Chinese Vegetable Tallow–Characterization and Contamination by Stillingia Oil

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Samples of seed of the Chinese Vegetable Tallow (CVT) tree, *Sapium sebiferum*, obtained from trial plantings in the U.S., together with materials obtained directly from a small-scale production unit in China, have been examined. The major glycerides of the CVTs have been characterized and a wide variation in the tripalmitin and 2-oleodipalmitin levels has been observed.

The contamination of CVT by stillingia oil has been examined. The major source of contamination occurs at the isolation/separation stage of CVT production. Only very low amounts of stillingia oil are actually present in the seed coat. The wider exploitation of this fat will depend upon satisfactory methods being available to ensure that estolide-type glycerides (from stillingia oil) are essentially absent from CVT.

KEY WORDS: Characterization, Chinese vegetable tallow, safety evaluation, seeds (U.S.), small-scale process (China), stillingia oil contamination/removal.

The history of the Chinese Vegetable Tallow tree goes back many centuries. Ancient Chinese maps suggest that the use of Chinese Vegetable Tallow (CVT) in candle-making was well known. In relatively modern times Benjamin Franklin was responsible for introducing the tree into North America in 1763. He wrote "Tis a most useful plant" (1). The use of CVT as an edible oil is documented (2), but available evidence is very limited and suggests that this is not common practice. Stillingia oil, on the other hand, is definitely regarded as being toxic by the Chinese. Industrial quantities of CVT entered Europe in 1894 and some typical export figures from Guangzhou (Canton) during the early part of the 20th century are given in Table 1 (3). Even today there are forecasts of prolific amounts of CVT being potentially available from China.

The fruit of the Chinese Vegetable Tallow tree, *Sapium sebiferum*, consists of an outer husk enclosing three seeds. These seeds are unusual in that they contain both a highly saturated fat and a highly unsaturated oil, which are physically separated in the seeds and may be independently isolated. The kernel, containing the highly unsaturated "drying oil" (stillingia oil), is surrounded by a fibrous waxy coating which contains the vegetable tallow fat. The seeds produce 20–30% w/w of tallow fat and 10–17% w/w of stillingia oil (4).

Interest in CVT has also been aroused on the North American Continent, and its commercial exploitation is being pioneered by Simco (Weston, CT). This activity was initiated at the time of the petroleum oil crisis. Interest was further stimulated by the realization that the yields [~2 metric tons (MT) per acre of tallow plus stillingia oil] approach those obtained for palm oil. A patent (5) describes a novel method of cultivating and harvesting CVT.

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TABLE 1

Exports of CVT from Guangzhou

Year	Tons
1909	9029
1910	9482
1911	4776
1912	10735
1913	13236
1914	10672
1915	10976
1916	17830
1917	10944
1918	11657
1919	13302

Experiments by the Simco group have demonstrated that the CVT tree can grow extremely rapidly in a wide variety of conditions. Plantings from seed in Hawaii have borne fruit after 18 months. There is no commercial production of CVT in Hawaii or North America at the present time, but trial plantings continue. If these are successful they potentially could lead to a significant source of CVT outside China.

Examination of the Houston seeds therefore served two purposes, the first being the characterization of material available outside China, the second being an examination of oil isolated from seeds of different quality. This is a very important consideration for commercial production of CVT, as both stillingia contamination and oil quality are likely to vary with the quality of the seed prior to extraction.

CVT is potentially of great interest to the confectionery industry. The fat is essentially a mixture of tripalmitin (5–30%) and 2-oleodipalmitin (~70%). A detailed glyceride composition is given in Table 2. However, a major problem with the use of CVT in edible products is the possibility of its contamination by stillingia oil. A method of determining stillingia oil in CVT was therefore developed and used to characterize CVT isolated from seeds, and also quantities of CVT produced on a larger scale in China.

