less than 20 μg/kg of aflatoxins in the ginned seed. Only 14 trailers of the 146 examined had more than 10 BGYF spots. Trailers with a high count of spots on one side usually had about the same number on the other side. The highest toxin level detected was in seed ginned from a trailer with 12 spots on one side and 14 on the other. The average toxin content on meats from this trailer was 1371 μg/kg; standard deviation (SD) between the 4 subsamples was 236.07 with a coefficient of variation (CV) of 17%. Two other trailers with 12 and 14 spots on one side and 10 and 15 on the other had seed with 284 and 49 μg/kg with SD of 67.34 and 55.17 and CV of 24 and 112. One exception to the trend were the trailers with the highest number of BGYF spots (19 and 18); seed had only 97 μg/kg of toxins, SD=107.11, CV=110. Six trailers had seed with low levels of toxins when no BGYF spots were detected. Such toxin-containing seed cotton could have come from the center of these trailers.

Results from module sampling paralleled those from trailers though BGYF spots on modules were more difficult to count. Dust on the sides and the apparent compression of fluorescent fibers so that spots could not be seen easily made observations difficult. Moreover, because of compacting of seed cotton to form the modules, the sides of the modules represented less of the total volume than did the respective sides of the trailers. No module had over 7 BGYF spots. Only one contained 7 spots and another 6. In all cases, ginned seed with levels of toxin below 20 μg/kg (and often ND) were from modules with a single or no fluorescent spots, although toxin levels in 18 modules with 0-1 fluorescent spots were above 20 ppb.

A Pearson correlation between number of BGYF spots on trailers and toxin in seed was 0.41, which is significant at a 0.0001 level, whereas a similar correlation on modules was 0.29, significant at only a 0.05 level. These results show that night or simulated nighttime examination for BGYF spots could be used as a practical procedure to divert trailers with seed potentially high in toxin. The procedure is not as effective for modules.

ACKNOWLEDGMENTS

We thank the National Cottonseed Products Association for financial support; Franzoy, Corey Consulting Engineers for the BGYF detection and sample collection; S. Buco for statistics.

REFERENCES

1. Marsh, P.B., and M.E. Simpson, Plant Disease Rptr. 52:671 (1968).
2. Marsh, P.B., M.E. Simpson, R.J. Ferretti, T.C. Campbell and J. Donoso, J. Agric. Food Chem. 17:462 (1969).
3. Lee, L.S., A.F. Cucullu, W.A. Pons, Jr. and T.E. Russell, JAOCS 54:238A (1977).
4. Lee, L.S., and A.F. Cucullu, JAOCS 55:591 (1978).
5. Pons, W.A., Jr., A.F. Cucullu and A.O. Franz, Jr., J. Assoc. Offic. Anal. Chem. 55:768 (1972).
6. Pons, W.A., Jr., and A.O. Franz, Jr., Ibid. 60:89 (1977).

[Received October 8, 1983]

The Determination of Light Petroleum Residues in Refined Oils and Fats

M.J. DOWNES and **J.B. ROSSELL**, Leatherhead Food Research Association, Randalls Road, Leatherhead, Surrey KT22 7RY, England

ABSTRACT

A rapid, direct injection gas liquid chromatographic (GLC) method for determining residual light petroleum in edible vegetable oils has been developed. The response is linear at levels between 0.05-0.5 mg hexane/kg oil. A sample containing 0.2 mg hexane/kg oil was analyzed for repeatability, giving a standard deviation of 0.008 mg/kg, equilvalent to a coefficient of variation of 4%. Separation of pentane, hexane, heptane, octane and decane was obtained by this method. A survey of 23 samples of freshly refined vegetable oils obtained from 13 U.K. refiners in 1981 showed that these all contained less than 0.05 mg hexane/kg oil.

INTRODUCTION

Light petroleum (hexane fraction) is a preferred solvent for the extraction of oil from oilseeds. Unavoidably, residual solvent is in both the oil and the meal after extraction. For example, previous investigators have reported levels of 310 mg kg^{-1} (1) and 550-3500 mg kg^{-1} (2) in crude soybean oil.

In the refining process, residual solvents are predominantly removed at 2 stages—bleaching and deodorizing. Some will be adsorbed onto the bleaching earth and the remainder drawn off with other volatile matter during deodorization. Temperatures of up to 270 C and a vacuum of up to 0.3 torr are applied during processing. After this treatment, residual solvent should be completely removed.

