mercaptoethanol or urea, while treatment with buffer-1-none did not cause any change of gel hardness. The shapes of the gels were not changed significantly by the treatments. The decrease of gel hardness may be due to severance of the bonds involved in the structure of the gel networks by 2-mercaptoethanol or urea. The partial solubilization of gels may not be a major factor contributing to the change of gel hardness, since the change in gel hardness with time of the treatment did not parallel that found in solubilization of the gels shown in Figure 2. It is not clarified here whether either or both of noncovalent bonds, hydrophobic interactions and hydrogen bondings, contributes in stabilization of the network structure of gel-20, since urea destabilizes both types of bondings.

From the results obtained here, it is deduced that intermolecular bondings, mainly disulfide bondings and noncovalent bonds, probably hydrophobic interactions, and/or hydrogen bondings proceed within the strands of networks on subsequent heating after the gel network has once been established, and then the junction points of constituent strands within the gel network are formed through further formation of both types of bondings. Thus, stabilization of the gel network structure and concomitant increase of the gel hardness may occur in the late stage of the thermal gelation process of glycinin.

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High-Performance Liquid Chromatographic Method for the Quantification of Cholesterol Epoxides in Spray-Dried Egg

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A simple and sensitive method for the quantification of cholesterol epoxides in egg products was developed by using reversed-phase high-performance liquid chromatography (HPLC). This method involved the addition of total lipids to a disposable column containing silica to obtain the cholesterol fraction and the subsequent derivatization with *p*-nitrobenzoyl chloride prior to separation for HPLC. Cholesterol α- and β-epoxides were found in commercial spray-dried egg at levels about 0.1% and 0.2% of cholesterol, respectively.

Cholesterol is known to be susceptible to air oxidation by a free-radical mechanism similar to unsaturated fatty acid. There have been extensive studies on the autoxidation products of cholesterol (Smith, 1980). Among such products, 5α-cholestane-5,6α-epoxy-3β-ol (α-epoxide) has been suggested to be involved in carcinogenesis (Bischoff, 1969; Black and Douglas, 1973; Reddy and Wynder, 1977; Parsons and Goss, 1978; Kelsey and Pienta, 1979, 1981). Sevanian and Peterson (1984) have recently found that α-epoxide is a direct-acting mutagen in mammalian cells. This oxygenated cholesterol seems to be formed in vivo or incorporated as a food contaminant. Bowden et al. (1979) reported that a small percentage of α-epoxide and its metabolites was found in a wide variety of organs when administered to mice by gastric intubation, although most of the epoxide was excreted in the feces. Therefore, attention should be paid to the content of α-epoxide in foods (Shepard and Shen, 1980).

Spray-dried egg is a popular foodstuff containing a high concentration of cholesterol. Chicoye et al. (1968) detected 5β-cholestan-5,6β-epoxy-3β-ol (β-epoxide) in photooxidized spray-dried egg. Tsai and Hudson (1984) isolated α- and β-epoxides from egg products after the spray-drying process. However, no quantitative analysis of each epoxide in egg products has yet been carried out, although the contents of total epoxides in cheese and butter oil were recently estimated by thin-layer chromatographic analysis (Finocchiaro et al., 1984). Tsai et al. (1980) proposed the use of high-performance liquid chromatography (HPLC) with a differential refractometer for the resolution of α- and β-isomers in egg products, but the sensitivity was not high. In this experiment, we developed a reversed-phase HPLC analysis for the quantification of α- and β-epoxides through their *p*-nitrobenzoyl derivatives (Watabe et al.,

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1980), which was found to elevate the sensitivity of each epoxide. Thus, it is concluded that this analytical method is useful for quantifying the cholesterol epoxides in dried-egg products.

MATERIALS AND METHODS

Materials. Spray-dried egg powder was the commercial product from whole egg. This was stored at room temperature for 9 months in the market and then kept in the dark at 4 °C for 4 months after we obtained it in the laboratory. This sample was used as the starting material. A part of the spray-dried egg was incubated at 50 °C with and without adjusting water activity (A_w = 0.8). The standard compound of cholesterol α-epoxide was purchased from Sigma Chemical Co., and that of cholesterol β-epoxide was kindly provided by Professor Tadashi Watabe, Tokyo College of Pharmacy, Tokyo, Japan.

