J. Fac. Appl. Biol. Sci.,
Hiroshima Univ. (1996), 35: 163~171

Unsaponifiable Matter Constituents of Crude Shea Fat Extracted by Different Methods

Kwaku TANO-DEBRAH and Yoshiyuki OHTA

Faculty of Applied Biological Science, Hiroshima University, Higashi-Hiroshima 739, Japan

Received October 31, 1996

Abstract The unsaponifiable matters (UM) of chloroform-methanol, Soxhlet, enzyme-assisted aqueous and a rural aqueous extracted samples of shea fat were comparatively, separated by thin layer chromatography on Silica gel 60 plates. A band corresponding to a mixture of reference sterols, and another to lanosterol, of the Soxhlet extracted sample were eluted and analyzed by gas chromatography. The purpose was to investigate an earlier observation that the enzyme-assisted extracted samples had lower UM; and also to add to the otherwise scanty literature on shea fat unsaponifiables. The chloroform-methanol sample separated into 5 bands, compared to 3 apparent bands in each of the other samples. The bands had different color reactions with 50% H_2SO_4, and based on the reference standards, were distinguished into sterols, triterpene alcohols and hydrocarbons. The triterpene alcohols seemed to form the major components. The hydrocarbon fraction of the enzyme-assisted shea fat UM appeared to be significantly lesser than the others. Generally the observations suggested that the method of extraction could influence the UM of crude fats and oils. The sterol and triterpene alcohol fractions of the Soxhlet sample showed 6 and 5 major peaks respectively. Three sterols and two triterpene alcohols formed over 90% in each group. Based on the TLC and GC data, it appeared that cholesterol, ß-sitosterol, campesterol, fucosterol, ergosterol or most of the common phytosterols are not apparently present in the shea fat unsaponifiables.

Key words: shea fat unsaponifiable matters, sterols, triterpene alcohols, chromatographic separation.

INTRODUCTION

Crude shea fats are known to contain high amounts of unsaponifiable matters (UM). Percentage compositions ranging from 2 to 17 have been reported (TANO-DEBRAH and OHTA, 1994, 1995a; PESQUET, 1992; SALUNKHE and DESAI, 1986; SWERN, 1979; ATA, 1978; CODD *et al*, 1975). These UM are thought to contribute to the numerous dermatological properties of shea fat; (LOZANO *et al*, 1993; PESQUET, 1992; ATA, 1978; NÉEMAN *et al*, 1970). However, the high UM content is also considered to be a limitation to the use of the fat in soap making (SALUNKHE and DESAI, 1986).

Generally, most of the UM of crude fats and oils are removed during refining; suggest-

ing a preference for crude fats and oils with lower UM contents. In a previous publication (TANO-DEBRAH and OHTA, 1995a), we reported that the UM content of shea fat apparently decreased in enzyme-assisted extracted samples. This may be a desirable product characteristic in enzyme-assisted shea fat extraction, as the low levels would require lesser treatment during refining. We have made further investigation into how the decrease seems to occur; and have also partially determined the constituents of the UM to add to the scanty literature on shea fat. The findings are presented in this report.

MATERIALS AND METHODS

Shea fat samples analyzed were extracted in the laboratory by (i) Chloroform-methanol (FOLCH *et al*, 1957), (ii) Soxhlet extraction (using hexane), (iii) enzyme-assisted aqueous extraction (TANO-DEBRAH and OHTA, 1994, 1995a; TANO-DEBRAH, YOSHIMURA and OHTA, 1996) and (iv) a typical rural aqueous extraction (TANO-DEBRAH and OHTA, 1994) method. These fat samples were designated as T.L., S, E and R respectively.