Hirayama and Imai (1) have reported "none detected" for residual hexane in deodorized oils. Their limit of detection was ca. 1 mg kg^{-1}.

The aim of the current study was to optimize analytical techniques and to obtain a lower detection limit. The method was then used to determine the residual levels of solvent remaining after refining, to provide evidence for the Seed Crushers' and Oil Processors' Association for submission to the (U.K.) Ministry of Agriculture, Fisheries and Food in connection with the Proposed EEC Directive on Extraction Solvents (3).

Many workers have described the determination of solvents in oilseed meals (4-8), in oils (1,2,12-17) and in other foods and biological tissues (9-11). The analytical methods described in the above papers can be classified into three groups: (a) headspace gas analysis; (b) solvent extraction and (c) direct injection into a gas chromatograph (GC). These methods are described below.

Headspace Gas Analysis

A suitable amount of sample is weighed into a septum vial, and the vial is sealed and placed in an oven. Low-boiling-point hydrocarbons pass into the headspace above the sample until equilibrium is achieved. Aliquots of the headspace gas are injected onto a GC column and peak areas compared with standards prepared in a similar manner.

This procedure has been described by various workers (4,8,11). It works reasonably well for lipids at levels of 10 mg kg^{-1} and above, with an absolute detection limit of 1 mg kg^{-1}. Several disadvantages can be cited for this method. (a) Exact replication of the headspace volume is essential for accurate results at low levels. This is extremely difficult to achieve because the size of the septum vials varies. Very often the volume taken up by the sample and by standards are also different. (b) Standards are often made up in another solvent. This can lead to masking of the hydrocarbon by a large diluent peak. (c) The solvent equilibrates between a sample and the headspace above it. This equilibrium will vary with the nature of the foodstuff. In the case of an oil, the equilibrium occurs at a much lower headspace concentration than in the case of other foods, resulting in a less satisfactory detection limit. (d) Judging when equilibrium conditions are attained is difficult. (e) The equilibrium is reached by heating the sample for a set time and temperature. For example, Dupuy and Fore (4) used 110 C for 2 hr, although Saxby and Pratten (11) reduced this to 70 C for 1 hr. Wolff (8), in a collaborative study, used different times for different seeds. However, the heating necessary for equilibration can result in the formation of interfering low-boiling compounds, e.g., pentane or hexanal, because of autoxidation of the oil and decomposition of peroxides already present. (f) In the authors' experience, to obtain a detection limit of 1 mg kg^{-1}, a volume of 2 mL headspace gas needs to be injected onto the column. This gives rise to a very short septum life. (g) Commercial hexane contains at least 4 isomers, each having a different volatility. The equilibrium mixture of isomers present in the headspace will vary with changes in the imposed conditions and may be different from the mixture of isomers in the sample. The total contribution of all the separate isomers must be determined for an assessment of the true level of residual solvent.

Solvent Extraction

A sample is blended with a suitable extraction solvent, and the 2 layers are separated. An aliquot of the solvent layer is then injected onto the GC column. Theoretically, a low detection limit should be possible by this method. The limitations include the following. (a) The large diluent peak from the extraction solvent could cause masking. (b) The choice of solvents can change results. Obtaining a solvent free from interfering low-boiling-point volatile matter is difficult. Rectified squalene and rectified cyclohexanone have been used (15) but the original proponents of this method later abandoned it, because of this difficulty, and subsequently favored a direct-injection method. Zinn and Edwards (10) applied the solvent-extraction technique to the determination of petroleum residues in animal tissue using Freon 113 as solvent; they did not report any problems with solvent purity.

Direct Injection

The oil or molten fat is injected directly into the GC. By heating the injection port to a comparatively high temperature, the solvent is rapidly evaporated and swept through the column. If samples are injected directly onto the column, deterioration may occur. This disadvantage is overcome by using a precolumn system (15-17).

Dupuy et al. (2) extended the technique to solid samples by packing the injection point with sample, which was held in place by a glass-wool plug. However, this is too cumbersome for routine use. Published limits of detection are at the 0.1 mg kg^{-1} level (2,15).

The advantage of this method is minimum downtime on the GC because waiting for the samples to equilibrate is not necessary.

A danger with direct injection of the sample onto the column is that it can shorten the column life. This is a particularly serious danger if crude oils with high free fatty acid (FFA) levels and other nontriglyceride components are analyzed. However, any shortening of column life would be offset by the time saved over the headspace technique with its equilibration stage. Moreover, we guarded against the danger by providing a precolumn that was changed frequently.

