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# DETERMINATION OF FATTY ACID COMPOSITIONS BY THE DIRECT TRANSESTERIFICATION OF SEED LIPIDS

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*The possibility of using the direct alkaline transesterification of seed lipids for the express analysis of fatty acid compositions has been shown on 11 samples of plant seeds.*

As a rule, the fatty acid (FA) compositions of lipids are determined by the GLC analysis of their volatile derivatives — methyl esters (MEs). The current generally adopted method of isolating the FAs and obtaining the MEs is fairly laborious and requires a large amount of initial material and time [1]. Numerous investigations have shown that "direct" (*in situ*) transesterification can be used widely for the express analysis of the FA compositions of numerous biological materials [2].

Both acid and alkaline catalysts are used for the direct transesterification of lipids [3]. A large number of investigations have been devoted to the direct transesterification of biological material using acid catalysts ($BF_3$, $H_2SO_4$, HCl) in methanol [3]. However, the reaction conditions in these cases (acid medium, temperature 60-90°C, lengthy process) may lead to a distortion of the results and, in some cases, cause a degradation of polyunsaturated fatty acids [4].

Direct transesterification under the conditions of alkaline catalysis has been described in only two cases, with soybeans, rapeseed, and maize as examples [3, 5], and this under conditions different from those that we use. This is possibly connected with the fact that when using direct esterification under the conditions of alkaline catalysis the free fatty acids (FFAs) present in the lipid material are not analyzed and there are difficulties in using this method for moist materials [3]. However, this method is attractive because of its rapidity (from a few minutes to an hour), and the possibility of performance at a temperature ranging from room temperature to 50°C.

TABLE 1. Characteristics of Samples of Seeds and Oils of Cultivated and Wild Plants

| Sample | Oil content, % | Moisture content, % | Acid No., mg KOH |
|---|---|---|---|
| Aconitum septentrianale | 33.7 | 6.9 | 4.8 |
| Viburnum opulus | 11.1 | 5.0 | 1.7 |
| Crambe abyssinica | 47.3 | 3.9 | 0.4 |
| Solanum lycopersicum | 5.9 | 7.3 | 2.9 |
| Umbilifera coriandrum sativum | 12.4 | 3.6 | 2.7 |
| Yuglans regia | 66.9 | 2.5 | 0.3 |
| Arachic hypogaea | 48.8 | 3.9 | 0.4 |
| Gossypium hirsutum | 32.5 | 6.0 | 1.9 |
| Allium cepa | 16.4 | 8.5 | 4.4 |
| Rosa canina | 7.2 | 5.7 | 6.7 |
| Hellanthus annuus | 63.1 | 4.1 | 3.0 |

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TABLE 2. Conditions of Performance of the Transesterification Reaction and Fatty Acid Compositions* of the Seed Lipids of Cultivated and Wild Plants Obtained by the Direct Transesterification of the Seed Lipids (1) and of the Oils (2)

| Sample | | Amount of 0.5% $CH_3ONa$, ml | Amount of toluene, ml | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 22:0 | 22:1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aconitum | 1 | 10† | 6 | – | 2.8 | – | – | 62.5 | 34.7 | – | – | – | – |
| septentrianale | 2 | 10 | 2 | – | 2.8 | – | – | 61.3 | 35.9 | – | – | – | – |
| Viburnum | 1 | 16 | 14 | – | 0.8 | – | – | 43.0 | 56.2 | – | – | – | – |
| opulus | 2 | 10 | 2 | – | 0.6 | – | – | 43.5 | 55.9 | – | – | – | – |
| Crambe | 1 | 10 | 3 | 0.1 | 1.0 | – | 0.3 | 13.6 | 7.2 | 7.7 | 0.6 | 0.5 | 69.0 |
| abyssinica | 2 | 10 | 2 | 0.1 | 0.9 | – | 0.3 | 12.7 | 6.4 | 7.7 | 0.8 | 0.5 | 70.6 |
| Solanum | 1 | 24 | 24 | 0.1 | 11.4 | – | 4.8 | 19.8 | 60.6 | 2.9 | – | – | – |
| lycopersicum | 2 | 10 | 2 | 0.1 | 9.9 | – | 4.6 | 20.3 | 62.1 | 2.5 | – | – | – |
| Umbilifera | 1 | 16 | 8 | – | 1.9 | – | – | 85.9 | 12.2 | – | – | – | – |
| coriandrum sativum | 2 | 10 | 2 | – | 1.5 | – | – | 87.5 | 11.0 | – | – | – | – |
| Yuglans | 1 | 10 | 2 | – | 3.7 | 0.2 | 2.5 | 16.8 | 68.4 | 8.4 | – | – | – |
| regia | 2 | 10 | 2 | – | 3.7 | 0.2 | 2.5 | 16.5 | 69.1 | 8.0 | – | – | – |
| Arachic | 1 | 10 | 2 | – | 8.5 | – | 2.3 | 35.6 | 46.6 | 1.9 | – | 5.1 | – |
| hypogaea | 2 | 10 | 2 | – | 8.7 | – | 2.2 | 35.9 | 47.2 | 1.7 | – | 4.3 | – |
| Gossypium | 1 | 11 | 2 | 0.6 | 19.4 | 0.7 | 2.4 | 18.7 | 58.2 | – | – | – | – |
| hirsutum | 2 | 10 | 2 | 0.6 | 19.9 | 0.7 | 2.6 | 18.3 | 57.9 | – | – | – | – |
| Allium | 1 | 10 | 4 | – | 6.6 | 0.2 | 1.5 | 25.5 | 66.2 | – | – | – | – |
| cepa | 2 | 10 | 2 | – | 6.0 | 0.3 | 1.5 | 27.1 | 65.1 | – | – | – | – |
| Rosa | 1 | 14 | 10 | – | 2.0 | 0.1 | 0.9 | 15.2 | 35.6 | 46.1 | – | – | – |
| canina | 2 | 10 | 2 | – | 1.8 | 0.2 | 1.0 | 15.7 | 36.5 | 44.8 | – | – | – |
| Hellanthus | 1 | 10† | 2 | – | 4.1 | – | 2.4 | 18.8 | 74.7 | – | – | – | – |
| annuus | 2 | 10 | 2 | – | 3.8 | – | 3.5 | 18.8 | 73.9 | – | – | – | – |