EXPERIMENTAL PROCEDURES

Materials and methods. CVT was obtained from a small scale, cottage-type industry (2 MT/day) operating in Hubei Province close to Huang Gang on the river Yangtse. The process had the following stages. The seeds were heated to between 70–90°C (estimate, the operators could not give a figure). This was achieved by gravity feeding the seeds through a coil which passed through a drum approximately 70 cm deep × 40 cm diameter. High pressure steam (120°C) was passed through the drum. The seeds were therefore heated indirectly. The hot seeds were fed directly and continuously into a

TABLE 2

Major Glycerides in CVT

Source	SSS[a]	SOS[b]	SSO	SLinS[c]	Other[d]
Houston (A) 1984	24	70	1	5	5
Houston (B) 1984	23	71	1	5	5
China (commercial) 1985	12	79	TR[e]	4	5
China (commercial) 1985	9	88	TR	1	2
China (commercial) 1986	7	78	TR	5	10
China (intact seed) 1986	10	81	TR	4	5

[a]S, saturated fatty acids.
[b]O, oleic acid.
[c]Lin, linoleic acid.
[d]Other, more unsaturated glycerides.
[e]TR, trace amount.

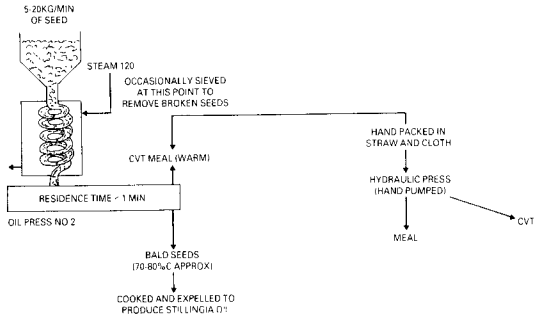

FIG. 1. Schematic Diagram of CVT seed processing as observed in China.

mechanical oil press No. 2, of the type that might well be used in processing olives to obtain olive oil. This was a continuous operation with a throughput of 5–20 kg seed/min. This machine removed the outer layer of the CVT seed without breaking the kernels. In this way two streams were obtained—"bald seeds" and "CVT meal". The operators could not elaborate on the mechanism in the press and as it was in operation, they could not open it.

It was difficult to establish to what extent any seeds were actually broken during the process. Virtually no shell could be detected in the CVT meal by quick visual inspection (one broken shell was found in 20 kg of meal). A sample of this meal was obtained for more detailed examination.

The CVT meal was manually wrapped in straw and bamboo and packed into a small hydraulic press which was pumped by hand (maximum pressure 200 kg/cm^{-2}). The pressure gauges were not working. The press operated horizontally, the piston being about 15 cm in diameter with a pressure plate about 30 cm diameter—similar to the diameter of the cakes. The expressed CVT was run into wooden containers where it was allowed to set in 50 kg blocks and was then demolded.

A sample of the outer seed coating (CVT meal), which is an intermediate in the process (see Fig. 1), was also taken together with a sample of sound seeds. CVT was extracted from the "meal" by Soxhlet extraction. Material isolated from sound seeds in the laboratory was used as a control. The outer coating of the seeds was carefully removed in such a way as to prevent contamination by stillingia oil from the kernel. The CVT was extracted from the separated outer layer with hexane. Triglyceride composition is given in Table 2. In a separate series of experiments the outer coating fats from a number of different seed samples (provided by S. Mason, Simco) were also characterized. These seeds, collected from trees growing in the geographic area to the southeast of Houston, TX, were approximately three-years-old, of varying qualities and classified A-F according to appearance (Table 3). CVT was recovered by simple extraction of the whole seeds with hexane in a Soxhlet extractor.

Stillingia oil was obtained from seed kernels remaining after the removal of vegetable tallow fat by Soxhlet extraction. The kernels were ground up with a little isooctane (containing 1% w/v butylated hydroxy-anisole) using a pestle and mortar. The liquid portion was filtered through anhydrous sodium sulphate and the solvent was removed under nitrogen.