Choice of Method

The headspace gas technique has been shown to be the most suitable for use with low-fat solid foods, e.g., oilseed meals (8) or ground cereal products (11). However, this technique is not sufficiently sensitive for oils and fats work. Therefore, the direct-injection method has been developed in this study.

EXPERIMENTAL PROCEDURES

Method

The method involved in detecting light petroleum residues in an oil or fat was direct injection of the oil into a GC. A glass precolumn, packed with silanized glass wool, was used to trap the oil and prolong column life. According to the EEC definition of light petroleum (3), light petroleum includes mixtures of saturated hydrocarbons containing 5, 6, 7 and 8 carbon atoms per molecule, or mixtures of these, distilling completely between 25 and 120 C. (NB: Commercial light petroleum is essentially normal hexane, although the amount of hexane present, and the composition of the other hydrocarbons, vary with the boiling-point of the fraction used.)

Materials

Reagents. Reagents used in this study were n-pentane analytical reagent (AR), n-hexane AR, n-heptane high pressure liquid chromatography (HPLC) grade, n-octane AR, n-decane pfs (Sigma Chemical Co., St. Louis, MO), chloroform AR, carbon tetrachloride AR, silanized glass wool, ethyl acetate AR, acetone AR, methanol AR and 1,2-dichlorethane (ethylene chloride).

Refined oils. Twenty-three samples of refined, deodorized edible oil were analyzed, all of which had been extracted commercially with light petroleum during August 1981. These samples were representative of the range of oils processed in the United Kingdom and were supplied by U.K. oil processors through the Seed Crushers' and Oil Processors' Association (SCOPA).

A drum of refined, deodorized palm oil known to have had no contact with light petroleum was also supplied for calibration purposes.

The oils were sent in glass containers filled to the brim with freshly refined oil and closed with a screw cap, to minimize any possible losses into the headspace.

Standard solutions. Solutions were prepared from a soybean oil shown to have a negligible residual solvent content. Standard amounts of hexane were added by microsyringe to provide solutions containing 0.0 mg, 0.2 mg, 0.3 mg, 0.4 mg and 0.5 mg hexane/kg oil.

Equipment. Equipment used consisted of a glass syringe, capacity 5 μL; glass syringe, capacity 1 μL; packed column GC fitted with a flame ionization detector (FID) and capable of accepting a precolumn (a Perkin-Elmer Sigma 2 instrument was used).

GC conditions. The precolumn was glass, 10 cm × 1.5 mm i.d., packed with silanized glass wool. The column was glass, 2 m × 0.4 mm i.d., packed with 3% OV-17 on 80-100 mesh Gas Chrom Q. The injector and precolumn temperature was 185 C. The detector temperature was 250 C, initial oven temperature was 70 C, the initial hold was 3 min. The heating rate was 12 C min^{-1} and the final oven temperature was 175 C. The final hold was 5 min. Nitrogen carrier gas at 40 mL min^{-1}, hydrogen at 40 mL min^{-1} and air at 400 mL min^{-1} were used.

Procedure

Sample preparation. Oils were well mixed and sampled directly. Fats were melted at the lowest temperature possible, well mixed and sampled immediately.

Column preparation and sample injection. The packed precolumn was placed in the inlet of the GC and was changed every 3 determinations to prevent oil getting onto the column. A 5-μL aliquot of sample was injected into the injection port of the GC.

Calibration. Solutions of hexane in oil of the listed concentrations were injected onto the column by the above procedure. All blank determinations were carried out using a 5-μL syringe that was kept especially for this purpose (see Sample Contamination in the Discussion section).

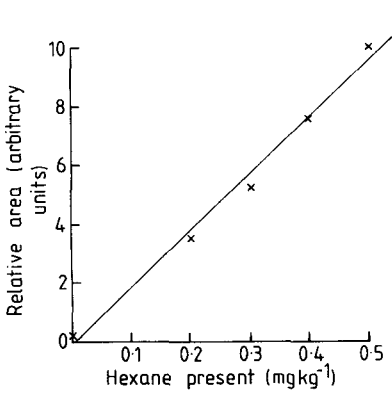
An oil was treated with 1 mg kg^{-1} each of n-pentane, n-hexane, n-heptane, n-octane and n-decane. This oil was analyzed as above to demonstrate the separation of these hydrocarbons. A number of other solvents that might be found in an oil were also studied, i.e., ethyl acetate, acetone and ethylene dichloride; good separations of these solvents from one another were obtained under the conditions used.

