Quality Assessment of Used Frying Fats: A Comparison of Four Methods

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ABSTRACT

The level of petroleum ether insoluble oxidized fatty acids in used frying fats is one of the recommended criteria for assessing their quality in Germany; however, the method of determination is time consuming and inaccurate. Gel permeation chromatography (GPC) allows the determination of dimeric and oligomeric triglycerides in a heated fat irrespective of the presence of oxidized material. An indication of the total amount of polar and oxidized compounds can be obtained by liquid chromatography (LC) on a silica gel column in connection with a moving-wire detector. A comparatively simple and quick method is the separation of polar and unpolar components in a used frying fat by means of column chromatography (CC) on silica gel. Over a large number of investigations, a good correlation was seen between the results obtained with GPC, LC, and CC and the amount of petroleum ether insoluble oxidized fatty acids isolated from used frying fats. Limits of the analytical data obtained by the new methods are proposed which indicate deterioration of used frying fats.

INTRODUCTION

In 1973, the German Society for Fat Research proposed the following recommendations for the quality assessment of used frying fats (1):

"A used frying fat is deteriorated

if, without any doubt, odor and taste are not acceptable;

if, in case of a doubtful sensory assessment

the concentration of petroleum ether insoluble oxidized fatty acids is 0.7% or higher and if the smoke point is lower than 170 C,

the concentration of petroleum ether insoluble oxidized fatty acids is 1.0% or higher."

A similar statement was published by a working group of federal health authority chemists (2). Both recommendations regard sensory evaluation as being most important. These evaluations are often contradictory and depend strongly upon the qualifications of the tester. Analytical data should be used to support the sensory evaluation especially if the results are doubtful.

One of these methods is the determination of the petroleum ether insoluble oxidized fatty acids. Figure 1 shows the principle of this method. The frying oil is saponified with potassium hydroxide in ethanol, and the soap solution is acidified and thoroughly extracted with ether. After evaporation of the solvent, the mixture of fatty acids is treated with boiling petroleum ether; thereby, most of the material is dissolved: the fatty acids, the unsaponifiable matter, and even part of the oxidized fatty acids. Only a small part of the oxidized fatty acids remains insoluble in petroleum ether, and their weight is determined. The amount of petroleum ether insoluble oxidized fatty acids in a used frying oil is a good indicator of the deterioration of such oils. The method does not need expensive equipment, but is labor intensive and time consuming. More than two days are needed to arrive at the final result, and a skilled technician cannot perform more than eight determinations in parallel runs.

Figure 1 shows the main disadvantage of the method: since only a small part of the oxidized material is isolated, whereas much more of the oxidized fatty acids still remains soluble in petroleum ether, the accuracy of this method is low. ("Oxidized fatty acids" will be used throughout the text, and these will refer only to those which are insoluble in petroleum ether. The soluble part will not be discussed.)

The determination of "oxidized fatty acids" was checked in a collaborative study involving 21 laboratories. The statistical evaluation (3) of their results showed a "Wiederholbarkeit" of 0.08 and a "Vergleichbarkeit"[1] of 0.33 (4). With regard to the above recommendations, the values of as low as 0.7% and 1.0%, respectively, already indicate fat deterioration. The performance of this method must not be regarded as satisfactory. Therefore, new methods were explored for the assessment of oil quality.

During the heating of frying oils, their composition changes gradually, and polymerization as well as oxidation reactions occur. As a consequence, dimeric and oligomeric triglycerides are formed, and their concentration can be determined easily by gel permeation chromatography

