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**CANTHAXANTHIN AS A LIPID SOLUBLE ANTIOXIDANT IN AN OXYMYOGLOBIN LIPOSOME MODEL SYSTEM**

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**BACKGROUND:** Canthaxanthin, a lipid-soluble carotenoid, has been shown to function as a chain-breaking antioxidant in a liposome model system (Lim et al., 1992). In meat, both oxymyoglobin and lipid oxidize over time resulting in discoloration, and development of off flavors and undesirable odors, respectively (Greene, 1969; Kanner and Harel, 1985). Vitamin E, like canthaxanthin is lipid-soluble, and has been shown to be a potent antioxidant in meat and meat-related systems (Faustman, 1993). Carotenoids are typically used for pigmentation of muscle-based foods(e.g. salmonids); their potential efficacy for delaying oxidation of oxymyoglobin and lipid in muscle foods is unclear.

**OBJECTIVES:** The objective of this study was to determine the effectiveness of canthaxanthin as an antioxidant in an oxymyoglobin-liposome model.

**METHODS**

**Liposome Preparation** Canthaxanthin (CX) and egg yolk phosphatidylcholine (PC) were obtained commercially. Liposomes were prepared according to New (1990) in chloroform/methanol (2:1; v/v). Three mg dicetyl phosphate and 12 mg cholesterol were dissolved in a round bottom flask containing 30 mg PC, the appropriate concentration of CX, and 5 mL solvent. The solvent was removed under nitrogen with a rotary evaporator to form a thin lipid film.

**Oxymyoglobin Preparation** Oxymyoglobin (OxyMb) was prepared according to Brown and Mebine (1969). Metmyoglobin (MetMb) was prepared in 10 mL sodium citrate buffer (50 mM, pH 5.6) and chemically reduced with sodium hydrosulfite. The OxyMb solution was oxygenated, and residual hydrosulfite removed by mixed-bed ion exchange chromatography. The concentration of OxyMb was adjusted to 2.5 mg/mL ($E_{525}$=7.6 $mM^{-1}cm^{-1}$). The OxyMb preparation or sodium citrate (control) was added to the previously prepared lipid films. With one mg glass beads (1 mm dia.). OxyMb-liposomes were formed by mechanical shaking at 5°C for 20 min on a table top shaker and allowed to stand for 20 min before oxidation measurements.

**Measurement of OxyMB-Liposome Oxidation** All assays were incubated at 30°C for a maximum period of 5 hrs. Aliquots (1mL) were removed from each assay hourly and oxidation of OxyMb and lipid determined. The Krzywicki (1982) method was used to determine metmyoglobin formation as previously described (Yin and Faustman, 1993). For lipid oxidation, the TBARS assay of Schmedes and Holmer (1989) was used. Briefly, 1 mL myoglobin-liposome solution was combined with 2 mL 20% trichloroacetic acid. The resulting solution was centrifuged at 2000 g for 10 min at 4°C. One mL of the supernatant was added to 1 mL 20 mM aqueous thiobarbituric acid and incubated at 25°C for 20 hrs. The absorbance at 532 nm was recorded and reported as TBARS.

**Statistical Analysis** Data were treated by analysis of variance (ANOVA) and computed using the SAS General Linear Model (GLM) procedure (SAS, 1985).

**RESULTS AND DISCUSSION**

Triplicate assays of each treatment were analyzed on three different days. Treatment effects are described below. There were no differences found between days ($p>0.05$). Therefore, the figures presented are a single day's results representative of observed behavior.

Figures 1 and 2 illustrate a typical oxidation sequence of control and 2 uM CX treatment. At 2 uM, CX treatment resulted in a reduction in overall MetMb formation between hours 2 and 3 of incubation at 30°C ($p<0.05$; Fig. 1). At hours 2 and 3, the percent MetMb in the CX assays was 9% and 6% less than in the control, respectively. The induction period was prolonged in lipid oxidation ($p<0.05$; Fig. 2). There appeared to be a stronger antioxidant effect of CX on lipid oxidation than on inhibition of MetMb formation. Palozza and Krinsky (1992) also showed that CX was effective as an antioxidant on chemically initiated lipid peroxidation in rat liver microsomes as monitored by malonaldehyde (MDA) production.

The effect of CX at varying concentrations on MetMb formation and lipid oxidation are presented in Figures 3 and 4, respectively. When the concentration of CX was varied from 2 to 10 uM, its antioxidant activity was increased ($p<0.05$). At hours 2 and 3, the antioxidant activity was concentration-dependent (2 uM < 5 uM < 10 uM). There was 18% and 12% percent lower MetMb at hours 2 and 3, respectively, observed with 10 uM CX than with the control (Fig. 3). Again, CX appeared to have a stronger effect in inhibiting lipid oxidation (Fig. 4). There was approximately a 4-fold decrease in TBARS at hour 2; and at hour 3, a 2.5-fold decrease in the 10 uM CX treatment when compared to the control. The effects of CX agree with results of Mayne and Parker (1989) which showed that CX was effective in protecting against lipid oxidation in chicks supplemented with CX. The authors used chemically intiated peroxidized liver homogenates and measured oxidation at varying partial oxygen pressures; no attention was given to heme protein oxidation.

