Evaluation of the methods of preparation of fatty acid methyl esters for their determination by gas chromatography

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A comparative review is given of the method of preparation of methyl esters of fatty acids with respect to the accuracy of their determination by means of gas chromatography.

THE METHOD OF PREPARATION OF FATTY ACID ESTERS

Gas chromatography is one of the modern analytical methods used in agricultural and food research. It is known that a direct analysis of lipids by the means of gas chromatography is difficult
because of a high number of theoretically possible components in a sample, because of their low volatility and because of their possible secondary decomposition occurring during analyses at high temperatures. Gas chromatography analyses of free fatty acids obtained by saponification of the sample have been, until now, very rarely used (Reference No. 1). Gas chromatography of low-molecular alcohol esters of fatty acids is most suitable. Such esters are prepared either by esterification of fatty acids released by alkaline hydrolysis of triglycerides, or by their direct transesterification. The procedure used for ester preparation may be the principal source of errors occurring during analyses in addition to errors possibly occurring during the separation of the lipid component from a natural material and in addition to errors of the chromatographic determination itself.

**Esterification**

Triglyceride hydrolysis, performed most frequently by alcohol solutions of alkaline hydroxides, is a prerequisite of the esterification methods. Potassium hydroxide (2-4) is more suitable than sodium hydroxide (5). Esterification itself is performed by the use of a number of reagents; different authors differ substantially in the amounts of esterification alcohol and of catalyst used. Customary is the esterification by methanol under catalysis by a mineral acid. In addition to the usually used hydrochloric acid (6) and to methanolic hydrogen chloride (7-10), also esterifications in the presence of acetyl chloride (3,11,12), sulphuric acid (1,13,14) and hydrobromic acid (15) have been described. Methyl esters are further prepa-
red by the action of diazomethane on free fatty acids (10,16 - 21).
This reaction is relatively fast and simple. Boron fluoride has been
used as a catalyst for esterification (5,22 - 24); a non-polar
solvent (25,26) is in this case added into the reaction mixture.
An esterification by the mixture of 2,2-dimethoxy-propane with benzene
and methanolic hydrogen chloride has been described (27).

Preparation of methyl esters of fatty acids from their silver
salts by a 30-minute treatment with methyl iodide is considered as
quantitative and has been developed for both the macro- and micro-
volumes (28). As also quantitative is characterized the procedure in
which tetra-methyl ammonium salts are prepared from free fatty acids and
these are decomposed to methyl esters following their application into
the chromatographic instrument and their subsequent heating up to
365°C (1a).

A different principle is employed in the method which uses a
reduction of free fatty acids to alcohols by lithium-ammonium hydride
(29). Alcohols are subsequently treated with a mixture of hexa-
methyl-di-silazane and trimethyl-chlorosilane; thus produced trimethyl-
silyl derivatives are analyzed.

Trans esterification

The apparent simplicity of this method brings disadvantages in
the form of balast compounds present in the mixture of esters; these
must be separated from the mixture, otherwise they would interfere with
the measurement. Alcoholates of alkaline metals, most frequently sodium
methanolate (30,31), are commonly used. Canic et al. (32) used 0.2 M
solution of sodium methanolate in methanol, and Glass & Christopher-son (33) developed the procedure for micro-determinations. Other authors (24,34,35) have used solutions of sodium methanolate ranging in concentration from 0.15 to 0.8 M and extracted the resulting methyl esters with either petroleum ether (36,37) or ether (36,38).

Guyot (39), Oette (19) and Craig (40,41) also used sodium methanolate to prepare methyl esters of fatty acids; only very small amounts of methanol are used when the procedure is carried out in sealed ampules (42). The use of potassium methanolate (43) and of lithium methanolate (23) is described in addition to that of sodium methanolate. A transesterification using a mixture of methanol with potassium hydroxide has also been described (44-47).

Acids were also used to catalyze transesterification. Methods differ only in the concentration of the acid and in the molar excess of methanol. Hydrochloric acid is used in concentrations 2-6% (14, 48-51), sulphuric acid is usually used as 1% solution (52,53). Boron fluoride has also been used as a catalyst (54).

Methods which use other esters of fatty acids for gas chromatography were also developed; for example propyl esters (55) prepared by transesterification with sodium propanolate or ethyl esters prepared by transesterification of the lipid component by the action of 40% solution of ethyl carbonate in 0.015 N sodium ethanolate (51).

