# Detection of Adulteration of Cottonseed Oil by Gas Chromatography

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## ABSTRACT

To detect adulterant vegetable oils in cottonseed oil, soybean, rapeseed, and ricebran oils were mixed into cottonseed oil extracted experimentally from seeds. These adulterated oils and the component oils were analyzed for sterols, fatty acids, and triglycerides by gas chromatography. In sterol analysis, stigmasterol was determined for adulteration with soybean and ricebran oils. Brassicasterol content seemed to be reliable as the indicator of adulteration for rapeseed oil. In fatty acid analysis, erucic acid for rapeseed oil and linolenic acid for soybean and ricebran oils were proof of adulteration. Triglyceride analysis was not so reliable as sterol analysis for detecting contamination, except that triglycerides with carbon-58, 60, and 62 indicate adulteration with rapeseed oil. Rapeseed oil (5%) and soybean and ricebran oils (10%) were the limits of detection for adulteration in cottonseed oil. Analysis of cottonseed oil from six refineries did not show positive indications of adulteration.

## INTRODUCTION

In Japan, cottonseed oil was popular as salad oil for mayonnaise and dressings, but recently, because of the decrease in availability, it has been replaced gradually by other vegetable oils, such as soybean, rapeseed, and ricebran. As these other oils become less expensive and more available than cottonseed oil, there is a possibility of adulteration into cottonseed oil.

In this article, sterol, fatty acid, and triglyceride compositions determined by gas chromatography are discussed as means of detecting these oils as adulterants in cottonseed oil.

The detection of adulteration of oils and fats by sterol analysis have been reported for: vegetable fats in milk fat (1), margarine in butter (2), palm oil in lard or fish oil in vegetable oils (3), other vegetable oils in olive oil (4), and animal fats in vegetable fats and oils (5). For the detection of other vegetable oils in cottonseed oil, however, a suggestion was given (6); but details are lacking. It was reported that stigmasterol was not contained in cottonseed oil (2,3,7) or contained in small amounts (8). Brassicasterol was considered as an indicator for rapeseed oil which contains ca. 10%, whereas cottonseed oil contains less than 1% (3,4,8). In fatty acid compositions, gadoleic and erucic acids, contained peculiarly in rapeseed oil, and linolenic acid, contained abundantly in soybean oil (9) mainly were discussed. In triglyceride compositions, it was observed that triglycerides with large carbon numbers, such as 58, 60, and 62 were peculiar to rapeseed oil (10). In addition to adulterated cottonseed oils and component oils, the triglyceride profiles of commercial cottonseed oils from six refineries were analyzed.

TABLE I

Sterol Compositions of Cottonseed Oils with Different Origin
Percentage of sterols

| Origin | Campesterol | Stigmasterol | β-Sitosterol |
|---|---|---|---|
| Nigeria | 9.6 | - | 90.4 |
| South Africa | 8.4 | - | 91.6 |
| Central Africa | 9.2 | 1.0 | 89.8 |
| Niger | 7.8 | trace | 92.2 |
| U.S.A. | 8.2 | - | 91.8 |
| Thailand | 8.2 | 1.0 | 90.8 |
| Average | 8.6 | 0.3 | 91.1 |

## MATERIALS

Six kinds of cottonseed oil from seeds of Nigeria, South Africa, Central Africa, Niger, U.S.A., and Thailand origin were extracted experimentally, refined, decolorized, winterized, and deodorized. Four samples each of soybean, rapeseed, and ricebran salad oils commercially produced were obtained from different refineries. The adulterated oils with soybean oil were prepared by the addition of soybean oil from a refiner into the cottonseed oil of Nigeria origin, so that the contents of soybean oil were 5, 10, 20, and 40% in final adulterated oils. The adulterated oils with rapeseed and ricebran oils were prepared by the same way. The above 6 cottonseed oils, 4 different kinds of soybean, rapeseed, and ricebran oils, 12 blends of adulterated oil, and cottonseed oil from 6 different refineries served for the analysis of sterol, fatty acid, and triglyceride by gas chromatography.