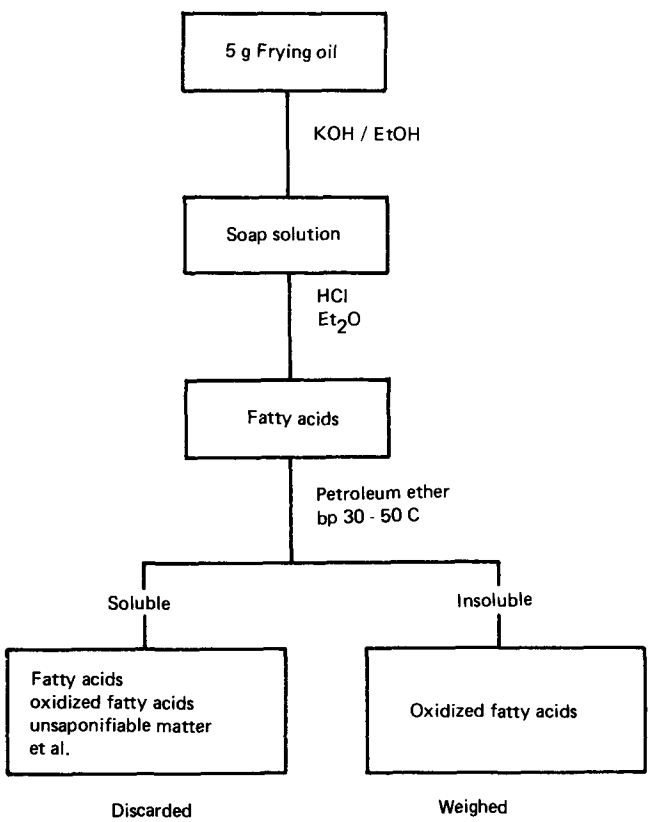

FIG. 1. Principle of the method "petroleum ether insoluble oxidized fatty acids."

[1]Definition according to Deutsche Industrie Norm. Similar but not exactly corresponding terms are "repeatability" and "reproducibility," respectively.

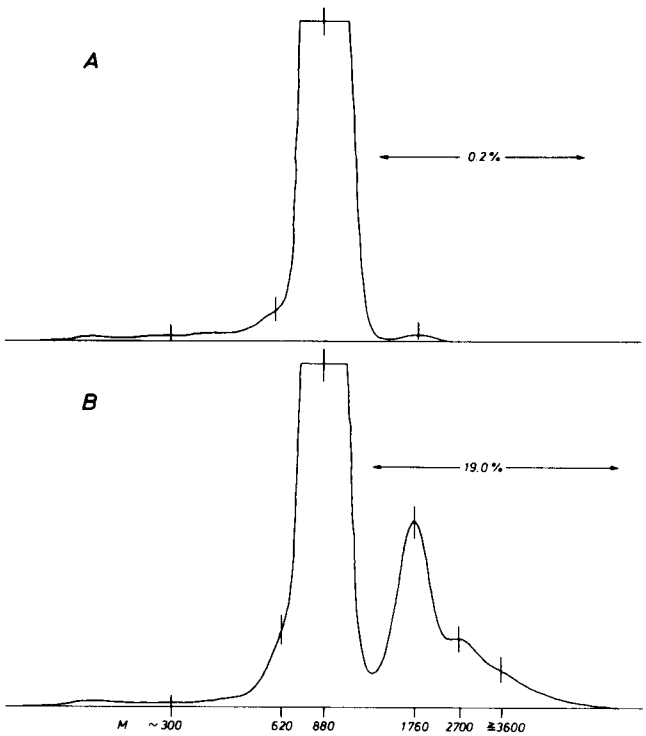

FIG. 2. Gel permeation chromatograms: A. refined soybean oil; B. soybean oil heated 64 hr up to 180 C.

(GPC), a method which indicates molecular size. Oxidized triglycerides, on the other hand, are adsorbed more strongly on silica gel due to their higher polarity. The total polar artefacts in a used frying oil can be measured by use of liquid chromatography (LC) on silica gel, which is a very fast method especially if a moving-wire detector is applied. Alternatively, a simple column chromatography (CC) on silica gel separates unaltered triglycerides from the total polar artefacts, and the weight of both fractions is determined.

