Investigation on the Efficiency of Agrotechnical Treatments Applied to Oilseed Plants by Chromatographic Analysis of the Fatty Acid Composition

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This paper presents the use of gas-chromatographic technique of compositional analysis of oils for the examination of the efficiency of different fertilization treatments applied to sunflower and rapeseed. Two series of oilseeds (sunflower and rapeseed) simultaneously grown in the same geographic aria and of the same variety but treated with different fertilizers have been analyzed. The efficiency of the agrotechnical treatments was determined on the basis of the oilseed production increase (kg/ha), oil production increase (kg/ha) and of some quality indices of oils (ω-3 fatty acid content, ω-3/ ω-6 ratio).

Keywords: vegetable oils, foliar fertilizers, fatty acid composition, gas-chromatography, oil content.

Vegetable oils represent a major economic resource in Romania, being mainly used in the food industry field or as raw material for the biodiesel production.

Oil quality is associated with fatty acid composition, mainly with percentage of oleic and linoleic acids [1]. The health authorities of many countries promote the intake of foods containing high amounts of ω-3 fatty acids and a favorable ω-3/ ω-6 fatty acid ratio[2, 3], due to the fact that ω-3 fatty acids play very important roles in physiology, especial during fetal and infant growth, especially in the formation of the central nervous system and retina [4, 5] and for the prevention of cardiovascular diseases (having antithrombotic and anti-inflammatory action) [6, 7].

Romanian agriculture has a long tradition in oilseed production, its geographical conditions generally offering soils with good to medium fertility [8]. As a result of an intensive agriculture in some regions, even highly fertile soils can grow poor in nutrients such as N, P or K, which negatively affects soil quality. This drawback is generally overcome by using different types of fertilizers. For more than 60 years, there is a permanent concern among Romanian agrochemists to find new efficient formulations for radicular or foliar fertilizers in order to contribute to the crop improvement [9-13].

Variations in fatty acid composition have been mainly related to plant genotype [3], temperature [2, 14-16] and planting date [17, 18]. A better understanding of agrotechnical treatments, plant genotype or environmental effects on fatty acid composition is needed to further improve oil quality [1].

Gas-chromatography is probably the most common method for determining the fatty acid composition of vegetable oils. For this purpose, oils are converted to their corresponding methyl esters prior to analysis. The method is very sensitive and allows both qualitative and quantitative fatty acid profiling of oils [19]. In the plant breeding gas-chromatography was successfully used to study the variation or hereditary transmission of the fatty acid profiles in breeded rapeseed [20, 21], soybean [22] or sunflower [23].

This paper deals with the analysis of the agrotechical treatments of two oilseed species (sunflower and rapeseed), by correlating the fatty acid profile with the corresponding seed oil content, crop production and fertilizer efficiency. Oilseed plants have been foliarly treated with different types and amounts of fertilizers and the variation of the fatty acid composition in each case was chromatographically determined.

Experimental part

Two series of oilseeds (sunflower SF-1 to SF-6 and rapeseed R-1 to R-8), each of them obtained from the same plant varieties simultaneously cultivated in the same geographic area (experimental fields of Feteşti, Ialomiţa county), with the same soil characteristics, foliarly treated with different types and amounts of fertilizers were provided by the "Gheorghe Ionescu Sisesti" Academy of Agricultural and Forestry Sciences (Bucharest, Romania). The agrotechnical treatments (fertilizers, number of treatments, amount of active substance, volume) and the agronomic efficiency (seed production, production increase, and efficiency) are presented in table 1 and 2.

Vegetable oils were extracted from oilseeds according to standard Soxhlet protocol [24].

The standard mixture of 37 fatty acid methyl esters (Supelco™ 37 Component FAME Mix) used for the gas-chromatographic analyses was purchased from Supelco.

Fatty acid methyl esters (FAME) of oils were prepared by transesterification of the triglyceride oils with methanol, using BF_3-MeOH complex as catalyst, according to the standard method [25].

The gas-chromatograms of the fatty acid methyl esters mixtures were recorded on an Agilent Technologies 6890 N instrument with FID detection. Separation into components was made on a capillary column especially designed for the FAME analysis (Supelco SP™ 2560, with

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Sample	Fertilizer	Active substance (g/L)	Volum (L/ha)	Treat-ments	Seed production (kg/ha)	Seed production increase (kg /ha)	Efficiency (%)
SF-1	Unfertilized sample	-	-	-	1840	-	100.0
SF-2	Fertifam 111-PP	235	400	2	2720	880.0	147.8
SF-3	Fertifam 311-BH_3	240	400	2	2786	946.0	151.4
SF-4	Fertifam 111-BH_3	303	400	2	2506	667.0	136.2
SF-5	Bihumat 3	329	400	2	2880	1040.0	156.5
SF-6	Bihumat 2	225	400	2	2653	813.0	144.2

