LIPIDS OXIDATION AND VITAMIN E SUPPLEMENTATION IN FRESH AND PROCESSED PORK

Zanardi E., Novelli E., Campanini G., Madarena G., Chizzolini R.
Istituto di Scienza e Tecnologia degli Alimenti, Universitá di Parma, 43100 Parma, Italy.

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BACKGROUND

Lipid oxidation products, and a few cholesterol oxides (COPs) in particular, are considered atherogenic agents and appear to have mutagenic, carcinogenic and cytotoxic properties. Human subjects can absorb oxysterols from food sources and COPs appear to be able to replace cholesterol molecules in membranes, perturbing permeability, stability and other membrane properties (Emanuel *et al.*, 1991; Guardiola *et al.*, 1996).

The research which has been undertaken aimed at improving the nutritional quality of pork through changes of the pigs' diets. The composition of deposit fat has been modified increasing the amount of oleic acid in the feeds and the diet has been supplemented with two levels of vitamin E.

METHODS

A group of 84 pigs was divided in 4 subgroups of which one, 12 animals, was the control (Diet 1), one group , 24 animals, received a diet enriched with 6% sunflower oil (78% oleic acid) (Diet 2) and two groups, 24 animals each, were raised on a diet with 6% sunflower oil supplemented with 100 ppm α-tocopheryl acetate for one group (Diet 3) and with 200 ppm α-tocopheryl acetate for the other (Diet 4). All animals were kept on experimental diets for about 6 months. Lipid oxidation analyses were carried out on chops, raw and cooked, on salame Milano, produced with shoulders, ham trimmings and bellies, and on the hams processed as Parma hams. The determinations of thiobarbituric reactive substances (TBARS), total cholesterol, cholesterol oxides (COPs) and vitamin E content were carried out as described by Zanardi *et al.* (1998).

RESULTS AND DISCUSSION

Vitamin E (Table 1) data were expressed on a dry matter basis without NaCl to avoid the distorting effects of moisture and NaCl contents. Values increased in all dietary groups compared with controls. A remarkable increase has taken place with diet 2, with oil but without vitamin E supplementation, where the vitamin content was more than twice that of control. Vitamin contents of the groups supplemented with vit.E were higher than groups 1 and 2 and the 200mg vit.E supplemented group had a vitamin content about three times higher than control. In most cases the content of vitamin E was not significantly different between the groups supplemented with 100 and 200 ppm α-tocopheryl acetate. Vitamin content of processed products was variable and not directly related with fat content. The latter was about 3% in chops, 30% in salame and 6% in ham. Vitamin E contents, therefore, should have been higher in salame and hams, in order, compared with chops. Whereas ham content of vitamin E can be considered more or less in line with fat content, it would appear that vitamin values of salame are lower than expected. The finding could suggest that salame processing technology has caused a significant consumption of the vitamin. The data, reported below, on oxidation (TBARS and oxysterols) seem to confirm the hypothesis.

TBARS measurements (Table 2) have not shown significant differences between the diets in samples analysed immediately after cutting. Exposure to fluorescent light, whether in the presence of oxygen (chops) or under vacuum (hams) produced higher and significantly different malonaldehyde values in the samples of the control group compared to the other groups. No differences were observed among the oil supplemented groups (Diets 2, 3 and 4) which could be related to vitamin E supplementation. Similar results were obtained with cholesterol oxides content (Tables 3, 4, 5). The oxides regularly found in all samples were 5,6α-epoxycholesterol and 7ketocholesterol; 7β-hydroxycholesterol has not been found in all samples, especially in chops, while 20α-hydroxycholesterol, 25-hydroxycholesterol and triol have never been detected. The percentage of oxidised cholesterol varied from 0.12 to 0.04. Differences between dietary groups were not significant due to considerable variation among samples. There appeared to be a clear tendency, though, for values gradually decreasing from the control group to the one supplemented with 200 ppm vitamin E, more evident in salame Milano than in hams and cooked chops. The observation appear to confirm TBARS values, higher in salame than in chops and hams and would also be in line with what has been remarked about vitamin content in salame, lower than expected.

The results substantially agree with what is known from the literature. Asghar et al. (1991) observed a reduction of TBARS values during refrigerated storage of chops frozen and thawed with vitamin E contents of 0.5, 2.6 and 4.7 ppm. Monahan et al. (1992a), with pigs supplemented with up to 200 ppm vitamin E from 30 kg to 98 kg, observed a significant reduction of TBARS values of chops frozen for 4 months, thawed and exposed under refrigeration for 8 days. Pie *et al.* (1991) studied oxidation of cholesterol in beef, veal and pork, fresh, 3 months frozen and cooked. On the whole, the percentage of oxysterols to cholesterol varied on average from 0.3 to 0.8% and the most dangerous oнes, cholestanetriol and 25-hydroxycholesterol, were either absent or present at very low amounts. Zubillaga e Maerker (1991) observed three oxysterols, namely 7-keto, 5,6α-epoxy and 5,6β-epoxy, in fresh meat (veal, pork, beef) and chicken with pork containing the lowest amounts of oxides, the three of them summing up to about 100 ppb. Monahan *et al.* (1992b) observed three oxysterols, namely 5,6α-epoxy-, 7-keto- and 7β-hydroxycholesterol in cooked ground pork. On the whole it would appear that in normal conditions vitamin E content of control diet fed animals would be sufficient to protect fat from oxidation but, if conditions of oxidative stress come in, higher vitamin contents would be needed to keep oxidation under control. It seems, moreover, that there might be room for reduction in cholesterol oxidation if the tendency towards lower oxidation levels in high vitamin E meats observed in the present study were confirmed by further investigations.