## PROCEDURES

### Sterol

Sample oil (5 g) in 50 ml alcoholic 1 N potassium hydroxide was refluxed in a water bath at 85 C for 1 hr. After cooling, the reaction mixture was transferred into a separatory funnel with 30 ml distilled water, and unsaponifiable matter was extracted with three 50 ml portions of diethyl ether. The combined ether extracts were washed with water until neutral and dried with unhydrous sodium sulfate. After concentration in a rotary evaporator, unsaponifiable matter was separated by Florisil column chromatography, according to Eisner and workers (2), and sterol fraction eluted with 25% ether in hexane was concentrated on a steam bath. After drying in a vacuum drier, sterol fraction was dissolved with 0.2 ml ether and analyzed under the following conditions. Sample solution (1 μliter) was injected into a Shimazu GC-5A gas chromatograph with a flame ionization detector. A 1.5 m long x 3 mm inside diameter glass column, packed with Chromosorb W, AW, DMCS, 80-100 mesh with 1% OV-17, was operated at 231 C with nitrogen of 40 ml/min as the carrier gas.

### Fatty Acid

Ten ml 0.5% sodium methoxide alcoholic solution were added to 1 g sample oil in a large test tube (200 mm long x 30 mm inside diameter) and heated in a glycerine bath at 65 C under a water condenser for 30 min bubbling with oxygen-free nitrogen. After clearing, the test tube was removed from the bath, and 8 ml N/5 acetic acid was added. The content was transferred to 100 ml separatory funnel. After adding 50 ml diethyl ether, the funnel was shaken vigorously and allowed to settle, and water layer was removed. Ether layer was washed with three 20 ml portions of distilled water. Then the ether layer was transferred to

TABLE II

Sterol Compositions of Soybean, Rapeseed, and Ricebran Oils from Different Refineries
Percentage of total sterols

| Kind of oil | Refiner | Brassicasterol | Campesterol | Stigmasterol | β-Sitosterol |
|---|---|---|---|---|---|
| Soybean[a] | A | - | 19.8 | 17.6 | 62.6 |
| | B | - | 19.0 | 23.2 | 57.8 |
| | C | - | 20.7 | 21.6 | 57.7 |
| | D | - | 21.2 | 20.2 | 58.2 |
| | Average | - | 20.2 | 20.7 | 59.1 |
| Rapeseed[b] | E | 11.0 | 29.2 | - | 59.8 |
| | B | 10.2 | 27.0 | - | 62.8 |
| | F | 11.4 | 30.9 | - | 57.7 |
| | C | 11.8 | 28.2 | - | 60.0 |
| | Average | 11.1 | 28.8 | - | 60.1 |
| Ricebran[c] | G | - | 19.8 | 17.6 | 62.6 |
| | H | - | 23.7 | 19.9 | 56.4 |
| | I | - | 20.8 | 18.9 | 60.3 |
| | J | - | 21.0 | 17.2 | 61.8 |
| | Average | - | 21.3 | 18.4 | 60.3 |

[a]All American origin.
[b]All Canadian origin, not LEAR type.
[c]All Japanese origin.

200 ml beaker, ca. 10 g unhydrous sodium sulfate added to remove moisture, and filtered through a filter paper. After concentration in nitrogen gas, residue was dissolved with 5 ml ether and served for the sample of fatty acid determination. Solution (1 μliter) was injected into a Shimazu GC-1C gas chromatograph with a flame ionization detector. A 2.5 m long x 3 mm inside diameter stainless steel column, packed with Chromosorb W, AW, 100-120 mesh with 20% diethylene glycol succinate, was operated at 190 C with nitrogen of 40 ml/min as the carrier gas.

### Triglyceride

Sato's procedure (10,11) was modified as follows: 2 g sample oil was dissolved with 100 ml acetone, and 1 μliter solution was injected into a Shimazu GC-5A gas chromatograph with a flame ionization detector and temperature programer TP-5.