Repeatability study. Ten separate determinations of a solution containing 0.2 mg hexane/kg oil were carried out on the same day and the results examined statistically.

Analysis of samples of refined oils. Aliquots of 5 μL were injected directly into the injection port of the GC. The samples were analyzed immediately on receipt to preclude the formation of interfering hydrocarbons that could result from autoxidation on storage or decomposition of peroxides already present.

RESULTS

The area under the hexane peak has a linear relationship to the amount present over the concentration range 0.05-0.50 mg kg^{-1} (Fig. 1). Under the analytical conditions used, the limit of detection is ca. 0.05 mg kg^{-1}. The refined oils listed in Table I were studied and shown to contain less than the detection limit of 0.05 mg kg^{-1}. The 10 analyses carried out on a sample of oil containing 0.2 mg kg^{-1} were subjected to statistical treatment, shown in Table II. A reasonable separation from levels of pentane, heptane, octane and decane, similar to the level of hexane, is obtained (see Table III). Figure 2 shows the separation of hexane from heptane.



Hexane (mg kg⁻¹)	Rel. response
0	<0·1
0·2	3·53
0·3	5·26
0·4	7·55
0·5	10·00

FIG. 1. Relation between hexane GC peak area and hexane content of oil.

In our work at these very low solvent levels, we did not detect any isomers of n-hexane as all residues were below the detection limit in the analytical samples studied. How-

TABLE II

Results for 0.2 mg kg^{-1} Sample and Statistical Treatment[a]

Determination number	Relative peak area
1	3.3
2	3.5
3	3.6
4	3.8
5	3.7
6	3.5
7	3.5
8	3.4
9	3.5
10	3.5

Average = 3.53 area units ≙ 0.2 mg kg^{-1}; range = 3.3-3.8 area units; standard deviation = 0.008 mg kg^{-1}; 95% confidence limits = 0.184-0.216 mg kg^{-1}; coefficient of variation = 4.0%.

[a]Ten aliquots examined on the same day.

TABLE I

Refined Oils Under Study

Oil type	Refining treatment (described on label)	Number of samples
Palm oil	Refined and deodorized (no contact with light petroleum)	1
Maize oil	Refined and deodorized	2
Palm oleine	Refined and deodorized	1
Palm-kernel oil	Refined and deodorized	2
	Refined, hardened and deodorized	1
	Refined, hardened and deodorized (36/38 C)	1
Rapeseed oil	Refined and deodorized	4
	Refined, hardened and deodorized	1
Soybean oil	Refined and deodorized	8
	Refined, bleached and deodorized	1
Sunflower oil	Refined and deodorized	1
	Refined and deodorized (80% soft oil)	1

In all samples examined, light petroleum residues were not detected by the proposed method (i.e., less than 0.05 μg kg^{-1}).

TABLE III

Relative Retention Time for Various Low-Boiling Solvents

Solvent	Relative retention time
Methanol	0.77
n-Pentane	0.88
n-Hexane	1.00
n-Heptane	1.27
Acetone	1.27
Chloroform	1.29
Ethyl acetate	1.32
Carbon tetrachloride	1.46
1,2-Dichlorethane	1.67
n-Octane	1.90
n-Decane	5.49

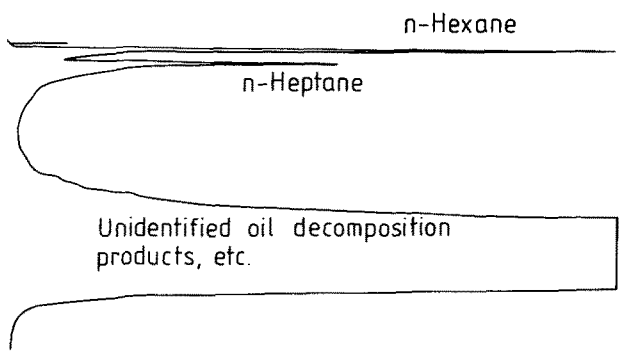


FIG. 2. Typical chromatogram showing separation of n-hexane from n-heptane.

ever, resolution and quantification of the separate isomers should cause no problem when the total residual solvent is present at higher levels.

DISCUSSION

We decided to inject the oil directly into the injection port of a GC fitted with a precolumn packed with silanized glass wool. Apart from the normal considerations encountered when optimizing a GC method, other problems had to be taken into account, as discussed below.

