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A Rapid Gas Chromatographic Method for Direct Determination of Free Sterols in Animal and Vegetable Fats and Oils

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ABSTRACT

In this study, a rapid and simple method without a derivatization process for determination of the free sterols in vegetable and animal oils was developed. A direct injection gas chromatography with a non-polar megabore column (DB-1, 5 *m* x 0.53 *mm*, 1*μm*) was used. The recoveries of cholesterol, campesterol, stigmasterol and *β*-sitosterol from lard were at the range of 89%-106% with a coefficient of variation less than 12%. Total sterols were determined using the same method after saponification of the oil sample. Fourteen samples, including olive oil, sunflower oil, peanut oil, soybean oil, sesame oil, lard and egg yolk oil, were quantitatively analyzed for total and free sterols. The contents of total cholesterol, brassicasterol, campesterol, stigmasterol, *β*-sitosterol, Δ^5-avenasterol, Δ^7-stigmastenol and Δ^7-avenasterol in test samples were at the range of 193-34384, 0-1222, 0-2356, 0-1365, 0-5414, 0-608, 0-1296 and 0-129 μg/g, respectively; while the contents of free cholesterol, brassicasterol, campesterol, stigmasterol, *β*-sitosterol, Δ^5-avenasterol, Δ^7-stigmastenol and Δ^7-avenasterol were 98-30340, 0-1057, 0-634, 0-724, 0-3087, 0-404, 0-558 and 0-23 μg/g, respectively. The amounts of esterified sterols were calculated by subtracting the free sterol contents from total sterol contents. Results showed olive oil, sunflower oil and peanut oil primarily contain esterified sterols; while soybean oil, sesame oil, lard and egg oil contain mostly free sterols. The developed method is recommended as a rapid method to determine if animal fats/oils are adulterated with vegetable oils.

Key words: **total sterol, free sterol, esterified sterol, fats and oils, gas chromatography, direct injection.**

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INTRODUCTION

Acylglycerols make up 98% (by weight) or more of animal fats/oils and vegetable oils. The remaining two percent of fats and oils are non-acylglycerol fractions composed of a mixture of compounds including hydrocarbons, tocopherols, esterified sterols and free sterols. Among them, the esterified and free sterols are the two predominant non-acylglycerols lipids in vegetable and animal fats and oils[1-8].

Traditional methods for analysis of sterols in the lipids of animals or vegetables require a saponification process, which is for conversion of the acylglycerols into fatty acid soaps. The non-saponification fraction is separated from the above soaps by organic solvent extraction[1-14] and can be analyzed using gas chromatography (GC)[5-14] or high performance liquid chromatography (HPLC)[15-23]. These classic methodologies[1-14], however, can only determine the total sterols, which are the sums of esterified and free sterols. The contents of esterified sterols and/or free sterols are still unable to be identified and quantified. Important information with respect to the effects of extraction processes and refining methods on the contents of free and esterified sterols will be thus lost if saponification process is conducted. In addition, the free and esterified sterol contents in fats or oils from different sources could vary. It is therefore important to establish a rapid method to analyze those sterols and monitor the esterified and free sterols in the above fats/oils.

Some analytical methods for determination of esterified and free sterols in animal fats have been reported[6-8, 24-27]. These methods which involve silica gel column chromatography[6-8, 24-25] or Sep-Pak cartridge clean-up[26] for removing acylglycerols followed by partitioning esterified and free sterols with different polarity solvents are, however, time-consuming and impractical. A simple and rapid GC method to analyze free and total cholesterol in animal fats was established in our previous study[28]. The esterified cholesterol can be calculated by subtracting free cholesterol from total cholesterol. However, neither the literature nor our laboratory were able to document the analytical method for determination of free or esterified sterols (β-sitosterol, campesterol and stigmasterol) in vegetable oils.

The purpose of this study was to develop a simple method based on the GC technique to analyze the free and esterified sterols in animal fats and vegetable oils. The contents of free and esterified sterols in both animal and vegetable fats/oils from different sources or varieties were the focus of investigation. This method was expected to be simple and rapid enough to determine if the animal fats can possibly be adulterated with vegetable oils.

MATERIALS AND METHODS

I. *Materials*

One sample each of olive oil, sunflower oil, and peanut oil, 2 samples of soybean oil, 4 samples of sesame oil, 1 sample of lard, and 1 egg oil capsule sample were purchased from supermarkets of Tainan. Standards of 5 α-cholestane, cholesterol, brassicasterol, β-sitosterol and α-tocopherol were obtained from Sigma Chemical Co. (Munich, Germany). Other reagents and solvents were reagent grade and purchased from ALPS Chemical Co. (Taipei, Taiwan).