A 0.3 m long x 3 mm inside diameter glass column, packed with Chromosorb W, AW, DMCS, 80-100 mesh with 1% OV-17, was operated in the range of 200-300 C risen 10 C/min and 300-340 C risen 3 C/min with nitrogen of 120 ml/min as the carrier gas.

## RESULTS AND DISCUSSION

### Sterol

Sterol compositions of experimental cottonseed oils;

TABLE III

Sterol Compositions of Cottonseed Oils Adulterated with Soybean, Rapeseed, and Ricebran Oils
Percentage of total sterols

| Adulterant Oil | Percent of adulterant oil | Brassica-sterol | Campe-sterol | Stigma-sterol | β-Sito-sterol | β-Sitosterol/ (Campesterol + Stigmasterol) |
|---|---|---|---|---|---|---|
| Soybean | 5 | - | 10.1 | trace | 89.9 | 8.90 |
| | 10 | - | 11.0 | 1.6 | 87.4 | 6.94 |
| | 20 | - | 12.2 | 3.5 | 84.3 | 5.37 |
| | 40 | - | 13.6 | 6.5 | 79.9 | 3.97 |
| Rapeseed | 5 | trace | 11.0 | - | 89.0 | 8.09 |
| | 10 | 1.1 | 11.6 | - | 87.3 | 7.89 |
| | 20 | 2.0 | 14.0 | - | 84.0 | 6.00 |
| | 40 | 4.1 | 17.6 | - | 78.2 | 4.43 |
| Ricebran | 5 | - | 11.0 | trace | 89.0 | 8.09 |
| | 10 | - | 10.6 | 1.4 | 88.0 | 7.33 |
| | 20 | - | 11.3 | 3.8 | 84.9 | 5.62 |
| | 40 | - | 14.4 | 7.2 | 78.4 | 3.63 |

TABLE IV

Fatty Acid Compositions Cottonseed Oils with Different Origin
(Area %)

| Origin | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 |
|---|---|---|---|---|---|---|---|
| Nigeria | 0.7 | 24.1 | 0.9 | 2.3 | 19.8 | 51.1 | 1.1 |
| South Africa | 1.1 | 27.6 | 1.1 | 2.3 | 16.7 | 50.1 | 1.1 |
| Central Africa | 1.2 | 27.9 | 2.3 | 2.2 | 15.5 | 49.7 | 1.2 |
| Niger | 0.7 | 20.7 | 1.4 | 2.4 | 18.4 | 56.4 | - |
| U.S.A. | 1.0 | 21.0 | 1.3 | 2.7 | 17.3 | 56.0 | 0.7 |
| Thailand | 0.8 | 20.2 | - | 2.7 | 22.0 | 54.3 | trace |
| Average | 0.9 | 23.6 | 1.2 | 2.4 | 18.3 | 52.9 | 0.7 |

TABLE V

Fatty Acid Compositions of Soybean, Rapeseed, and Ricebran Oils from Different Refineries (Area %)

