• *Letter to the Editor*

# Effect of Extraction Temperature and Refining on the Halphen-Test Response of Cottonseed Oil

Cottonseed oil of higher Halpen-acid-moiety concentration can be obtained by hot hexane extraction of cottonseed meats from which the oil has already been partially extracted at room temperature. The intensity of the Halphen-test color solution is affected by many nitrogen-containing substances including soybean phosphatides.

IN THE HEXANE EXTRACTION of cottonseed for an investigation into the quantitative aspects of the Halphen test it was observed that Soxhlet-extracted oil gave a stronger Halphen test than oil extracted at room temperature. This led to the assumption, now confirmed by experimental results here reported, that an oil giving an even stronger test could be obtained by hot re-extraction of cold-extracted seed.

The cottonseed was dehulled and the meats extracted for one hour at room temperature using approximately three liters of commercial hexane Skellysolve B) per kilogram of meats. They were then re-extracted with fresh solvent in a Soxhlet apparatus for 15 hours at a syphoning rate of once an hour. The Soxhlet was wrapped with an electrical heating tape to provide means for keeping the liquid in the extraction chamber just below its boiling point. In both instances the solvent was removed by distilling under a stream of nitrogen at reduced pressure. The cold extraction yielded 28% of the total amount of oil extracted (Fraction 1), and re-extraction with hot solvent yielded 72% (Fraction 2).

The Halphen determinations were made by a modi-tracted for one hour at room temperature using approximately three liters of commercial hexane (Skel-response is expressed in terms of the color intensity of the final test solution at 50 ml. dilution; that is, in terms of its absorbance as measured in a 1 cm. cell with a Cary Model 14M spectrophotometer at the absorption maximum in the 490–500 mμ region.

The Halphen response of Fraction 2 was almost three times that of Fraction 1. The two oil fractions differed also in their free fatty acid and phosphatide contents as shown in Table I.

Previous experiments had shown that the Halphen response of refined cottonseed oil is unaffected by the presence of free fatty acid (oleic), but that it is enhanced by the addition of commercial soybean phosphatides which themselves gave no Halphen test when dissolved in refined peanut oil. This phosphatide mixture had no significant effect on the Halphen response of crude cottonseed oil.

TABLE I

Analyses of cottonseed oil fractions before and after "refining"

| Oil sample | Halphen acid response[a] | Free fatty acid % | Phosphatides % |
|---|---|---|---|
| Fraction 1[b] | | | |
| Crude | 0.53 | 1.00 | 0.30 |
| Refined | 0.25 | 0.21 | 0.08 |
| Fraction 2[c] | | | |
| Crude | 1.51 | 2.00 | 1.18 |
| Refined | 0.90 | 0.29 | 0.08 |

[a] Average of six determinations.
[b] Obtained by cold hexane extraction of cottonseed meats.
[c] Obtained by hot re-extraction of cold-extracted meats.

The percentages of phosphatides and free fatty acids were reduced to a minimum by "refining" the oils by the A.O.C.S. method (2) for determining *neutral oil content*; i.e., by passing an ether-methanol solution of the oil through a column of activated alumina and removing the solvent at 60°C. under partial vacuum. This procedure had been found to have no significant effect on the Halphen response of refined cottonseed oils. Although as expected, the Halphen responses of both crude oil fractions were markedly reduced by this procedure (Table I), Fraction 2 still had a response over three times as great as Fraction 1.

It had already been observed that when 2% or even 4% of the soybean phosphatides were added to cottonseed oil refined in this procedure the response was never greater than 80% of that of the parent crude oil. All phosphatides do not have the same effect; cephalin separated chromatographically from egg phosphatides, for example, caused no significant change. Cottonseed phosphatides would be expected to contain some combined Halphen acid. If however the enhancing effect *per se* of the cottonseed phosphatides is the same as that of the soybean phosphatides, and if no other interfering substances are involved, the results indicate that the concentration of the Halphen-acid-moiety in the cottonseed phosphatides would have to be considerably greater than in the cottonseed glycerides.