II. *Preparation of Egg Yolk Oil*

Chicken eggs were boiled and left to cool. One hundred grams of egg yolk were weighed and extracted with 500 mL of *n*-hexane, which was then filtered and evaporated under vacuum to obtain the egg yolk oil.

III. *Preparation of Standard Solution*

The standards of 5 α-cholestane (internal standard, IS), cholesterol, brassicasterol, campesterol, stigmasterol and β-sitosterol (0.1 ± 0.0001 g) were separately weighed into a 100-mL of volumetric bottle. The solution of *n*-hexane/acetone (1: 1, v/v) was then added to the volume to make a 0.1% (w/v) standard solution.

IV. *Determination of Relative Response Factor (RRF) of Sterols to 5 α-Cholestane*

The mixtures of various ratios of 0.1% sterols (cholesterol, brassicasterol, campesterol, stigmasterol and β-sitosterol) to 0.1% 5 α-cholestane (1:2, 1:1, and 2:1, v/v) were injected in triplicate for GC analysis. The RRF was calculated as follows:

$$RRF_{sterol} = \frac{A_{sterol}}{W_{sterol}} \div \frac{A_{IS}}{W_{IS}} \quad \text{.....(1)}$$

Where RRF_{sterol} is the RRF of sterols to IS; A_{sterol} is the peak area of sterol; W_{sterol} is the sterol weight (mg); A_{IS} is the peak area of IS, 5α-cholestane; W_{IS} is the IS weight (mg).

V. *Determination of Free Sterols (FS)*

An oil sample (0.1 g) was weighed and placed in a 7-mL vial. One mL of 0.1% 5 α-cholestane solution was spiked into the same vial and mixed, and 0.1 μL of which was then injected into GC for analysis. Analysis of each sample was carried out in duplicate. The contents of FS were calculated according to the following equation (2):

$$\text{Sterols}(\mu g/g) = \frac{A_{sterol}}{A_{IS}} \times \frac{W_{IS}}{RRF_{sterol}} \times \frac{1}{W_{sample}} \quad \text{.....(2)}$$

Where W_{sample} is the sample weight.

VI. *Determination of Total Sterols (TS)*

Oil sample (0.5 g) was weighed and placed in a 50-mL round bottle containing 1 mL of 0.1% 5α-cholestane solution. Five mL of 1 N KOH/methanol solution was then added and the solution was refluxed at 100°C for 30 min. A non-saponification portion was extracted twice with 50 mL of diethyl ether each time. The combined ether layer was collected and washed with water to remove the saponification portion. Organic phase was then concentrated under vacuum to a volume of 1-2 mL and 0.1 μL of the final solution was injected into GC for analysis. The contents of TS were calculated according to the equation (2). Analysis of each sample was carried out in duplicate.

VII. *Calculation of Esterified Sterols (ES)*

ES (μg/g) = TS (μg/g) - FS (μg/g)

VIII. *Detection Limit of Sterols on GC-FID*

Standard solutions (0.1%, w/v) of cholesterol, campesterol, stigmasterol and β-sitosterol were separately diluted to concentrations of 10.0, 5.0, 2.5 and 1.0 μg/mL. Each concentration of standard solution was injected into GC-FID (the parameters of both range and attenuation were set at 1) in triplicate to determine the detection limit of the above sterols.

IX. *Fortification Recovery Test*

The solutions (0.442%, w/v) of cholesterol, campesterol, stigmasterol, and β-sitosterol were diluted by 1 or 4 fold to make the concentrations of 1.10, 2.21, and 4.42 mg/mL standard solutions. One mL of above the solutions was individually added into a 7-mL vial containing 0.5 g lard and 1 mL of 0.1% 5 α-cholestane solution. After mixing, 0.1 μL of the mixture was injected into GC for analysis. Analysis of each fortification sample was carried out in triplicate. A blank sample containing 0.5 g lard, 1 mL of *n*-hexane/acetone solution, and 1 mL of 0.1% 5 α-cholestane solution was also injected into GC for analysis. Recoveries were thus calculated when the peak areas of test samples were compared with those of the blank sample.

X. *GC Conditions*

A Hitachi GC (Model G-3000) equipped with a flame ionization detector (FID, H_2 flow = 30 mL/min, air flow = 300 mL/min) was used in this study. The analytical column was a DB-1 megabore capillary column (0.53 mm x 5 m, 1.0 μm, J&W Scientific). The temperatures of injection port and detector were 280° and 360°C, respectively. The initial oven temperature was set at 190°C for 3 min. The temperature was then programmed to 210°C at 1.5°C/min, held at 210°C for 2 min, increased to 230°C at 2°C/min, and finally raised to 300°C at 30°C/min. The carrier gas was H_2 at a flow rate of 20-mL/min. The sample injection volume was 0.1 μL, and the direct injection mode was used. The dimension of the

glass liner in injection port was 67 cm x 6 mm.