**Comparison of the different techniques used for preparation of esters of fatty acids**

It is difficult to compare the different methods of production
of esters on the basis of information available from different authors. Several such attempts are already known in the literature (20 - 26).

Esterification under the catalytic action of boron fluoride has been compared to that under the action of sulphuric acid (26). Technique using boron fluoride seems to be more reliable for both the short-chain and the long-chain fatty acids, in spite of the fact that namely in the case of caprylic acid a negative deviation in the determined values of almost 15% has been observed. For long-chain fatty acids (for example palmitic acid) variations of about 1% were observed. The method which uses sulphuric acid as an esterification catalyst seems to be very distorting; deviations are often more than 20% for short-chain fatty acids. The results become more accurate with increasing number of carbons and an error of about 3% is then observed.

Somewhat more comprehensive comparison is given for the preparation of methyl esters by the two methods of esterification catalyzed by hydrochloric acid, for esterification catalyzed by boron fluoride and for esterification using diazomethane (20). The use of diazomethane seems to be most suitable for preparation of methyl esters from short-chain fatty acids. Method which uses boron fluoride can be described as relatively less suitable (deviation of ±13.8%). The use of hydrochloric acid gives distorted results and it is less suitable for esterification of short-chain fatty acids (20).

A different evaluation applies to long-chain fatty acids (caproic to linoleic). All the compared procedures show a similar degree of accuracy; the method which uses diazomethane can be considered to be the best one since it shows the least deviations (±0.25%). However, esterification catalyzed by boron fluoride (±0.46%) and both the methods
using hydrochloric acid, i.e. Stoffel (12) ± 0.52% and Hornstein (7) ± 0.77%, are not significantly worse. A relatively very small deviation (± 0.72 %) has been found (20) when diazomethane had been used for esterifications of fatty acids of wide variations in their carbon chain length.

In spite of the fact that the technique of preparation of fatty acid methyl esters via their silver salts has been described as quantitative (28), it has also been proposed that propyl esters are more suitable for gas chromatography of short-chain fatty acids (55). Up to date, the material available on some of the techniques (for example on transesterification by alkaline methanolates not even mentioning some other newer techniques of ester preparation) is too scarce to allow for formulation of generally valid conclusions on reliability of different techniques. We have therefore attempted to perform comparisons of some esterification and transesterification methods using equipment standard for serial analyses. Techniques already investigated, as well as techniques not yet compared and widely used, became components of our investigations.

EXPERIMENTAL

Chemicals used

Methanol and other routinely used chemicals were of commercially available purity ("Lachema" Co.), etherate of boron fluoride was before use always freshly distilled in the absence of air humidity. Methanol and eventually other liquids were dried by the means of a molecular filter (Nalsit) for at least 48 hours. Caproic, lauric, myristic, palmitic, stearic, oleic, linoleic and erucic acids ("Lachema" Co.) used to pre-
pare the model mixture were of at least 90% purity; contaminants were found and identified by gas chromatography of methyl esters of individual authentic samples (esterification carried out by the diazomethane method). The observed contents of other fatty acids were re-calculated and taken into consideration when the individual components of the model mixture were calculated.

The oil sample was obtained from a specimen of rape seed of the Trebic variety. Free fatty acids were prepared from an aliquot of the oil and were subsequently used for model esterifications. Samples of the model mixture as well as samples of oil and those of fatty acids isolated from the rape seed oil sample were all stored at -2°C. They were homogenized by heating them in a water-bath (50°C) and shaking prior to taking an aliquot for determination (usually 0.1 - 0.2 g).

Gas chromatography analyses were performed on a CHROM II instrument, column 140 x 0.6 cm, 15% polyethylene-glycol adipate (sebacate, respectively) on Chromosorbe NAW, temperature of the column 190°C, pressure in the column usually 0.5 - 0.8 atp N₂. Carrier gas was nitrogen of a nitrogen-lamp purity, flow-rate was approx. 150 ml/min.

