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Analytical Methods Section

TECHNICAL NOTE

Qualitative and comparative studies of cholesterol oxides in commercial and home-made Indian ghees

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Cholesterol oxides are reported to be cytotoxic, mutagenic and many be atherogenic. Cholestane triol and epoxide are particularly important and are studied qualitatively in ghee samples obtained from different sources. In home-made ghee, both cholesterol oxides are identified whereas in commercial ghees they are absent. It is also observed that subjecting butter to high temperatures causes cholesterol to be converted into a high content of cholesterol oxide in ghee. Thus, dietary exposure of these cholesterol oxides from home-made ghee might lead to adverse effects and be a risk factor for atherosclerosis.

INTRODUCTION

Cholesterol oxides are of current interest due to the reports of their adverse human health effects (Addis *et al.*, 1983). Cholestane triol was shown to be most active in its cytopathic effects on cells in culture (Baranowski *et al.*, 1982) and cholesterol 5,6 epoxide was shown to be mutagenic to V79 Chinese hamster cell cultures (Savanian & Peterson, 1984). In addition, cholesterol oxides are known to be cytotoxic (Peng *et al.*, 1971; 1985; Hill *et al.*, 1984) and angiotoxic (Smith *et al.*, 1979). There has also been speculation that a link may exist between ingested and cholesterol oxidation products and coronary heart disease (CHD). Cholesterol oxides are oxidized products of cholesterol, formed after exposure to air at elevated temperatures, free radical initiators, light or combinations of these. Such oxidation has been observed in cholesterol-containing foods such as bleached butter oil, cheeses (Finocchiaro *et al.*, 1984) and spray-dried eggs (Chicoye *et al.*, 1968). In 1987 Jacobson reported the presence of the cholesterol oxides (12·3% sterols) in Indian home-prepared ghee (Jacobson, 1987). These oxides include cholestane triol, 7α-hydroxy cholesterol, 7β-hydroxy cholesterol, 25-hydroxy cholesterol/epoxide and 20α-hydroxy cholesterol. Nath and Murthy (1988) reported no cholesterol oxides in ghees that were laboratory-made, home-made or commercially available in India, having peroxide values below 12, but did identify cholesterol oxides in ghees having peroxide values above 12. The present study was aimed to identify (qualitatively) cholesterol oxides, especially cholestane triol 5,6 epoxide in freshly prepared home-made ghee and commercially available ghees.

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MATERIALS AND METHODS

Materials

Ghee samples were obtained from two commercial sources, Vita and Delhi Milk Scheme, a home-made source and fresh butter was used as control. The standard cholesterol oxides were obtained from Sigma, USA and other required solvents were obtained from BDH.

Saponification and extraction of cholesterol oxides

Cholesterol oxides were isolated and identified by following the procedure of Jacobson (1987). Two-gram

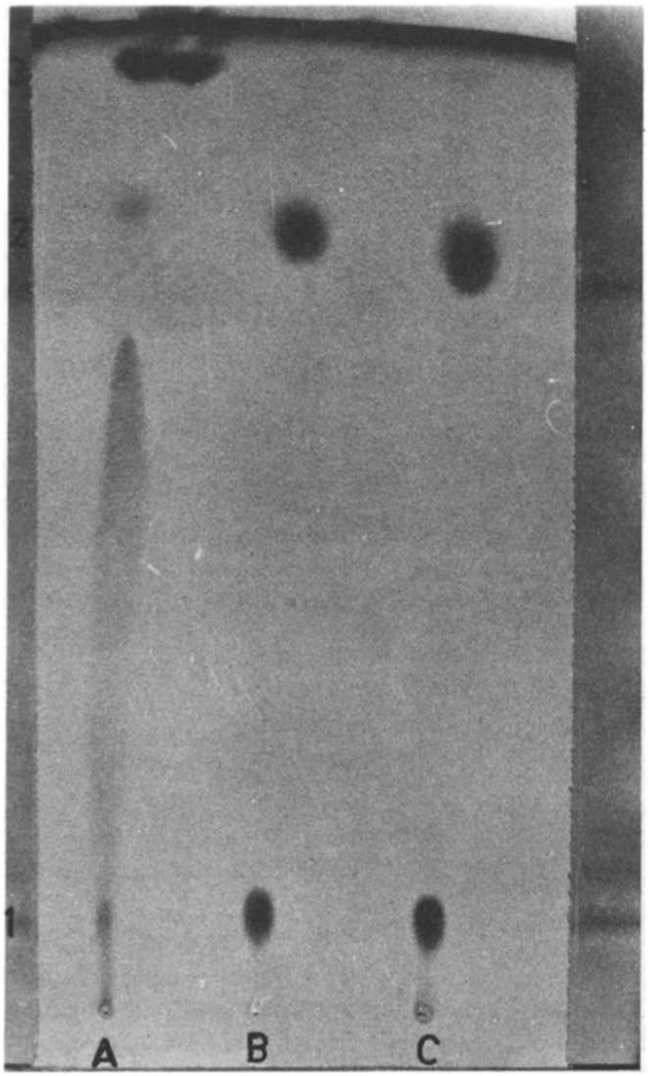

Fig. 1 Separation patterns of cholesterol oxides. (A) Sample (home-made ghee); (B and C) standard cholesterol oxides. (1) Cholestane triol; (2) 5,6 epoxy cholesterol; (3) cholesterol.

samples of ghee or butter were taken and saponified/refluxed in 15% potassium hydroxide under nitrogen for 2 h. After addition of distilled water, the non-saponifiable components were extracted with diethyl ether and washed with distilled water two to three times. Then the sample was concentrated by evaporating the diethyl ether under nitrogen, finally dissolved in 1 ml of hexane, and stored under nitrogen.