Precolumns

Metal must not be used because high-molecular-weight components of the oil (e.g., peroxides), are liable to decompose rapidly in the presence of metals, liberating volatile compounds (Telling and Rossell, unpublished data). By this means, hexanal may be produced and will interfere with the light petroleum peaks. In this work, a glass precolumn was used throughout to avoid hexanal formation.

Column Life

When oil is injected directly onto a column, the packing material deteriorates and decomposition of the oils may lead to interfering artefacts, reduced detector sensitivity and so forth. To overcome this, we used a glass precolumn packed with silanized glass wool to trap the oil. This was placed in a heated injection port (185 C) so that the hexane evaporated very rapidly and was carried into the column by the carrier gas. The glass wool became saturated with oil very quickly and had to be changed every 3 determinations. This may sound cumbersome but is accomplished with ease in ca. 2 minutes. The system was programmed to remove volatiles with a higher boiling point before the next determination. Only 1 column has been used for this work and this has, at the time of writing, been used for over 200 determinations without loss of sensitivity. Other columns suitable for this type of work, e.g., Carbowax 20M, Porapak Q and Phasepack Q, may not be as stable when treated in this way.

Preparation of Standards

Because of the complex nature of an oil, preparing standards in an oil base to obtain a standard matrix as similar to the sample as possible is necessary. This is extremely difficult and requires considerable care. Headspace must be eliminated to prevent loss of solvent by evaporation. Because evaporation losses cannot be avoided completely, standards should not be kept more than 24 hr. Traces of solvent can also be absorbed by the oil. Therefore, analytical calibration standards should be prepared and handled in an atmosphere free from solvent vapors. Whenever possible, the method of standard additions should be used. For these reasons, the organization of a collaborative trial may require particularly detailed precautions, instructions, and so on.

Sample Contamination

For most chromatographic purposes, an injection syringe would be cleaned by flushing out with a hydrocarbon solvent with a low boiling point, e.g., hexane or heptane. For this work, this method is obviously undesirable and keeping separate syringes especially for this type of analysis is necessary. Cleaning the syringe is also difficult. Flushing out the syringe with the oil under test was tried. Doing this with a solvent-free oil required at least 30 flushes to remove all contamination. Chloroform and carbon tetrachloride have also been used for cleaning the syringes because of their comparatively low response in the FID. Chloroform separates from hexane but elutes with heptane, whereas carbon tetrachloride elutes between heptane and octane (see Table III). Therefore, carbon tetrachloride is the preferred cleaning agent.

Silicone 'O' rings and ferrules can contaminate the GC system and give rise to artefact peaks. If used, they should first be baked at 200 C for several hours (2). No problems occur when graphite ferrules are used.

Sampling

Changes may occur in the stored sample. For instance, solvent vapors may evaporate into the surrounding headspace; autoxidation may lead to hexanal formation; solvents can be adsorbed from the laboratory atmosphere and so forth. Sample containers sealed with a lid in which a septum is embedded can also give rise to low recoveries because of absorption of solvent by the septum and consequent swelling of the septum, which may become permeable as a result.

Sample Volume

Oil is a relatively viscous material. As a result, measuring small volumes by microsyringe is difficult. Accurate withdrawal of a given volume of oil by microsyringe is successful only if the plunger is withdrawn very slowly.

Volatile Matter

Some fragrant oils contain large amounts of volatile matter. Tagaki and Yamasaki (15) found up to 120 mg kg^{-1} volatile matter in sesame-seed oil, which they claim interferes with analysis for solvents with low boiling points. We encountered no such problems in the present work.

Internal Standard

An internal standard is obviously desirable as an alternative, or back-up, to quantification by standard additions, especially when an integration system is available. A method using n-decane as internal standard was supplied by Shell Research, Amsterdam, The Netherlands. Table II shows that n-decane could be used, but it has a long retention time (5.49 times that of hexane). Under our experimental conditions, octane (relative retention time 1.90) may be more suitable as an internal standard. The addition of an internal standard would involve similar precautions and difficulties as those discussed above (see Preparation of Standards). Because of limited funding, this aspect was not considered in detail.

Other Uses

To extend this technique to other liquid foodstuffs and to oil-soluble materials, such as lecithin, may be possible. In particular, solvents used to extract oleoresins, e.g., 1,2-dichlorethane, ethyl acetate and acetone, could be determined by a similar approach.

Scope of the Technique

While this paper has studied low concentrations of light petroleum, i.e., less than 0.5 mg kg^{-1}, other workers (16, 17) have used a similar experimental procedure to determine the much higher levels found in crude oil. Italian workers (16,17) claim a linear response of 5-20,000 mg kg^{-1}. The combination of our results with these indicates that a linear range is probably 0.05-20,000 mg kg^{-1} for determining residual solvents by the technique of direct injection.