Preparation of methyl esters of fatty acids

Preparation of methyl esters from silver salts of fatty acids (28). Approx. 0.5 g of fatty acid mixture was dispersed in a mixture of 25 ml of water with 10 ml of ethanol. Suspension was titrated to neutrality by 1 N potassium hydroxide using phenolphthalein as indicator; 0.8 - 1.0 g of silver nitrate as a saturated water solution was subsequently added to the neutralized suspension. The dark-brown reaction mixture was evaporated
to dryness under vacuum and left over night. 0.8 g of methyl iodide in 5 ml of hexane was then added and the mixture was shaken vigorously for 30 minutes, followed by filtration. Filtrate was then evaporated at room temperature under a vacuum of 20 mm Hg.

**Preparation of methyl esters by esterification catalysed by sulphuric acid** (2). 10 ml of methanol and 0.2 ml of concentrated sulphuric acid were added to the mixture of fatty acids (approx. 0.2 g). The reaction mixture was boiled in a water-bath for 1 hour, transferred into 25 ml of water and the water phase agitated into 10 ml of petroleum ether three times. The combined organic phases were washed three times by 10 ml of water, dried by sodium sulphate and the final solution was concentrated at room temperature under a vacuum of 20 mm Hg.

**Esterification of fatty acids by diazomethane** (19). N-methyl-N-nitroso urea, which is required for the preparation of diazomethane, has been prepared from methyl ammonium chloride in such an amount as to be utilized within 24 hours. Ether solution of diazomethane was prepared by the decomposition of N-methyl-N-nitroso urea (in the form of water solution covered by a layer of ether) by the action of 30% potassium hydroxide at 0°C. The resulting solution was dried by solid potassium hydroxide and was immediately used for the reaction.

Diazomethane dissolved in ether was added drop by drop to the fatty acid mixture for as long as nitrogen had been released and the yellow color of diazomethane solution kept disappearing. Subsequently, under continuous stirring, enough of the etheric solution of diazomethane was added (usually 2 ml) to keep the mixture yellow in color for at least additional 30 minutes. After standing over night, the samples were concentrated at room temperature under a vacuum of 20 mm Hg.
Esterification by a mixture of methyl iodide with potassium carbonate (56). Mixture of fatty acids (approx. 0.2 g) was stirred into 2 ml of methyl iodide and after homogenization 1.4 g of anhydrous sodium carbonate was added. The resulting mixture was left overnight at room temperature and concentrated under vacuum. The concentrate was washed with 10 ml of petroleum ether, solid salts were removed by filtration and the filtrate was concentrated at 25°C under a vacuum of 20 mm Hg.

Esterification catalyzed by etherate of boron fluoride (38). Approx. 0.2 g of the fatty acid mixture was added into 5 ml of 1% solution of etherate of boron fluoride in methanol. After 60 minutes of standing at room temperature the mixture was concentrated at 50°C under vacuum, the evaporated residue was extracted into 5 ml of petroleum ether and the extract was evaporated under a vacuum of 20 mm Hg.

Esterification and transesterification catalyzed by acetyl chloride (11). 0.5 of the fatty acid mixture or 0.4 g of oil were dissolved in 40 ml of methanol. 4 ml of acetyl chloride were subsequently added and the mixture was boiled for 2 hours in a water-bath. 10 ml of petroleum ether were layered over thus obtained solution, the extract was dried by sodium sulphate, filtered and evaporated at 25°C under a vacuum of 20 mm Hg.

Transesterification by a mixture of methanolic hydrogen chloride with 2,2-dimethoxy propane (27). 0.1 g of oil was added into 7 ml of 8% solution of 2,2-dimethoxy propane in benzene. 5 ml of 10% methanolic hydrogen chloride was then added to the mixture and the resulting mixture was left to stand at room temperature for 12 hours. A solid titrating agent composed of sodium sulphate, sodium carbonate and sodium hydrogen
carbonate \((2:2:1)\) was used to neutralize the mixture with the use of methyl red as an indicator. Crystalline components were spun down and the clear supernatant was evaporated at \(50^\circ\) C under a vacuum of 20 mm Hg.

**Transesterification by sodium methanolate** \((37)\). 0.2 g of oil was added to 5 ml of 0.5\% solution of sodium methanolate in methanol. After 12 hours of standing the reaction mixture was neutralized by 5 ml of 12\% solution of potassium dihydrogen phosphate; the neutralized mixture was then extracted by 25 ml of petroleum ether, the extract was dried by sodium sulphate and it was finally evaporated at \(25^\circ\) C under a vacuum of 20 mm Hg.

