PAPER

INFLUENCE OF FERTILISATION TYPE ON THE QUALITY OF VIRGIN RAPESEED OIL

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ABSTRACT

The aim of the study was to determine whether, and to what the type of fertilisation employed can affect the quality of the harvested spring rape and the oil extracted therefrom through virgin oil pressing. The article presents the results of research conducted with the use of ATR-FTIR absorption spectroscopy of selected cold pressed vegetable oils. The study included three types of rapeseed oil from spring plants grown with traditional NPK mixture fertilisation and digestate. The oxidative stability of the pressed oil was also determined and soil parameters such as: content of absorbable nutrients and acidity in the experimental plots.

Keywords: ART-FTIR spectroscopy, cold pressed oils, fat, fatty acids, fertiliser, oxidative stability

1. INTRODUCTION

With the view of increasing rapeseed yields, numerous artificial fertilisers have been used in recent years to modify the soil content of micro-organisms and drastically alter the general environmental conditions of the soil. This situation has a negative impact on both the sustainable development of soil fertility and, above all, quality of the natural environment (RAMIREZ et al., 2012). Health and environmental concerns force societies to support a revival of traditional methods of food production that are consistent with the guidelines for sustainable agriculture (CETIN and SEVIK, 2016). The trend led to the development of the concept of the so-called eco-efficiency, which entails the use of natural fertilisers produced through natural processing of agricultural waste in agricultural biogas plants (CZEKALA *et al*., 2012; DENNEHY *et al.,* 2016). As observed by MÖLLER and MÜLLER (2012), the use of digestate containing large amounts of macro- and micro-nutrients normally found in the natural environment provides a viable alternative to mineral fertilisers, capable of improving soil fertility and quality of farm produce by providing plants with easily absorbable elements (such as nitrogen, phosphorus and potassium). Depending on the quality and nutritional status, digestate application has been shown to be advantageous in terms of crop yield, plant nutrient uptake (TERHOEVEN-URSELMANS *et al*., 2009; ANDRUSCHKEWITSCH *et al.,* 2013) soil health (VANEECKHAUTE *et al.,* 2013), and enhancement observed in dehydrogenase activity (GARCÍA-SANCHEZ *et al*., 2015).

The technological and nutritional value of rapeseeds is determined by their chemical composition, in particular the content of nutrients and anti-nutrients, which in turn is closely related to the qualities of the plant species itself and primarily defined by genetic factors. Under specific conditions, the nutrient content can, however, vary significantly depending on the cultivar, soil type, fertilisation, weather conditions, and technological processing procedures employed (heating, steaming, autoclaving, etc.) (KRASUCKI *et al.,* 2001). Low quality raw material – containing immature seeds, contaminated, damaged, excessively moist, affected by the processes of fat hydrolysis and oxidation – will produce low quality oil characterised by short shelf life. Consequently, the most valuable oils are those extracted from the freshest, highest quality material in which the oxidative processes are still relatively unadvanced. The process of fat oxidation is the primary cause of diminished quality manifested in the loss of oil's organoleptic and nutritional value (ZIEMLAŃSKI and BUDZYŃSKA-TOPOLOWSKA, 1991; POYATO *et al.,* 2014). The oxidation rate is conditioned by a number of factors, including the content of fatty acids, presence of pro- and antioxidants and, particularly in the case of cold pressed oils, the storage conditions (WRONIAK and ŁUKASIK, 2007).

The growing interest in natural and safe food, including oils and fats, is a testament to its value as a rich source of healthy nutrients, whose consumption facilitates prevention of numerous, particularly digestive diseases and is beneficial to the overall health of organisms (HAREL *et al*., 2002). The seeds of both spring and winter rape are used in the production of cooking oil and provide a key raw material in the production of (increasingly popular and sought-after) biofuels for compression-ignition engines, particularly in Europe (VAN DUREN *et al.,* 2015, ATABANI *et al*., 2013) and certain countries in Africa and Asia, including China (WU and LEUNG, 2011; SHIN *et al*., 2012). It can be safely assumed that Europe is currently the leading global producer of rapeseed (USDA, 2016). Compared to other oils such as sunflower oil, corn oil, sesame oil or olive oil, rapeseed oil is characterised by a particularly rich chemical composition. The high content of fat and protein as well a wide range of fatty acids, including large quantities of

n-3 fatty acids such as α-linolenic acid (C18:3), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid, as well as its n-6/n-3 ratio, make it an attractive offering for various consumer groups. Said acids are very beneficial from the perspective of human health. However, high content of α-linolenic acid, often exceeding 10%, increases the rapeseed oil's susceptibility to autooxidation, thus negatively affecting its oxidative stability (SHAHIDI, 2005; PARRY *et al*., 2005). The presence of lipids, carbohydrates, sterols, aliphatic alcohols, tocopherols and a combination of pigments ensures antioxidative activity and high quality, both in terms of nutritional and sensory value (DAMERAU, 2015; TSAKNIS *et al.*, 1997; SZYDŁOWSKA-CZERNIAK *et al.*, 2013). Notably, as reported in a number of studies comparing cold pressing to other methods of oil extraction, due to the presence of anti-oxidants (carotenoids, tocopherols), as well as other phenolic compounds whose small quantities are introduced into the oil in the process of pressing the seeds, cold pressed oils are characterised by one of the highest values of oxidative stability (CICHOSZ and CZECZOT, 2011).

It is noteworthy that the final quality of both the seeds themselves and the oil extracted therefrom is dependent on a variety of diverse factors, including climate, type of plantation, methods of harvest, storage, preliminary processing and, above all, the process of cold pressing itself.

The increased awareness of the value of oil consumption stimulated the development of better quality products and renewed interest in methods of extracting vegetable oil without the use of chemical reagents, or at least with minimum use thereof. Due to the above, in recent years, we have observed a turn back to the methods of oil extraction involving cold pressing and virgin oil pressing (SZYDŁOWSKA-CZERNIAK *et al.*, 2013) under which, due to the low temperatures under which the process takes place, the extent of degradation of bioactive compounds can be substantially reduced (RAMADAN, 2013). The simplicity of the process of extracting the oils selected for the present study simultaneously allows them to preserve much of their original colour, high nutritional value, high bioactive compounds content, as well as the characteristic taste and smell.

The aim of the study was to: 1. determine and compare selected growing conditions based on soil analysis after fertilisation with a mineral NPK mixture and digestate. 2. analyse the quality of seeds in terms of fat and protein content in spring rape seeds after using fertilisers containing different amounts of nitrogen. 3. analyse selected quality parameters of seeds intended for oil extraction and the quality of the thus obtained virgin rapeseed oil. The scope of the study included: determination of the content of moisture, fats, fatty acids, and protein in the respective seeds, an analysis of the oil extracted therefrom and determination of its oxidative stability. A comparative analysis of ATR-FTIR spectra was employed for the purposes of identifying/highlighting differences in the chemical makeup of the respective oils.

2. MATERIALS AND METHODS

The research material comprised three spring rape cultivars: 'Markus' – 'M', 'Bios' – 'B' and 'Feliks' – 'F' obtained from Hodowla Roślin Strzelce Sp. z o.o. The aforementioned rapeseed cultivars were grown in two combinations on experimental plots of 27 m^2 located in the Lublin Voivodship (51°16'19.8"N 22°24'34.0"E), Poland (Fig. 1). In the first version of the experiment, natural fertiliser (digestate) obtained from the agricultural biogas farm Wikana Bioenergia Sp. z o.o. in Piaski, Lublin Voivodship, was used, while in the second

version, the traditional multi-compound fertilizer Yara NPK 5-14-28 was applied (ammonium nitrogen - 5 %, P- 14% K - 28 %, SO_3 – 12.5%, CaO - 3%). The digestate in the amount of 97 L was distributed in a plot of 27 m^2 (36,000 l/ha). The pH of the digestate used in the cultivation of spring rape was 8.73. The cultivars grown in the control plot – C did not receive any of the aforementioned fertilisers. The products included in the study were obtained from a two-year experimental plot cultivation (2015 and 2016). After harvest, the seeds were cleaned and stored in laboratory conditions for a period of one month in 20°C ambient temperature and 70% ambient humidity to equalise the moisture content. Subsequently, the seeds were cold pressed to extract oil. The oil was then refrigerated for a period of one week in 4°C, in dark bottles, to separate the oil from impurities. Calculations were conducted for triplicate mean values. All the results obtained in the course of the two-year experiment were averaged and tabularised (see tables below).