It was the aim of this work to check the usefulness of GPC, LC, and CC for the assessment of frying oil quality. The data thus obtained should be compared with the concentration of "oxidized fatty acids."

EXPERIMENTAL PROCEDURES

Materials

The oil samples were taken from various commercial fryers used in snack bars for deep-fat frying. As indicated in the following figures, four different oil blends were in use. There was a great variety and throughput of frying goods. Heating times and habits were quite different – only the equipment was somewhat similar and of modern design. The samples were taken at random, some of them at that time when the used oil was discarded.

Petroleum Ether Insoluble Oxidized Fatty Acids

The original procedure as published in 1968 was still more complicated since it made necessary the separation of the unsaponifiable matter. Throughout our work, however, a revised version was used (Fig. 1), which was published only recently (4).

Gel Permeation Chromatography (GPC)

The oils were analyzed with the GP-Chromatograph 200, Waters Associates Framingham, MA, applying the method of Unbehend and Scharmann (5,6).

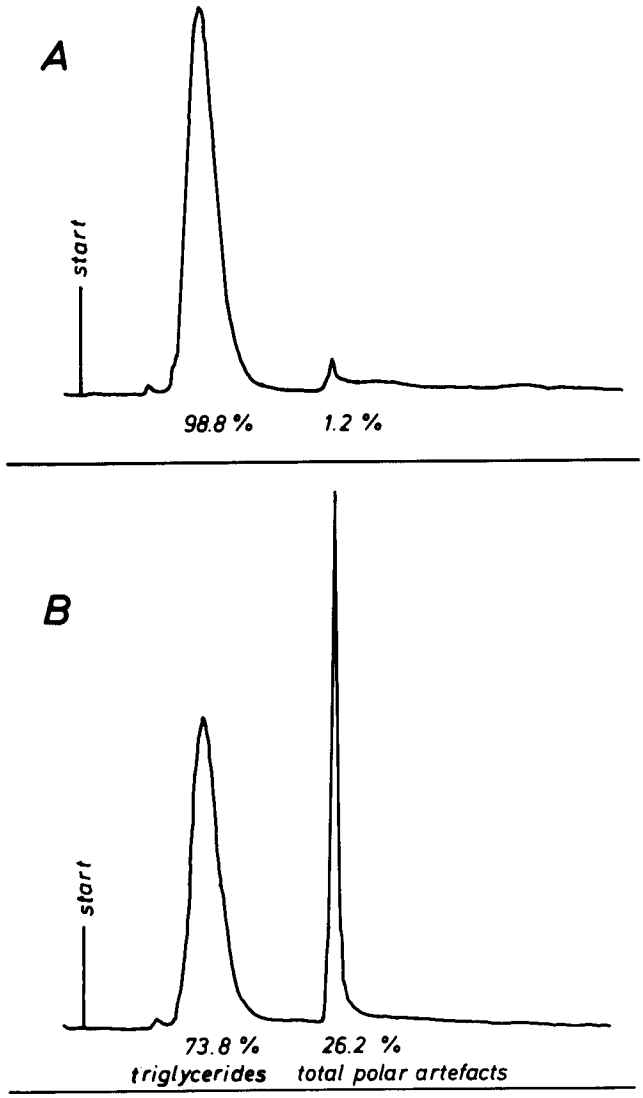

FIG. 3. Liquid chromatograms: A. refined soybean oil; B. soybean oil heated 64 hr up to 180 C.

Figure 2A shows the GPC of a refined soybean oil. Whereas natural fats and oils do not contain higher polymerized material, in this case, during deodorization, heating up to 190 C caused the formation of 0.2% dimeric triglycerides. This figure shows that GPCs are a sensitive means of detecting the history of an oil as far as preheating is concerned. Figure 2B shows the chromatogram of the same oil after heating for 64 hr up to 180 C. There is a considerable increase of compounds with higher molecular weight. Predominantly, dimeric triglycerides with a molecular weight of around 1800 are formed, but higher polymeric material is also present.