Table 1
EXPERIMENTAL CHARACTERISTICS AND RESULTS FOR THE FERTILIZATION OF SUNFLOWER (DATA REFER TO THE SEED PRODUCTION)

Sample	Treatment	Active substance (kg/ha)	Seed production (kg/ha)	Seed production increase (kg/ha)	Efficiency (%)
R-1	Unfertilized sample (blank)	-	2620	-	100.0
R-2	Complex N-40 kg/ha; P_2O_5-40 kg/ha; foliar fertilization -10 L/ha	90	3200	580	122.1
R-3	Complex N-40 kg/ha; P_2O_5-40 kg/ha; K_2O-50 kg/ha; foliar fertilization -10 L/ha	140	3400	780	129.7
R-4	Complex humic fertilizer N-40 kg/ha; P_2O_5-16 kg/ha; K_2O-20 kg/ha; humic acids-36 kg/ha; foliar fertilization -10 L/ha	86	3000	380	114.5
R-5	Complex N-20 kg/ha; P_2O_5-20 kg/ha; K_2O-20 kg/ha; MgO-10 kg/ha; foliar fertilization -10 L/ha	70	3040	420	116.0
R-6	R-311 (N-54 kg/ha; P_2O_5-18 kg/ha; K_2O-15 kg/ha); foliar fertilization -10 L/ha	97	3020	400	115.2
R-7	R-311 (N-54 kg/ha; P_2O_5-18 kg/ha; K_2O-15 kg/ha); foliar fertilization-10 L/ha; 3% B	97	2820	200	107.6
R-8	R-111 (N-21 kg/ha; P_2O_5-30 kg/ha; K_2O-21 kg/ha); foliar fertilization -10 L/ha	82	2920	300	111.4

Table 2
EXPERIMENTAL CHARACTERISTICS AND RESULTS FOR THE FERTILIZATION OF RAPESEED (DATA REFER TO THE SEED PRODUCTION)

the following characteristics: 100 m length, 0.25 mm inner diameter, 0.2 μm film thickness). The ready for injection solutions were prepared in CH_2Cl_2 of HPLC purity grade. Fatty acids identification was made by comparing for each peak the retention time with those of a standard mixture of 37 fatty acid methyl esters (Supelco™ 37 Component FAME Mix). In the standard mixture the exact concentration of each component is known. Both standard mixture and each of the fatty acid methyl esters of the analyzed oils were chromatographically separated under the same conditions, using the same temperature program, according to Supelco specifications. The calibration of the signals was made by taking into account the exact concentration of each component of the standard mixture, correlated with the detector response.

Results and discussions

Seed production depending on fertilization treatment

The fertilization treatments and the corresponding amounts of active substances are described in table 1 (sunflower) and table 2 (rapeseed). There are also presented data referring to the seed production (kg/ha), seed production increase (kg/ha) and efficiency (%) of the fertilization treatment. Experimental data were provided by the "Gheorghe Ionescu Siseşti" Academy of Agricultural and Forestry Sciences.

It can be noticed from table 1 that the maximum sunflower seed production has been obtained in the case of the Bihumat-3 foliar fertilizer, the seed production increase being 1040 kg/ha, corresponding to an efficiency of 156.5% (indicating a more than 50% production increase).

Regarding rapeseed, the maximum seed production was obtained in the case of the R-3 sample. The seed production increase was 780 kg/ha, corresponding to a treatment efficiency of 129.7% (a production increase of about 30%).

Oil content depending on fertilization treatment

The significant oilseed production increase due to fertilization treatment (more than 50% for sunflower and about 30% for rapeseed) justifies the research on improving

Sample	Active substance (kg/ha)	Oil content (%)	Oil production (kg/ha)	Oil production increase (kg/ha)	Efficiency (%)	Efficiency of active substance (kg oil increase/kg a.s.)
SF-1	-	44.8	824.3	-	100	-
SF-2	94	46.7	1270.2	445.9	154.2	4.7
SF-3	96	47.2	1315.0	490.7	159.6	5.1
SF-4	121	45.5	1140.2	315.9	138.4	2.6
SF-5	132	44.3	1275.8	451.5	154.8	3.4
SF-6	90	43.1	1143.4	319.1	138.8	3.5

Table 3
ANALYSIS OF THE EFFICIENCY OF DIFFERENT FERTILIZATION TREATMENTS APPLIED ON SUNFLOWER (DATA REFER TO THE OIL PRODUCTION)