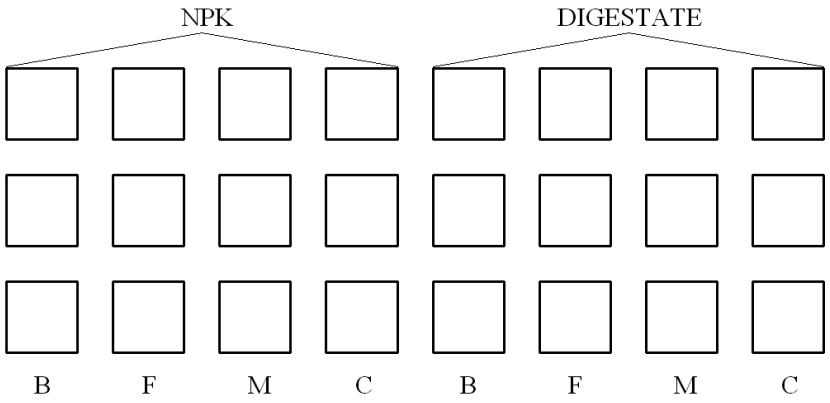

Figure 1. Distribution of experimental spring rape plots.

Weather information relating to the period from March to September 2015 and 2016 was obtained from the Institute of Meteorology and Water Management National Research Institute IMGW-PIB as recorded at the weather station Lublin-RADAWIEC.

The measurements of soil microelement content prior to the application of the selected fertilisers and after the harvest were conducted at the Regional Chemical-Agricultural Station in Lublin. Soil samples were tested for the available nutrient content, in particular phosphorus and potassium, in accordance with the applicable norms with respect to those nutrients, i.e. PN-R-04024:1997, as well as magnesium in mg per 100g od soil, in accordance with PN-ISO 10390:1997. Soil acidity, pH (KCl) and potential liming requirements were also considered.

The digestate content of major nutrients and heavy metals was determined. The laboratory tests were conducted at the Regional Chemical-Agricultural Station in Lublin in accordance with KQ/PB-17-76-77 rev. 04 of 02.07.12. The biogas plant feedstock used in the production of biogas and digestate consisted of: corn silage, whey and plant waste.

Rape seeds were analysed in terms of fat content. The fat content was determined using Soxhlet method in accordance with PN- EN 1163:1999, i.e. by multiple, continuous extraction from pulverised and pre-dried product using an organic extraction solvent, followed by removal of the solvent and determination of the fat content by weight. The

tests were conducted at the Central Agro-Energy Laboratory of the University of Life Sciences in Lublin.
Rapeseeds were tested for fatty acids content in accordance with PM-EN ISO 5509:2001. "*Vegetable and animal fats and oils*" – The analysis of fatty acid methyl esters was performed by way of gas chromatography at the Central Agro-Energy Laboratory of the University of Life Sciences in Lublin.
Rape seeds were analysed in terms of protein content. The protein content was determined using the Kjeldahl method, in accordance with PN – A – 04018/A z 3:2002, i.e. by way of sulphation of organic nitrogen compounds using concentrated sulphuric acid in the presence of a catalyst, and subsequent alkalization of the solution, distillation and titration with sulphuric acid of ammonia bound by boronic acid.
The oil was extracted using a continuous action screw press with exchangeable nozzles, 8 mm in diameter with a set of microscopic sieves, manufactured by Farmet DUO. Before activation, the press was preheated to 60°C ±10°C. Once the press was active and the pressing temperature equalised, the pressing process itself was initiated. Stabilisation was achieved after pressing oil from approximately 1kg of seeds, with the temperature stabilised at approximately 70°C. The pressing temperature was measured using an ama-digit thermometer. After the extraction, the oil was stored in dark-glass bottles at 5°C to achieve natural decantation over a period of 6 days. Afterwards, the oil was ready for laboratory analyses.
The oxidative stability was measured using a Rancimat 670 apparatus (Metrohm AG, Herisau, Switzerland). Oil samples (2.5 g) were weighed into reaction vessels and heated to 120°C under a dry air flow of 20 l h^{-1}. The volatile compounds released during oxidation were collected into a cell containing distilled water, and the increasing water conductivity was continually measured. The time taken to reach the conductivity inflection point was recorded as the induction period (IT) expressed in hours. All determinations were carried out in triplicate. The normal time was calculated using StabNet software, which controlled the Rancimat apparatus.
Infrared absorption spectra were recorded with a Vertex 70 FTIR (Bruker) spectrometer. The attenuated total reflection (ATR) configuration was used with 20 internal reflections of the ZnSe crystal plate (45° cut). Typically, 16 scans were collected, Fourier-transformed, and averaged for each measurement. Absorption spectra at a resolution of one data point per 1 cm^{-1} were obtained in the region between 4000 and 600 cm^{-1}. The instrument was continuously purged with N_2 for 40 min. before and during measurements. The ZnSe crystal plate was cleaned with ultra-pure organic solvents from Sigma-Aldrich. The spectral analysis was performed with Grams/AI software from ThermoGalactic Industries (USA). All experiments were carried out at 25°C.
In the recent years, chemometric methods have played an important role in studies on food quality as well as the identification and quantification of major constituents of foods. The use of chemometric methods combined with spectroscopic techniques has been analysed in previous research. The literature is extensive and details the theoretical aspects of applying such methods as e.g. Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA) or Partial Last Squares Regression (PLS). Examples are the analysis pork fat or lard as a whole of matter extracted from pork (ROHMAN *et al*., 2011), the determination of fat in milk samples (INON *et al*., 2004), analysis of extra virgin olive oil adulterated with palm oil (ROHMAN and CHE MANA, 2010). Principal Component Analysis (PCA) is the most popular mathematical method of reducing data dimensionality with a minimum loss of information. PCA allows us to visualize and interpret data. Another multivariate method with which we can hierarchize the data is

Hierarchical Cluster Analysis (HCA), which searches for objects, which are close together in the variable space. HCA and PCA methods combined with spectroscopic techniques have been successfully used for the purposes of determining food quality as well as identifying and quantifying major constituents of foods.
The objective of the present study was to overview the similarities and differences observed between oil samples extracted through cold pressing and also involved the content of fatty acid composition in rapeseeds.
The results obtained for the selected oil products were analysed statistically by way of mean value and ± standard deviation determination, as wells as post–hoc Tukey's test, with the level of significance of $p < 0.05$, using Statistica 10 software. Furthermore, correlations between the respective variables were evaluated using PCA and HCA. PCA transforms the original, measured variables into new uncorrelated variables. HCA was applied to the data in order to identify similarities between different oils samples. HCA calculates the distances (or correlations) between all oils samples based on Euclidean distance.

3. RESULTS AND DISCUSSION

Table 1 presents the relevant weather conditions including atmospheric pressure, air temperature and rainfall in the years of cultivation, 2015 and 2016, for the period from March (sowing) to August when the crop was collected by way of two-stage harvest. As shown in the relevant data, 2015 was characterised by lower rainfall (10.08 mm H_2O) relative to 2016, when the total rainfall in the analysed period was (12.59 mm H_2O). The mean daily air temperature in 2015 was higher than in 2016, with the exception of April and May, respectively 7.75 and 12.37°C.

Table 1. Weather conditions in 2015 and 2016.

Month	Year 2015			Year 2016		
	Pressure (hPa)	Temperature (°C)	Rainfall (mm H_2O)	Pressure (hPa)	Temperature (°C)	Rainfall (mm H_2O)
3	991.41	4.70	1.31	985.26	3.46	2.04
4	986.86	7.75	1.20	983.69	8.91	1.20
5	986.75	12.37	2.20	985.82	14.44	1.09
6	989.60	26.63	0.63	986.30	18.34	1.78
7	986.81	21.60	1.42	987.12	18.91	4.49
8	990.38	25.15	0.30	990.73	18.07	1.52
9	989.19	14.63	3.02	990.73	15.15	0.47

An important gap given that a significant popularisation of inorganic N fertilizers in the coming decades is expected to occur in developing countries as a consequence of population growth and increasing food demands (FARNWORTH *et al*., 2017). Oil and nitrogen (N) content in soil and rapeseed is an important quality indicator affecting oil yield and protein content of the meal (GRANT *et al*., 2011). Nitrogen content has a significant impact on protein synthesis and must be supplied in adequate amounts to

ensure optimum crop yield. Too much N in the soil can lead to a nutrient imbalance that can restrict protein synthesis and reduce rapeseed growth and seed yield (GRANT *et al*., 2012; LEMKE *et al*., 2009). According to RATHKE et al. (2005), the yield of oilseed rape involves balancing the synthesis of oil and crude protein in the seeds, as well as the energy and carbon dioxide (CO_2) budget of the photosynthetic pool.
Table 2 presents the results of the analysis of major nutrient and heavy metal content in the digestate used for the spring rape cultivation. The same confirm the presence of numerous major nutrients. However, one should not that with respect to primary major nutrients such as: nitrogen, phosphorus and potassium, the levels observed in the applied digestate (respectively: 0.119; 0.12; 5.37 g/L) were significantly lower than those present in industrial fertilisers. ODLARE et al. (2008) reported in his research high content of mineral nutrients (nitrogen, phosphorus, potassium) in fermentation wastewater, while Rehl (2011) observed that in terms of response time digestate was in fact comparable to mineral fertilizers due to that fact that the N, P, and K nutrients were more readily accessible and available for the plants. Based on the subsequent analyses of the obtained results it can be reported that the tested digestate samples contained virtually no heavy metals, while the overall high major nutrient content supported the usability of digestate as a potential alternative fertiliser. Furthermore, COMPARETTI (2013) and KOUŘIMSKÁ *et al*. (2012) observed that digestate contains organic matter that has a significant positive influence on the physicochemical quality of the fertilised soil.