About 5-g of fat samples were refluxed gently with 50 mL of 0.5 N ethanolic KOH for 90 minutes. The unsaponifiable matters were then extracted with diethyl ether, as described in the AOAC method (AOAC, 1984, method 28.038). The diethyl ether was completely evaporated using a rotary evaporator. The residues were redissolved in chloroform and used for thin layer chromatographic (TLC) and gas chromatographic (GC) analyses. *Analysis by TLC.* TLC was done on Silica gel 60 plates, 20×20 cm, 0.25 mm layer thickness (MERCK). Cholesterol, ß-sitosterol, stigmasterol, ergosterol, fucosterol, lanosterol, and squalene (all from NACALAI TESQUE INC, Kyoto, Japan) were used as reference (standard) compounds. Ten μL of each UM solution and chloroform solutions of the standards (the standards were dissolved in chloroform to concentrations of about 10 μg/mL), were spotted on same plate, on an imaginary line. The sterols were also mixed in aliquots and spotted together with lanosterol and the Shea fat UM solutions on other plates. The plates were developed using chloroform/diethyl ether (9:1) or Hexane/diethyl ether (7:3), in one-dimensional TLC. Developed plates were sprayed with 50% H_2SO_4 and oven dried (100°C, 10 min) for observation. The chloroform solutions of samples S and E were also each applied on separate plates, developed in two-dimensions with hexane diethyl ether and chloroform/diethyl ether (9:1) and sprayed with 50% H_2SO_4 for observation. Sample S was again applied in very closed spots, apparently forming a band, and developed two times in one-dimension with hexane/diethyl ether (7:3), or once with chloroform/diethyl ether (9:1). The plates were dried in air and observed with a UV light, Transilluminator UVP (FUNAKOSHI, Tokyo, Japan). The individual bands were scraped and the components extracted with chloroform. The extracts were evaporated to dryness under vacuum below 50°C and redissolved in hexane. The components which corresponded to the sterols mixture or the lanosterol standards were used for gas chromatography (GC). These were taken as the sterols and triterpene alcohols fractions.

Analysis by GC. GC analysis was done using a Hitachi 263−30 Chromatograph, equipped with an flame ionization detector (FID) and an integrator (D-2500 Chromato-integrator, HITACHI Co. Tokyo Japan), on a glass column (2.0 m×3 mm i.d.) packed with Silicone OV-105 (2%) material on Uniport HP 80/100 support. Nitrogen was the carrier gas, at

the flow rate of about 45 mL per minute. The injection temperature was 280°C; the column temperature was kept constant at 270°C. The retention times of the reference samples were determined under the same conditions as the experimental samples, for the identification of the unknown sample constituents. Relative retention times (RRT) of peaks were calculated with the retention time of cholesterol as reference.

RESULTS AND DISCUSSION

The UM of all the samples were similarly high, and consistent with the previously reported data (TANO-DEBRAH and OHTA, 1994, 1995a). The mean values for the samples were: T.L., 6.75%; S, 6.89%; E, 5.30%; and R, 6.08%. The mean value for the enzyme-assisted aqueous extracted samples was again, comparatively lower than the others.

The typical thin-layer chromatograms of the shea fat unsaponifiables are shown in Figs. 1 to 3. Figure 1 shows the resolutions of all the 4 samples, and of the reference compounds. Figures 2 and 3 show the two-dimensional chromatograms of samples S and E respectively. The pattern of separation of the UMs were similar for all samples. There were three major bands (or trails) from each sample, except for sample TL., which had two apparent additional bands. Each of the bands had a different color reaction with the 50% H_2SO_4. The first band for all samples had Rf of about 0.366 and corresponded well to bands of the reference sterol mixture. The second band for the UM samples (Rf of about 0.545) also corresponded to the triterpene alcohol. In another setup in which squalene was also spotted (chromatogram not shown) the Rf of the squalene (0.870) was within the range (0.782−1.0) for the trailing fraction of the UM samples, suggesting that these trailing fractions were the hydrocarbon fractions. In the two-dimensional TLC these hydrocarbon trails separated into many smaller bands or trails (Figs. 2 and 3) and the content seemed to vary for samples S and E. Separation with the hexane-diethyl ether showed similar constituents as described, except that the Rf values for the various fractions were comparatively lower (Data not shown); and it was necessary to develop a plate a second time to obtain a better separation of bands when this solvent was used. The triterpene alcohol fraction seemed to be in higher concentrations than the sterols fraction. There seemed also to be a comparatively lower concentration of hydrocarbons in sample E. It is not certain however whether this caused the observed lower UM in the enzyme-assisted extracted samples. But it is possible that some hydrocarbons are lost in the aqueous extraction process, unlike in the extraction with hexane or chloroform-methanol where the hydrocarbons would remain in the organic solvent with the oil, and consequently in the oil, after evaporation of the solvent. This seems to be the principle, as the rural aqueous extracted samples (R) also had quite a lower UM content and apparently lesser hydrocarbon fractions compared to samples T.L. and S. SWERN, (1979) reported the sterol content of 0.09% for a refined shea fat. In the analyses of the UMs of 18 other vegetable oils, FEDELI *et al*, (1966), observed rather higher proportions of sterol than triterpene alcohols in all oils. ITOH *et al*, (1973) also made similar observations in the analyses of 19 vegetable oils. The lower sterol composition observed on shea fat may thus be a peculiar characteristic of the fat.