For the assessment of oil quality, the total peak area of "polymeric triglycerides," consisting of compounds with a molecular weight higher than that of monomeric triglycerides, was measured.

Liquid Chromatography (LC)

LC analyses were performed according to the method of Aitzetmüller and Guhr (7) using the detection system supplied by Pye Unicam. Part of the effluent was deposited on a fast moving wire, the solvent was evaporated, and the residue was combusted. The pyrolysis products, after reduction to methane, were fed to a flame ionization detector and registered as peaks.

Figure 3A shows the LC of a refined soybean oil. The

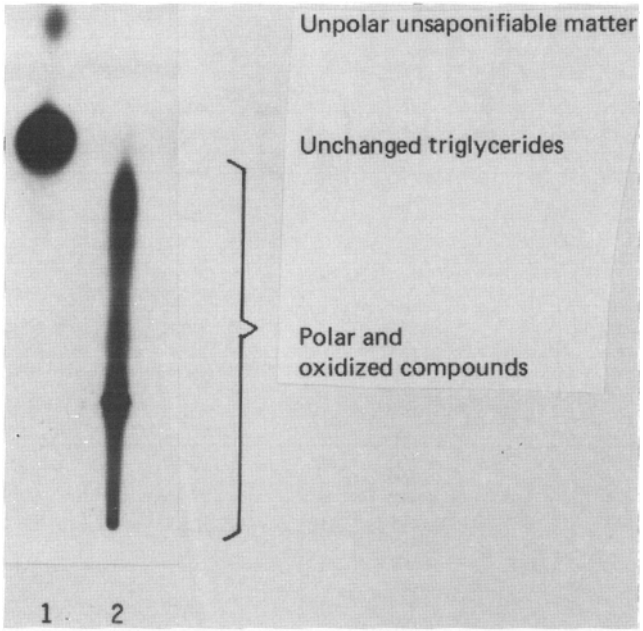

FIG. 4. Thin layer chromatogram of fractions 1 and 2. Fraction 1: unpolar components. Fraction 2: polar components.

first peak indicates triglycerides. Material more polar than triglycerides is collected in the second peak. This fraction of total polar artefacts is considerably increased after heating the oil for 64 hr up to 180 C, as is seen from Figure 3B. Measurement of the peak areas allows a semiquantitative determination.

Column Chromatography (CC)

A method for the separation of phosphatides from triglycerides previously published by Sen Gupta (8) gave, after some modification and standardization, the best separation of polar and unpolar components in a used frying oil.

Silica gel as obtained from the supplier (Merck, Darmstadt, Germany, particle size 0.063-0.200 mm, product no. 7734) was dried at 160 C for at least 4 hr. After cooling to ambient temperature in a desiccator, the gel was weighed into a 500-ml flask, and sufficient water was added to make a 5% (w/w) mixture, and the flask was shaken for 1 hr.

Two different solvents were used for elution of the column. Solvent 1 was a mixture of petroleum benzene (Haltermann, Hamburg, Germany), boiling point 40-60 C, and diethyl ether (Merck, product No. 921) in the ratio 87:13, v/v. Solvent 2 was pure diethyl ether (Merck, product No. 921). About 30 ml of solvent 1 was introduced into a glass chromatography column (inside diameter 2.1 cm, length 45 cm) with a ground glass joint on top. A wad of cotton wool was tightly packed over the outlet of the column, and the residual air in the wad was removed by means of a glass rod. The deactivated silica gel (25 g) was slurried in solvent 1 (80 ml) and introduced through a funnel into the column. The surface of gel in the column was leveled by tapping. The gel was then covered with a 1-cm layer of acid washed and calcined sea sand (ca. 4 g) (Merck, product No. 7712). The supernatant solvent was drained off to the level of the sand.