All the reactions involved in the listed methods were simultaneously performed five times and each from the prepared methyl ester mixtures was then chromatographed five times. Hence 25 analyses were obtained for each method. Two from the results which exhibited highest deviation from the mean were deleted and the remaining values were used to calculate the means given in the Tables. Each from the individual peaks on a chromatogram was measured and expressed as follows:

\[
\text{area} = \text{height of the peak} \times \text{width of the peak determined in the middle of its height.}
\]

**RESULTS**

Methods based on saponification, isolation of fatty acids and their subsequent esterification are time-consuming for serial determinations. Their importance can be limited only to instances where the time spent is compensated for by the accuracy and reproducibility of the results, and also to the determinations of free fatty acids directly in
a sample because in such instances the direct esterification is necessary. One of the factors which contribute to a considerable error incurred during these methods can be the losses of low-molecular-weight fatty acids occurring during their isolation. Not only how laborious and how time consuming is each of the methods, but also the accuracy by which it approaches the true values, were taken into consideration in our evaluation of the esterification methods. Fatty acids are represented by numerical symbols customary in food industry (the first number represents the number of carbon atoms; the second represents the number of double-bonds).

Esterification catalyzed by sulphuric acid (Method A).

Results shown in Table I demonstrate that, in agreement with the literary data (25), no satisfactory level of accuracy is reached by this method; errors of the determination reach up to 25% for the individual acids—values observed for the contents of high-molecular-weight fatty acids were higher, and those of low-molecular-weight fatty acids were lower than the real content. In addition, the method is considerably time-consuming.

Esterification by diazomethane (Method B).

Results (Table I) demonstrate that in our hands, in contrast to the literary data (18), some differences were found between the real and the determined values; especially so for caproic and linoleic acids. We could not explain this disagreement to our satisfaction. In the case of other acids the method equals the other methods as far as the accuracy is concerned. The unstability of N-nitroso-N-methyl urea and the fact that the diazomethane preparation is rather laborious seem to be a hindrance of this method when it is compared to the other techniques.
### Table I.

<table>
<thead>
<tr>
<th>Kyselina</th>
<th>$C_{6:0}$</th>
<th>$C_{8:0}$</th>
<th>$C_{10:0}$</th>
<th>$C_{12:0}$</th>
<th>$C_{14:0}$</th>
<th>$C_{16:0}$</th>
<th>$C_{18:0}$</th>
<th>$C_{18:1}$</th>
<th>$C_{18:2}$</th>
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<td>Naváženo</td>
<td>21.8</td>
<td>0.9</td>
<td>0.8</td>
<td>8.4</td>
<td>18.0</td>
<td>14.5</td>
<td>5.2</td>
<td>20.1</td>
<td>10.3</td>
</tr>
<tr>
<td>Nalezeno</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>metoda A</td>
<td>14.9</td>
<td>2.5</td>
<td>1.0</td>
<td>8.7</td>
<td>16.7</td>
<td>12.6</td>
<td>7.7</td>
<td>20.8</td>
<td>15.4</td>
</tr>
<tr>
<td>metoda B</td>
<td>9.3</td>
<td>1.1</td>
<td>1.2</td>
<td>10.8</td>
<td>20.2</td>
<td>17.1</td>
<td>12.4</td>
<td>25.4</td>
<td>2.4</td>
</tr>
<tr>
<td>metoda C</td>
<td>14.3</td>
<td>0.6</td>
<td>0.5</td>
<td>9.1</td>
<td>19.5</td>
<td>15.4</td>
<td>6.0</td>
<td>24.9</td>
<td>10.2</td>
</tr>
<tr>
<td>metoda D</td>
<td>21.3</td>
<td>0.9</td>
<td>0.8</td>
<td>8.6</td>
<td>17.1</td>
<td>13.3</td>
<td>6.7</td>
<td>21.8</td>
<td>6.8</td>
</tr>
<tr>
<td>metoda E</td>
<td>22.1</td>
<td>1.0</td>
<td>1.0</td>
<td>8.4</td>
<td>16.8</td>
<td>14.6</td>
<td>5.6</td>
<td>21.8</td>
<td>8.2</td>
</tr>
</tbody>
</table>

1) Fatty acid content (as %) determined in the model mixture by different methods; 2) acid; 3) weighed; 4) found; 5) method A.