TABLE 3

Classification of Seeds According to Appearance

Seed code	Description
A (best)	White
B	Less white
C	Grey/brown—few twigs/hulls
D	Brown—man twigs/hulls
E	Grey/white/moldy
F (poorest)	Very brown/black

Stillingia oil is known to contain ~77% w/w of normal triglycerides (6,7) (comprising one saturated and two unsaturated fatty acids), and ~23% of a tetraester fraction (7,8) in which the triglycerides contain two normal fatty acids (*sn*-1 and *sn*-2) and one estolide fatty acid (*sn*-3). This 3-position fatty acid is 8-hydroxy-5, 6-octadienoic acid joined to *trans*-2, *cis*--4-decadienoic acid by an estolide linkage, the former being attached to the glycerol moiety by an ester linkage. The major nonestolide component of stillingia has also been characterized (9).

The estolide fatty acid itself is rare in oils (7,8), and the estolide triglyceride is a useful "marker" for the presence of stillingia oil in vegetable tallow. Crossley and Hilditch (10,11) and Devine (12) were able to isolate about 5% w/w of the decadienoic acid from stillingia oil, and to demonstrate that it had a characteristic absorption band at 260 nm. Maier and Holman (6) demonstrated that the estolide triglycerides were the only fraction from stillingia oil to contain the 2, 4-decadienoic acid.

The absorption band at 260 nm is also characteristic of the estolide triglycerides, and this unique feature was used as the basis of our analytical approach. Payne-Wahl and Kleiman (13) showed that the estolide triglycerides can be separated from normal triglycerides by high performance liquid chromatography (HPLC) on silica columns, the run time being about 10 min. However, they used a comparatively insensitive infra-red detector system (Du Pont, Boston, MA) set at 1750 cm^{-1} (carbonyl band). For our work, we chose to make use of the (comparatively) strong absorption at 260 nm in the ultraviolet spectrum of the estolide. Devine (12) found a molar extinction coefficient approximating to 26,000 at 260 nm for the decadienoic acid itself.

Estolide triglycerides (and hence stillingia oil contents) in the vegetable tallows were determined by a "standard

addition" approach in which we used the authentic stillingia oils extracted from seed. The stillingia oils were added back to the CVT in a limited range of known amounts (w/w basis). These known CVT/stillingia oil mixtures were made up as solutions (10% w/v) in solvent of the same composition as that used for HPLC (10% v/v tetrahydrofuran in hexane). Twenty μL aliquots of these solutions were injected onto the HPLC column [5 micron Lichrosorb Si-60 (ex. Merck), 150 mm × 4.6 mm i.d.] running under isocratic conditions with the same solvent system; detection was via a Perkin-Elmer LC-55 (Norwalk, CT) variable wavelength instrument set at 260 nm, and data collection was via a Multichrom 5000 (PDP 11/73+ based) system.

At a solvent flow-rate of 1 mL/min, four characteristic estolide peaks, following Payne-Wahl, were seen to elute between retention times of 3.8 and 5.0 min (Fig. 2). Total run time was no longer than 10 min, this extra time was allowed to enable triglyceride hydroperoxides (which elute after the estolides, and are detected at 260 nm) to clear the system before the next injection. Stillingia contents were quantified by summing the total estolide peak areas, and plotting this value graphically against the amount of stillingia oil actually added to the samples by the analyst. By extrapolation to the "x" axis (Fig. 3), the stillingia oil present in the original vegetable tallow sample could be determined.

An alternative method is to estimate stillingia oil in CVT on the basis of C18:3 fatty acid content, assuming 35% linolenic acid in neat stillingia oil. This approach has been compared with the more sophisticated HPLC method.

RESULTS AND DISCUSSION

Major glycerides in CVT. The glyceride composition of the various CVTs analyzed (14,15) confirms previous observations that the fat is essentially a mixture of tripalmitin and 2-oleodipalmitin. The natural variation in composition has been previously noted (16). In fact, we also see a remarkable variation in the relative proportions of the two major glycerides (Table 2).