| Kind of oil | Refiner | 14:0 | 16:0 | 16:1 | 18:0 | 18:0 | 18:2 | 18:3 | 20:1 | 22:1 |
|---|---|---|---|---|---|---|---|---|---|---|
| Soybean | A | tr[a] | 12.2 | - | 4.2 | 23.7 | 52.3 | 7.6 | - | - |
| | B | - | 10.5 | - | 3.2 | 21.2 | 58.8 | 6.3 | - | - |
| | C | tr | 10.7 | - | 3.7 | 22.5 | 55.0 | 8.1 | - | - |
| | D | tr | 10.2 | - | 4.1 | 23.4 | 54.8 | 7.5 | - | - |
| | Average | tr | 10.9 | - | 3.8 | 22.7 | 55.2 | 7.4 | - | - |
| Rapeseed | E | - | 2.9 | 0.7 | 1.0 | 30.9 | 17.9 | 6.9 | 12.8 | 26.9 |
| | B | - | 2.8 | 0.4 | 1.7 | 28.9 | 17.7 | 8.7 | 14.5 | 25.3 |
| | F | - | 2.8 | tr | 1.1 | 28.7 | 17.7 | 8.8 | 14.3 | 26.6 |
| | C | - | 2.8 | 0.5 | 1.4 | 28.4 | 17.3 | 8.5 | 15.1 | 26.0 |
| | Average | - | 2.8 | 0.4 | 1.3 | 29.2 | 17.7 | 8.2 | 14.2 | 26.2 |
| Ricebran | G | 0.4 | 21.0 | tr | 1.3 | 44.1 | 30.1 | 3.1 | - | - |
| | H | 0.3 | 17.4 | tr | 1.6 | 46.8 | 27.9 | 6.0 | - | - |
| | I | 0.4 | 20.1 | tr | 1.4 | 43.3 | 31.4 | 3.4 | - | - |
| | J | 0.3 | 17.4 | tr | 1.2 | 40.5 | 38.1 | 2.5 | - | - |
| | Average | 0.4 | 18.9 | tr | 1.4 | 43.7 | 31.9 | 3.7 | - | - |

[a]tr = trace.

TABLE VI

Fatty Acid Compositions of Cottonseed Oils Adulterated with Soybean, Rapeseed, and Ricebran Oil (Area %)

| Adulterant oil | Percent of adulterant oil | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:1 | 22:1 |
|---|---|---|---|---|---|---|---|---|---|---|
| Soybean | 5 | 0.7 | 23.3 | 0.9 | 2.5 | 18.9 | 51.4 | 1.3 | - | - |
| | 10 | 0.6 | 22.7 | 0.8 | 2.5 | 20.3 | 51.5 | 1.6 | - | - |
| | 20 | 0.6 | 21.7 | 0.8 | 2.6 | 20.6 | 51.3 | 2.4 | - | - |
| | 40 | 0.5 | 19.5 | 0.7 | 3.1 | 21.2 | 21.2 | 3.4 | - | - |
| Rapeseed | 5 | 0.7 | 23.4 | 0.9 | 2.2 | 20.2 | 49.8 | 1.5 | tr[a] | 1.3 |
| | 10 | 0.6 | 21.7 | 0.9 | 2.4 | 21.0 | 47.7 | 2.1 | 1.3 | 2.4 |
| | 20 | 0.5 | 19.9 | 0.9 | 2.0 | 22.2 | 43.3 | 2.8 | 3.0 | 5.4 |
| | 40 | 0.4 | 15.6 | 0.8 | 1.8 | 24.5 | 38.5 | 3.4 | 4.3 | 10.7 |
| Ricebran | 5 | 0.7 | 23.9 | 0.9 | 2.2 | 21.0 | 50.1 | 1.2 | - | - |
| | 10 | 0.6 | 23.8 | 0.8 | 2.1 | 22.3 | 49.1 | 1.3 | - | - |
| | 20 | 0.5 | 23.5 | 0.7 | 2.1 | 24.7 | 47.0 | 1.5 | - | - |
| | 40 | 0.5 | 22.9 | 0.6 | 1.9 | 29.5 | 42.7 | 1.9 | - | - |

[a]tr = trace.

TABLE VII

Fatty Acid Compositions of Commercial Cottonseed Oils (Area %)

| Refiner | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:1 | 22:1 |
|---|---|---|---|---|---|---|---|---|---|
| A | 0.9 | 20.4 | - | 2.5 | 20.0 | 56.2 | - | - | - |
| B | 0.7 | 19.3 | - | 3.1 | 22.5 | 52.7 | 1.7 | - | - |
| K | 1.1 | 22.0 | 0.3 | 1.7 | 19.2 | 55.3 | 0.4 | - | - |
| D | 0.4 | 19.9 | - | 2.9 | 22.6 | 54.2 | - | - | - |
| L | 0.8 | 20.8 | 0.9 | 2.5 | 20.4 | 54.6 | trace | - | - |
| M | 0.6 | 18.5 | -- | 2.2 | 20.8 | 57.9 | - | - | - |

