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Cholesterol oxidation products in small sun-dried fish

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Cholesterol oxidation products (COPs) in small sun-dried fish *Spratelloides gracilis* and *Decapterus maruodsi* were extracted, separated by thin-layer chromatography, and then quantified by capillary gas chromatography after trimethylsilylation. Peak identifications were confirmed by mass spectrometry. Four major COPs including 7α- and 7β-hydroxycholesterol, 7-ketocholesterol, and 5, 6α-epoxycholesterol, were identified in small sun-dried fish which were stored at ambient temperature for *c.* 3 months under air. The total concentration of COPs in small sun-dried fish ranged from 4·82 ppm to 65·7 ppm, with 7α-hydroxycholesterol appearing as the predominant product.

INTRODUCTION

Cholesterol undergoes spontaneous oxidation when in contact with air (Smith, 1981; Finocciaro & Richardson, 1983; Maerker, 1987) or photoxidation under fluorescent light (Chicoye *et al.*, 1968; Herian & Lee, 1985; Bekbolet, 1990). Cholesterol oxidation products (COPs) may possibly cause sterol metabolism interruption, cytotoxicity, atherogenicity, mutagenicity and even carcinogenicity (Maerker, 1987; Hurrard *et al.*, 1989). This has intensified interest in investigating cholesterol oxidation and its magnitude in foods such as eggs (Hurst *et al.*, 1985; Tsai & Hudson, 1985; Morgan & Armstrong, 1989), meat products (Park & Addis, 1985*a*, *b*; Sander *et al.*, 1989*a*; Pie *et al.*, 1991), heated tallow (Park & Addis, 1986); milk and dairy products (Nourooz-Zadeh & Appelqvist, 1988*a*, *b*; Sander *et al.*, 1989b). Few reports have, however, been made about the traditional oriental foods produced by different traditional food processing methods. Various small sun-dried fish, such as *Spratelloides gracilis* or *Decapterus maruodsi*, have been one of the most commercially traditional foods in Chinese culture for a long time. These products are prepared under sunlight for dehydration and without special packaging during storage or retail. Therefore, the autoxidation of cholesterol in these products is possible and cannot be ignored from the point view of food safety. Determining the significance of COPs present in various small sun-dried fish which remain popular with Chinese people is the focus of this study.

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MATERIALS AND METHODS

Reagents

Sylon BTZ, trimethylsilylation reagent, and 5α-cholestane were purchased from Supelco Inc. (Bellefonte, PA, USA). Silica gel plates (0·25 mm thick, non-fluorescence) were obtained from Merck Co. (Germany). All other reagents were ACS grade from ALPS Chem. Co. Ltd, (Taiwan) or Merck Co. (Germany).

Samples

All small sun-dried fish were purchased from local retail stores and supermarkets in Taichung, Taiwan. These samples were roughly stored 3 months before analysis. All the small sun-dried fish sold in the market were exposed to air at ambient temperature and did not have any package covering. Two batches of small sun-dried fish were purchased. The first batch of small sun-dried fish contained four species of small fish, three species were *Spratelloides gracilis*, labelled as samples A, B and C, and one was *Decapterus maruadsi*, labelled as sample D which came from Peng-Hwu county in

Taiwan. The second batch which contained only one species of small sun-dried fish, *Spratelloides gracilis* labelled as sample E, was imported from Japan.

Sample preparation

Total lipids were extracted by the modified method of Folch *et al.* (1957). Cholesterol and its oxidized derivatives were extracted from the cold saponified small sun-dried fish according to the method of Park and Addis (1986). The dried non-saponifiables obtained were treated to concentrate the COPs by TLC and then through trimethylsilylation as described by Park and Addis (1985b, 1986, 1987).

Thin layer chromatography

Plates were dried in a 105°C air oven for 4 h prior to use. Plates containing samples were developed with benzene-ethyl acetate-acetic acid (60:40:1, v/v/v). Air-dried plates were lightly sprayed with 50% sulphuric acid, then placed in a 150°C air oven to produce a maximum colour display of the COPs according to the method of Maerker and Bunick (1986). After complete charring on a 200°C hot plate, the plates were removed from the hot plate, cooled, and R_f values were measured. The COPs were removed from the TLC plates with 30 ml acetone containing 40μg 5α-cholestane as internal standard for quantitation. The COPs were quantified by GC following vacuum evaporation.

Quantitation of COPs

Gas chromatography analyses of the COPs isolated by TLC were performed with a Shimadzu GC-14A equipped with a flame ionisation detector as trimethylsilyl (TMS) ether sterols derivatised with Sylon BTZ according to Park and Addis (1985*b*). Briefly, GC conditions were as follows: a silica capillary column DB-1 (15 m × 0·25 mm id, 0·1 μm film thickness; J & W Scientific Inc., Rancho Cordova, CA, USA); temperature programming from 180°C to 250°C at 3°C/min increase; injector at 250°C detector at 300°C and split ratio of 100:1.