Combining the Rf values and the observed color reactions, (Fig. 1) it is quite certain to suggest that cholesterol, ß-sitosterol, fucosterol and squalene are apparently not present in

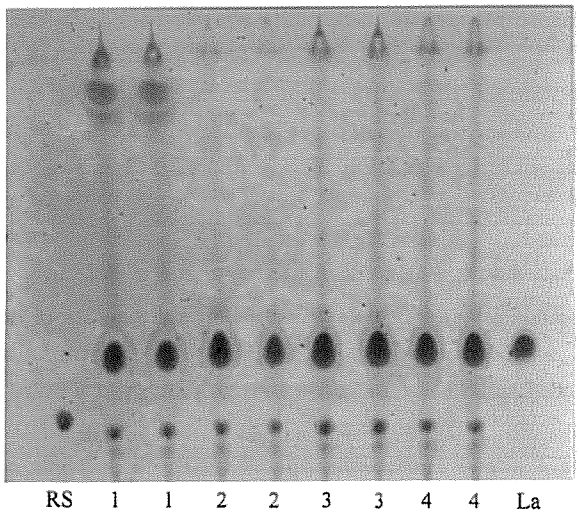

Fig. 1 One dimensional thin-layer chromatography separations of the unsaponifiables of shea fat samples extracted by the different methods, and some reference sterol compounds, on Silica-gel 60 plates with hexane-diethyl ether (7:3, vol/vol) as eluant. [RS, a mixture of cholesterol, ß-sitosterol, campesterol, stigmasterol, ergosterol and fucosterol; 1, total lipid (TL); 2, enzyme extracted (E); 3, Soxhlet extracted (S); 4, rural sample (R); La, lanosterol].

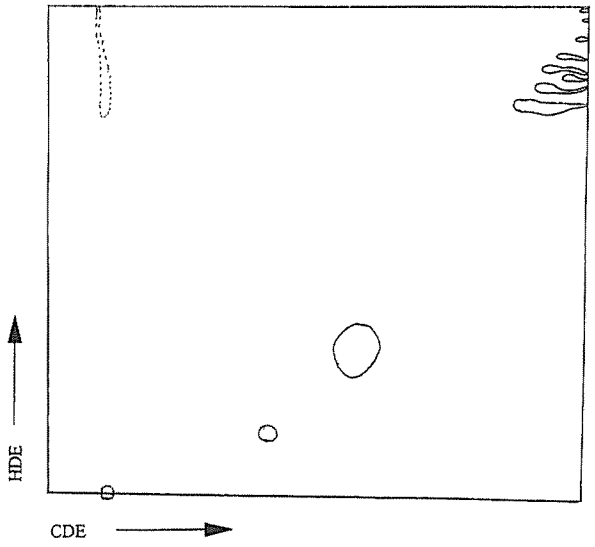

Fig. 2 Two dimensional thin-layer chromatophy separations of a Soxhlet-extracted shea fat unsaponifiables; on Silica-gel 60 plates with the solvents: (1) HDE, n-hexane/diethyl ether (7:3, vol/vol) and (2) CDE, chloroform/diethyl ether (9:1, vol/vol).

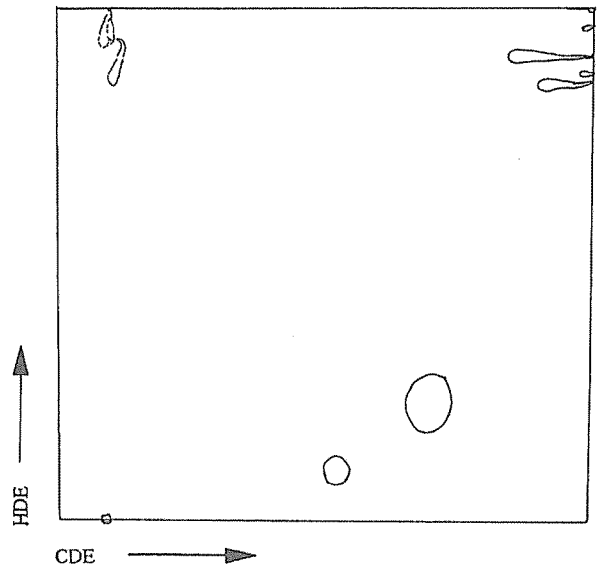

Fig. 3 Two-dimensional thin-layer chromatography separations of the unsaponifiables of crude shea fat extracted by an enzyme-assisted aqueous process; on Silica-gel 60 plates with the solvents: (1) HDE, n-hexane/diethyl ether (7:3, vol/vol) and (2) CDE, chloroform/diethyl ether (9:1, vol/vol).