One gram (± 1 mg) of the filtered frying fat was weighed into a 25-ml beaker and dissolved in 10 ml solvent 1. If the sample has to be melted prior to filtration, the temperature should be kept as low as possible. After careful introduction of the fat solution through a small funnel onto the packed column, the solution was drained off until its level reached the top of the sand layer and was collected in a 250-ml beaker (as fraction 1). The 25-ml beaker (which contained the fat) was then rinsed with 5 ml of solvent 1 and the rinsings were added to the column. The solvent was again drained off to the level of the sand. This procedure was repeated again with 5 ml of solvent 1.

The column was eluted with 150 ml solvent 1 followed by 150 ml solvent 2. Special care was taken to ensure that the first portion (2-4 ml) was added without disturbing the surface of the adsorbent. Further quantities were added from a 250-ml dropping funnel which was connected to the column via a ground glass joint. The flow rate was adjusted to ca. 150 ml in 60 min. The first 150 ml of eluate were collected in the same beaker (fraction 1). This fraction contained the unaltered triglycerides. The further 150 ml of

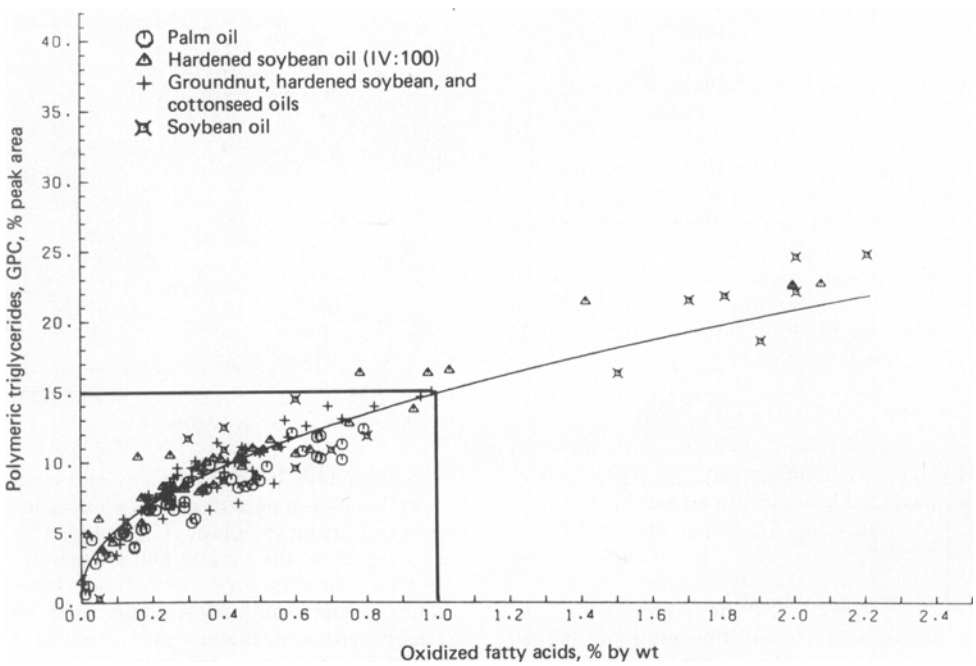

FIG. 5. Oxidized fatty acids vs. GPC results.