RESULTS AND DISCUSSION

I. *The Effect of GC Conditions*

Sample injections with the split/splitless mode are routinely used for GC analysis. The cool on-column injection mode, which is a newly developed technique, can offer a high precision analysis in quantification[29-30]. This technique, however, is not widely used due to the complexity of its maintenance. The direct injection mode, in which the sample is retained in a deactivated glass liner, can replace cool on-column injection mode for GC analysis. Our previous study showed the insertion of glass wool in the glass liner or cleaning the glass liner periodically was capable of preventing contamination of non-volatiles in the analytical column so as to prolong the life of the column and improve the column resolution as well [28, 31]. Based on the direct injection technique, a rapid analytical method for determination of FS in fats/oils was developed. An oil sample (0.1 g) diluted with *n*-hexane/acetone solution containing 0.1% (w/v) 5 α-cholestane was directly injected into GC for analysis without further sample preparation. The analytical column and GC conditions are the two factors that needed attention.

Test samples of sunflower, soybean, sesame, and egg yolk oils were directly injected into GC installed with a non-polar DB-1 megabore column. Using the GC conditions as described in Methods X, the GC chromatograms of TS and FS from test samples were obtained as shown in Figures 1~4. In total, ten peaks appeared on the GC chromatograms. Peaks 4~10 were tentatively identified as brassicasterol, campesterol, stigmasterol, β-sitosterol, Δ^5-avenasterol, Δ^7-stigmastenol and Δ^7-avenasterol, respectively, as listed in Table 1. Peak 3 was identified as a mixture of cholesterol and α-tocopherol, since their retention times were overlapped. Peaks 1 and 2 were designated as unknown 1 and 2, respectively, because the identification of these two peaks was unsuccessful. A compound identification was made by com-

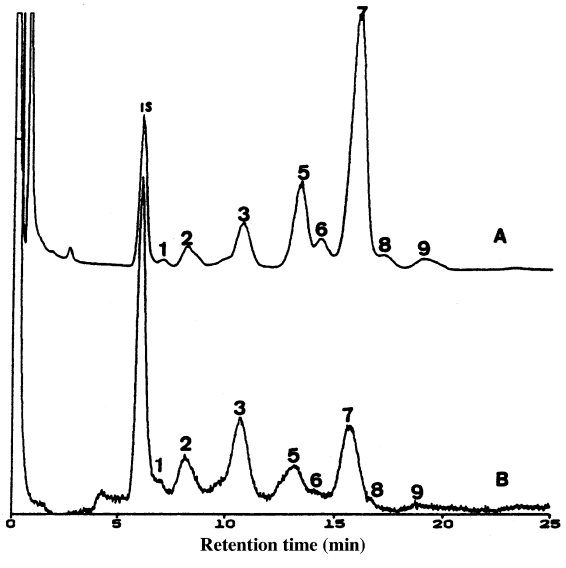

Figure 1. Gas chromatograms of (A) total sterols and (B) free sterols of sunflower oil by direct injection method. Peaks : IS = 5α-cholestane, 1 = unknown 1, 2 = unknown 2, 3 = cholesterol + α-tocopherol, 4 = brassicasterol, 5 = campesterol, 6 = stigmasterol, 7 = β-sitosterol, 8 =Δ^5-avenasterol, 9 =Δ^7-stigmasterol, 10 =Δ^7-avenasterol.

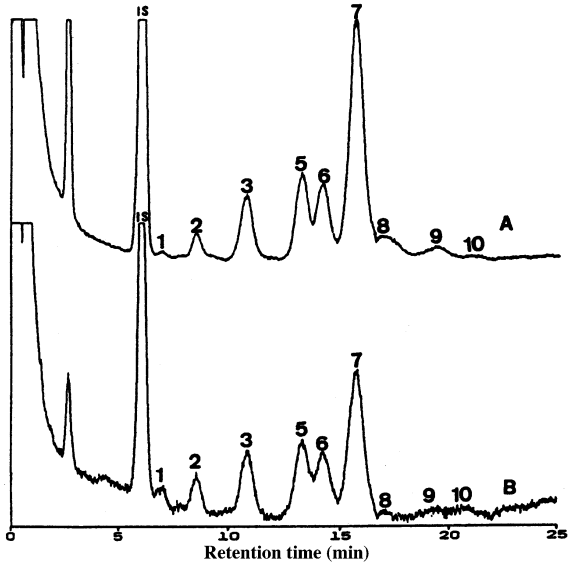

Figure 2. Gas chromatograms of (A) total sterols and (B) free sterols of soybean oil by direct injection method. Peaks : IS = 5α-cholestane, 1 = unknown 1, 2 = unknown 2, 3 = cholesterol + α-tocopherol, 4 = brassicasterol, 5 = campesterol, 6 = stigmasterol, 7 = β-sitosterol, 8 =Δ^5-avenasterol, 9 =Δ^7-stigmasterol, 10 =Δ^7-avenasterol.

paring the retention times (RT) and relative retention times (RRT) of standards or soybean sterols reported in the literature[1-7, 28] under the same GC conditions. RRT of compound was determined by comparing the RT of the target compound to that of β-sitosterol.