Peak identification

GC-mass spectrometry (MS) was performed with a Hewlett-packard 5895. GC conditions were the same as those for quantitation of COPs except for a splitless injection. MS conditions were as follows: electron impact ionization at 70 eV; ion source at 200 °C; electron multiplier 2800 V; and mass spectra scanning from mass/charge (*m/z*) 100–600.

RESULTS AND DISCUSSION

Two batches of small sun-dried fish were investigated for COPs content. The first batch contained four species of small sun-dried fish from Taiwan. The second batch contained only one species of small sun-dried fish imported from Japan.

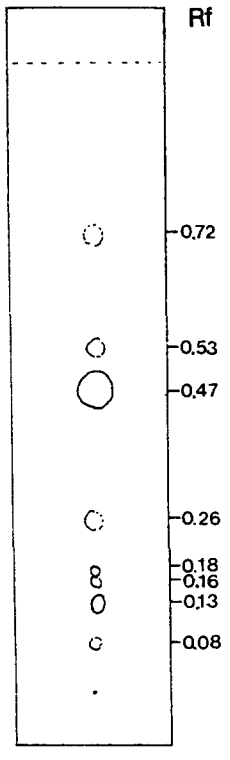

Fig. 1. TLC chromatogram of cholesterol oxidation products in small sun-dried fish. R_f 0·47, cholesterol; R_f 0·26, 7-ketocholesterol; R_f 0·18, 7α-hydroxycholesterol; R_f 0·16, 7β-hydroxycholesterol.

GC analysis has been directly employed to quantitate the COPs in various foods after saponification (Park & Addis, 1985b, 1986, 1987). COPs concentration of the sample was found from the preliminary study to be too low to make a direct measurement by GC analysis. Another separation method for determination of COPs in small sun-dried fish was therefore necessary.

TLC proved to be useful in assessing the oxidation of cholesterol dispersions made with various surfactants and buffered to different pH values (Christie, 1982). The COPs could be effectively separated by TLC with this developing solvent system. A typical TLC chromatogram is shown in Fig. 1. In the nonsaponifiables, cholesterol at R_f 0·47 and relatively trace amounts of COPs were visible at low R_f values. Initially, after spraying with 50% sulfuric acid, the cholesterol spot appeared as a red-violet colour within a few seconds; the isomeric 7-OH spots, 7α-OH and 7β-OH, then appeared as a light blue color with R_f 0·18 and 0·16, respectively. The 7-keto spot appeared as a quite light brownish colour at R_f 0·26.

TLC, however, provided an indication of COPs, whereas capillary GC permitted quantification of COPs as shown in Fig. 2. Noteworthy is the cleaniness of the 7α-OH (peak a) which was overlapped by the proceeding cholesterol peak as described in previous reports (Park & Addis, 1985b, 1986, 1987). Therefore, when there were few COPs in food, as the GC chromatograms showed, it was advantageous to quantitate

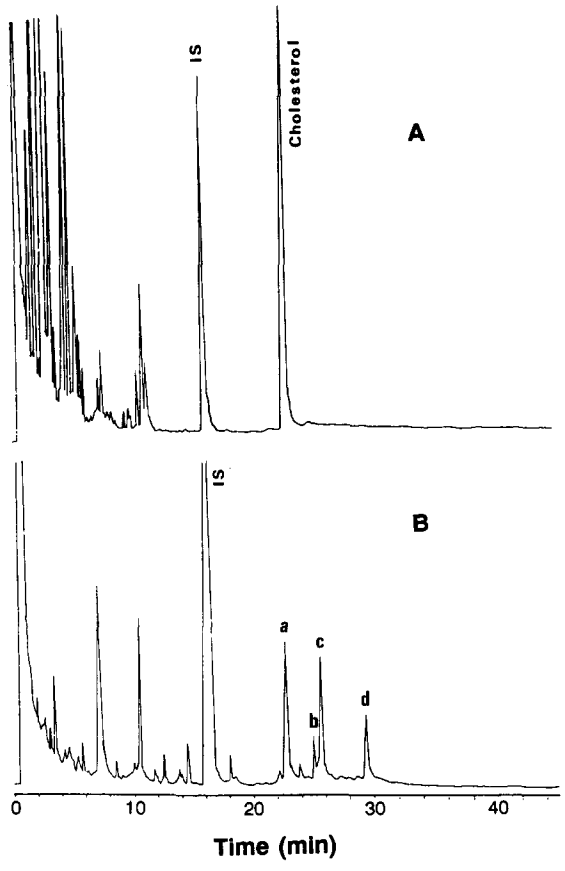

Fig. 2. Chromatogram of cholesterol oxidation products in small sun-dried fish, (A) before separation by TLC, (B) after separation by TLC. IS represents 5α-cholestane used as internal standard. As trimethylsilyl ether sterols, peaks a to d were identified as 7α-hydroxycholesterol, 5, 6α-epoxycholesterol, 7β-hydroxycholesterol and 7-ketocholesterol, respectively.

the COPs if the COPs were separated by the TLC prior to GC analysis.