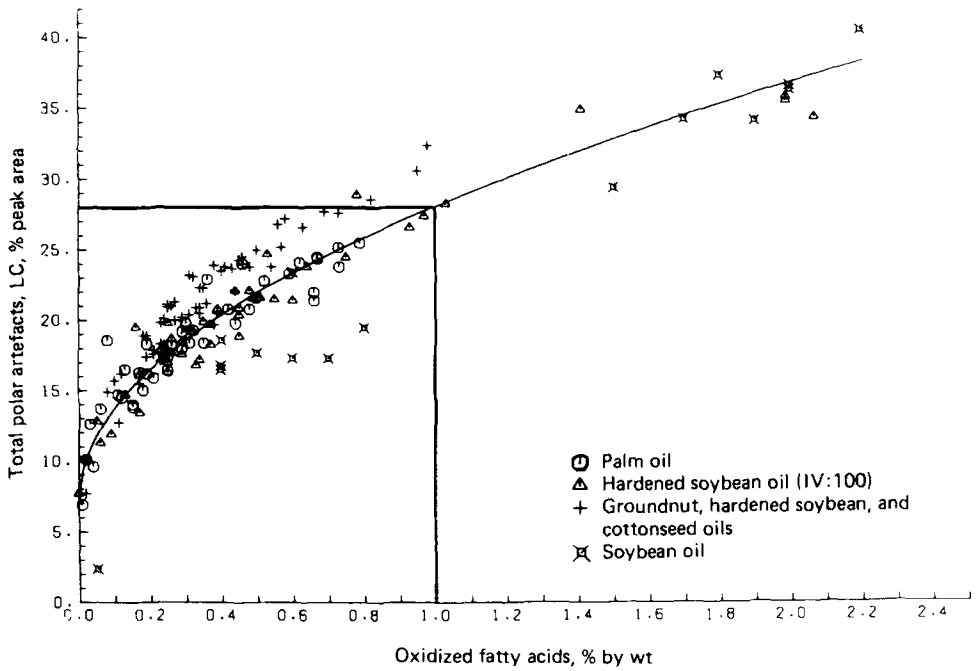

FIG. 6. Oxidized fatty acids vs. LC results.

eluate was collected in a second beaker (fraction 2), which contained all other substances, including the oxidized material.

The two fractions were separately freed from solvent by adding them in portions to preweighed 100-ml round bottomed flasks and distilling the solvent off at a maximum temperature of 60 C using a rotary vacuum evaporator. Shortly before termination of distillation, nitrogen was introduced into the system from a rubber bulb. The residue was cooled to ambient temperature, and nitrogen was introduced into the flasks.

The flasks were weighed and the contents calculated as a percentage of the total fat weight. Fraction 1 is the amount of unaltered triglycerides. Fraction 2 contains all other substances, including the oxidized material.

The performance of the separation can be checked by thin layer chromatography on silica gel plates, 0.25 mm thick (Merck, product No. 5721), developed in petroleum ether (Merck, product No. 1775)-diethyl ether (Merck, product No. 921)-acetic acid (100%, Merck, product No. 900:63) 70:30:2, v/v). Ten percent solutions of the substances in chloroform were applied as spots of 2 μl. After development, the dried plate was sprayed with a 10% solution of molybdatophosphoric acid and heated to 120-130 C. A typical chromatogram is shown in Figure 4.

RESULTS

Oxidized Fatty Acids vs. GPC Results

In Figure 5, the content of "oxidized fatty acids" in more than 150 samples of used frying oils is compared with the amount of "polymeric triglycerides" in the same oils as determined by GPC.

A parabola-like curve was obtained, which begins almost at zero, because fresh oils do not contain either "polymeric triglycerides" or "oxidized fatty acids." The regression line is not linear but declines somewhat in cases of high oxidation. This is quite understandable because "oxidized fatty acids" can be present in monomeric triglycerides, i.e., can be hidden in the main peak of the gel permeation chromatogram.

The important limit of 1.0% "oxidized fatty acids" corresponds to 15% peak area of "polymeric triglycerides."

Oxidized Fatty Acids vs. LC Results

Figure 6 shows the correlation between "oxidized fatty acids" and total polar artefacts as determined by LC. Again a parabola-like curve was obtained.

The curve starts at 5% total polar artefacts which are present even in fresh oils, e.g., free fatty acids, mono- and diglycerides, and others. Fresh oils, of course, do not contain "oxidized fatty acids" at all, or less than 0.1%.

As far as the quality assessment is concerned, only the region around 1.0% "oxidized fatty acids," a value which now corresponds with 28% of total polar artefacts, is of importance, and in this region the regression line is almost linear.