II. *The RRF of Sterols to 5 α-Cholestane*

To accurately quantify the sterols in fats/oils, the first step is to determine the RRF of sterols including cholesterol, brassicasterol, campesterol, stigmasterol, β-sitosterol, Δ^5-avenasterol, Δ^7-stigmastenol and Δ^7-avenasterol to internal standard 5α-cholestane. The contents of sterols are then calculated by the equation (2). The coefficients of linearity of five standard curves plotted by peak area ratios of sterols (cholesterol, brassicasterol, campesterol, stigmasterol, β-sitosterol) to internal standard versus concentrations were > 0.9. The RRFs of the above five commercially available sterols were calculated to be 0.86, 0.81, 0.75, 0.75 and 0.77, respectively. The RRFs of the other three sterols (Δ^5-avenasterol, Δ^7-stigmastenol and Δ^7-avenasterol), which are not commercially available, were estimated to be 0.75. Table 2 lists the RRFs of sterols to 5 α-cholestane.

III. *Detection Limit of Sterols on GC-FID*

The detection limit of sterols including cholesterol, campesterol, stigmasterol, and β-sitosterol on GC-FID were determined to be 2.5-5.0 μg/mL.

IV. *Fortification Recovery Test*

Fortification recoveries of cholesterol, campesterol, stigmasterol and β-sitosterol (fortification levels were 1.10, 2.21 or 4.42 mg) from 0.5 g lard containing 1 mL of 0.1% internal standard (5α-cholestane) solution were at the range of 94-107, 91-94, 90-104 and 92-103%, respectively, with the coefficients of variation (CV%) between 5-12% as listed in Table 3. These results indicate the developed method is simple, fast and precise enough to quantify the FS in fats/oils. This

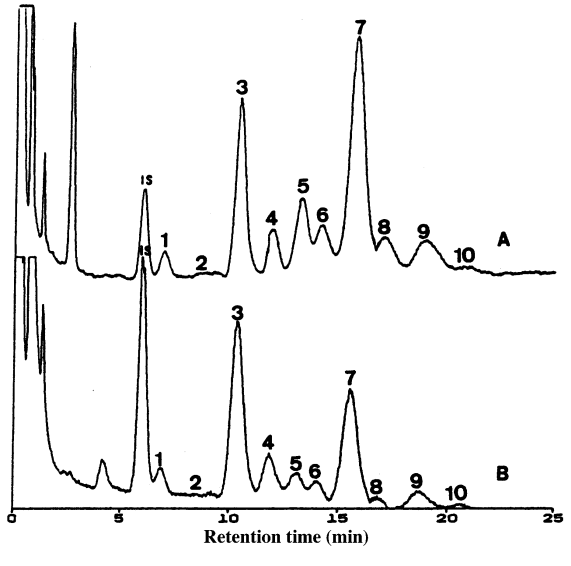

Figure 3. Gas chromatograms of (A) total sterols and (B) free sterols of sesame oil by direct injection method. Peaks : IS = 5α-cholestane, 1 = unknown 1, 2 = unknown 2, 3 = cholesterol+ α-tocopherol, 4 = brassicasterol, 5 = campesterol, 6 = stigmasterol, 7 = β-sitosterol, 8 = Δ^5-avenasterol, 9 = Δ^7-stigmasterol, 10 = Δ^7-avenasterol.

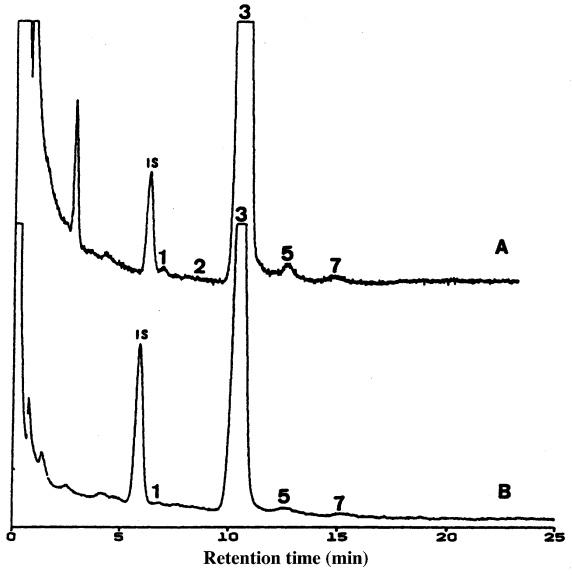

Figure 4. Gas chromatograms of (A) total sterols and (B) free sterols of egg yolk oil by direct injection method. Peaks : IS = 5α-cholestane, 1 = unknown 1, 2 = unknown 2, 3 = cholesterol + α-tocopherol, 4 = brassicasterol, 5 = campesterol, 6 = stigmasterol, 7 = β-sitosterol, 8 = Δ^5-avenasterol, 9 = Δ^7-stigmasterol, 10 = Δ^7-avenasterol.