Table 2. Content of major nutrients and selected heavy metals in digestate used for cultivation of spring rape.

Element	Unit	Content
Nitrogen	(g/l)	0.119
Phosphorus	(g/l)	0.12
Potassium	(g/l)	5.37
Calcium	(g/l)	0.28
Magnesium	(g/l)	0.07
Cadmium	(mg/l)	<0.43
Lead	(mg/l)	<0.43
Nickel	(mg/l)	<0.43
Chromium	(mg/l)	<0.43
Copper	(mg/l)	0.43
Zinc	(mg/l)	2.00
Manganese	(mg/l)	2.26
Iron	(mg/l)	70.82

Table 3 presents the averaged results obtained in the course of the two-year study in terms of major nutrient content in the soil prior to an after the application of selected fertilisers for the purposes of spring rape cultivation. Based on the obtained, data it was observed that the pH of the soil in both cases remained relatively stable and did not change significantly after the sowing. With respect to nutrient content in plants relative to the levels or phosphorus, potassium and magnesium, the values varied significantly in the samples selected for the study. In all cases, the content of said nutrients was significantly

higher in the fertilised plots when compared to the control plots, both prior to sowing and after the harvest.
The analysis of soil content after the harvest revealed similar values for both experimental variants, which in both cases remained higher than those observed for the control, and were e.g.: 64.5 mg /100g of phosphorus in the plot fertilised with digestate compared to 25.1 mg /100g in the control plot. In the case of potassium, its concentration in the control plot was 20.04 mg/100g, while in the digestate plot it was 60.05 mg/100g. Data related to magnesium content analyses returned very similar results, i.e. 9.6 mg/100g in control plots and 21.8 mg/100g in lots fertilised with digestate. Given the above, the primary agricultural use of digestate, given its physical and chemical properties, is biofertilisation. Such usability of fermentation wastewater was also suggested, based on obtained research results, by KOUŘIMSKÁ *et al*., (2012), EICKENSCHEIDT *et al*., (2014), VÁZQUEZ-ROWE *et al*., (2015). KOUŘIMSKÁ *et al*., (2012) reported that digestate used in fertilisation improved soil fertility, as well as quality of the crop and the plants' resistance to biotic and abiotic factors.
The statistical analysis using the post-hoc Tukey test at the significance level of $p<0.05$ also revealed statistically significant differences in the content of the respective nutrients between the control plots and the experimental fertilised plots. Statistically significant differences were also observed between the content of the analysed nutrients in the soil prior to sowing and after the harvest, with the exception of control plots relate to the use of traditional NPK fertilisation.

Table 3. Content of available nutrients and soil acidity.

Experimental variant	Plot type	Acidity		Liming needed	Content of available nutrients (mg 100/g)		
		pH in KCL	reaction		Phosphorus P_2O_3	Potassium K_2O	Magnesium Mg
Prior to sowing							
Digestate	Control	6.97	neutral	no	23.8a	16.3a	10.3a
	Exp. plot	7.22	alkaline	no	42.17b	53.17b	13.75b
NPK fertilizer	Control	7.06	alkaline	no	21.1c	15.7a	9.5a
	Exp. plot	5.51	acidic	yes	23.57a	20.55c	14.05b
After harvest							
Digestate	Control	6,99	neutral	no	25.1d	20.04c	9.6a
	Exp. plot	7.52	alkaline	no	64.5e	60.5e	21.8c
NPK fertilizer	Control	7.06	alkaline	no	21.6c	16.7a	9.55a
	Exp. plot	5.37	acidic	yes	26.5d	23.3d	17.2b

a, b, c, d, e – statistically significant cultivar differences relative to the control; mean values marked with the same letter are not statistically significantly different ($p > 0.05$).

The yield obtained from spring rape plants in 2015/2016 was below expectations and the unfavourable weather conditions observed in the summer in both years resulted in yield discrepancies. It is assumed that the correct moisture content of rapeseed suitable for further storage ought to be between 5 and 9%, with 10% being within the admissible range in some countries, e.g. Canada (GAWRYSIAK-WITULSKA *et al*., 2012). Table 4 presents

the values of initial moisture during harvest, moisture reported during analyses, as well as fat and protein content of the rapeseeds. The initial moisture content during harvest was between 8.3 and 10% dry mass, respectively for all the samples. During storage, the value dropped on average by 1% relative to the initial moisture content, which rendered it acceptable by oil and fat industry standards, i.e. the harvested seeds would be accepted for further potential storage.

The tests conducted with the aim of determining the fat content in seeds revealed that this particular characteristic was primarily dependent on genetic conditions. Seeds of spring rape are characterised by slightly lower fat and protein content (respectively 48.6% and 24%) compared to winter rapeseed (WARMIŃSKI *et al*., 2001). In our study, the protein concentration wasn't strongly affected by the application of N, regardless of the fertilizer used. Since N is a major structural component of protein, increasing N supply frequently leads to an increase in protein concentration (MALHI and GILL, 2007). In both experimental cases, the fat content turned out to be fairly similar, varying between 40.23 and 42.57% for digestate cultivations, with the lowest value recorded for 'Feliks' control, and between 41.33 and 42.53% for 'Feliks' control cultivar on traditional NPK fertiliser.

The protein content is undoubtedly an important parameter influencing the usefulness of various oils, which is why its measurement is necessary when determining the potential usability of the fat-free leftover material found in rapeseeds as e.g. raw material in the production of dietary protein supplements (GAWRYSIAK-WITULSKA *et al.*, 2012). The analysis of protein content revealed that the same was slightly higher in seeds cultivated using natural fertiliser (digestate) and was 23.30% (for 'Bios' cultivar) and between 23.23% and 22.47% (for 'Feliks' cultivar). We concluded that the application of digestate had a subtle but noticeable influence on yield and quality compared to mineral fertilization. The obtained results in terms of fat and protein content were consistent with values reported by other authors (MURAWA and WARMIŃSKI, 2005). In the research of RATHKE *et al*. (2005) analyzing the effect of applying cattle manure, it was observed that seed yield of winter oilseed rape tended to increase with increasing N fertilization rate, while the oil content in the seeds declined. CHEEMA *et al.*, (2001) and MASON and BRENNAN, (1998) reported the negative influence of N fertilization on the oil (fat) content in seeds.

The statistical post-hoc Tukey test conducted at the significance level of $p<0.05$ revealed no statistically significant differences in terms of the protein content between the respective rapeseed cultivation variants. At the same time, such significant differences were observed for the fat content. MENSINK and KATAN (1990) say that high fatty acids content in oils is desirable because of their health benefits. The fatty acid content varies significantly between vegetable oils obtained from different sources, particularly depending on the actual plant cultivar used and plant maturity. Other factors with significant influence include: the region in which the plants are grown and specific climatic conditions (MURKOVIC *et al*., 1996).

Given the above, Table 4 presents the primary fatty acids content in spring rapeseeds during the first stage of storage treated as the point of reference for the subsequent extracted oil measurements. The fatty acid content in oils was determined for specific rape cultivars (as reflected by the obtained measurement results) and returned the approximate percentages of 60% oleic acid, 21% linoleic acid, and 10% linolenic acid (KRYGIER, 1997). Comparable results were obtained in the cultivation and harvest of winter rape, where the highest reported content of MUFAs and PUFAs was respectively 68.33% and 34.55% (TYS *et al*., 2006). In terms of the analysed seeds, the fatty acid content was within the range approved by the CODEX ALIMENTARIUS (2011).

Table 4. Moisture, fat and protein content in seeds.