Oxidized Fatty Acids vs. CC Results

Figure 7 shows the correlation between "oxidized fatty acids" and polar components isolated by CC as described above. There is a good, but not linear, correlation. In Figure 7, the interesting number of 1.0% "oxidized fatty acids" is now connected with the corresponding value of polar components. One percent of "oxidized fatty acids" corresponds with 27% polar components.

The methods of LC and CC rely upon the same principle of separation; only the registration of the results is different. This is indicated by the great similarity of the curves in Figures 6 and 7 as well as in Figure 8, where the contents of polar components (CC) are compared with the total polar artefacts (LC).

DISCUSSION

Four different methods of quality assessment of used frying oils have been tested and compared with each other. Equipment is inexpensive for "oxidized fatty acids" and column chromatography (CC) and rather expensive (ca. $10,000) for the instrumental analyses LC and GPC. However, these methods are much more efficient, since one analysis is performed in less than 1 hr. Parallel runs are not possible, but altogether they are much less labor intensive. As far as CC is concerned, the given 3.5 hr refer to the complete procedure as described above, including the

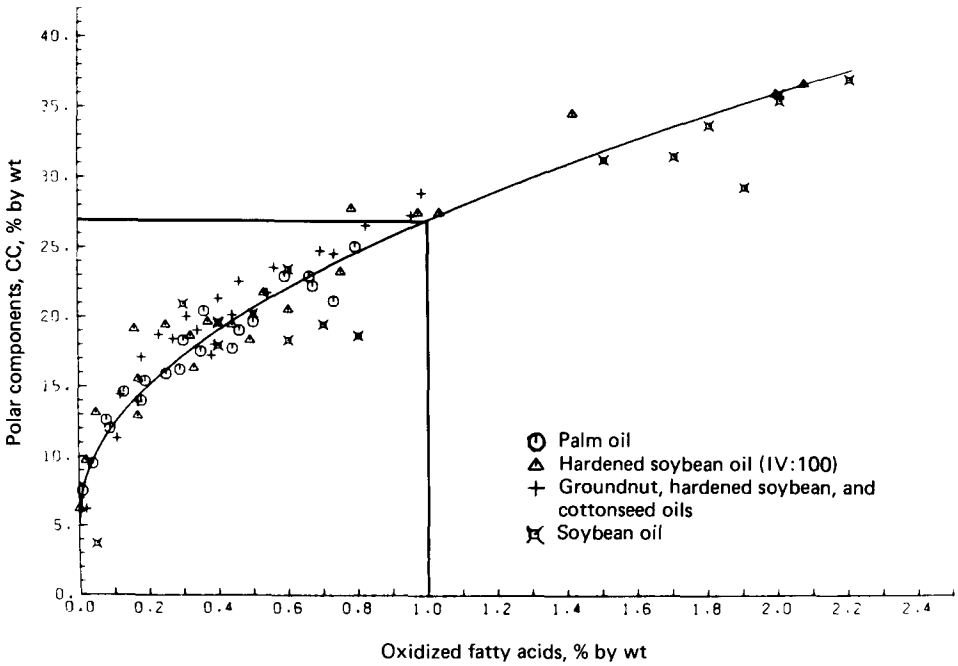

FIG. 7. Oxidized fatty acids vs. CC results.