**CONCLUSIONS**

In a myoglobin liposome model system, CX delayed oxidation of OxyMb and lipid. When incubated at 30°C at varying concentrations, CX was more effective at 10 uM than at lower concentrations during the second and third hour of incubation.

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Fig. 1. The effect of 2 uM canthaxanthin (Cx) on MetMb formation in a liposome model system incubated at 30°C, pH 5.6.

Fig. 2. The effect of 2 uM Cx on lipid oxidation in a liposome model system following incubation at 30°C, pH 5.6.

Fig. 3. The effects of 0, 2, 5, and 10 uM CX on MetMb formation at hours 2 and 3 of 30°C incubation, pH 5.6.

Fig. 4. The effects of 0, 2, 5, and 10 uM CX on lipid oxidation at hours 2 and 3 of 30°C incubation, pH 5.6.

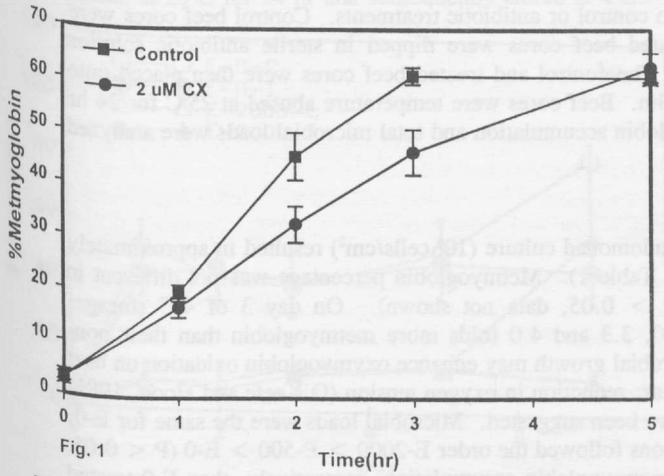


Fig. 1

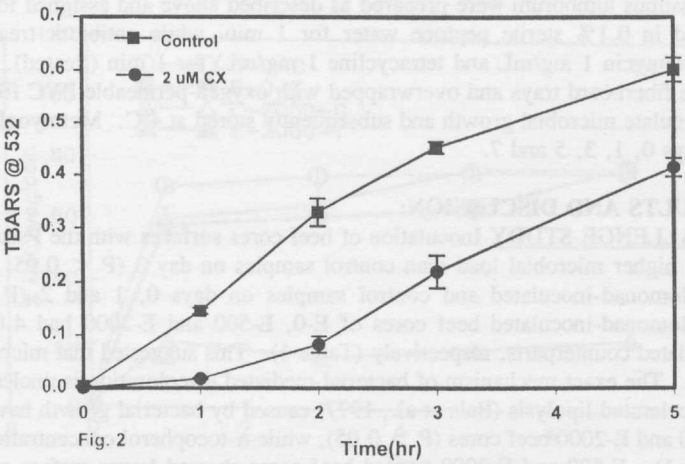


Fig. 2

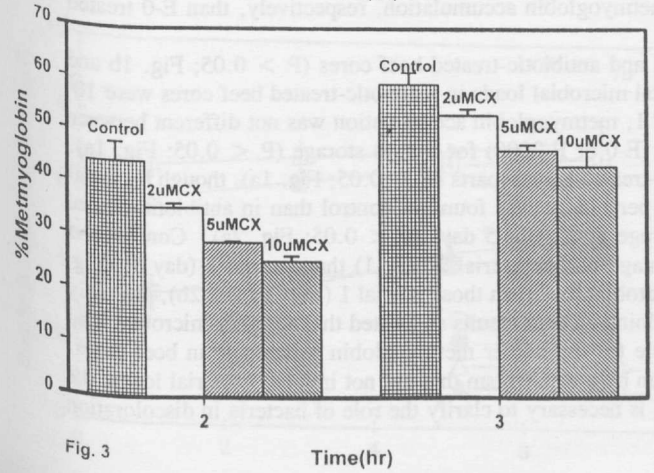


Fig. 3

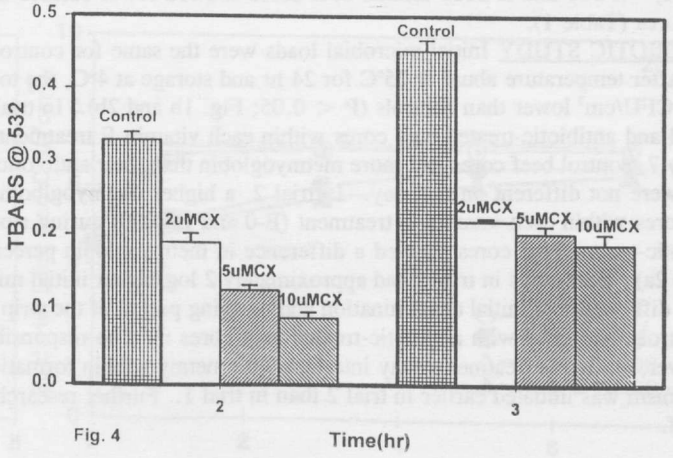


Fig. 4