The Houston seeds show, not unexpectedly, compositional differences which reflect the observed "visual qualities" (Table 4). Thus free fatty acid contents increase and triglyceride contents decrease with increasing deterioration. The seed sample judged to possess the best "visual" quality (seed A) did not produce the best triglyceride content (seed B). Only seed F furnished fat which was largely composed of free fatty acids. Seeds A-C produced fats with useful contents of SOS triglycerides (Table 5), and which might furnish useful feedstocks for fractionation. Fatty acid distributions in fat extracted from seed A-E are given in Table 6.

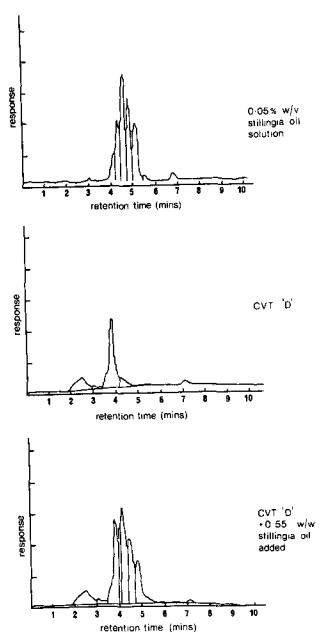

FIG. 2. Representative HPLC/UV chromatograms of stillingia oil, vegetable tallow and vegetable tallow with added stillingia oil.

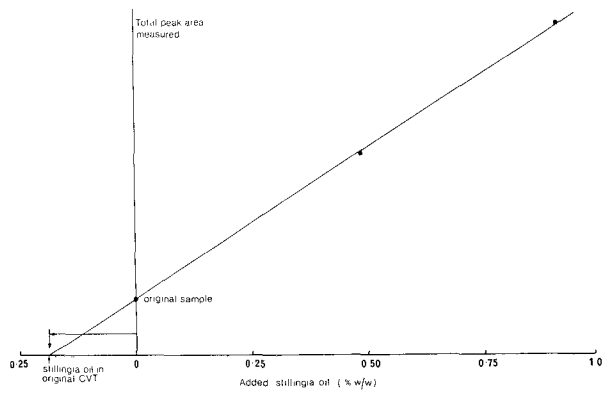

FIG. 3. The graphical approach to the determination of stillingia oil content of vegetable tallows extracted from seeds.

TABLE 4

Free Fatty Acid and Triglyceride Content of CVT Isolated from Houston Seeds

Seed code	FFA (% w/w)	Triglyceride (% w/w)
A	29.2	68.0
B	18.3	76.3
C	32.7	61.3
D	39.0	55.6
E	31.7	66.0
F	85.2	14.0

TABLE 5

Major Triglycerides in Houston Seeds

Seed code	% of Triglycerides[a]			
	SSS	SOS	SSO	Others
A	24.0	70.0	0.7	5.3
B	22.6	70.9	0.5	6.0
C	21.9	71.0	0.8	6.3
D	29.9	63.3	0.9	5.9
E	36.5	57.3	0.7	5.5
F	81.7	14.5	0.7	3.1

[a]See Table 2 for abbreviations.

TABLE 6

Fatty Acid Composition of Houston CVT

Code	14:0	16:0	18:0	18:1	18:2	18:3	20:0
A	—	73.4	1.0	24.8	0.7	tr[a]	tr
B	—	75.3	1.1	22.9	0.6	tr	tr
C	—	73.9	1.1	24.3	0.6	0.01	—
D	—	76.7	1.1	21.1	0.7	0.3	0.1
E	tr	77.6	1.5	20.1	0.8	tr	0.1
F	n.d.[b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a]tr < 0.03. [b]n.d., not determined.