Experiment	Cultivar	Initial moisture (%)	Moisture content (%)	Fat content (% dry mass)	Protein content (% dry mass)
Control	'Bios'	10.00±0.06aA	9.93±0.06aA	42.57±0.15aA	23.35±0.35aA
	'Feliks'	9.50±0.06aA	8.80±0.10bB	40.23±0.85cB	22.47±0.21aA
	'Markus'	9.90±0.08aA	8.93±0.21bA	40.73±0.64cB	23.37±0.15aB
Digestate	'Bios'	9.20±0.05bA[a]	8,17±0.06aA[a]	41.37±0.12bA[a]	23.30±0.10aA[a]
	'Feliks'	8.30±0.06bA[a]	7.27±0.06aA[a]	41.67±0.38bA[a]	23.23±0.06aA[a]
	'Markus'	9.03±0.07bA[a]	7.97±0.12aA[a]	41.97±0.06bA[a]	22.83±0.21aA[a]
NPK fertilizer	'Bios'	8.75±0.05bA[a]	7.97±0.06aA[a]	41.80±0.10aA[a]	22.73±0.06aA[a]
	'Feliks'	8.60±0.05bA[a]	7.57±0.06aA[a]	42.00±0.44bA[a]	23.57±0.31aA[a]
	'Markus'	8.95±0.09bA[a]	7.67±0.06aA[a]	41.33±0.06bA[a]	22.37±0.40aA[a]

a, b, c - statistically significant cultivar differences relative to the control;
A, B - statistically significant differences between cultivars under the same field experiment;
[a, b,] - statistically significant differences between the respective fertilizer type experiments relative to the same parameters; mean values marked with the same letter are not statistically significantly different ($p > 0.05$).

Based on the obtained results (Table 5) it can be concluded that in the case of seeds grown with the use of digestate, the content of oleic acid (C18:1) and linoleic acid (α-C18:3) was lower in the control than in the "ecologically" grown samples of 'Bios', 'Markus' and 'Feliks' cultivars. An analogous situation was also observed in seeds cultivated using traditional NPK fertilisation. The statistical Tuckey's post-hoc analysis revealed statistically significant differences in terms of acid content between the cultivars and their respective control, in terms of C18:1n9c for 'Bios' and 'Feliks', in terms of C18:2n6c (only for 'Feliks'); and in terms of C18:3n3 again for 'Bios' and 'Feliks', where the experimental plots were cultivated on digestate. Statistical differences were observed in terms of the C18:1n9c content between particular seed cultivars from the cultivation fertilized traditionally with NPK, specifically between 'Bios' seeds on the one hand, and 'Feliks' and 'Markus' seeds on the other. Differences were also observed in terms of C18:3n3 acid between seed cultivars grown on digestate, specifically between Bios' seeds on the one hand, and 'Feliks' and 'Markus' seeds on the other.
In terms of SFAs in the 'Bios' cultivar, the highest values were observed in the control, where it reached 7.85%, compared to 7.53% for traditionally fertilised crops. In terms of the other two cultivars, the highest Saturated Fatty Acids content was observed in the digestate cultivations 7.99% followed by the control 7.76% ('Feliks') and 7.76% for traditional fertilisation ('Markus'). The lowest content of those fatty acids was observed in 'Markus' seeds grown on digestate: 7.55%.
A very similar situation could be observed with respect to Monounsaturated Fatty Acids. After analysing the results obtained for Omega 3 acids, it can be concluded that the same fluctuated between 6.22 and 7.64%, with the lowest values observed for Feliks seeds both in the control (6.90%) and the experimental cultivations, respectively 6.22% for digestate and 6.31% for NPK. The highest content of the same was observed for 'bios' seeds cultivated using the biofertilizer (7.64%).
The content of Omega 6 acids in the seeds of analysed cultivars varied between 17.90 and 20.98%. The highest values were observed for the control 'Feliks' cultivar (20.48%) and the lowest for 'Markus' at 17.90% (on traditional fertiliser).

The statistical analysis using Tukey's post-hoc test at the level of significance of $p<0.05$ revealed clear statistical difference in the content of MUFAs between the control plots and plots fertilised with digestate for the 'Bios' cultivar, as well as between the control plots and experimental plots fertilised with either digestate of NPK mixture for the 'Feliks' cultivar. The statistical analysis also revealed significant differences in terms of the content of MUFA acids in the analysed seeds, specifically for 'Bios' vs. 'Feliks' and 'Markus' grown on digestate, and between 'Bios' and 'Markus' in the NPK cultivation.
In terms of PUFAs, statistical differences were also observed for the 'Bios' and 'Feliks' cultivars relative to seeds obtained from their respective control plots. The statistical analysis revealed the existence of certain differences between the respective analysed cultivar types. Significant differences in the content of PUFAs were observed in the control cultivation between 'Bios' and 'Markus' seeds on the one hand and 'Feliks' seeds on the other. Moreover, Discrepancies were observed between the three cultivars grown on digestate as well as between 'Bios' on the one hand and 'Markus' and 'Feliks' on the other in the NPK cultivation.
Statistical differences were also observed in the content of Omega 6 acids for the 'Feliks' cultivar when comparing the control plots to the experimental plots, regardless of the fertilisation method. Furthermore, significant differences in terms of their levels were noted in the control between 'Bios' and 'Markus' seeds on the one hand and 'Feliks' seeds on the other.
Simultaneously, the statistical analysis revealed no statistical differences in terms of the content of Omega 3 acids between the experimental cultivations and the control. Differences of borderline statistical significance were observed between particular cultivars fertilized with digestate, specifically between 'Bios' seeds on the one hand and 'Feliks' and 'Markus' on the other.
According to JELEŃ *et al.* (2000) major components of vegetable oils are fatty acids, both saturated and unsaturated, mainly bound to glycerol as triacylglycerols. The allyl groups in unsaturated fatty acids are highly susceptible to free-radical reactions. DE LEONARDIS and MACCIOLA (2012) reported that the influence of fatty acid composition on oil stability has been varied between respective cases. In general, oils that are more unsaturated oxidise more readily than their less unsaturated counterparts. In the presence of oxygen, unsaturated fatty acids undergo decomposition even at low temperatures. The process of lipid oxidation results in deterioration of food quality, which can manifest itself through unpleasant odour leading consumers to reject the product (SHAHIDI, 2005). It is the decomposition of hydroperoxides that constitute the main non-volatile compounds directly involved in the decomposition of volatile compounds, such as alkenes, aldehydes, alcohols, esters, acids and carbohydrates, that deteriorates the taste of food and is responsible for the rancid smell of soap. Moreover, the oxidative degradation of lipids can damage biological membranes and protein, and as such can pose a direct threat to human life (MALHEIRO *et al*., 2013).

Table 5. Fatty acid content of the rapeseeds.