The identities of COPs peaks, labelled as a, c and d (Fig. 2), were confirmed by combined GC-MS analysis. Mass spectra for these COPs were the same as the data that had already been presented by other previous reports (Park & Addis, 1985*a*, *b*, 1986, 1987). The mass spectrum of 7β-OH was the same as the spectrum of 7α-OH. The molecular ion (*M*) of 7α-OH (*m/z* 546) as the TMS ether was not found, but the peak at *m/z* 456 was the base peak that corresponded to the loss of trimethylsilanol (M-90) as in a previous report (Park & Addis, 1986). The concentration of COPs was possibly too low to observe another small peak that was observed at *m/z* 472 which is the molecular ion (*M*) of the 7-keto TMS ether. Moreover, the base peak was at *m/z* 129 with no other peaks with a relative intensity greater than 10%. The spectrum of the 7-keto was also the same as that reported previously (Park & Addis, 1986). Because there were no detectable peaks in the mass spectrum of the 5, 6α-epoxide, the identify of 5, 6α-epoxide, labelled as b (Fig. 2) was determined according to the relative retention time on the GC chromatogram according to other reports where the same GC condition was used (Park & Addis, 1985*b*, 1986, 1987).

Table 1 shows the COPs content of various small sun-dried fish obtained from a local market. All the COPs in small sun-dried fish sterols were based on the response of 5α-cholestane used as an internal standard. Some of the major COPs, i.e. 7α-hydroxy- cholesterol, 7β-hydroxycholesterol, 5, 6α-epoxycholesterol and 7-ketocholesterol, were detected at a limit of 0·01 ppm.

Table 1. Cholesterol oxidation products in small sun-dried fish

Sample[b]	Cholesterol oxidation products (ppm)[a]			
	7α-OH	7β-OH	5,6 α-epoxide	7-keto
A	2·23 ± 0·45	1·33 ± 0·08	0·33 ± 0·05	0·93 ± 0·40
B	2·80 ± 0·08	1·75 ± 0·11	0·45 ± 0·08	0·05 ± 0·04
C	4·15 ± 0·18	1·60 ± 0·15	0·25 ± 0·08	0·25 ± 0·09
D	26·8 ± 1·53	12·6 ± 1·01	6·15 ± 1·02	20·2 ± 1·75
E	19·6 ± 1·51	24·0 ± 1·60	5·25 ± 0·95	7·25 ± 0·98

[a] Data are the mean ± SD of three separate determinations.
[b] Samples A, B, C and E are *Spratelloides gracilis*; sample D is *Decapterus meruadsi*.

Obviously, the concentration of COPs from different batches of small sun-dried fish were markedly different. The total concentration in small sun-dried fish ranged from 4·82 ppm (*Spratelloides gracilis*) to 65·7 ppm (*Decapterus maruadsi*). The total concentration of COPs in small sun-dried fish (*Spratelloides gracilis*) (sample E) which was imported from Japan was 56.1 ppm. 7α-Hydroxycholesterol was the predominant cholesterol oxidation product in these samples. 7α-Hydroxycholesterol was formed readily by the reduction of corresponding 7α-hydroxyproxycholesterol (Finocchiaro & Richardson, 1984). The hydroperoxide forms by a free radical reaction. This occurs despite the fact that 7β-hydroperoxide is thermodynamically more stable and expected to predominate. However, 7α-hydroxycholesterol was indicated to have formed in a greater amount than 7β-hydroxycholesterol. Thus, thermodynamic stability alone did not determine the relative amounts of the COPs. The predominant COPs in dried-egg products formed under photoxidation with fluorescene were 7α- and 7β-hydroxycholesterol (Herian & Lee, 1985). The traditional processing method of small sun-dried fish in Taiwan has been conducted by drying under sunshine for one or two days on the seashore. The processing and storage conditions should be the primary factors for the formation of COPs in small sun-dried fish, since these samples had been kept under abusive conditions, i.e. in the presence of air, uncontrolled relative humidity, exposure to fluorescent light and a long storage time. Further studies, including improvement of the processing and packaging methods and reduction of the concentration of COPs in traditional Chinese foods, such as small sun-dried fish, are necessary and currently underway.

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