each packaging condition only few but no significant increases for COPS levels were observed over display time. The

COPS concentration found at the present study (Table 2) are in agree with those related by Paniangvait et al. (1995). Five oxysterols were identified in raw pork meat (7β-OH, α-Epoxi, Triol, 25-OH and 7-ketocholesterol). α-Epoxi and 7β-OH levels supposed 57% and 17% of the overall cholesterol oxides concentration, respectively. Both total COPS levels and rate COPS/Cholesterol were significantly correlated with TBARS numbers (r=0.406, r=0.370; p<0.05). However, higher correlation coefficients were observed between TBARS values and 7β-OH (r=0.704) or 7-keto (r=0.677). These results are consistent with those reported by (Nourooz-Zadeh et al., 1988). Both 7-keto and 7-β-OH also showed stronger correlation coefficient with total COPS amount (r=0.658, P<0.001), which justify their application as COPS production index (Addis et al., 1985). α-Tocopherol muscle levels showed a negative and significant correlation (p<0.05) with 7-β-OH (r=-0.468), 25-OH (r=-0.308) and rate COPS/Cholesterol (r=0.329)

Table 1: Analysis of variance (Probability of F values)

	TBARS	COPS
Day	0.073	0.670
Packaged[1]	0.000	0.460
Diet[2]	0.000	0.330
Day*Packaged	0.148	-
Day*Diet	0.592	-
Diet*Packaged	0.000	-
Day*Packaged*Diet	0.641	-

[1] C, 100 ppm, 200 ppm; [2] AV, AP

Table 2: COPS values (Mean±SD) in meat

	DAY 1[1]	AP[2]	VP[3]
7β-Hidroxy	0.04±0.015	0.04±0.016	0.04±0.020
α-Epoxi	0.13±0.051	0.13±0.048	0.15±0.060
Triol	0.01±0.009	0.02±0.015	0.02±0.007
25-Hidroxy	0.04±0.033	0.03±0.014	0.03±0.024
7-Keto	0.01±0.016	0.02±0.017	0.02±0.014
TOTAL COPS	0.24±0.070	0.23±0.064	0.26±0.101
COPS/CHOL (%)	0.04±0.013	0.04±0.012	0.04±0.018

[1] Unpacked meat; [2] Storage time 7 days; [3] Storage time 21days

Figure 1. TBARS values (Mean±SEM) in AP pork chops

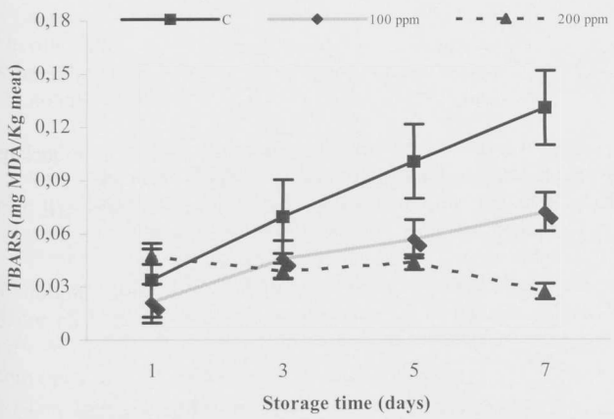
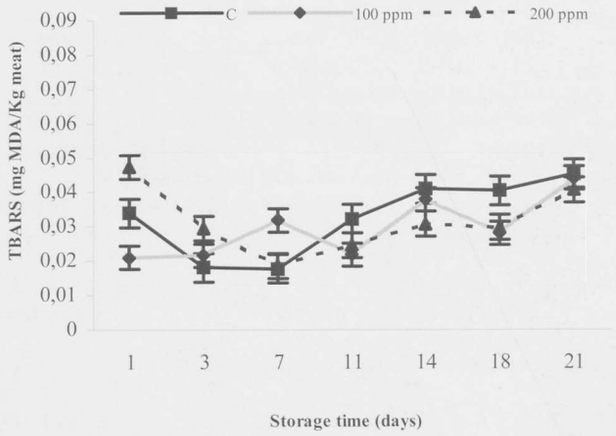


Figure 2. TBARS values (Mean±SEM) in VP pork chops



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