Extraction of Lipids. Water (1.0 mL) was added to spray-dried egg (100 ± 5 mg), and the mixture was blended with a Vortex mixer for 30 s. Chloroform/methanol (1/2, v/v, 3.75 mL), which contained 0.04 mg of butylhydroxytoluene to prevent lipid peroxidation, was added during the extraction procedure. Then, 0.1 mL of chloroform solution containing 0.14 mg of stigmasterol (internal standard) was added to the mixture and blended again for 30 s. Chloroform (1.25 mL) and water (1.25 mL) were added, and the mixture was shaken for 30 s. The mixture was then centrifuged at 3000 rpm for 5 min. After centrifugation, the upper layer was washed with 2 mL of chloroform three times. The lower layers were combined and dried with a rotary evaporator at 30 °C. The residue was dissolved in 2 mL of *n*-hexane and diethyl ether (97.5/2.5, v/v).

Fractionation of Cholesterol and Cholesterol Epoxides. The lipid extract was applied to a Bondelut (Analytichem International Co., Harbor City, CA), which is a disposable silica packed column (unbonded silica, column volume 2.8 mL), to obtain cholesterol fraction. The column was equilibrated with 30 mL of *n*-hexane in advance. The sample solution (2.0 mL) was charged on the column and then eluted with 20 mL of *n*-hexane/diethyl ether (97.5/2.5, v/v) followed by 30 mL of *n*-hexane/diethyl ether (40/60, v/v). The fraction eluted with the last solvent contained the cholesterol fraction which was subjected to derivatization.

Derivatization of the Cholesterol Fraction. The derivatization with *p*-nitrobenzoyl chloride was carried out according to the method of Watabe et al. (1980). The cholesterol fraction was evaporated by rotary evaporator at 30 °C and then placed in desiccator under vacuum for 30 min. The residue was heated at 80 °C for 1 h in a mixture of 20% aqueous potassium hydroxide (30 μL) and pyridine (150 μL) containing 10% *p*-nitrobenzoyl chloride. The reaction mixture was diluted with 5.0 mL of water and extracted with 1.0 mL of *n*-hexane three times. The hexane layer was collected and concentrated to dryness by rotary evaporator at 30 °C. The residue was dissolved in 5 mL of *n*-hexane/2-propanol (4/1, v/v) and subjected to HPLC analysis.

HPLC. A Shimadzu liquid chromatograph LC-4A was used with a YMC packed ODS column (6 × 160 mm, 5-μL particle size, Yamamura Kagaku Co., Japan.). Fifty microliters of the sample was injected into the HPLC. The sample was eluted with 2-propanol/acetonitrile (10/90, v/v) for the first 5 min; then, the solvent was changed linearly from the initial composition to 2-propanol/acetonitrile (50/50, v/v) for 40 min. Flow rates was kept at 3 mL/min for the first 5 min and then maintained at 1.0 mL/min. The eluent was monitored at 254 nm on a Shimadzu SPD-2A variable-wavelength UV detector (aufs = 0.01). Drift of the base line was corrected by a Shimadzu Chromatopack C-R1B data processor.

Identification of Cholesterol Epoxides. The peaks corresponding to cholesterol epoxides were fractionated, evaporated by rotary evaporator at 30 °C, and then heated at 80 °C for 15 min in a mixture of 0.25 mL of ethanol and aqueous solution of potassium hydroxide (33%, 0.01 mL). The reaction mixture was diluted with 0.25 mL of water and extracted with *n*-hexane three times. Each extract was subjected to gas chromatography–mass spectrometry (GC–MS). For GC–MS analysis, the extract was trimethylsilylated with Me_3Si–HT (Tokyo Kasei Co., Tokyo, Japan) at 60 °C for 5 min. Mass spectrum was obtained with a Shimadzu LKB 9000 gas chromatograph–mass spectrometer. The column used was a glass column packed with 2% OV-17 (3 mm × 1 m) on Chromosorb W (60/80 mesh). Flow rate of helium gas was 30 mL/min. The temperature of the oven was programmed from 260 to 280 °C (2 °C/min). The mass spectrometer was operated at a trap current of 60 μA, an accelerating voltage of 3.5 kV, and an ionization electron energy of 22 eV. The temperature of the injection port, separator, and an ion source were 280, 290, and 310 °C, respectively.