The Halphen test has been found to be sensitive in various degrees to a large number of nitrogen-containing substances. Small amounts (5–7%) of primary amines: cyclohexylamine, ethanolamine, or benzylamine, added to cottonseed oil reduced the Halphen response whereas pyridine and acetanilide had no appreciable effect. On the other hand, similar additions of urea, thiourea, and the secondary amines: morpholine, dibenzylamine, diethanolamine, and piperidine increased the Halphen response of the oil by as much as 100% in most instances and incidentally suppressed the formation of a second pigment absorbing at 540 mμ usually formed with refined oils. Even commercial refined peanut oil and corn oil, which do not give the Halphen test, tend to have an enhancing effect when added to refined cottonseed oil. It is therefore possible that the phosphatides are not the only interfering substances removed in the refining procedure.

It can be concluded that crude cottonseed oils, since they contain phosphatides similar to the soybean phosphatides, give fictitiously high Halphen responses. It can also be concluded that a cottonseed oil of high Halphen-acid-moiety concentration can be obtained by hot hexane re-extraction of cold-extracted cottonseed meats. It may be possible to obtain a hot-extracted oil of even higher concentration by removing a larger proportion of the oil by cold extraction. This would be of special importance in the preparation of starting materials for concentrating or isolating Halphen acid from cottonseed oils.

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## • *Letter to the Editor*

WE NOTED the letter from Fryer, Ormand, and Crump, describing the behavior of triglyceride oils when subjected to gas chromatography (1). We confirm this but find further that conditions can be found such that mixtures of relatively pure tripalmitin and tristearin give sharp separations with moderate retention times.

Figure 1 shows results on a synthetic mixture made from relatively pure triglycerides. These results were obtained with a column containing ¾% SE-30 on 80–100 mesh GAS-CHROM P, at 300°C. A 2-ft. coiled column of ¼-in. stainless steel was used, with

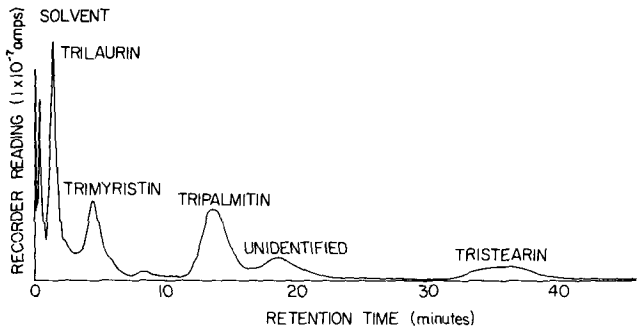


FIGURE 1

an argon rate of 300 ml. per minute and the entire flow passing through an argon ionization detector. The retention time of tristearin was 36 min. The broad peak to the right of the tripalmitin on Figure 1 was also present in chromatograms of the tripalmitin starting material before it was placed in the synthetic mixture.

Figure 2 is a composite chromatogram, showing results obtained with individual triglycerides with 16.7% SE-30 on 60–80 mesh GAS-CHROM P at 284°C in an 18-in. column. All the data give straight lines when carbon number is plotted *versus* log retention-time. Apiezon wax and relatively heat-stable polyester phases, such as ethylene glycol isophthalate and neopentyl succinate, gave unsatisfactory results.

Because of the nonpolar nature of SE-30 a separation of triolein from tristearin was not expected, but there was some indication that triolein has an appreciably shorter retention time than tristearin on SE-30. A polar silicone such as QF-1 (2) may well be preferable for this purpose, but temperature programming or further reduction of the silicone percentage may be necessary further to reduce retention times and minimize decomposition or other reactions of the triolein.

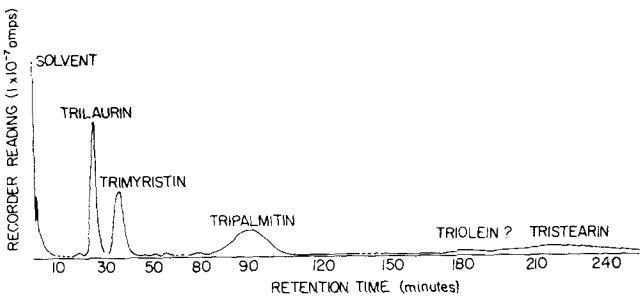


FIGURE 2

No collection of effluents or studies of decomposition or other losses have been made, but it is judged from peak sizes that 90% of the 0.4 lambda samples of the higher triglycerides emerged from the column and showed on the recorder chart. Quantitative interpretation of the chromatograms would be premature until decomposition and calibration studies have been made. At present the procedure is chiefly useful for indication of impurities in triglycerides from various sources and for examination of relatively simple mixtures. The examination of natural oils presents difficulties probably because of the multiplicity of mixed glycerides that are present.

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