TABLE 7

Composition of CVT Fractions[a]

	Stearine	Mid fraction	Oleine	CVT
Yield %wt	12	66	22	
Glyceride composition[b]				
SSS	67	2	2	13
SOS	31	94	40	78
SSO	1	1	4	2
SLinS	1	2	13	3
SOO	TR	TR	26	2
Others	TR	1	15	2
	100	100	100	100
Stillingia Oil	<0.02	<0.02	0.5	0.1

[a]CVT fractionated from acetone at 0°C and 19.5°C. Approximately 15% w/w of insoluble, uncharacterized material was removed prior to fractionation.

[b]Abbreviations as in Table 2.

TABLE 8

Solid Fat[a] Content of CVT and Related Fats

	CVT Mid fraction	Palm mid Fraction	A cocoa butter
N_{20}	97	84	76
N_{25}	96	74	69
N_{30}	90	50	32
$N_{32.5}$	49	22	8
N_{35}	7	8	0
N_{40}	0	0	—

[a]Measured by Brucker Minispec NMR after stabilization at 26°C/40 hr.

Fractionation of CVT fat from solvent yields a stearine rich in tripalmitin and a lower melting fraction rich in 2-oleodipalmitin. The latter fraction has potential use as a cocoa butter equivalent (CBE) (17) (Tables 7 and 8). Currently the major source of 2-oleodipalmitin for CBE manufacture is fractionated palm oil, but in this latter case the material contains a significant amount of the asymmetrical isomer 1-oleodipalmitin. Our major concern, however, has been the potential for stillingia oil contamination of CVT prior to considering its use in edible products.

TABLE 9

Unsaponifiable, Total GLC Sterol Content and the Composition of the Three Sterol Fractions of a CVT

	%		mg/kg
Unsaponifiable	0.42		4200
Total GLC sterol	0.07		716.6
Ratio of the fractions			
4-Demethyl sterols	63.4		454.4
4α-Methyl sterols	2.6		18.6
4,4-Dimethyl sterols	34.0		243.6
Composition of the 4-demethyl sterol fraction			
Cholesterol	1.00	0.8	3.8
Campesterol	1.33	8.7	39.3
Stigmasterol	1.47	4.1	18.6
β-Sitosterol	1.69	77.8	353.6
Δ5 Avenasterol	1.91	7.2	32.8
	2.08	1.4	6.3
Composition of the 4-α methyl sterol fraction			
	1.45	7.0	1.3
Obtusifoliol	1.60	10.2	1.9
	1.72	13.4	2.5
Grammisterol	1.89	31.7	5.9
24-Ethyl lophenol	2.38	2.2	0.4
	2.67	21.5	4.0
	3.33	2.7	0.5
	3.63	11.3	2.1
Composition of the 4,4-dimethyl sterol fraction			
	1.16	2.1	5.0
	1.40	10.8	26.2
	1.59	2.5	6.1
	1.72	1.1	2.7
	1.87	6.9	16.8
cyclo Artenol	2.11	6.1	14.9
	2.20	5.3	13.0
24-Methylene cyclo artanol	2.38	57.0	138.9
	2.55	8.2	20.0

We have not characterized any other minor components. However, for completeness we report some previously unpublished data (private communcations: L. Schouten; Unilever Research, Vlaardingen, Netherlands; and J. Rossell, Food Research Association, Leatherhead, U.K.) on the sterol composition of CVT (Table 9). No tocopherol was detected in CVT in this work.

Stillingia oil contamination. We conclude that CVT extracted under ideal conditions contains <0.05% C18:3. On this basis, contamination even at relatively low levels can be detected by the overall C18:3 content. This approach must be used with caution, however, especially with older seeds where we have observed a significant reduction in total unsaturation as a consequence of oxidation. At very low levels of contamination we prefer to use the HPLC/UV detector method.