RESULTS AND DISCUSSION

Total lipids corresponding to 38.9 ± 1.1 wt % of the spray-dried egg were extracted from the egg before incubation at 50 °C. Total lipids were found to be separated to 73.8 ± 0.5 wt % of simple lipids, including triacylglycerol and cholesterol, and 26.2 ± 0.7 wt % complex lipids, including phospholipids, by the acetone precipitation according to the procedure of Kates (1972). In the treatment with the disposable silica column (Bondelut), triacylglycerol was eluted almost completely with the first solvent (*n*-hexane/diethyl ether, 97.5/2.5). Cholesterol epoxides were found to be eluted along with cholesterol during the elution with the second solvent (*n*-hexane/diethyl ether, 40/60 v/v), when standard cholesterol epoxides were charged on the column with the total lipids. Phospholipids were retained in the column throughout the elution. Thus, pretreatment with an unbonded silica cartrige column seems to be convenient and highly effective to concentrate cholesterol epoxides from lipid extracts of spray-dried egg.

Figure 1 shows the typical HPLC pattern of *p*-nitrobenzoyl derivatives of the mixture of the standard materials and that of cholesterol fraction obtained from spray-dried eggs before incubation at 50 °C. Standard cholesterol α-epoxide (**2**) and cholesterol β-epoxide (**1**) were eluted separately from each other. Stigmasterol (**4**), an internal standard, was eluted after cholesterol (**3**). The lowest level for each injection was estimated to be 10 ng for cholesterol α-epoxide and β-epoxide (1 μg/100 mg of spray-dried egg). The retention times of peaks A and B in the chromatogram of cholesterol fraction of spray-dried egg coincided with those of the standard compounds, cholesterol β-epoxide (1) and cholesterol α-epoxide (2), respectively. Both peaks were fractionated and trimethylsilylated after alkaline treatment for the identification by GC–MS. The peaks whose retention times agreed with those of the trimethylsilyl derivative of standard cholesterol β-epoxide and cholesterol α-epoxide appeared on the gas chromatogram, although cholesterol epoxides were shown to be partially decomposed by alkaline treatment (Tsai et al., 1980). In the mass spectrum obtained from peak A, molecular ion (M) appeared at m/z (relative intensity) 474 (65). Principal ions were present at m/z 459 [M − 15, loss of CH_3] (11), 456 [M − 18, loss of H_2O] (11), 445 [M − 29, loss of CHO] (65), 384 [M − 90,

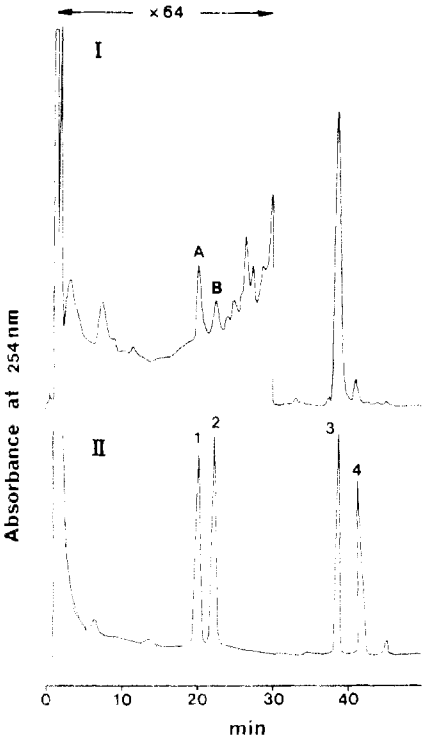

Figure 1. HPLC of *p*-nitrobenzoyl cholesterol fraction from spray-dried egg (I) and the mixture of standard compounds (II). The operation conditions are shown in the Materials and Methods. In I, the sensitivity was lowered to $^1/_{64}$ after a 30-min run: 1, β-epoxide; 2, α-epoxide; 3, cholesterol; 4, stigmasterol.

loss of trimethylsilanol] (100), 369 [M − (90 + 15)] (45), and 366 [M − (90 + 18)] (74). The mass spectrum from peak B also gave molecular ion at *m/z* 474 (26) and principal ions at *m/z* 459 (53), 456 (28), 384 (100), 369 (45), and 366 (45). These fragmentation patterns were in fair agreement with those of respective standard cholesterol epoxides. Thus, peaks A and B were identified as cholesterol β-epoxide and cholesterol α-epoxide, respectively. These data support the results of Tsai and Hadson (1984) that cholesterol epoxides were found in dehydrated commercial egg powders.

