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EFFECT OF DIETARY α-TOCOPHERYL ACETATE SUPPLEMENTATION ON THE FORMATION OF CHOLESTEROL OXIDES IN COOKED CHICKEN

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BACKGROUND

Lipid oxidation in meats has long been a concern in terms of food safety and keeping quality. More recently attention has focused on cholesterol oxidation products (COPs) in foods, as they may play a key role in the development of atherosclerotic lesions. COPs are generated via a free radical-initiated mechanism, involving attack by fatty acid radicals. Disruption of the integrity of muscle membranes by mincing and restructuring alters cell compartmentalization, facilitating free radical propagation and COPs formation. The use of α-tocopherol to prevent oxidative deterioration in meats is well-established. Dietary α-tocopherol supplementation increases membrane α-tocopherol levels, resulting in greater membrane stability and improved storage stability of meat . Evidence suggests that dietary supplementation with α-tocopheryl acetate may also play a key role in reducing COPs formation in muscle foods. The objectives of the present experiment were to determine the effect of storage and α-tocopheryl acetate supplementation on the formation of COPs in cooked ground chicken breast and thigh meat.

METHODS

Seventy two Cobb 500 broiler chicks were randomly divided into 3 groups and were fed diets supplemented with 20, 200 or 800mg α-tocopheryl acetate /kg diet. Birds were slaughtered after 6 weeks. Breast and thigh muscle was removed, vacuum-packed and stored at -20°C until required. Prior to the storage stability study, meat was thawed overnight. Following grinding, 1% salt and 5% water were added. Breast and thigh samples were formed into patties and cooked in a conventional oven at 160°C for 40 minutes. Following cooling, samples were wrapped in plastic film and placed in a retail display unit under fluorescent light, at 2°C. Lipid oxidation was assessed by the 2-thiobarbituric acid method of Ke *et al.* (1977). Total lipid extracts for COPs analysis were prepared using the dry column extraction method of Marmer & Maxwell (1981). 6-Ketocholesterol (50μg) was incorporated into lipid extracts as an internal standard. COPs were separated from cholesterol and other muscle lipids using the sample clean-up procedure of Park & Addis (1985). Acetone extracts were evaporated to dryness by rotary evaporation and redissolved in 4ml ethyl acetate. Prior to GC analysis, 1ml of sample was evaporated to dryness under nitrogen. Trimethylsilyl (TMS) derivatives of COPs were prepared (Monahan *et al.*, 1992).

The TMS ether sterols were then evaporated to dryness under nitrogen and redissolved in 100μl ethyl acetate. A Shimadzu (Model GC-14A) gas chromatograph with flame ionization detection, equipped with a Shimadzu (Model C-R6A) Chromatopac integrator was used to quantify cholesterol oxides. The column used was a methylsilicone column (15m x 0.32mm i.d., film thickness 0.25μm, S.A.C. Chromatography Ltd., Letchworth, Herts., England). The carrier gas was helium (0.7kg/cm^2). Oven temperature programming was as follows: 190°C to 220°C at 10°C/min.; 220°C to 234°C at 0.4°C/min.; 234°C to 255°C at 1.5°C/min. and held at 255°C for 20 min. Injector port and detector temperatures were 275°C and 330°C, respectively.

RESULTS AND DISCUSSION

25-Hydroxycholesterol was present in all samples immediately after cooking, and on all days of analysis. In addition, 20α-hydroxycholesterol was present in breast at day 6, and 7-ketocholesterol was detected in breast and thigh at 12 days (data not shown). COPs were slow to increase during storage. Total COPs did not increase appreciably until day 12 (Fig. 1). Harsh processing and storage conditions are required for extensive COPs formation to occur. The conditions used here may not have been severe enough to induce extreme oxidative stress and a high level of COPs production. Initially, there was no difference in COPs between the 3 dietary treatments in breast. At 12 days, supplementation with 200 and 800mg α-tocopheryl acetate reduced COPs levels by approximately 40-50% and 60-70% respectively in both breast and thigh. There were some discrepancies in thigh at days 0 and 6. These may have been due to the low variability in some samples, resulting in statistically significant differences. The effect of α-tocopherol on COPs appears to be related to the protection of membrane fatty acids. Free radicals from phospholipid oxidation may initiate cholesterol oxidation in the membranes of ground muscle. α-Tocopherol supplementation also reduced TBARS in meat samples (Fig. 2), indicating protection of membrane fatty acids. This would have resulted in a reduction in free radical formation and hence a reduction in the formation of COPs.

CONCLUSIONS

COPs were present in cooked, ground chicken meat and increased during storage. Dietary α-tocopheryl acetate supplementation resulted in a reduction in the level of COPs formed during storage and may be an effective means of controlling the formation of COPs in chicken.

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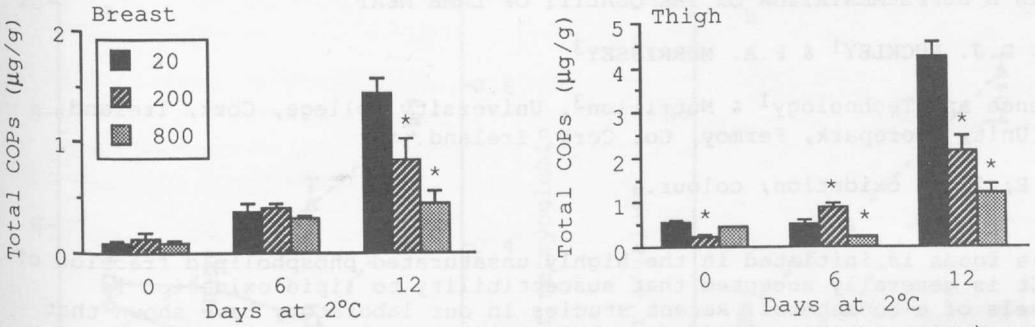

Fig. 1. Effect of storage and dietary α-tocopheryl acetate supplementation (20,200 or 800mg/kg diet) on COPS formation in cooked ground breast and thigh meat.
Mean ± SEM of 4 analyses performed in duplicate.
* For each day, means are significantly different from '20'group (P<0.05) (t-test).

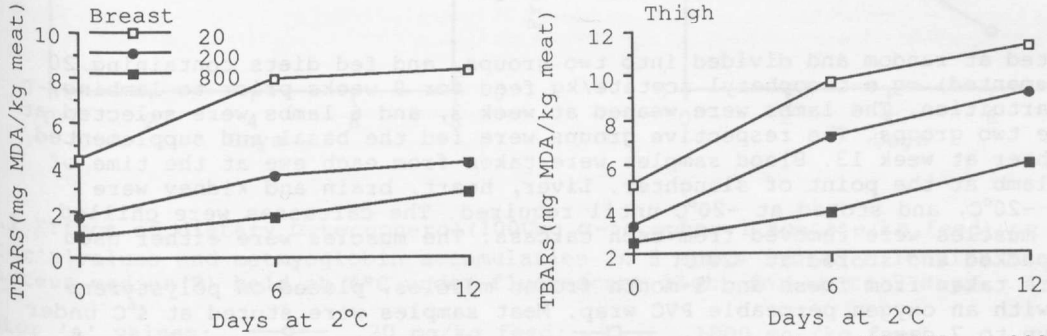

Fig. 2. Effect of dietary α-tocopheryl acetate supplementation (20,200 or 800mg/kg diet) on the storage stability of cooked ground breast and thigh meat.
Means ± SEM of 6 analyses performed in duplicate.
MDA = Malondialdehyde.