Specification (%)	Rapeseeds								
	Control ‚B'	'B'- digestate	'B' – NPK fertilizer	Control 'F'	‚F' - digestate	'F' – NPK fertilizer	Control 'M'	'M' - digestate	'M' – NPK fertilizer
C14:0	0.07±0.02 aA[a]	0.08±0.02aA[a]	0.07±0.02aA[a]	0.09±0.02aA[a]	0.08±0.02aA[a]	0.09±0.02aA[a]	0.09±0.02aA[a]	0.08±0.02aA[a]	0.07±0.02aA[a]
C16:0	4.47±0.71aA[a]	4.24±0.68aA[a]	4.26±0.68aA[a]	4.74±0.76aA[a]	4.66±0.74aA[a]	4.52±0.72aA[a]	4.39±0.70aA[a]	4.46±0.71aA[a]	4.39±0.70aA[a]
C16:1	0.27±0.01aA[a]	0.25aA[a]	0.28aA[a]	0.31±0.01aA[a]	0.30±0.01aA[a]	0.28aA[a]	0.27±0.01aA[a]	0.27aA[a]	0.26aA[a]
C17:0	0.05±0.01aA[a]	-	-	0.05±0.01aA[a]	-	-	0.05±0.01aA[a]	-	-
C17:1	0.07±0,01aA[a]	0.10±0.01aA[a]	0.08aA[a]	0.08±0.01aA[a]	0.08±0.01aA[a]	0.08aA[a]	0.09±0.01aA[a]	0.07aA[a]	0.07±0.01aA[a]
C18:0	2.05±0.21aA[a]	1.99±0.21aA[a]	2.01±0.21aA[a]	1.71±0.18aA[a]	2.04±0.21aA[a]	2.08±0.22aA[a]	1.91±0.20aA[a]	1.87±0.19aA[a]	2.11±0.22aA[a]
C18:1n9c +C18:1n9t	65.01±0.60aA[a]	63.81±0.48bB[a]	64.24±0.52aA[a]	62.41±0.33cB[b]	65.76±0.67aC[a]	65.56±0.65aC[a]	64.60±0.56aA[a]	64.69±0.57aB[a]	65.24±0.62aC[a]
C18:2n6c+C18:2n6t	18.19±0.57aA[a]	18.74±0.74aA[a]	18.52±0.67aA a	20.48±0.27aB[b]	17.99±0.51aA[a]	18.04±0.53aA[a]	18.49±0.66aA[a]	18.78±0.75aA[a]	17.90±0.48aA[a]
C18:3n6 (gamma)	-	-	-	-	-	-	-	-	-
C18:3n3 (alpha)	6.72±0.19aA[a]	7.64±0.22aA[a]	7.14±0.20aA[a]	6.90±0.20aA[a]	6.22±0.18aB[a]	6.31±0.18aA[a]	7.17±0.20aA[a]	6.38±0.18aB[a]	6.50±0.18aA[a]
C20:0	0.69±0.06aA[a]	0.68±0.06aA[a]	0.67±0.06aA[a]	0.63±0.06aA[a]	0.69±0.06aA[a]	0.72±0.06aA[a]	0.64±0.06aA[a]	0.65±0.06aA[a]	0.72±0.06aA[a]
C20:5	-	-	-	-	-	-	-	-	-
C20:1	1.50±0.23aA[a]	1.54±0.23aA[a]	1.65±0.25aA[a]	1.66±0.25aA[a]	1.38±0.21aA[a]	1.44±0.22aA[a]	1.45±0.22aA[a]	1.65±0.25aA[a]	1.77±0.27aA[a]
C22:0	0.35±0.03aA[a]	0.34±0.03aA[a]	0.35±0.03aA[a]	0.37±0.03aA[a]	0.36±0.03aA[a]	0.37±0.03aA[a]	0.33±0.03aA[a]	0.35±0.03aA[a]	0.33±0.03aA[a]
C22:2	-	-	-	-	-	-	-	-	-
C24:1	0.17±0.03aA[a]	0.14aAa	0.15aA[a]	0.19±0.01aA[a]	0.14aA[a]	0.14aA[a]	0.14±0.03aA[a]	0.16±0.03aA[a]	0.10aA[a]
SFA	7.85aA[a]	7.49aA[a]	7.52aA[a]	7.76aA[a]	7.99aA[a]	7.95aA[a]	7.56aA[a]	7.55aA[a]	7.76aA[a]
MUFA	67.13aA[a]	65.98bA[a]	66.68aA[a]	64.71bB[b]	67.66aB[a]	67.49aA[a]	66.67aA[a]	67.15aB[a]	67.74aB[a]
PUFA	25.10aA[a]	26.60bA[a]	26.03bA[a]	27.53aB[b]	24.27bB[a]	24.41bB[a]	25.87aA[a]	25.53aC[a]	24.70aB[a]
OMEGA 3	6.72aA[a]	7.64aA[a]	7.14aA[a]	6.90aA[a]	6.22aB[a]	6.31aB[a]	7.17aA[a]	6.38aB[a]	6.50aA[a]
OMEGA 6	18.19aA[a]	18.74aA[a]	18.52aA[a]	20.48bB[b]	17.99aA[a]	18.04aA[a]	18.49aA[a]	18.78aA[a]	17.90aA[a]

a, b - statistically significant cultivar differences relative to the control; A, B - statistically significant differences between cultivars under the same field experiment; [a, b,] - statistically significant differences between the respective fertilizer type experiments relative to the same parameters; mean values marked with the same letter are not statistically significantly different ($p > 0.05$).

The oxidative stability of the analysed oil samples was determined using the Rancimat method. Said stability is affected by a number of factors, including above all: the content of fatty acids, antioxidants and a whole range of other minor compounds. Table 6 presents the results of analyses performed in relation to the oxidative stability of the studied oils. In the case of oils extracted from plants cultivated on digestate, the shortest induction time was observed for the 'Bios' cultivar and was 5.21 h, while in the case of the respective control the same was three hours longer (8.31 h). The longest period of oxidative stability was recorded for the 'Feliks' oil, specifically 8.17 h, the same result as that observed in the respective control – 8.17 h. The results of measurements conducted for oil extracted from seeds cultivated using NPK and digestate fertilisation revealed relatively high oxidative stability compared to that reported by other authors. In a study by CICHOSZ and CZECZOT (2011), the induction time of oxidative processes (Rancimat test) in pressed and refined rapeseed oil was respectively: 4.5 and 4.7 h. Results obtained by WRONIAK and ŁUKASIK (2007) with respect to the oxidative stability of cold-pressed rapeseed oils with various antioxidative additives varied between 3.76 and 4.38 h.

In the case of oils obtained from traditionally fertilised cultivations, the longest induction time was observed for control 'Markus' oil – 9.30 h, and the shortest for 'Bios' oil – 8.41. In terms of oxidative stability of oils analysed relative to normal time, a discrepancy was observed for 'Bios' oil obtained from seeds grown on digestate, NPK mixture and the respective control, where it was 24.4 h (control), 20.96 h (digestate) and 22.02 h (NPK). A similar situation was recorded for 'Markus' oil (Tale 6).

The conducted statistical analysis using Tukey's post-hoc test for the significance level of $p<0.05$ also revealed slight statistical induction time differences between 'Bios' grown on digestate and the respective control plants. In the case of the 'Markus' cultivar, slight statistical differences were observed between the control and oil from the NPK plots. The results of the statistical analysis also indicated the presence of significant differences between the respective cultivars from particular field experiments. Seeds grown on digestate showed differences in terms of the time of oil induction, specifically between 'Bios' seeds on the one hand and 'Markus' and 'Feliks' seeds on the other. Significant differences were also observed between the fertilizer types for the respective rape cultivars. Specifically, for 'Bios' seeds in the case of the control and 'Bios' and 'Markus' seeds grown on digestate and NPK.

In the discussed case it should be noted that the seeds were subjected to no heating processes prior to extraction. The obtained oxidative stability values were considerably higher than those reported in other studies (4.35 or 4.39h) (KONDRATOWICZ-PIETRUSZKA, 2014; CIEMNIEWSKA-ŻYTKIEWICZ *et al.*, 2014).

Fig. 2 presents the ATR-FTIR spectra for selected oil samples. For easier interpretation of the results, Table 7 provides a breakdown of all characteristic bands (maximums) for selected samples together with the corresponding vibration of the respective functional groups. Samples were placed on a ZnSe crystal under N_2 atmosphere (as described above in Materials and Methods).

Spectra in the infrared region (ATR-FTIR) have well resolved bands that can be assigned to functional groups of particular components of food and biodiesel. Edible fats and oils as well as some biodiesel substances consist basically of triglycerides groups with different substitution patterns, which are mainly differentiated by the degree and form of unsaturation of the acyl groups and their length (GUILLEN and COBO, 1997; HONG *et al.*, 2005; SCHOLZE and MEIER, 2005; ROHMAN and CHE MAN, 2010; Van de VOORT, 1992). Fig. 2 shows the infrared spectrum of selected oil samples chosen for the spectroscopic research.

Table 6. Analysis of the induction time for oils.

Parameter		Experiment	Cultivar	Measurements
Oxidative Stability (h)	Induction time	Control	'Bios'	8.31±0.02aA[a]
	Normal time			24.40±0.53aA[a]
	Induction time		'Markus'	8.58±0.55aA[a]
	Normal time			21.39±0.46aB[a]
	Induction time		'Feliks'	8.17±0.04aA[a]
	Normal time			21.11±0.93aB[a]
Oxidative Stability (h)	Induction time	Digestate	'Bios'	5.21±0.13bA[b]
	Normal time			20.96±0.02bA[b]
	Induction time		'Markus'	7.71±0.21aB[a]
	Normal time			21.42±0.11aA[a]
	Induction time		'Feliks'	8.17±0.05aB[a]
	Normal time			21.66±0.38 aA[a]
Oxidative Stability (h)	Induction time	NPK fertilizer	'Bios'	8.41±0.07aA[a]
	Normal time			22.02±0.23bA[c]
	Induction time		'Markus'	9.30±0.08cA[b]
	Normal time			23.89±0.65aB[a]
	Induction time		'Feliks'	8.51±0.18aA[a]
	Normal time			21.77±0.77aB[a]

a, b, c - statistically significant cultivar differences relative to the control;
A, B - statistically significant differences between cultivars under the same field experiment;
[a, b, c] - statistically significant differences between the respective fertilizer type experiments relative to the same parameters; mean values marked with the same letter are not statistically significantly different ($p > 0.05$).