method is also superior to the coupled LC-GC method proposed by Grob, *et al*[8, 27], the on-column GC method by Smith[13], HPLC methods by Bonge[22] and Murata, *et al*.[25], and a method involving the Sep-Pak cartridge for separation of FS from ES followed by GC analysis by Chen and Sun[26].

V. *Determination of FS in Fats/Oils*

Table 1. Relative retention data of sterols by direct injection gas chromatographic method

Sterols	Peaks[a]	Retention time (min)	Relative retention time[b] of sterol standard	Relative retention time[b] of sterol in oil sample
5α-Cholestane	IS	5.62	0.378	--
α-tocopherol	3	9.57	0.645	0.645
Cholesterol [c] (Δ^5- cholesten-3β-ol)	3	9.58	0.645	0.646
Brassicasterol [(24S)-24-ethyl-$\Delta^{5,22}$- cholestadien-3β-ol]	4	11.11	0.748	0.750
Campesterol [(24R)-24-methyl-Δ^5- cholesten-3β-ol)]	5	12.45	0.838	0.839
Stigmasterol [(24R)-24-ethyl-$\Delta^{5,22}$- cholestadien-3β-ol]	6	13.25	0.892	0.893
β-sitosterol [(24R)-24-ethyl-Δ^5- cholesten-3β-ol]	7	14.86	1.000	1.000
Δ^5-avenasterol [(24Z)-24-ethylidene-Δ^5- cholesten-3β-ol]	8	15.81	-- [d]	1.064
Δ^7-stigmasterol [(24R)-24-ethyl-Δ^7- cholesten-3β-ol]	9	17.78	-- [d]	1.197
Δ^7-avenasterol [(24R)-24-ethyl-$\Delta^{5,22}$- cholestadien-3β-ol]	10	19.36	-- [d]	1.303

[a] See method for the operating condition of GC.

[b] Relative retention time for β-sitosterol taken as 1.000.

[c] Overlapping with α-tocopherol.

[d] Unavailability of authentic compounds.

Table 2. The relative response factor (RRF) of various sterols to 5 α-cholestane

Sterols	RRF
5α-Cholestane[a]	1.00
Cholesterol (Δ^5- cholesten-3β-ol)	0.86
Brassicasterol [(24S)-24-ethyl-$\Delta^{5,22}$- cholestadien-3β-ol]	0.81
Campesterol [(24R)-24-methyl-Δ^5- cholesten-3β-ol)]	0.75
Stigmasterol [(24R)-24-ethyl-$\Delta^{5,22}$- cholestadien-3β-ol]	0.75
β-sitosterol [(24R)-24-ethyl-Δ^5- cholesten-3β-ol]	0.77
Δ^5 -avenasterol [(24Z)-24-ethylidene-Δ^5- cholesten-3β-ol]	0.75[b]
Δ^7 -stigmasterol [(24R)-24-ethyl-Δ^7- cholesten-3β-ol]	0.75[b]
Δ^7 -avenasterol [(24R)-24-ethyl-$\Delta^{5,22}$- cholestadien-3β-ol]	0.75[b]

[a] Used as internal standard.

[b] Assumed value due to unavailability of authentic compounds.

Table 3. Recoveries of the spiked sterols from lard by direct injection gas chromatographic method

Sterols	Blank (mg) (A)[a]	Spiked amount (mg) (B)	Amount determined (mg) (C)[b]	Recovery (%)[c]	CV (%)[d]
Cholesterol	0.45	1.10	1.65	106.45	11.57
		2.21	2.52	93.66	9.86
		4.42	4.98	102.49	6.04
Campesterol	0.00	1.10	0.96	90.91	8.23
		2.20	1.99	90.45	7.89
Stigmasterol	0.00	1.10	1.03	93.64	10.16
		2.21	1.97	89.14	8.74
		4.42	4.59	103.85	6.41
β-Sitosterol	0.00	1.11	1.02	91.89	10.87
		2.22	2.28	102.70	5.16

[a] The amount of free cholesterol in 0.5 g of lard, average of 5 tests.

[b] Average of triplicates.

[c] Recovery (%) = (C - A) / B x 100%.

[d] Coefficient of variation.