The effectiveness of the extraction procedure was determined by measuring the recoveries of cholesterol epoxides and cholesterol from spiked spray-dried egg. Chloroform solution of the standard mixture (cholesterol 0.38 mg, α-epoxide 0.39 mg, β-epoxide 0.40 mg) was evaporated, and the residue was injected to HPLC after derivatization (standard samples). The same volume was added to spray-dried eggs (44 ± 4 mg), and the cholesterol fraction was obtained by lipid extraction followed by the treatment of the silica column. After derivatization, this preparation was analyzed by HPLC (extracted samples). Recoveries of α-epoxide and β-epoxide were measured by the ratio of the peak areas in extracted sample to those in standard sample on the chromatogram. In the case of cholesterol, the peak area of extracted sample was obtained by substracting the peak area of cholesterol present in unspiked spray-dried egg from that in extracted sample. The mean values of the recoveries for four samples were estimated to be 100.1 ± 9.6% (cholesterol α-epoxide), 96.0 ± 7.9% (cholesterol β-epoxide), and 111.4 ± 30.2% (cholesterol). The present procedure is considered to be suitable for the extraction of cholesterol epoxides from spray-dried egg.

Cholesterol epoxides were shown to be completely converted to *p*-nitrobenzoyl derivatives by TLC analysis of the derivatives of standard cholesterol epoxides (silica gel PF 254; solvent system benzene/ethyl acetate, 3/2, v/v). Epoxide formation during this derivatization procedure could be negligible because the derivative of standard cholesterol with the level in spray-dried egg did not give any peaks corresponding to cholesterol epoxides on the chromatogram. Tsai et al. (1980) showed that recoveries of the cholesterol epoxides were lowered by alkaline treatment such as saponification. However, alkaline solution (aqueous potassium hydroxide) is necessary to neutralize hydrogen chloride generating from *p*-nitrobenzoyl chloride during the derivatization procedure (Watabe et al., 1980). Figure 2 shows the calibration curves for α-epoxide, β-epoxide, and cholesterol. Good correlation between the ratio of amount of *p*-nitrobenzoyl derivatives of each epoxide to that of the internal standard, stigmasterol, and the ratio of their peak areas (correlation coefficient 0.997 for α-epoxide, 0.9994 for β-epoxide, 0.9903 for cholesterol) was observed.

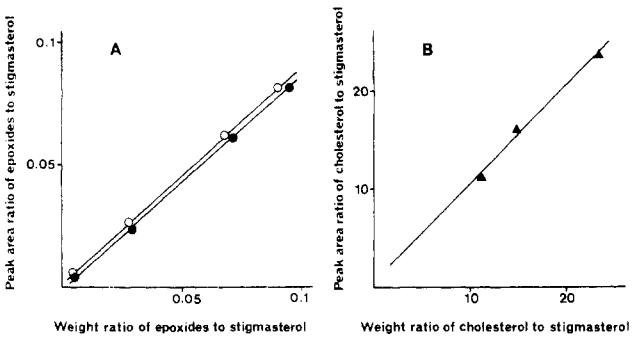

Figure 2. Calibration curves for epoxides (A) and cholesterol (B): ●, α-epoxide, ○, β-epoxide; ▲, cholesterol.

Table I. Contents of Cholesterol Epoxides in Spray-Dried Egg[a]

	cholesterol epoxides, μg/g			cholesterol, mg/g
	α-epoxide	β-epoxide	α/β	
before incubation	17.4 ± 1.9	31.8 ± 1.7	0.55	15.7 ± 1.3
40 days	27.1 ± 1.9	57.3 ± 3.7	0.47	15.9 ± 1.4
80 days	27.3 ± 4.4	58.1 ± 8.5	0.47	15.1 ± 0.6
70 days[b]	31.9 ± 3.3	47.0 ± 2.5	0.68	17.2 ± 0.8

[a] Average value ± standard deviation for four samples. [b] Water activity (A_w) was adjusted at 0.8.