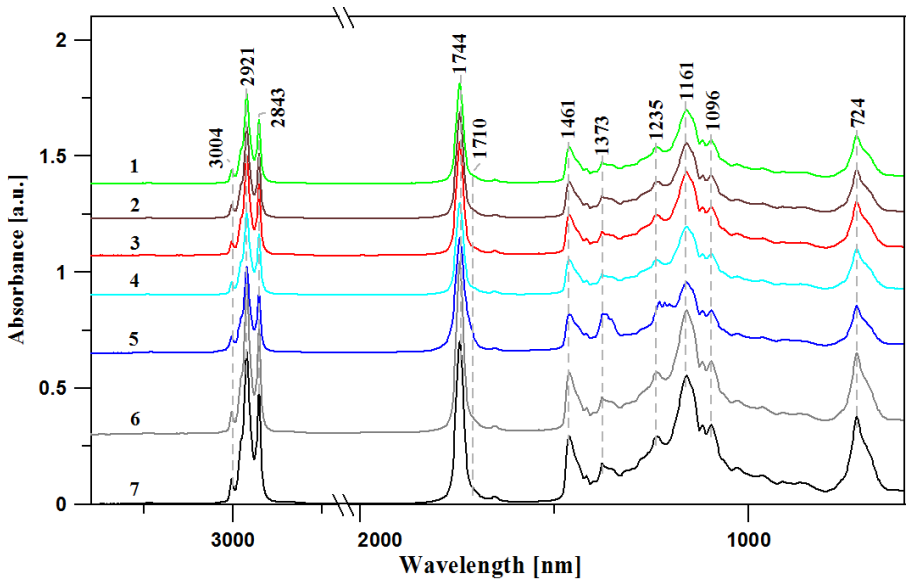

Figure 2. ATR-FTIR spectra for selected rapeseed oil samples for the following cultivars: 'Bios', 'Feliks' and 'Markus', respectively, 'Bios' NPK– (7) solid black line, 'Bios' digestate – (6) solid grey line, 'Feliks' NPK – (5) solid blue line, Feliks digestate – (4) solid light-blue line and 'Markus' NPK – (3) solid red line, 'Markus' digestate – (2) solid brown line. The spectra are presented in the spectral range of 600 – 3800/cm.

Although some authors have suggested specific assignments of spectral bands in oils and fats (GUILLEN and COBO, 1997; LI *et al.*, 2013; QIAN *et al.*, 2007), there are some, which are notoriously difficult to decidedly assign to a specific group. Table 7 shows the frequencies of the characteristic bands or shoulders in the spectra of 7 oil samples (GUILLEN and COBO, 1997), as well as their assignment to respective functional groups (GUILLEN and COBO, 1997; YANG *et al.*, 2005; HONG *et al.*, 2005; SCHOLZE and MEIER, 2005; ROHMAN and CHE MAN, 2010), their vibration mode and intensity in spectra typical for the IR region. It should be noted that the assignment of bands corresponding to the stretching vibration in FTIR modes is usually easier than the assignment of bands corresponding to the bending vibration modes due to overlap or vibration of this group. Thus, in the absorption spectra of our oil samples, we can observe that some bending vibrations of the methylene group are produced between 1350 and 1150/ cm. This stretching vibration originates from –C-H vibrations in $–CH_3$ (~1350/cm) groups and deformation vibrations in this group (~1150 cm^{-1}).

On the other hand, stretching vibrations of the C-O bond of esters are composed of two coupled asymmetric vibrations, C-C(=O)-O and O-C-C, the first being more important (GUILLEN and CABO, 1997; HONG *et al.*, 2005; ROHMAN and CHE MAN, 2010); these bands occur in the region between 1300 (some C-C(=O)-O) and 1000/cm (of this combination's group). The C-C(=O)-O band of saturated esters appears between 1240 and 1163/cm, and in unsaturated esters the vibration is produced at lower frequencies. On the other hand, the O-C-O band of esters derived from primary alcohols appears in the zone between 1064 and 1031/cm, while for those derived from secondary alcohols the band appears approximately at 1100/cm (ROHMAN and CHE MAN, 2010; LI *et al.*, 2013). Both kinds of esters are present in triglyceride molecules. However, some authors assign the band at about 1238/7 /cm exclusively to out-of-plane bending vibrations of the methylene group (GUILLEN and CABO, 1997; LI *et al.*, 2013; QIAN *et al.*, 2007). Two bands in Table 7 have been assigned in a different way: the bands at approximately 1400/cm and 1319/cm. The first band, at about 1400/cm, has been assigned by some authors to terminal methyl groups on the aliphatic chains of oil components (GURDENIZ and OZEN, 2009). The second band, at about 1319/cm, was observed in all samples where absorption reached between 967 and 914/cm. In this context, it must be pointed out that the band at about 914/cm, which appears in all oil samples, has been related to the bending vibration of *cis*-disubstituted olefinic groups (GUILLEN and CABO, 1997; QIAN *et al.*, 2007) and to vinyl groups. Although the oil spectra examined in this articles seem to be similar, they show differences in the intensity of their bands as well as in the exact frequency at which the maximum absorbance is produced in each case, due to the different nature and composition of the respective oil samples under study. Further characteristic areas of vibration are found in the bands with the maximum of approximately 1740/cm, typically associated with vibrations of the C=O carbonyl group in esters (HONG *et al.*, 2005; SCHOLZE and MEIER, 2005). At the same time, a very weak band, or more specifically extension of the band with the maximum at approximately 1705/cm, reveals very weak vibrations of the carbonyl group in acids. In turn, bands with the maximum at approximately 1650/cm correspond to vibrations originating from stretching vibrations of the –C=C- group (due to *cis*- transformation). A characteristic area is also found in bands with the maximum of approximately 1460/cm originating from deformation vibrations in the -C-H groups of CH_2 and CH_3 (bending (scissoring)). The subsequent area of bands from 890 to 660/cm represents the characteristic deformation vibrations of -HC=CH- groups (*cis*- conformation, extra plain) and ring vibrations of the groups (δ(-(CH2)n- and –

HC=CH- (cis-)) (GUILLEN and CABO, 1997; SCHOLZE and MEIER, 2005; LI *et al.*, 2013; QIAN *et al.*, 2007).
As we progress to vibrations within higher wavenumber ranges, one should mention the significant stretching vibrations =C-H (*trans*-) with the maximum at approximately 3060/cm which originate from the vibrations of triglyceride fractions. A very characteristic feature of stretching =C-H vibrations in the *cis*- configuration are the relatively intensive vibrations at approximately 3006/7 /cm. Next, a number of vibrations with the maxima at approximately 2955, 2920 and 2855/cm originate from the stretching vibrations of–C-H in the respective -CH_3, CH_2 groups belonging to the aliphatic groups in triglycerides (HONG *et al.*, 2005; SCHOLZE and MEIER, 2005; QIAN *et al.*, 2007). Characteristic vibrations associated with specific functional groups were described in detail for all studied samples in Table 7.
Furthermore, it is noteworthy that in the spectra of the studied samples of oil extracted from rape grown on digestate and traditional fertilizer mixture (Fig. 2), there are clearly visible differences in terms of the shape of spectra in the 1770 – 1670/cm area. In most of the studied samples there is apparent, slight amplification of the band at 1742/3/cm (responsible for vibrations of the C=O group) on the side of lower wave numbers, with the clearly defined extreme at approximately 1710/cm (with the relatively lowest intensity in the control spectrum), which can be associated with the formation of a hydrogen bond between the C=O···H-O-H groups in oil samples selected for the study. Simultaneously to the appearance of the band at 1710/cm (under both fertilisation regimens), there is a clear increase in the intensity of bands at approximately 1360, 700/cm, which is associated to stretching vibrations in C-O and C-C groups (as described above).
The area between 1100 – 1300/cm is also responsible for the stretching vibrations of the C-O group, but the same varied only slightly between the studied oil samples, regardless of their origin (i.e. cultivation with the use of digestate or traditional fertiliser) (LI *et al.*, 2013; QIAN *et al.*, 2007).
With the reducing affinity of the constituent molecules for the formation of the hydrogen bond between C=O···H-O-H, the bands show a slight increase in intensity. A similar correlation can be observed both in rapeseed oil samples originating from traditionally fertilised plots and from plots fertilised with digestate.
Moreover, it would be prudent to once again mention bands from the range of 2960 to 2840/cm, which are responsible for the symmetrical and asymmetrical vibrations of CH_2 and CH_3 groups in the aliphatic sections of triglycerides. Their rates vary significantly depending on the origin of the respective samples, i.e. from rapeseed grown in plots fertilised traditionally or with the use of digestate. The variations observed in this area are very well correlated with variations in the fatty acids profile presented in Table 5. The aforementioned spectral changes also confirm the slight discrepancies in the overall fat content in the studied oil samples, as presented in Table 4 above. One should also point out the clear discrepancies in terms of band intensity between the particular rapeseed cultivations, relative to the method of fertilisation. In all spectrum-wide band intensity measurements the same was clearly lower in in the case of plots fertilised with digestate as compared to traditional fertilisation. With regard to a comparison between specific cultivars, the most intensive bands were observed for 'Bios', followed by 'Markus', with the lowest values observed for 'Feliks'. The described differences correspond to the respective overall nutrient content in the studied samples of rapeseed oil. The main band differences for all samples, relative to the method of fertilisation, can be observed in bands with the respective maxima at 1745, 1709, 1368, 1220, 1154 and 1146/cm, which confirms the discrepancies in the overall fatty acids profile of the analysed samples.