the shea fat UM. The bands (Rf 0.782 to 1.00) of samples S and E seemed to vary in composition, as shown by the separation in the two-dimensional TLC, (Figs. 2 and 3). There were many constituents in the fraction of sample S than sample E. These observations were made in all replications and they seem to suggest that the method of fat extraction could influence the composition of the UM.

The gas-chromatograms of fraction 1 (corresponding to the sterols) of sample S on the Silicone OV-105 is represented in Fig. 4. The relative retention times (RRTs) for the reference samples and for the peaks in the chromatograms are also presented in Tables 1 and 2 respectively. A later analysis of the sample on Silicone OV-1 on uniport HP 100/120 showed similar separation pattern (Data not shown). Consistently, no peak corresponded to cholesterol. Cholesterol was therefore used as an internal standard for the RRT calculations. The data also suggested that ß-sitosterol, campesterol, ergosterol and fucosterol were apparently absent. Stigmasterol however, seemed to be present (peak 1 in Fig. 4) but in relatively smaller concentration. Unfortunately, the main components could not be identified based on the available reference standards. When the mixed sterols standard was analyzed, ß-sitosterol and fucosterol could not be separated on the OV-105 column; and also campesterol and stigmasterol on the OV-1. This made the identification rather difficult, as the RRT of the combined compounds did not correspond to any of the peaks for the individual compounds.

The chromatogram of Fraction 2 of sample S, on Silicone OV-105, showed 5 major peaks (Fig. 5). The RRTs of the peaks are also shown in Table 3. In this case also, based on the data obtained, each component could not be identified. Both the TLC and GC

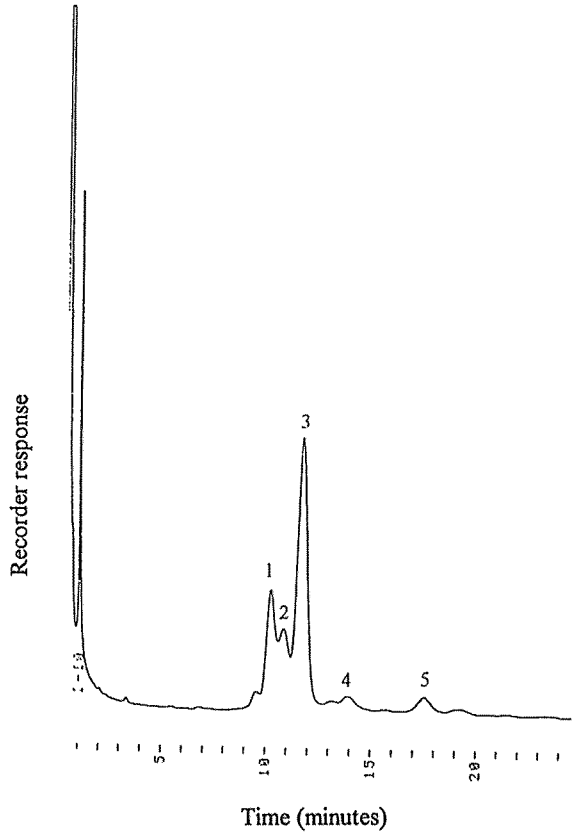

Fig. 4 A typical gas chromatography separations of the sterol fraction of Soxhlet-extracted crude shea fat unsaponifiables; on a glass column packed with 2% Silicone OV-105 material on Uniport HP 80/100 support. Carrier gas was nitrogen at 45 mL/min; injection temperature, 280°C; Column temperature, 270°C.

analyses suggested the presence of lanosterol; however, this could not be confirmed for the lack of other triterpene alcohols. ITOH *et al* (1982) observed similar RRTs for two or more different triterpene alcohols. The patterns of the chromatograms suggest that two compounds are predominant in the triterpene alcohol fraction, constituting over 90% of the fraction.