The levels of stillingia oil found in the outer coatings of the Houston seeds are given in Table 10. The data show that stillingia oil is present in all the samples, albeit at relatively low levels in the pure CVT (0.02–0.05%). The stillingia oil present in "pure" CVT isolated carefully from sound seed is compared with values found in the CVT meal and commercially produced CVT from China (Table 11). Despite taking reasonable care, the cottage

TABLE 10

Stillingia Oil in CVT Isolated from Houston Seeds

Seed code	% Stillingia oil in vegetable tallows	
	HPLC/UV	From 18:3 content
A	0.03	0.05
B	0.06	0.05
C	0.03	0.08
D	0.02	0.45
E	0.02	0.05
F	0.05	—

TABLE 11

Characteristics of CVT from China (Hubei Province)

CVT	% FFA	% 18:3	% Stillingia oil[a]
Careful isolation	2.0	0.06	0.04
CVT meal	8.3	0.09	0.14
Commercial CVT	7.8	0.23	0.60

[a]By HPLC/UV.

industry, using a mechanical device to remove the outer coating, produced material which was significantly contaminated with stillingia oil.

Under ideal conditions the level of stillingia oil in CVT is therefore very low. The actual level of the dienoic acid and the hydroxyallenic acid are of the order of 0.001%–0.0025% (w/w), respectively. This is also confirmed by the analyses on the seed coats from the samples from Houston.

The presence of stillingia oil in CVT must be regarded as nutritionally undesirable. We have considered various methods of removing stillingia oil, e.g., fractionation, partial hydrogenation and use of activated carbon and bleaching earths.

Fractionation of the stillingia oil into an oleine fraction, although largely successful (Table 7), is regarded as commercially unattractive. Partial hydrogenation, although removing the conjugated acids, also partially isomerizes oleic acid, generating unwanted elaidic glycerides—a problem if the product is to be used as a cocoa butter replacer. We have not characterized the by-products of the partial hydrogenation.

Stillingia oil estolides were reduced when CVT was treated with excessive amounts of either activated carbon or bleaching earth, especially at elevated temperatures (100–180°C). Chromatographic adsorption on silica can be used to isolate laboratory scale quantities of normal triglyceride and estolide.

Safety evaluation. Chinese vegetable tallow containing less than 1% stillingia oil was fed to rats for four weeks at dietary levels of 5% and 15% to obtain evidence of its harmlessness and nutritional adequacy. The only effect observed was an increase in the weights of livers and spleens in male rats, and livers in female rats, fed 15% Chinese vegetable tallow. The "no effect" level was therefore 5% material (equivalent to 5 g/kg body weight/day) but examination of the liver and spleens, together with other organs and tissues, showed no histological differences between rats fed 15% Chinese vegetable tallow and control rats. When stillingia oil was fed to rats, food consumption and, consequently, growth are reduced due to palatability of the diet if the dietary level exceeded 0.5%. However, organ weight changes (increased liver and thyroid weights) noted at lower dose levels require that further studies are necessary before Chinese vegetable tallow can be accepted as a wholesome fat.

The major source of stillingia contamination occurs at the isolation/extraction stage of CVT production. As far as we are aware, a commercially attractive way of removing stillingia oil from CVT has not been described. The wider exploitation of this fat will depend on satisfactory methods being available to ensure that estolide-type glycerides are essentially absent from CVT.

ACKNOWLEDGMENT

Technical support was provided by H. Nash, G. Sassano, M. Dallas (Unilever Research, Colworth House, U.K.) and A. Rozendaal (Unilever Research, Vlaardingen, Netherlands); safety evaluation was carried out by R. Wilson (Unilever Research, Colworth House); S. Mason of Simco (Weston, CT) provided samples of seeds for analysis and information on the development of CVT technology in North America and Hawaii; D.C.Y. Tung (Yi Fen Trading) arranged the visit to China and the opportunity to obtain authentic commercial samples; and Loders Croklaan gave permission to publish this work.

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[Received July 15, 1990; accepted November 23, 1990]