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TABLE 1. Vitamin E content (mg/kg on dry matter for chops, mg/kg on dry matter and no NaCl for salame Milano and ham) (Mean ±Standard Deviation). Different superscripts stand for significant differences at the Scheffé's test (P≤0.05)

DIET	1	2	3	4
CHOPS - RAW	5.18±0.69^{c}	12.31±2.23^{b}	15.48±2.36^{a}	16.87±2.81^{a}
CHOPS - COOKED	5.91±0.96^{d}	13.37±2.03^{c}	17.06±2.26^{b}	18.64±2.25^{a}
SALAME MILANO	6.45±0.71^{c}	13.57±1.82^{b}	16.73±2.23^{ab}	19.65±2.06^{a}
HAM	5.98±1.48^{c}	14.17±2.72^{b}	18.73±1.98^{a}	19.41±3.55^{a}

TABLE 2. TBARS (mg MDA/kg) (Mean±Standard Deviation). Different superscripts stand for significant differences at the Scheffé's test (P≤0.05)

DIET	1	2	3	4
CHOPS - RAW	0.11±0.03	0.10±0.03	0.10±0.03	0.12±0.06
CHOPS - RAW - EXPOSED 7 DAYS	0.47±0.22^{a}	0.31±0.16^{ab}	0.24±0.11^{b}	0.35±0.20^{ab}
CHOPS - COOKED	0.07±0.04	0.06±0.02	0.05±0.02	0.04±0.02
CHOPS- COOKED - EXPOSED 7 DAYS	0.94±0.18^{a}	0.56±0.18^{b}	0.55±0.08^{b}	0.43±0.07^{b}
SALAME MILANO	0.40±0.12	0.28±0.10	0.27±0.13	0.24±0.07
HAM	0.18±0.03	0.15±0.02	0.16±0.04	0.15±0.03
HAM - UNDER VAC. EXPOSED 90 DAYS	0.29±0.03^{a}	0.23±0.03^{b}	0.24±0.03^{b}	0.23±0.03^{b}

TABLE 3. Cholesterol (mg/100g) and cholesterol oxides (μg/g) content in cooked chops (Mean±Standard Deviation) (N.D.= Not Detected, *= only one sample).

DIET	1	2	3	4
CHOLESTEROL	71.8±13.3	77.6±5.2	74.3±13.1	74.4±7.3
7β-OHCHOLES.	N.D.	0.03*	0.02±0.01	0.02*
5,6α-EPOXYCHOL.	0.50±0.12	0.31±0.16	0.32±0.10	0.24±0.06
7-KETOCHOL.	0.30±0.19	0.29±0.12	0.20±0.10	0.18±0.16
% CHOL-OXIDISED	0.11	0.11	0.07	0.06

TABLE 4. Cholesterol (mg/100g) and cholesterol oxides (μg/g) content in salame Milano (Mean±Standard Deviation).

DIET	1	2	3	4
CHOLESTEROL	110.5±8.9	97.2±7.5	96.8±4.6	94.8±4.7
7β-OHCHOLES.	0.56±0.84	0.22±0.33	0.10±0.08	0.11±0.06
5,6α-EPOXYCHOL.	0.23±0.21	0.14±0.11	0.10±0.09	0.19±0.17
7-KETOCHOL.	0.52±0.71	0.23±0.39	0.19±0.23	0.12±0.08
% CHOL-OXIDISED	0.12	0.06	0.04	0.04

TABLE 5. Cholesterol (mg/100g) and cholesterol oxides (μg/g) content in Parma ham (Mean±Standard Deviation).

DIET	1	2	3	4
CHOLESTEROL	76.4±7.4	61.6±9.2	72.2±11.7	74.6±13.6
7β-OHCHOLES.	0.21±0.14	0.18±0.33	0.17±0.33	0.13±0.06
5,6α-EPOXYCHOL.	0.31±0.16	0.25±0.17	0.15±0.11	0.17±0.12
7-KETOCHOL.	0.32±0.18	0.22±0.15	0.18±0.14	0.23±0.12
% CHOL-OXIDISED	0.11	0.10	0.07	0.07