Table 7. Position of the maxima of absorption spectra in relation to the respective vibrations for selected spring rapeseeds grown with the use of traditional and digestate fertilisation, within the spectral range of 3800-550/cm.

FTIR Position of the maximum (/cm)									Types and origin of vibration
NPK fertilizer			Digestate			Control			
'B'	'F'	'M'	'B'	'F'	'M'	'B'	'F'	'M'	
–	3463	3476	3476	3474	3476	3474	3476	3474	-C=O_w (overtone)
3005	3066	3064	3063	3061	3066	3067	3181	–	and v(=C-H_{vw}, *trans*-)
–	3006	3007	3007	3007	3007	3006	3059/ 3007	3062/ 3007	v(=C-H_m, *cis*-)
–	2969	2954	2954	2953	2955	2954	–	–	v_{as}(-C-H_m, -CH_3)
–	2955	2923	2922	2921	2922	2922	2953	2956	v_{as}(-C-H_{vst}, -CH_a)
2922	2923	–	–	–	–	–	2922	2923	and
2854	2871/ 2853	2853	2853/ 2872	2853/ 2870	2853/ 2872	2852/ 2872	2854	2852	v_s(-C-H_{vst}, -CH_a) (aliphatic groups in triglycerides)
2731	2731	–	2731	2730	–	2729	2730	2729	
2676	2677	2676	2635/ 2680	2675	2674	2678	2678	2679	
–	2584	–	–	–	–	2335/ 2361	–	2339/23 60	-C=O_{vw} Fermi Resonance
2360/ 2336	2362/ 2337	2361	–	–	2362	–	–	–	
1743	1743	1744	1743	1743	1743	1743	1743	1744	v(-C=O_{vst}) in ester
1704	1704	1704	1705	1705	1705	1704	1702	1705	v(-C=O_{vw}) in acid
–	1654	1654/ 1559	1654	1654	1654	1653/ 1558	1653	1653	v_{vw}(-C=C-, *cis*-)
1462	1462	1462	1462	1462	1462	1463	1462	1462	δ_{vw}(-C-H) in CH_2 and CH_3 group, bending (scissoring)
1417	1441/ 1418	1440/ 1417	1416	–	1418	1440/ 1418	1439/ 1418	1440/ 1418	v_{vw}(-C-H, *cis*-) bending (rocking)
–	1401	1400	1400	–	1400	1400	1400	1401	
1377	1376	1377	1377	1377	1351/ 1377	1377/ 1350	1376/ 1349	1377	
	1367	–	–	–	–	–	–	1365	$v_{w, m, vw}$(-C-H, -CH_3), bending
1349	1353	1350	1349	1350	1318	1316	1317	1351	
1317	1318	1318	1317	1318/ 1302	1301	1300	1300	1300	
1300	1301	1299	1279	1277	1278	1278	1277	1279	
1278	1278	1277	–	–	–	–	–	–	
–	1265	1262		1264	1261		1262	–	v_m(-C-O) or δ_m(-CH_2-)
1237	1229	1237	1237	1237	1236	1237	1237	1234	
1161	1160	1160/ 1141	1160	1143/ 1159	1141/ 1160	1161	1142/ 1160	1160/ 1143	v_{st}(-C-O) or δ_{st}(-CH_2-)
1120	1119	1119	1119	1119	1119	1120	1120	1119	v_m(-C-O)
1097	1097	1096	1097	1096	1096	1097	1097	1097	
1066	1060	1061	1062	1062	1059	1060	1063	1060	$v_{m,vw}$(-C-O)
1030	1030	1030	1030	1031	1030	1030	1030	1031	
966	965	967	966	914/965	913/966	913/ 967	912/ 965	913/969 /949	δ_w(-HC=CH-, *trans*-) bending out of plane

913/ 867/ 851	911	912/ 867/ 849	848/ 868/ 913	–	870	689/ 867	694/ 869	791	δ_{vw}(-HC=CH-, *cis*-) bending out of plane
766	767	765	765	–	766	770	765	765	δ(-$(CH_2)_n$- and –HC=CH- (*cis*-) bending (rocking)
723	722	722	721	–	722	722	722	722	
–	689	691	694	–	687	662	664	690	

ν – stretching, δ – deformation, s – symmetrical, as – asymmetrical, st – strong, vst – very strong, w – weak

PCA is a mathematical tool, which performs a reduction in data dimensionality and allows the visualization of underlying structure in experimental data and relationships between data and samples. FTIR spectra data of 7 oil samples were used in Principal Component Analysis (PCA). PCA of FTIR spectra using absorbencies at 16 wavenumber regions was carried out using Statistica 13 advanced statistics software. The PCA score plot (Fig. 3) was obtained from the correlation matrix of peak absorbencies at 16 frequency regions, namely 3007, 2921, 2853, 1744, 1654, 1461, 1418, 1373, 1235, 1161, 1119, 1096, 1031, 966, 913, 724/cm. In PCA, the first principal component (PC1) and the second principal component (PC2) account the largest and the next largest of variable variation. PC 1 explain 99,32% variance, meanwhile PC 2 accounted 0,62%; therefore the variance can be described by the first two PCs.

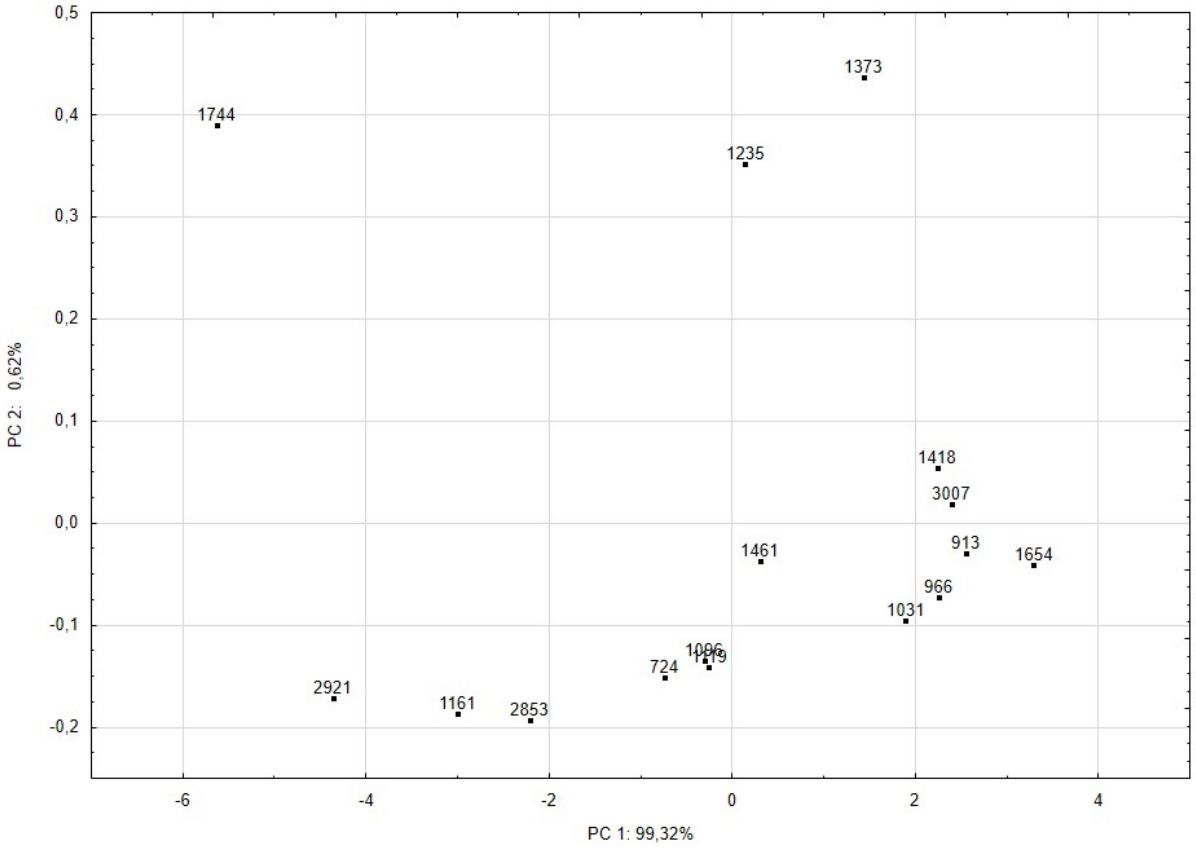

Figure 3. Projection of cases on the PC 1 and PC 2 plane.

