

## 10. Assay of Cholesterol Oxidation Products in Foods

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Cholesterol will oxidize spontaneously in air, yielding a variety of oxysterols. Many of these have potent biological effects. Thus cholesterol epoxide causes sarcomas in mice, mixtures of oxysterols cause necrosis of rabbit aorta cells, and several oxysterols strongly suppress cellular cholesterol synthesis and LDL receptor activity.

We have recently developed a sensitive and specific analysis method for oxysterols in foods. A chloroform-methanol extract of the food is fractionated over two silica columns, with particle sizes of 63-200 and 30-60  $\mu\text{m}$ , respectively. Fractions containing oxysterols are analysed by gas chromatography on a 25 m x 0.25 mm capillary Sil 5 column, using on-column injection. In egg yolk powder we identified 6 oxysterols; the identity of cholesterol 5,6 epoxide, cholestane 3  $\beta$ , 5  $\alpha$   $\beta$  triol and 7  $\beta$  hydroxycholesterol was confirmed by mass spectrometry. The sensitivity was better than 1  $\mu\text{g/g}$  dry weight for egg yolk powder. Recovery of 5 pure oxysterols added to yolk at a level of 6.5  $\mu\text{g per g}$  was  $96 \pm 2\%$ .

Total oxysterol levels varied between egg yolk powders from 21  $\mu\text{g/g}$  (1.8 of which was epoxide) to 137.3 (9.4 epoxide). Fresh egg yolk yielded 1.7  $\mu\text{g}$  of total oxysterols per g dry weight, which shows that artefactual cholesterol oxidation during sample work-up was minimal.

Mixed human diets made up of raw and boiled foods only contained 4.5  $\mu\text{g}$  oxysterols per g dry homogenate (epoxide, 0.7  $\mu\text{g/g}$ ). If the same foods were fried or baked before homogenization the level of oxysterols was 3.5  $\mu\text{g/g}$ , showing that frying of cholesterol-containing foods does not necessarily cause formation of large amounts of oxygenated sterols.