TABLE VIII

Triglyceride Compositions of Cottonseed Oils with Different Origin (Area %)[a]

| Origin | C:50 | C:52 | C:54 |
|---|---|---|---|
| Nigeria | 10.6 | 50.5 | 38.9 |
| South Africa | 12.2 | 49.5 | 38.3 |
| Central Africa | 12.4 | 44.2 | 43.4 |
| Niger | 11.8 | 43.6 | 44.6 |
| U.S.A. | 10.5 | 47.0 | 42.5 |
| Thailand | 11.0 | 50.0 | 39.0 |

[a]Peaks appeared before C:50 were not calculated.

commercial soybean, rapeseed, and ricebran oils; and adulterated cottonseed oils are given in Table I-III. Of the 6 experimental cottonseed oils, the sterols contained about 90% of -sitosterol and ca. 10% of campesterol, but brassicasterol was not detected. Stigmasterol was detected in the range of trace to 1% in 3 samples of experimental cottonseed oil. In soybean and ricebran oils, ca. 20% stigmasterol and, in rapeseed oil, ca. 10% brassicasterol were detected. In these oils, the content of -sitosterol was only ca. 60% total sterols. In adulterated cottonseed oil, the sterol content varied in accordance with the percent of adulterant oil. From the view points of brassicasterol and stigmasterol, it is presumed that adulteration of 10% with these oils was the limit of detection. Karleskind, et al., (4) proposed the

TABLE IX

Triglyceride Compositions of Soybean, Rapeseed, and Ricebran Oils from Different Refineries (Area)[a]

| Kind of oil | Refiner | C:50 | C:52 | C:54 | C:56 | C:58 | C:60 | C:62 | C:64 |
|---|---|---|---|---|---|---|---|---|---|
| Soybean | A | 4.2 | 30.5 | 65.3 | - | - | - | - | - |
| | B | 5.4 | 29.6 | 65.0 | - | - | - | - | - |
| | C | 4.8 | 31.4 | 63.8 | - | - | - | - | - |
| | D | 6.3 | 31.7 | 62.0 | - | - | - | - | - |
| | Average | 5.2 | 30.8 | 64.0 | - | - | - | - | - |
| Rapeseed | E | - | - | tr[b] | 4.3 | 18.3 | 31.5 | 45.9 | tr |
| | B | - | - | - | 4.8 | 19.2 | 28.8 | 47.2 | tr |
| | F | - | - | tr | 4.2 | 17.4 | 33.2 | 45.2 | - |
| | C | - | - | tr | 4.6 | 18.2 | 31.1 | 46.1 | - |
| | Average | - | - | tr | 4.5 | 18.3 | 31.2 | 46.0 | tr |
| Ricebran | G | 11.0 | 38.2 | 50.8 | tr | - | - | - | - |
| | H | 10.2 | 39.5 | 50.3 | tr | - | - | - | - |
| | I | 11.4 | 38.8 | 49.8 | tr | - | - | - | - |
| | J | 10.5 | 38.2 | 51.3 | tr | - | - | - | - |
| | Average | 10.8 | 38.7 | 50.5 | tr | - | - | - | - |

[a]Peaks appeared before C:50 were not calculated.
[b]tr = trace.

TABLE X

Triglyceride Compositions of Cottonseed Oils Adulterated with Soybean, Rapeseed, and Ricebran Oils (Area %)[a]