Sample	Active substance (kg/ha)	Oil content (%)	Oil production (kg/ha)	Oil production increase (kg/ha)	Efficiency (%)	Efficiency of active substance (kg oil increase/kg a.s.)
R-1	-	45.3	1186.9	0.0	100.0	-
R-2	90	45.4	1452.8	265.9	122.4	3.0
R-3	140	43.6	1482.4	295.5	124.9	2.1
R-4	86	43.9	1317.0	130.1	111.0	1.5
R-5	70	46.7	1419.7	232.8	119.6	3.3
R-6	97	44.4	1340.9	154.0	113.0	1.6
R-7	97	45.1	1271.8	84.9	107.2	0.9
R-8	82	43.8	1279.0	92.1	107.8	1.1

Table 4
ANALYSIS OF THE EFFICIENCY OF DIFFERENT FERTILIZATION TREATMENTS APPLIED ON RAPESEED (DATA REFER TO THE OIL PRODUCTION)

the agricultural production. It remains to be established if and how the various agricultural treatments affect seed oil content, as well as the fatty acid composition. In this respect, the oil content of sunflower and rapeseeds (%) has been determined and the oil production (kg/ha), oil production increase (kg/ha) and treatment efficiency (%) have been extrapolated. The results are presented in table 3 (sunflower) and table 4 (rapeseed).

Surprisingly for the sunflower, although the seed crop increase has been maximum in the case of the SF-5 sample, the best results regarding the oil production have been obtained for the SF-3 (absolute oil production increase of 470.7 kg/ha, corresponding to a treatment efficiency of 159.6%. This fact is due to a bigger seed oil content obtained in the case of the SF-3 sample (47.2%) than in the case of the SF-5 sample (44.3%). A rich oil content has also been obtained for the SF-2 sample, but it is associated with a modest seed production increase (880 kg/ha), which leads to the decrease of the efficiency with respect to the oil production.

The efficiency of the fertilization treatments is also judged in relation with the amount of active substances used. From this point of view, the best results were obtained for the same SF-3 sample (when an increase of 5.1 kg oil / kg active substance was obtained).

It can thus be concluded that Fertifam 311-BH_3 fertilizer proved to be the most efficient for the sunflower crop.

Surprisingly in the case of rapeseed, the best results regarding the oil production have been obtained for the R-3 sample (an absolute production increase of 295.5 kg/ha), corresponding to an efficiency of 124.9%. Although the seed oil content of the R-3 sample is modest (43.6%), the efficiency of the fertilization treatment referred to the oil production is due to the fact that a big seed production increase has been obtained. The best values for the oil content (46.7%), as well as for the active substance efficiency (an increase of 3.3 kg of oil/kg of active substance) have been obtained for the R-5 sample, but these associate with a low seed production increase (420 kg/ha), which is an important drawback for the overall efficiency of the fertilization treatment in this case.

Fatty acid profile depending on fertilization treatment

The fatty acid profile of the sunflower and rape seeds has been analyzed by gas-chromatography, the fatty acid composition being shown in table 5 (sunflower) and table 6 (rapeseed).

It can be noticed that sunflower oils have similar compositions with respect to stearic, oleic and linoleic acids. There is no linolenic acid (ω-3 acid) in the sunflower samples; the palmitic acid content varies from 6.94 (SF-1) to 10.12% (SF-5).

The minor occurring fatty acids are arachidic acid (0.18-0.25% in SF-1, SF-2 and SF-3 samples), behenic acid (0.39-5.24% in SF-1, SF-2, SF-3 and SF-4 samples), *cis*-8,11,14-eicosatrienoic acid (0.44% in SF-1 sample) and *cis*-11-eicosenoic acid (0.28% in SF-3 sample).

In the case of the rapeseed oils, the main fatty acids are oleic (52.51-62.73%), linoleic (16.55-19.38%), linolenic (7.67-9.29%) and palmitic (4.81-5.80%). Among the minor fatty acids, it can be noticed the lack of stearic acid in R-7 and the relatively high content (6.94%) in R-6; in the rest of the rapeseed samples, it varies from 1.85 and 2.43%.

The R-6 sample shows also an atypical fatty acid profile with respect to behenic acid: 9.57% in comparison with the rest of the samples, where its presence was detected

No.	Fatty acids	SF-1	SF-2	SF-3	SF-4	SF-5	SF-6
1.	Palmitic acid	6.94	7.37	7.99	9.12	10.12	9.20
2.	Stearic acid	3.24	3.38	3.35	3.24	3.06	3.37
3.	Oleic acid	21.39	21.98	21.72	21.02	21.39	21.67
4.	Linoleic acid	62.5	66.62	66.13	66.23	65.43	65.76
5.	Arachidic acid	0.25	0.18	0.20	0	0	0
6.	Behenic acid	5.24	0.46	0.33	0.39	0	0
7.	*cis*-8,11,14-Eicosatrienoic acid	0.44	0	0	0	0	0
8.	*cis*-11-Eicosenoic acid	0	0	0.28	0	0	0