*In addition to the fatty acids shown, the 12:0 acid was detected in the dog rose seeds (0.1%), and the 17:1 acid in the tomato seeds (0.4%) and the tomato seed oil (0.5%). The given amounts of toluene and of methanolic sodium methanolate solution are calculated to 1 g of oil or seeds. The time of transesterification was 5 min for the oils and 10 min for the seeds.

†$CH_3ONa$ concentration 1%.

Our task was to study the possibility of using the method of direct alkaline transesterification for determining the FA compositions of plant seeds. The results obtained showed that, when the conditions of the reaction and of the post-reaction treatment were observed, the fatty acid methyl esters (FAMEs) had a representative composition and that the natural moisture content did not affect the results of the determination.

The seeds of 11 species of plants were investigated: wolfbane monkshood (*Aconitum lycoctonum* or *septentrianale*), European cranberrybush viburnum (*Viburnum opulus*), Spanish crambe (*Crambe abyssinica*), tomato (*Solanum lysopersicum*), coriander (*Umbilifera coriandrum*), Persian walnut (*Juglans regia*), peanut (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), garden onion (*Allium cepa*), dog rose (*Rosa canina*), and common sunflower (*Helianthus annuus*). FAMEs were obtained by the direct alkaline transesterification of the lipids of the ground seeds without preliminary extraction of the oil from them. To check the reprentativeness of the FA composition of the seeds, the oil from each sample was exhaustively extracted with petroleum ether in a Soxhlet apparatus and was then subjected to transesterification under the same conditions.

The transesterification of the oils and the direct transesterification of the seeds were conducted with stirring in a mixture of toluene and a 0.5% methanolic solution of $CH_3ONa$ at 50°C (Table 2). The oil and the toluene were taken in a ratio of 1:2, and for the seeds with a fairly high oil content — sunflower, cotton, peanut, walnut, crambe — only as much toluene was needed as for reaction with the oils; i.e., the ratio of seeds to toluene was 1:2 (Table 2); for seeds with a low oil content it was necessary to increase the amount of toluene. Approximately the same rule was observed in the ratio of sodium methanolate to seeds. The reaction times were 5 min for the oils and 10 min for the seeds in all the experiments except those involving the monkshood and sunflower; for these the reaction time both for the seeds and for the oils was 5 min.

In both cases, the course of the reactions was monitored by TLC, the completeness of the reaction being judged from the disappearance of the spot of triacylglycerols and the appearance of the FAMEs.

The FA compositions of the products obtained are given in Table 2, from which it can be seen that the difference between experiments 1 and 2 is small and lies within the limits of experimental error.

Some authors have reported [3] that the products of the direct alkaline transesterification of seed lipids, i.e., the fatty acid methyl esters, may undergo hydrolysis after their treatment under these conditions. Our investigations showed that samples of FAMEs obtained under the conditions described above could be stored at 5-10°C for more than six months without appreciable changes.

## EXPERIMENTAL

GLC analysis was conducted on a Chrom-5 apparatus with a flame-ionization detector. A 1.2-m column with 5% of polyethyleneglycol succinate on Chromaton N-AW-DMCS was used at 198°C.

TLC was conducted on Silufol plates in the solvent system hexane–diethyl ether (8:2).

The oil was extracted from the ground air-dry seeds with petroleum ether (40-60°C) in a Soxhlet apparatus for 18 h.

The indices of the seeds and oils were determined by generally adopted methods: oil and moisture contents [6] and acid No. [7].

**Transesterification of an Oil.** A solution 0.1 g of the oil in 0.2 g of toluene was transferred to a three-necked flask fitted with a reflux condenser provided with a calcium chloride tube and a thermometer. At 50°C, 1 ml of 0.5% methanolic sodium methanolate was added, and the reaction mixture was stirred with a magnetic stirrer for 2-5 min.

**The transesterification of the seeds** was conducted in a similar way to that of an oil, with an increase in the time of transesterification where necessary. The only difference was that the ground seeds were first stirred with the toluene at room temperature for 5-10 min.

After the performance of the reaction, the reaction mixture was filtered, and its following treatment was the same for both types of samples. It was diluted with water and neutralized with acetic acid, and the product obtained was extracted three times with petroleum ether. The combined ethereal extracts were washed with water, dried over sodium sulfate, and evaporated.

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