Table 4. Free sterols content in some animal and vegetable fats and oils

Sample [a]	Free sterols content (μg/g) [d]								
	Chol.*	Bras.	Camp.	Stig.	β-Sito.	Δ^5-ave.	Δ^7-stig.	Δ^7-ave.	Total
Ol	98.3	0.0	39.6	32.2	639.7	19.5	82.5	Trace[e]	911.8
Sf	713.0	17.0	192.1	106.3	1728.6	22.4	23.3	0.0	2802.7
Pn	217.3	41.3	223.9	127.4	1286.0	28.9	28.9	2.9	1956.6
Sb-1	277.7	0.0	355.6	352.7	1080.7	4.0	31.5	0.0	2102.2
Sb-2	491.6	0.0	543.6	372.6	1272.0	5.4	13.3	0.0	2698.5
Ss-1	1931.5	461.9	509.5	170.1	2197.4	21.4	109.7	Trace	5401.5
Ss-2	2763.0	1056.6	529.7	343.5	3086.7	27.2	78.7	0.0	7885.5
Ss-3	3092.0	389.8	584.4	724.3	3082.8	169.7	557.8	23.2	8624.0
Ss-4	2094.4	563.3	615.8	287.7	2890.7	40.6	7.8	0.0	6500.3
L	905.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	905.7
L+Sb[b]	576.7	0.0	289.8	123.5	477.8	0.0	24.9	0.0	1492.7
Eg-c	21194.3	4.1	633.9	383.3	1391.6	404.1	128.9	8.9	24149.1
Eg-H	30340.0	0.0	10.2	0.0	7.1	0.0	0.0	0.0	30357.3
Eg-H+Sb[c]	24384.1	0.0	301.8	216.7	587.4	0.0	21.4	0.0	25511.4

[a] Ol=olive oil, Sf=sunflower, Pn=peanut oil, Sb=soybean oil, Ss=sesame oil, L=lard, Eg-c=commercial capsulated egg oil,
Eg-H= egg yolk powder n-hexane extracts.

[b] Lard + soybean oil (1/1=w/w).

[c] Egg yolk powder *n*-hexane extracts + soybean oil (1/1=w/w).

[d] Average of duplicated analyses. Chol.*=cholesterol (contain α-tocopherol), Bras.=brassicasterol, Camp.=campesterol, Stig.=stigmasterol, Sito.= β-sitosterol, Δ^5-ave=Δ^5-avenasterol, Δ^7-stig.=Δ^7-stigmasterol, Δ^7-ave=Δ^7-avenasterol.

[e] Trace < 2.5 μg/g.

Table 5. Total sterols content in some animal and vegetable fats and oils

Sample [a]	Total sterols content (μg/g) [d]								
	Chol.*	Bras.	Camp.	Stig.	β-Sito.	Δ^5-ave.	Δ^7-stig.	Δ^7-ave.	Total
Ol	193.4	0.0	96.5	114.8	1497.2	130.4	810.8	27.4	2870.5
Sf	731.8	49.5	1142.4	367.6	4023.2	59.3	212.9	0.0	6586.7
Pn	230.9	99.4	319.0	177.1	635.9	58.4	77.5	16.0	8200.9
Sb-1	333.0	14.6	496.6	447.5	1720.1	8.1	73.2	4.1	3097.2
Sb-2	543.7	20.9	768.1	589.3	2019.5	14.9	53.4	4.8	4014.6
Ss-1	2316.6	568.7	755.7	349.8	3013.7	49.2	335.2	19.9	7408.8
Ss-2	2861.5	1199.1	1300.6	501.3	4618.5	42.2	167.0	8.5	10698.7
Ss-3	3166.1	916.5	2082.3	1365.3	5413.6	536.4	1296.2	128.6	14905.0
Ss-4	2630.0	1221.6	2364.8	462.8	4809.8	50.4	17.8	Trace[e]	11557.2
L	1182.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1182.0
L+Sb[b]	709.9	20.4	396.7	137.8	1092.1	0.0	37.1	0.0	2394.0
Eg-c	25116.5	27.1	1517.4	1132.6	2500.4	608.0	146.8	18.8	31067.6
Eg-H	34383.9	0.0	171.9	0.0	61.0	0.0	0.0	0.0	34616.8
Eg-H+Sb[c]	26911.6	3.7	359.6	280.5	1008.7	0.0	35.3	0.0	28599.4

[a] Ol=olive oil, Sf=sunflower, Pn=peanut oil, Sb=soybean oil, Ss=sesame oil, L=lard, Eg-c= commercial capsulated egg oil, Eg-H= egg yolk powder *n*-hexane extracts.

[b] Lard + soybean oil (1/1=w/w).

[c] Egg yolk powder *n*-hexane extracts + soybean oil (1/1=w/w).