Esterification catalyzed by acetyl chloride (Method C1).

This technique was used here only for a comparison; merit of its use is triglyceride transesterification. Results are given in Table I. When the determined values are compared to the theoretical amounts it is clear that this method is not suitable for analyses of samples which emphasize fatty acids of low-molecular-weight; content of these is negatively distorted. Starting with lauric acid, the accuracy of this method is relatively good with errors usually not exceeding 10%.

Esterification catalyzed by etherate of boron fluoride (Method D).

This method is approx. three times faster than that which uses sulphuric acid; elaboration of samples is simpler. As demonstrated by repeated experiments, the concentration of etherate of boron fluoride...
(1.5%) must be strictly adhered to, and the agent must be freshly distilled. Results are given in Table I; they demonstrate that this technique is one of the most advantageous from those we evaluated. There is an excellent agreement between the real and the determined values, especially so for the low-molecular-weight fatty acids; errors did not exceed 10% even when the compound concentration was varied between 1% and 20%.

Preparation of methyl esters from silver salts of fatty acids (Method E).

Disadvantages of this method are the fact that it is relatively laborious and the fact that it requires extreme accuracy. Results obtained (Table I) demonstrate its very high degree of accuracy - in fact it is the most precise from all the compared methods. It was used as the reference method for our rape-seed oil analyses. It is perhaps the most suitable technique for analyses of free fatty acid samples; it can only be replaced by esterification catalyzed by etherate of boron fluoride.

Esterification by methyl iodide and potassium carbonate (Method F).

This technically non-demanding and as yet unused method requires somewhat higher amount of sample (minimum of approx. 0.1 g). As illustrated by the results (Table I) it may be compared to the method which uses etherate of boron fluoride as far as its accuracy is concerned. Accuracy of the described method approaches the accuracy of the boron fluoride technique and, in the case of high-molecular-weight fatty acids, it even exceeds it; at the same time the method is more simple. To its disadvantage is the requirement that the analyzed samples must be anhydrous.
TABLE II.

Tabulka II

Obsah mastných kyselin (v %) stanovený v řepkovém oleji různými metodami 1)

<table>
<thead>
<tr>
<th>Kyselina</th>
<th>C16:0</th>
<th>C16:1</th>
<th>C18:0</th>
<th>C18:1</th>
<th>C18:2</th>
<th>C18:3</th>
<th>C20:1</th>
<th>C20:2</th>
<th>C22:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nalezeno 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>metoda E</td>
<td>4.6</td>
<td>1.8</td>
<td>1.5</td>
<td>14.4</td>
<td>16.7</td>
<td>9.8</td>
<td>9.9</td>
<td>0.9</td>
<td>46.2</td>
</tr>
<tr>
<td>metoda B</td>
<td>4.2</td>
<td>1.6</td>
<td>1.7</td>
<td>12.3</td>
<td>13.8</td>
<td>8.9</td>
<td>9.8</td>
<td>1.1</td>
<td>46.7</td>
</tr>
<tr>
<td>metoda C</td>
<td>4.8</td>
<td>1.8</td>
<td>1.6</td>
<td>12.2</td>
<td>13.8</td>
<td>8.7</td>
<td>9.6</td>
<td>1.1</td>
<td>45.9</td>
</tr>
<tr>
<td>metoda H</td>
<td>4.5</td>
<td>1.4</td>
<td>1.3</td>
<td>10.9</td>
<td>14.8</td>
<td>7.6</td>
<td>8.0</td>
<td>0.5</td>
<td>48.8</td>
</tr>
<tr>
<td>metoda I</td>
<td>4.1</td>
<td>1.8</td>
<td>1.5</td>
<td>12.0</td>
<td>13.1</td>
<td>8.3</td>
<td>9.7</td>
<td>1.1</td>
<td>46.2</td>
</tr>
</tbody>
</table>

1) Fatty acid content (as %) determined in the rape-seed oil by different methods; 2) acid; 3) found; 4) method E.

Elaboration of the rape-seed oil and fatty acids isolated from it.