Thin-layer chromatography (TLC)

The different samples were applied on the silica gel-G coated TLC plates and developed by using ether as mobile phase (Chicoye *et al.*, 1968). The standard cholesterol oxides were run in parallel with the samples. The components were visualized by charring the plates at 180°C after spraying with 10% $CuSO_4$ in 8% H_2PO_4 and the components were identified by comparing with the Rf values of standard compounds.

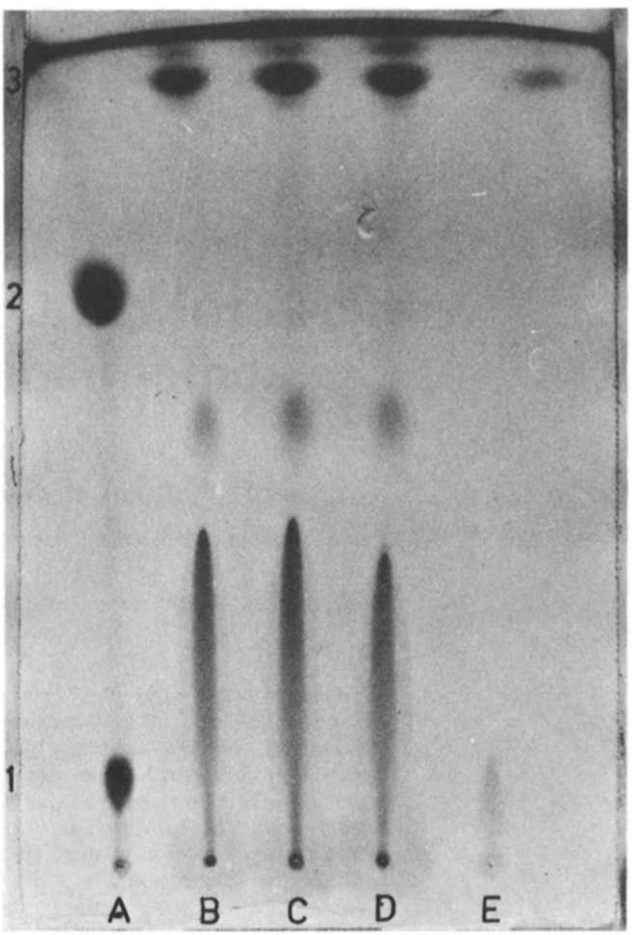

Fig. 2. Separation patterns of cholesterol oxides. (A) Standard cholesterol oxides; (B and C) sample (Delhi Milk Scheme ghee); (D) sample (Vita ghee); (E) fresh butter. (1) Cholestane triol; (2) 5,6 epoxy cholesterol; (3) cholesterol.

RESULTS AND DISCUSSION

The cholesterol oxides, 5,6 epoxide and cholestane triol, were identified in home-made ghee by their Rf values (corresponding to standard Rf values of 5,6 epoxide 0·79 and triol 0·10) run in parallel, whereas in commercial sources, Vita and DMS ghee, they were absent. (Figs 1 and 2).

With reference to the Rc values computed by Chicoye *et al.* (1968) using the same solvent as mobile phase, the Rc value 0·10 for cholestane triol was in agreement with the quoted Rc value 0·11. However, the Rc value for the 5,6 epoxide, 0·8 was near to the quoted Rc value, 0·71. Rc values quoted for other cholesterol oxides (0·64 for 7-ketocholesterol, 0·50 for 7β-hydroxy cholesterol, 0·35 for 7α-hydroxy cholesterol) were not checked in this study, but, by comparing with the quoted Rc values, there could be 7-ketocholesterol in all the samples, i.e. home-made ghee, Vita and DMS ghee. Regarding the presence of other cholesterol oxides, we cannot draw any conclusions as there are no clear-cut spots on the chromatograms.

In 1979 Smith *et al.* concluded that cholestane triol and 5,6 epoxides are secondary oxidation products as the 5,6 epoxide is formed when cholesterol is treated with either epimer of 7-OH cholesterol or with 5-hydroxy 6-ene in chloroform and cholestane triol is formed by hydration of this 5,6 epoxide (Smith *et al.*, 1979).

The presence of these oxides suggest that the extent of oxidation has reached secondary level. During the preparation of home-made ghee, the butter was subjected to heat at 150°C in an open vessel for about 20–25 minutes without antioxidant, and this might be the sole reason for the conversion of cholesterol to its secondary oxidation products cholestane triol and 5,6 epoxide. In commercial ghees these secondary cholesterol products are absent. However, the presence of 7-ketocholesterol in commercial ghees could be due to the storage for 2–3 months prior to buying. This suggests that either the commercial ghees are not subjected to high temperatures, or antioxidants have been used to prevent the formation of these oxides.

Based on the hypothesis that dietary exposure to cholesterol oxides from ghee may be an explanation for the atherosclerotic complications in Indian immigrants to London (Jacobson, 1987), consumption of home-made ghee may be more harmful than commercially prepared ghees. For proof epidemiological studies are needed and quantitative studies are also required to show that the amount present in home-made ghee is significant.

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