[d] Average of duplicated analyses. Chol.*=cholesterol (contain α-tocopherol), Bras.=brassicasterol, Camp.=campesterol, Stig.=stigmasterol, Sito.= β-sitosterol, Δ^5-ave=Δ^5-avenasterol, Δ^7-stig.=Δ^7-stigmasterol, Δ^7-ave=Δ^7-avenasterol.

[e] Trace < 2.5 μg/g.

Fourteen test samples including 1 olive oil (Ol), 1 sunflower oil (Sf), 1 peanut oil (Pn), 2 soybean oil (Sb), 4 sesame oil (Ss), 1 lard (L) and 1 lard mixed with soybean oil (L + Sb), 1 egg oil capsule (Eg-C), 1 egg oil (Eg-H) and 1 egg oil mixed with soybean oil (Eg-H + Sb) samples were tested for FS. Test results are shown in Table 4. The total FSs in Ol, Sf, Pn, Sb, Ss, L and Eg-H were found to be 912, 2803, 1957, 2102-2699, 5402-8624, 906 and 30357 μg/g, respectively. β-Sitosterol was shown to be the most abundant sterol in vegetable oils. Its concentration was at the range of 640-3078 μg/g. The contents of stigmasterol, campesterol, mixture of cholesterol and α-tocopherol, brassicasterol, Δ^5-avenasterol, Δ^7-stigmastenol and Δ^7-avenasterol were ranged at 32-724, 40-616, 98-3092, 0-1057, 4-170, 8-557 and 0-23 μg/g, respectively.

With respect to the sterols in animal fats, the major FS in animal fats were found to be cholesterol. Lard contained 906 μg/g mixture of cholesterol and α-tocopherol; while egg yolk oil, contained 30340 μg/g mixture of cholesterol and α-tocopherol, but contained only 10.2 μg/g campesterol and 7.1 μg/g β-sitosterol as listed in Table 4. Figure 4 shows the GC chromatograms of TS and FS from egg yolk oil. The developed method is also capable of detecting whether lard or egg yolk oil is adulterated with vegetable oils, since animal fats hardly contain phytosterol. Mixing vegetable oils with animal fats can significantly change the composition of FS. In this study, lard mixed with soybean oil (1:1) reduced the mixture of cholesterol and α-tocopherol of lard from 906 to 577 μg/g while increased the contents of brassicasterol, campesterol and β-sitosterol, which belong

Table 6. Sterol esters content in some animal and vegetable fats and oils

Sample[a]	Sterol esters content (μg/g) [d]								
	Chol.*	Bras.	Camp.	Stig.	β-Sito.	Δ^5-ave.	Δ^7-stig.	Δ^7-ave.	Total
Ol	95.1	0.0	56.9	82.6	857.5	110.9	728.3	26.2	1958.7
Sf	18.8	32.5	950.3	261.3	2294.6	36.9	189.6	0.0	3784.0
Pn	13.6	58.1	95.1	49.7	349.9	29.5	48.6	13.1	6244.3
Sb-1	55.3	14.6	141.0	94.8	639.4	4.1	41.7	4.1	995.0
Sb-2	52.1	20.9	224.5	216.7	747.5	9.5	40.1	4.8	1316.1
Ss-1	385.1	106.8	246.2	179.7	816.3	27.8	225.5	18.2	2007.3
Ss-2	98.5	142.5	770.9	157.8	1531.8	15.0	88.3	8.5	2813.2
Ss-3	74.1	526.7	1497.9	641.0	2330.8	366.7	738.4	105.4	6281.0
Ss-4	535.6	658.3	1749.0	175.1	1919.1	9.8	10.0	Trace[e]	5056.9
L	276.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	276.3
L+Sb[b]	133.2	20.4	106.9	14.3	614.3	0.0	12.2	0.0	901.3
Eg-c	3922.2	23.0	883.5	749.3	1108.8	203.9	17.9	9.9	6918.5
Eg-H	4043.9	0.0	161.7	0.0	53.9	0.0	0.0	0.0	4259.5
Eg-H+Sb[c]	2527.5	3.7	57.8	63.8	421.3	0.0	13.9	0.0	3088.0

[a] Ol=olive oil, Sf=sunflower, Pn=peanut oil, Sb=soybean oil, Ss=sesame oil, L=lard, Eg-c= commercial capsulated egg oil, Eg-H= egg yolk powder *n*-hexane extracts.

[b] Lard + soybean oil (1/1=w/w).

[c] Egg yolk powder *n*-hexane extracts + soybean oil (1/1=w/w).

[d] Average of duplicated analyses. Chol.*=cholesterol (contain α-tocopherol), Bras.=brassicasterol, Camp.=campesterol, Stig.=stigmasterol, Sito. = β-sitosterol, Δ^5-ave=Δ^5-avenasterol, Δ^7-stig.=Δ^7-stigmasterol, Δ^7-ave=Δ^7-avenasterol.