Table 5
FATTY ACID COMPOSITION OF THE SUNFLOWER OIL SAMPLES (MOLAR %)

No.	Fatty acids	R-1	R-2	R-3	R-4	R-5	R-6	R-7	R-8
1.	Palmitic acid	5.42	5.41	5.36	5.41	5.33	4.81	5.80	5.28
2.	Stearic acid	1.85	2.00	2.43	1.89	1.96	6.94	0	1.93
3.	Oleic acid	59.56	59.51	59.91	61.23	60.39	52.51	62.73	57.25
4.	Linoleic acid	18.97	19.01	19.09	18.13	19.38	16.55	19.04	17.9
5.	Linolenic acid	9.29	8.58	8.69	9.16	8.82	7.67	9.24	8.45
6.	Erucic acid	1.33	1.62	1.68	1.79	1.40	0	0	1.26
7.	Arachidic acid	0.6	0.63	0	0	0.57	0	0	0
8.	Behenic acid	0.68	0	0	0	0	9.57	0	0
9.	*cis*-11-Eicosenoic acid	2.30	3.24	2.85	2.39	2.15	1.94	3.19	2.27
10.	Heptadecanoic acid	0	0	0	0	0	0	0	5.65

Table 6
FATTY ACID COMPOSITION OF THE RAPESEED OIL SAMPLES (MOLAR %)

No.	Parameters	SF-1	SF-2	SF-3	SF-4	SF-5	SF-6
1.	Unsaturated fatty acid content (mass %)	83.8	89.0	88.6	87.8	87.6	88.1
2.	Unsaturated fatty acid production (kg/ha)	691.2	1131.0	1165.5	1001.6	1117.1	1007.4
3.	Unsaturated fatty acid production increase (kg/ha)	-	439.8	474.3	310.4	425.9	316.2
4.	Efficiency related to the unsaturated fatty acid production (%)	100	163.6	168.6	144.9	161.6	145.7
5.	ω-3 Fatty acids (kg/ha)	0	0	0	0	0	0
6.	ω-3/ ω-6 Ratio (mol/mol)	0	0	0	0	0	0

Table 7
QUALITY INDICES OF SUNFLOWER OILS WITH RESPECT TO THE UNSATURATED FATTY ACIDS

No.	Parameters	R-1	R-2	R-3	R-4	R-5	R-6	R-7	R-8
1.	Unsaturated fatty acid content (mass %)	91.7	92.4	92.7	93.2	92.5	77.4	94.2	87.8
2.	Unsaturated fatty acid production (kg/ha)	1088.7	1342.0	1373.9	1227.1	1313.8	1038.5	1198.1	1122.8
3.	Unsaturated fatty acid production increase (kg/ha)	-	253.3	285.2	138.4	225.1	-50.2	109.4	34.1
4.	Efficiency related to the unsaturated fatty acid production (%)	100	123.3	126.2	112.7	120.7	95.4	110.0	103.1
5.	ω-3 Fatty acids (kg/ha)	108.7	122.9	127.1	119.1	123.6	99.8	117.5	107.1
6.	ω-3/ ω-6 Ratio (mol/mol)	0.49	0.45	0.46	0.51	0.46	0.46	0.49	0.50

Table 8
QUALITY INDICES OF RAPESEED OILS WITH RESPECT TO THE UNSATURATED FATTY ACIDS

as traces or it was not detected at all. The *cis*-11-eicosenoic acid is present in all the investigated rapeseed samples, varying from 1.94 to 3.24; an abnormal high heptadecanoic acid content (5.65%) has been found in sample R-8.

Unsaturated fatty acid composition and correlation with total production

A series of oil quality indices have been determined for the sunflower and rapeseed samples with respect to the unsaturated fatty acids: unsaturated fatty acid content (mass %), production, production increase and efficiency of the fertilization treatments (extrapolated to ha),ω-3 fatty acids (kg/ha) and ω-3/ ω-6 ratio (mol/mol). These results are presented in tables 7 (sunflower) and table 8 (rapeseed).

Conclusions

In conclusion, the different fertilization treatments applied to sunflower and rapeseed proved different efficiencies, having different meanings, depending on the aimed feature.

From the sunflower seed production increase point of view, the best efficiency has been obtained for sample SF-5, with a production increase of 1040 kg/ha (156.5%).

From the oil production increase point of view, the best efficiency has been obtained in the case of SF-3, with an oil production increase of 490.7 kg/ha (159.6%).

If samples are examined from a quality index point of view (*e. g.* unsaturated fatty acid content), it can be concluded that sample SF-3 has suffered the most efficient treatment (an increase of 168.6% unsaturated fatty acids).

Other quality indices – such as ω-3 fatty acid content – do not highlight any efficiency of the agro technical treatments.

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