Even though it was not possible to identify completely the individual components of the Shea fat unsaponifiables, the study has revealed that triterpene alcohols are the predominant constituents. The sterols are rather present in relatively lower concentrations. Three sterols and two triterpene alcohols seem to be predominant in their groups, forming over 90% in each case. Data obtained have also suggested that cholesterol and many of the common phytosterols (ß-sitosterol, campesterol, fucosterol and ergosterol) are not present in the shea fat unsaponifiables. This seems to make Shea fat UM quite different from that of many other vegetable oils. The peculiar characteristics perhaps account for the peculiar pharmacological properties of shea fat.

Table 1. Rf values of separated bands of Shea fat unsaponifiables.

Sample	Rf for the different bands		
	Band 1	Band 2	Band 3
R	0.367	0.545	0.784–1.0
E	0.366	0.546	0.782–1.0
S	0.366	0.546	0.782–1.0
TL	0.369	0.543	0.778–1.0

Table 2. Rf values and the relative retention times (RRT) on OV-105 and OV-1 columns, of the reference standard compounds.

Standard	Rf	RRT	
		OV-105	OV-1
Cholesterol	0.38	1.0	1.0
ß-Sitosterol	0.384	1.525	1.480
Campesterol	nd	1.236	1.225
Stigmasterol	0.378	1.357	1.293
Ergosterol	0.371	1.167	1.147
Fucosterol	0.385	1.497	1.433
Lanosterol	0.54	1.540	nd
Squalene	0.87		

nd-not determined.

Table 3. Relative Retention Times (RRT) and Relative Concentrations (RC) of the major constituents of the Soxhlet extracted Shea fat Unsaponifiable Matters*.

Peak #	RRT	RC
Sterols fraction		
1	1.369	2.359
2	1.469	22.005
3	1.548	14.522
4	1.674	57.328
5	1.996	1.131
6	2.507	2.408
Triterpene alcohols fraction		
1	1.31	0.772
2	1.566	31.941
3	1.695	61.911
4	1.888	1.500
5	1.99	3.860

* RRT calculations were relative to retention time of cholesterol. The fat sample was extracted by Soxhlet method and the UM fractions separated on the Silicone OV-105 column.

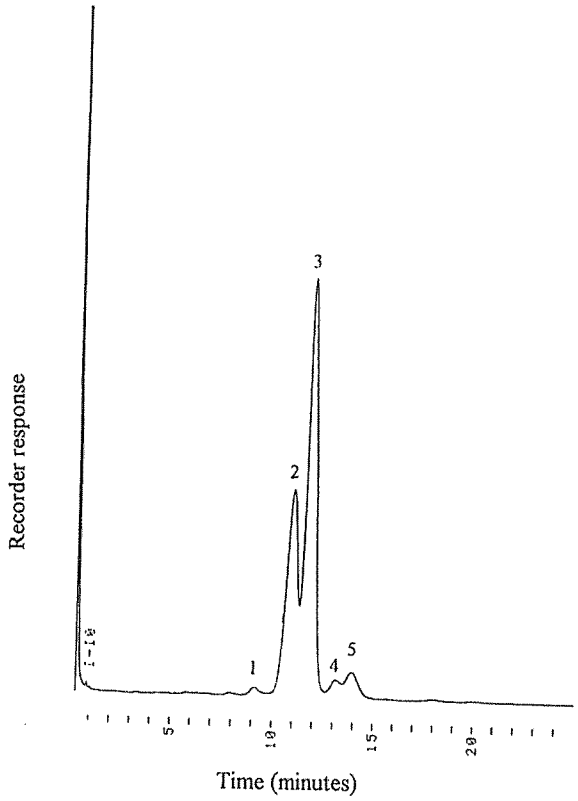

Fig. 5 A typical gas chromatography separations of the triterpene alcohols fraction of Soxhlet extracted crude shea fat unsapponifiables on a glass column packed with 2% Silicone OV-105 material on Uniport HP 80/100 support. Carrier gas was nitrogen at 45 mL/min; injection temperature, 280°C; Column temperature, 270°C.

REFERENCES

ATA, J.K.B.A., 1978. The Study of the Shea Kernel in Relation to the Traditional Process of Shea Fat, Ph.D. Thesis, University of Ghana, Legon-Accra.