[e] Trace < 2.5 μg/g.

to phytosterols and should not be in the lard. A relative abundance of phytosterols was found in commercial capsulated egg oils, indicating those products were adulterated with vegetable oils.

VI. *Determination of TS in Fats/Oils*

Oil samples containing an internal standard (5α-cholesane) were saponified, extracted with diethyl ether, and then analyzed by GC to determine the TS. As can be seen in Table 5, the TSs in Ol, Sf, Pn, Sb, Ss, L and Eg-H were found to be 2871, 6587, 8201, 3097-4015, 7409-14905, 1182 and 34617 μg/g, respectively. β-Sitosterol (concentration ranged from 636-5414 μg/g) was the major TS found in vegetable oils (Ol, Sf, Pn, Sb, and Ss). The other TS contents in tested vegetable oil samples were as follows: 193-3166 μg/g mixture of cholesterol and α-tocopherol, 97-2365 μg/g campesterol, 115-1365 μg/g stigmasterol, 0-1222 μg/g brassicasterol, 8-536 μg/g Δ^5-avenasterol, 18-1296 μg/g Δ^7-stigmastenol and 0-129 μg/g Δ^7-avenasterol. The mixture of cholesterol and α-tocopherol was found as the primary TS in animal fats/oils. The contents of TS in lard and egg yolk oil were determined to be 1182 and 34617 μg/g, respectively.

Quantification of FS and TS in fats/oils can be easily achieved using the direct injection GC method developed in this study. Esterified sterols (ES) can also be determined by subtracting FS from TS. The total ESs in Ol, Sf, Pn, Sb, Ss, L and Eg-H were calculated to be 1959, 3784, 6244, 995-1316, 2007-6281, 276, 4260 μg/g, (counted as 68.2, 57.5, 76.1, 32.1-32.8, 26.3-43.8, 23.4 and 12.3% TS) respectively, as shown in Table 6. These results reveal that more than half of sterols

in Ol, Sf and Pn are in an ester type; while FS constitutes more than half of sterols in Sb, Ss, L and Eg-H.

CONCLUSIONS

This report presents a direct injection GC method, which is rapid and precise and allows one sample to be analyzed in 20-25 min for free sterol (FS) analysis. The same method can also be applied to total sterol (TS) analysis where the fat/oil samples were saponified and extracted prior to GC injection. Sterol esters (ES) can be calculated by an equation of ES = TS - FS. Analyses of FS, TS and ES in animal fats and vegetable oils show olive oil, sunflower oil and peanut oil are mainly composed of sterol esters while the major sterols in soybean oil, sesame oil, lard and egg yolk oil are FS. This method can also be used to quickly analyze whether animal fats/oils are adulterated by vegetable oils.

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動、植物油脂中游離態固醇類之快速氣相層析分析

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摘　要

本研究建立了動、植物油脂中游離態固醇類之簡便、快速分析定量法。0.1 g之油脂樣品以1 mL 0.2%之5α-cholestane正己烷/丙酮混合溶液 (1/1, v/v) 溶解後，即可直接注入氣相層析儀分析之，使用廣口徑、非極性之DB-1短管柱 (0.53 mm × 5.0 m, 1 μm)。添加回收實驗中，cholesterol、campesterol、stigmasterol及β-sitosterol之回收率為89-106%，變異係數小於12%。經皂化後，油脂中的總固醇類含量亦可以本法分析定量之。以本方法測定14種動、植物油脂，包括橄欖油、葵花油、花生油、大豆沙拉油、芝麻油、豬油及蛋黃油等之總固醇類及游離固醇類含量。結果顯示，總cholesterol (含α-tocophenol)、brassicasterol、campesterol、stigmasterol、β-sitosterol、Δ5-avenasterol、Δ7-stigmasterol及Δ7- avenasterol含量分別依序為：193-34384、0-1222、0-2356、0-1365、0-5414、0-608、0-1296及0-129 μg/g；而游離態cholesterol (含α-tocophenol)、brassicasterol、campesterol、stigmasterol、β-sitosterol、Δ5-avenasterol、Δ7-stigmasterol及Δ7- avenasterol含量分別依序為：98-30340、0-1057、0-634、0-724、0-3087、0-404、0-558及0-23 μg/g；另外，酯化態之固醇類則可以總固醇類與游離固醇類含量之差值計算得之。結果顯示，橄欖油、葵花油及花生油中之固醇類主要以酯化態方式存在；而大豆沙拉油、芝麻油、豬油及蛋黃油則主要以游離態固醇類存在。本研究方法可作為動物油脂是否摻有植物油之簡便檢測方法。

關鍵詞：總固醇，游離態固醇，酯化態固醇，油脂，氣相層析，直接注入法。