The contents of α-epoxide, β-epoxide, and cholesterol in spray-dried egg were determined by using these analytical procedures, and the values are listed in Table I. The values of α- and β-epoxides correspond to about 0.1% and 0.2% of cholesterol present in spray-dried egg. The contents of cholesterol epoxides increased with length of the storage at 50 °C. It was believed to be formed during the spray-drying process (Tsai and Hudson, 1984). However, the increase in epoxide level after storage may be due to the occurrence of cholesterol oxidation by a free-radical process. The ratio of α-epoxide to β-epoxide in spray-dried egg was found to be in the range from 0.47 to 0.68. This ratio is consistent with that reported by Tsai and Hudson (1984).

The analytical method described above is sensitive and convenient and thus seems to be satisfactory technique for estimating cholesterol epoxides in spray-dried egg. Detection with the UV detector after *p*-nitrobenzoyl derivatization elevated the detectable level significantly as compared with that obtained with the refractive index (Tsai et al., 1980, 1984), and furthermore, this analytical technique provided simultaneous determination of cholesterol and its epoxides.

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Registry No. 5α-Cholestane-5,6α-epoxy-3β-ol, 1250-95-9; 5β-cholestane-5,6β-epoxy-3β-ol, 4025-59-6.

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Compositional Study of *Apios priceana* Tubers

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Apios priceana Robinson (Fabaceae), a reputedly edible tuber native to North America, contains 61.9% water, 5.0% fiber, 2.6% crude protein, 2.7% ash, 27.1% carbohydrate, and 0.7% fat. The amino acid content of the crude protein was of poor nutritional value because of a high proportion of nonessential amino acids. Removal of the nonprotein nitrogen fraction by alcohol extraction significantly improved the nutritional quality. The protein, after removal of nonprotein nitrogen, was limiting in threonine and sulfur-containing amino acids.

INTRODUCTION

The North American plant *Apios priceana* Robinson (Sadie Price's Potato Bean) has been classified as an underutilized, edible legume (National Academy of Sciences, 1979) with potential as a new crop. Cultivation of *A. priceana* would expand land utilization for food production because in the wild it produces a tuber in highly alkaline (pH >8) and wooded habitats. *A. priceana* produces significantly larger tubers than its widely distributed relative *Apios americana* Medicus, but *A. priceana* has only been collected from eight widely distinct habitats in Alabama, Mississippi, Tennessee, Kentucky, and Illinois (Seabrook, 1973; Medley, 1980). This plant is so rare that it has been proposed as a candidate for the Federal Endangered and Threatened Plants list (Federal Register, 1980).

Although rare and endangered plants have been protected in part because of their potential as new foods or medicines, to our knowledge this the first nutritional analysis to evalute a rare plant's potential for food. Although a rare plant, *A. priceana* can readily be propagated by chipping the seed coat (Seabrook, 1973) or by acid scarification.

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MATERIALS AND METHODS

***A. priceana* Tubers.** The tubers were harvested in February, near University, MI, packed in damp sphangum moss, and air-shipped to North Carolina State University for analysis. The tubers were received in excellent condition. Three tubers were selected for analysis. The tubers were a deep brown color with numerous wartlike projetions about 1 × 3 mm. They were 12–14 cm across and 7–8 cm high. The weights ranged from 303.6 to 438.7 g. The tubers were sliced into 2-mm-thick sections. Duplicate samples were removed for moisture measurements, and the remainder was freeze-dried and ground to pass through a 60-mesh screen.

Analyses. The moisture content was measured by drying tuber slices at 60 °C for 24 h, followed by drying at 100 °C until constant weight was attained. The moisture content was calculated from the differences in weight between fresh and dried samples. All other analyses were carried out on freeze-dried material. The moisture content of the freeze-dried powder was measured as described for tuber slices. The values given in this paper were converted to fresh weight and dry weight basis by appropriate factors. Crude protein, crude fat, and ash were determined by using AOAC (1975) methods. Acid detergent fiber was measured according to Van Soest (1963). The carbohydrate content was obtained by difference.

Alcohol-insoluble solid (AIS) content was obtained by extraction of the powder with boiling 80% ethanol three times (four parts by volume ethanol to one part by weight