CODD, L.W., DIJKHOFF, K. FEARSON, J.H., VAN OSS, C J., ROEBERTSON, H.G. and STANDFORD, E.G., (eds), 1975. *Materials and Technology*, Vol. VIII, Longman J.H. De Bussy, Amsterdam, pp. 60–61.

FOLCH, J.M., LEES, M. and STANLEY, G.S.H, 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226: 497.

ITOH, T., TANI, H., FUKUSHIMA, K., TAMURA, T. and MATSUMOTO, T., 1982. Structure-relationship of sterols and triterpene alcohols in gas chromatography on a glass capillary column. J. of Chromatography, 234: 65–76.

LOZANO, Y.F., DHUIQUE MAYER, C., BANNON, C. and GAYDOU, E.M., 1993. Unsaponifiable Matter, Total Sterol and Tocopherol Contents of Avocado Oil Varieties. *J. Am. Oil Chem. Soc.* 70: 561–565.

NÉEMAN, I., LIFSHITZ, A. and KASHMAN, Y, 1970. New Antibacterial Agents Isolated from the Avocado Pear. *Appl. Microbiology*, 19: 470–473.

OFFICIAL METHODS OF ANALYSIS OF THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC), 1984. Association of Official Analytical Chemists, Washington, D.C., Method 28.092.

PESQUET, J-J., 1992. Shea nuts. *Aproma/Bimonthly Review*, Nos. 27–29.

SALUNKHE, D.K. and DESAI, B.B., 1986. Post-Harvest Biotechnology of Oilseeds, CRC Press, Inc., Boca Raton, pp. 204–205.

SCHWARTZ, D.P., 1988. Improved Method for Quantitating and Obtaining the Unsaponifiable Matter of Fats and Oils. *J. Am. Oil Chem.* Soc. 65: 246–251

SWERN, D. 1979 (ed), Baileys Industrial Oil and Fat Products, Vol. 2, 4th edn., a. Wiley-Interscience Publication, John Wiley & Sons, New York, pp. 53–69.

TANO-DEBRAH, K. and OHTA, Y 1994. Enzyme-Assisted Aqueous Extraction of Fat from Kernels of the Shea Tree, *Butyrospermum parkii*. *J. Am. Oil Chem. Soc.* 71: 979–983.

TANO-DEBRAH, K. and OHTA, Y. 1995a. Enzyme-Assisted Aqueous Extraction of Shea Fat: A Rural Approach. *J. Am. Oil Chem. Soc.* 72: 251–256.

TANO-DEBRAH, K., YOSHIMURA, Y. and OHTA, Y., 1996. Enzyme-Assisted Extraction of Shea Fat: Evidence from Light Microscopy on the Degradative Effects of Enzyme Treatment on the Cells of Shea Kernel Meal. *J. Am. Oil Chem. Soc.* 73: 449–453.

シア脂中の不けん化物組成の抽出法による変化

Kuwaku TANO-DEBRAH・太田　欽幸

広島大学生物生産学部，東広島市 739

クロロフォルムーメタノール混合溶媒，ソックスレー法，酵素法，及び伝統的法の各方法で抽出したシア脂中の不けん化物をシリカゲル薄層クロマトで分離し比較した。標準ステロールとラノステロールに相当するバンドを抽出し，ガスクロで分析した。酵素法で抽出した脂試料中には低濃度の不けん化物が検出される著者らの結果の確認と，シア脂中の不けん化物の研究が少ないのでこの研究を行った。クロロフォルムーメタノールの抽出物には5バンドが検出されたが，他の試料では3バンドしか見られなかった。硫酸50%溶液でそれぞれ異なった呈色反応を示し，それぞれステロール，トリテルペンアルコールと炭化水素に識別された。特にトリテルペンアルコールと炭化水素分が主体であった。酵素抽出脂の試料では炭化水素分は，他の成分よりも少なかった。一般的に，抽出方法によって，不けん化物の組成が変わった。ソックスレー抽出物には，ステロールとトリテルペンアルコール分がそれぞれ6及び5ピークが検出された。3種類のステロールと，2種類のトリテルペンアルコールが全体の90%以上を占めていた。薄層クロマトとガスクロのデータから，コレステロールβ－シトステロール，キャンペステロル，フコステロール，エルゴステロールなどのステロール類はほとんど見出させなかった。

キーワード：シア脂の不けん物，ステロール，トリテルペンアルコール，クロマトグラフィ分離