ACKNOWLEDGMENTS

This work was assisted by the Seed Crushers' and Oil Processors' Association. Advice was received from D. Morgan, R. Crawford and D. I. Prior.

REFERENCES

1. Hirayama, S., and C. Imai, JAOCS 54:190 (1977).
2. Dupuy, H.P., S.P. Fore and E.T. Rayner, JAOCS 52:118 (1975).
3. European Economic Community, Proposed Directive EN 987 (1980).
4. Dupuy, H.P., and S.P. Fore, JAOCS 47:231 (1970).
5. Wan, P.J., M. Chittwood, C.M. Cater and K.F. Mattil, JAOCS 54:542 (1977).
6. House, R.J.R., A.O. Harcombe and R.G. Guinness, JAOCS 58:626 (1981).
7. Prevot, A., and J.L. Coustille, Rev. Fr. Corp. Gras 28:413 (1981).
8. Wolff, J.P., JAOCS 60:172A (1983).
9. Ghimenti, G., M. Galli and E. Galli, Annuar. Ist. Super. Sanit. 14:583 (1978).
10. Zinn, L.L., and W.C. Edwards, Bull. Env. Contam. Toxicol. 22:285 (1979).
11. Saxby, M.J., and L.J. Pratten, Leatherhead Food R.A. Technical Circular No. *766* (1982).
12. Dean, A.C., E. Bradford, A.W. Hubbard, W.D. Pocklington and J. Thomson, J. Chromatogr. 44:465 (1969).
13. Tous, J.G., and J. Martel, Gracas Aceites (Seville) 23:1 (1972).
14. Dupuy, H.P., S.P. Fore and L.A. Goldbatt, JAOCS 50:340 (1973).
15. Takagi, T., and M. Yamazaki, Yukagaku 24:367 (1975).
16. Marzo, S., and L. Giro, Riv. Ital. Sostanze Grasse 59:199 (1982).
17. Morchio, G., Riv. Ital. Sostanze Grasse 59:335 (1982).

[Received November 6, 1983]

A Comparative Study of Batch and Continuous Refining of Cottonseed Oil in the Sudan

K.M. WAGIALLA*, M.E. ABDUL RAHMAN and **E.A. ELAWAD**, Department of Chemical Engineering, College of Engineering, King Saud University, P.O. Box 800, Riyadh 11421, Saudi Arabia

ABSTRACT

This study is concerned with a comparison of some technical aspects regarding batch vs continuous refining (centrifugal alkali refining) of crude cottonseed oil. Implication of processing modes of operation were examined in light of their effect on the following performance criteria: (a) percentage of refining loss as a function of the initial crude-oil free fatty acid (FFA) content; (b) refined oil color as a function of initial crude-oil FFA; (c) caustic soda consumption as a function of initial crude-oil FFA; (d) bleachability characteristics of refined oil. The study shows, in quantitative terms, that continuous refining of cottonseed oil is more efficient in each of these performance criteria, particularly the percentage of refining loss.

INTRODUCTION

In recent years a trend has been growing toward replacing the traditional batch edible-oil refining techniques by what was believed to be superior continuous refining methods. Although the continuous methods proved to be more efficient on a commercial basis, little attention was paid toward quantifying this superiority (1-3).

*To whom correspondence should be addressed.

Kuroda et al. (4) reported satisfactory results for the commercial testing of a direct-fired, semicontinuous deodorizer (capacity 18 tons of oil per day), using refined cottonseed oil, for a period of more than 300 hr, including 200 hr of continuous operation. On the basis of laboratory tests, a technological scheme for the continuous adsorption purification of cottonseed oil was reported (5).

Figure 1 shows an outline of the 2 main processing schemes qenerally employed to produce edible oil: conventional (chemical) refining and physical refining.

In this study, a comparative analysis of batch and continuous conventional (chemical) oil processing operational plants is undertaken. The units, whose performance is investigated, are located at the Bittar Oil and Soap Factory in Khartoum North, Sudan. The emphasis of the study is on the heart of the processing scheme, the refining, washing and bleaching stages.

Alkali refining is practiced as a purifying treatment designed to remove free fatty acids (FFA), phosphatides, gums, coloring matter, insoluble matter and miscellaneous unsaponifiable materials. These impurities may be present in true solution or may be present as a colloidal suspension.