Methyl esters of fatty acids for the rape-seed oil analyses were prepared either from their silver salts or by esterification by diazomethane. Results obtained by both these methods (Methods E and B) are given in Table II. Contents of individual fatty acids, determined in such a manner, served as reference values for our evaluation of transesterification methods. The results obtained by the two methods did not differ to any large extent: average deviation of the diazomethane method from the silver salt method was 0.8%. This demonstrates a relatively high degree of accuracy (the concentration of the compounds varied between 1% and 47%).

Transesterification catalyzed by acetyl chloride (Method C2).

An average deviation of only 0.8% from the values determined by
the method of silver salts was also observed in this method (Table II). Larger deviations occurred only with fatty acids of a medium-length of the chain. Generally, it can be stated that this method is not hindered by errors and that its only disadvantage may be the fact that it is rather laborious and more time-consuming.

**Transesterification by sodium methanolate (Method H).**

It can be seen from the results (Table II) that this method is less accurate (average deviation 1.7%). It is worth mentioning that results of individual analyses differed only to a very low extent; deviation from the mean never exceeded 10%. The possibility to carry out this method in large series and also its little demands for laboratory equipment are certainly advantageous.

**Transesterification by the means of 2,2-dimethoxy propane (Method I).**

Also this technique is very simple and suitable for serial use. Accuracy of its results (Table II) approaches that of the transesterification catalyzed by acetyl chloride (average deviation of 0.9%). This method is very suitable for the use on mixtures containing fatty acids of more than 14 carbon atoms. When applied to analyses of fatty acids with shorter chains it is interfered with by the products of decomposition of 2,2-dimethoxy propane. A certain degree of improvement is reached when lower amounts of the agent are used.

**DISCUSSION**

Gas chromatography is often used to determine relative amounts
of fatty acids in fat and oil. It is best developed for procedures in which the original lipid material is transformed into methyl esters of fatty acids by the means of either hydrolysis of fat to fatty acids followed by their esterification, or by a direct transesterification. In this paper we tested some catalytically active compounds and their effect on the accuracy of the analytical determinations, and compared it with some known methods.

Esterifications started with mixtures of fatty acids of known composition. The following esterifications were performed: esterification catalyzed by the action of sulphuric acid, esterifications catalyzed by hydrogen chloride generated from acetyl chloride, by etherate of boron fluoride, and esterification by diazomethane. Esters were prepared from silver salts of fatty acids by the action of methyl iodide and by the action of a mixture of methyl iodide with potassium carbonate. It has been observed, in agreement with the literature (26), that the esterification catalyzed by sulphuric acid is not very suitable. Catalysis by hydrogen chloride generated from acetyl chloride is suitable also for analyses of fatty acids with low-molecular-weight.

Esterification catalyzed by etherate of boron fluoride is much faster and it is very accurate for analyses of low-molecular-weight fatty acids. Production of methyl esters by the action of methyl iodide on silver salts is suitable for analyses of samples with low variations in their fatty acid content and for analyses of highest possible accuracy. The esterification by methyl iodide and potassium carbonate was used here for the first time in this respect. It is the simplest from all the methods compared when applied to samples containing no water; on the other hand it is somewhat less accurate.

After evaluation of the esterification methods, we have performed
esterifications of fatty acids obtained from rape-seed oil together with transesterifications of the initial oil. Contents of fatty acids, as determined by analysis of methyl esters of their silver salts, served as the reference values for our evaluations. Esterification by diazomethane seems to be a very accurate method among the esterification techniques (in agreement with the literary data - 20) in spite of the fact that some deviations were observed. The method which uses acetyl chloride proved to be a satisfactory technique of transesterification; it is, however, more time-consuming, laborious and more difficult than other methods. Even though the use of methanolate somewhat increases error of the determinations, its procedure is very simple. The same applies to the use of 2,2-dimethoxy propane. With the exception of fatty acids with lower molecular weight, this technique is very accurate and, at the same time, it is not time demanding. If the accuracy of the chromatographic procedure itself were taken into consideration, all the investigated transesterification would seem equally suitable; in reference to their simplicity the transesterifications by methanolate and by 2,2-dimethoxy propane can be recommended for serial analyses.

BIBLIOGRAPHY

Literatura


1a. Forman, L., Prumysl Potravin (Food Industry) 21:121, 1970