| Adulterant oil | Percent of adulterant oil | C:50 | C:52 | C:54 | C:56 | C:58 | C:60 | C:62 | C:64 |
|---|---|---|---|---|---|---|---|---|---|
| Soybean | 5 | 10.5 | 49.8 | 39.7 | - | - | - | - | - |
| | 10 | 9.7 | 48.7 | 41.6 | - | - | - | - | - |
| | 20 | 9.1 | 47.0 | 43.9 | - | - | - | - | - |
| | 40 | 8.4 | 42.4 | 49.1 | - | - | - | - | - |
| Rapeseed | 5 | 10.1 | 48.4 | 36.6 | trace | 1.0 | 1.3 | 2.6 | - |
| | 10 | 9.8 | 45.0 | 35.6 | trace | 1.6 | 3.5 | 4.4 | - |
| | 20 | 8.2 | 40.8 | 31.7 | 1.0 | 3.6 | 6.5 | 9.2 | - |
| | 40 | 6.6 | 30.6 | 21.7 | 1.7 | 7.8 | 12.4 | 19.2 | - |
| Ricebran | 5 | 10.8 | 50.4 | 38.8 | - | - | - | - | - |
| | 10 | 10.6 | 49.0 | 40.4 | - | - | - | - | - |
| | 20 | 10.6 | 48.0 | 41.4 | - | - | - | - | - |
| | 40 | 10.8 | 44.6 | 44.6 | - | - | - | - | - |

[a]Peaks appeared before C:50 were not calculated.

ration of -sitosterol/(campesterol + stigmasterol) to determine other vegetable oils in olive oil. Experimental cottonseed oils had the values of 8.80-11.19 in this ratio, which were greater than the values of adulterated oils in Table III. Trace of stigmasterol, which was detected in a few of cottonseed oils in market, was insufficient to prove adulteration. Trace of brassicasterol was detected in one sample of commercial cottonseed oil. However, it was not thought that trace of brassicasterol gave evidence of adulteration with rapeseed oil, as Karleskind, et al., (3) reported that cottonseed oil sometimes contained 1% brassicasterol.

## Fatty acid

Fatty acid compositions of experimental cottonseed oils; commercial soybean, rapeseed, and ricebran oils; adulterated cottonseed oils; and commercial cottonseed oils are given in Table IV-VII. Remarkable peculiarities were observed in myristic and linolenic acids of cottonseed oils; in myristic, palmitic, and linolenic acids of soybean oils; and in gadoleic and erucic acids of rapeseed oils. Ricebran oils contained more oleic and linolenic acids and less myristic and palmitoleic acids compared to cottonseed oils; but these peculiarities were not so remarkable. In adulterated cottonseed oils with rapeseed oil, gadoleic and erucic acids were remarkable as the evidence of adulteration. However, in the adulterated oils with soybean and ricebran oils, the decrease of myristic acid and the increase of linolenic acid were not enough to prove the adulteration, considering the error on measure.

## Triglyceride

Triglyceride compositions of experimental cottonseed oils; commerical soybean, rapeseed, and ricebran oils; adulterated cottonseed oils; and commercial cottonseed oils are given in Table VIII-XI. Considerable differences were observed in triglyceride compositions between cottonseed oil and other oils, but it seemed to be difficult to prove the adulteration in small amounts, except the case of rapeseed oil. In rapeseed oil, the absence of triglycerides with carbon-50, 52, and 54 and the abundant presence of triglycerides with carbon-58, 60, and 62 were quite different from cottonseed oil, so it seemed to be possible that the adultera-

TABLE XI

Triglyceride Compositions of Commercial Cottonseed Oils (Area %)[a]

| Refiner | C:50 | C:52 | C:54 |
|---|---|---|---|
| A | 13.4 | 43.6 | 43.0 |
| B | 10.4 | 45.4 | 44.2 |
| K | 12.6 | 44.1 | 43.3 |
| D | 10.8 | 44.2 | 45.0 |
| L | 15.5 | 43.3 | 41.2 |
| M | 13.0 | 45.2 | 41.8 |

[a]Peaks appeared before C:50 were not calculated.

tion with rapeseed oil is proved even in small quantity.

Our tests show that ca. 5% rapeseed oil in cottonseed oil can be detected, and ca. 10% soybean or ricebran oil can be detected by the analysis of sterol, fatty acid, and triglyceride. From the results of the analyses for six kinds of commerical cottonseed salad oil, the adulteration with these oils was not proven conclusively.

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