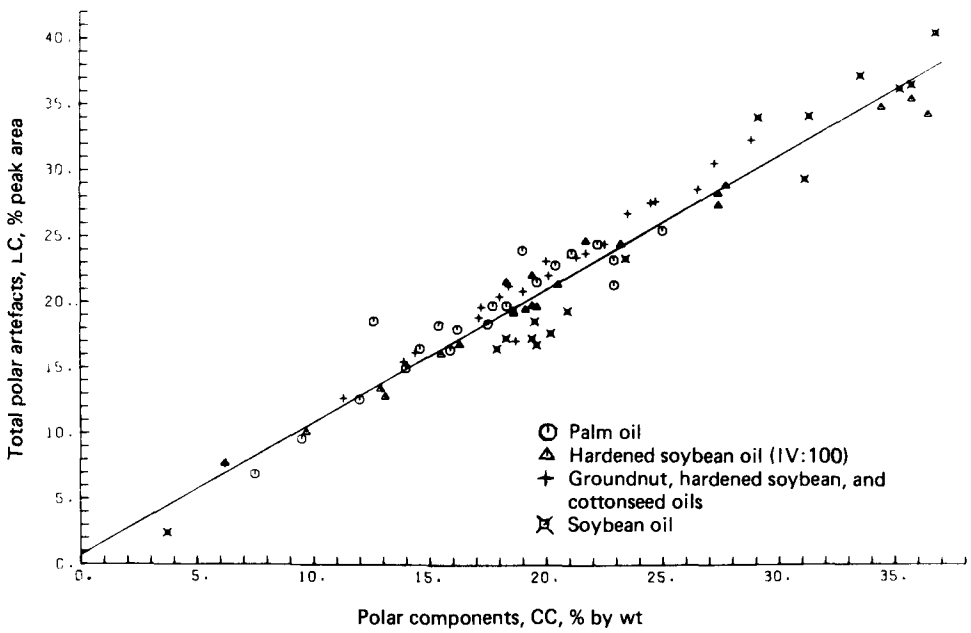

FIG. 8. Polar components (CC) vs. total polar artefacts (LC).

isolation of both fractions. Experience has shown that further time-saving down to 2.5 hr per run is possible if only fraction 1 containing the unaltered triglycerides is isolated and weighed. Fraction 2 which then is usually discarded with the spent silica gel adds up to 100%, and the amount of polar components is calculated.

The column chromatography (CC) showed the best reproducibility in this investigation. In a 12-fold determination performed by three persons, the following mean values were obtained: unaltered triglycerides, 71.6%; polar components, 26.9%; the standard deviation was 0.3 for each fraction. The fact that both fractions give only 98.5% of the weight of the sample indicates that a small part of very high polar material is not eluted from the column under the conditions as described above. Taking this into account, it seems to be justified to isolate only fraction 1 and to calculate the content of polar components.

An eightfold run with gel permeation chromatography (GPC) using a certain sample, which showed a mean value of 15.2% "polymeric triglycerides," the standard deviation was 0.5%, provided that the same equipment was used by the same person, on different days, however. With modern equipment one analysis can be performed in 50 min. This method seems to be well suited for routine analysis.

With liquid chromatography (LC) the reproducibility was not as good. The standard deviation was 0.7% after an 11-fold run on the same day with the same apparatus and a sample of 13.0% total polar artefacts as mean value. The reason might be that the rather sophisticated detection system depends on external factors which are not fully under control. But the method can still be recommended for a quick assay of a large series of samples, where the

utmost accuracy is not necessary.

As outlined in the introduction, it is the opinion of scientific bodies in Germany that objective analytical criteria should be used if the sensory assessment of a used frying oil does not give a clear indication that the oil is deteriorated. If the defects in taste and odor give rise to some doubts, analytical data should be evaluated to support a final decision. The recommended methods are the determination of the "oxidized fatty acids" and that of the smoke point. A correlation of the results of such analyses on several hundreds of used frying oils with a sensory assessment was performed by Mankel (9) and led to the proposition of the mentioned limits.

Federal health authorities and official chemists in Germany now apply these recommendations to check the oil quality in restaurants and snack bars. The first positive results of such actions have already been reported. After starting these actions, some cases have been brought to court, the average quality of the oils improved considerably.

However, a large scale application of the proposed procedures was hampered in many instances due to the fact that they are very time consuming and, as has been shown recently, not very accurate.

We hope that extensive use of the new and fast methods as described here will enable better control of oil quality and permit the processing industry to determine when they need to refresh their frying oil.

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