Hierarchical cluster analysis (HCA) was performed in order to observe similarities or dissimilarities between the 'Bios', 'Markus' and 'Feliks' oil samples from spring plants grown with traditional NPK mixture fertilisation and digestate (D). The dissimilarity of different clusters was defined by Euclidean distance and calculated by single linkeage method. The results obtained are presented in the dendrogram structure, showing the different groups. On the Fig. 4 we present the plot obtained from the 7 oil samples of 'Bios', 'Markus' and 'Feliks'. Considering the cut-off of 0.1 dissimilarity units we can distinguish three clusters (in red, green and blue colour, respectively). The first cluster, green coloured, is a small cluster, aggregated on the far left arm of the dendrogram. This cluster is formed by F-NPK oil of 'Feliks' sample. The second cluster, red coloured, is composed of all 'eco-digestate' oil samples including 'Bios', 'Markus' and 'Feliks' samples and samples of Control and of M-NPK. This result suggests that these oils have a physical-chemical properties more similar than the others. The sample of B-NPK oil is aggregated in third cluster (blue coloured) on the right arm of dendrogram.

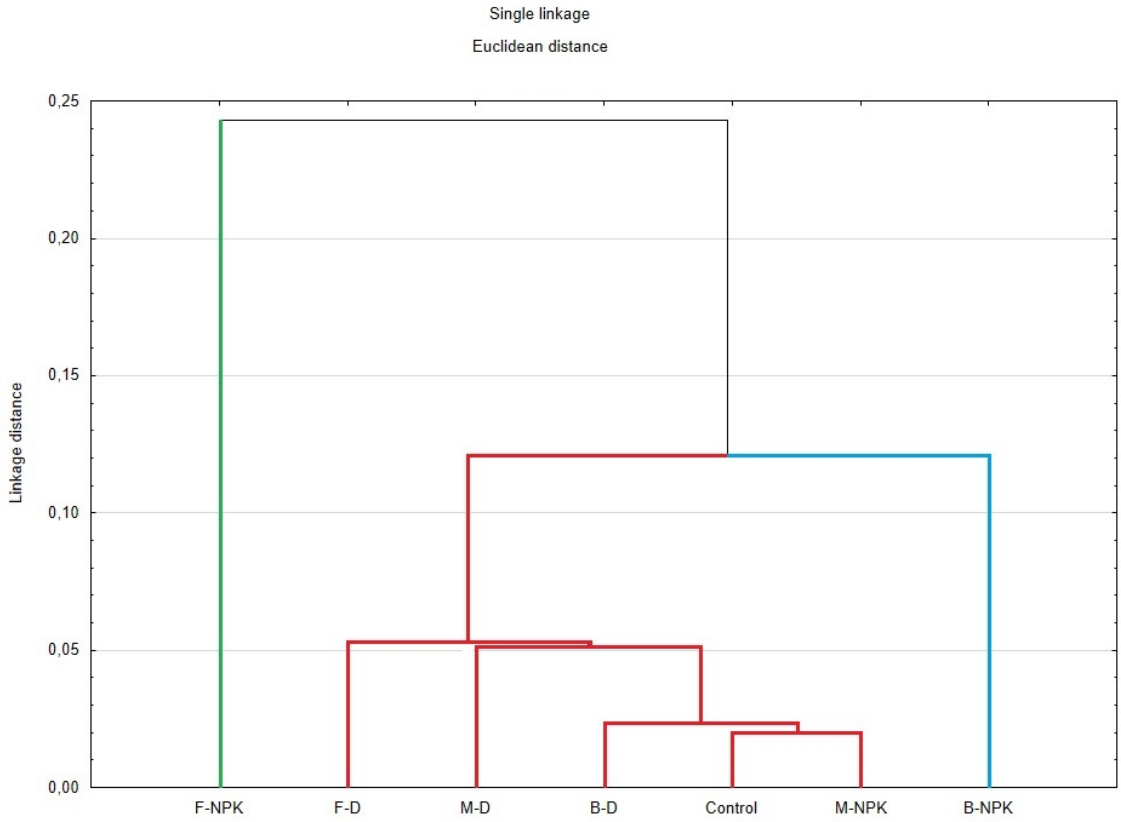

Figure 4. Dendrogram analysis (HCA) of all samples in the 3800-600/cm spectral region.

The PCA analysis was applied to fatty acid data. The obtained results are presented in Figs. 5-6. The greatest effect on PC 1 in PCA is positively correlated with the content of C18:1N9c +C18:1n9t (0.017), C16:0 (0.0127) and is negatively correlated with the content of PUFA (-0.0471). In turn, PC2 is negatively correlated with C18:2n6c+C18:2n6t and OMEGA 6 (-0,0091) and positively correlated with the content of C18:3n3 (alpha) and OMEGA 3 (0,0096).

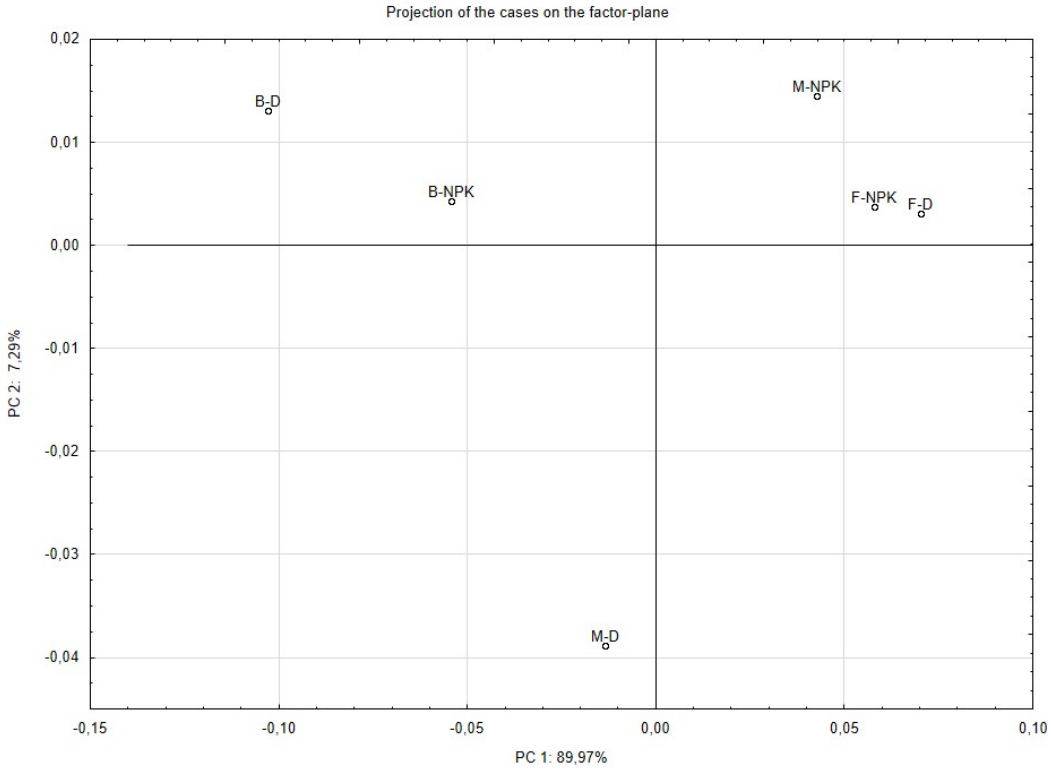

Figure 5. The plot of two first principal components after PCA analysis of fatty acid compositions. Projection of the cases on the PC 1 and PC 2 plane.

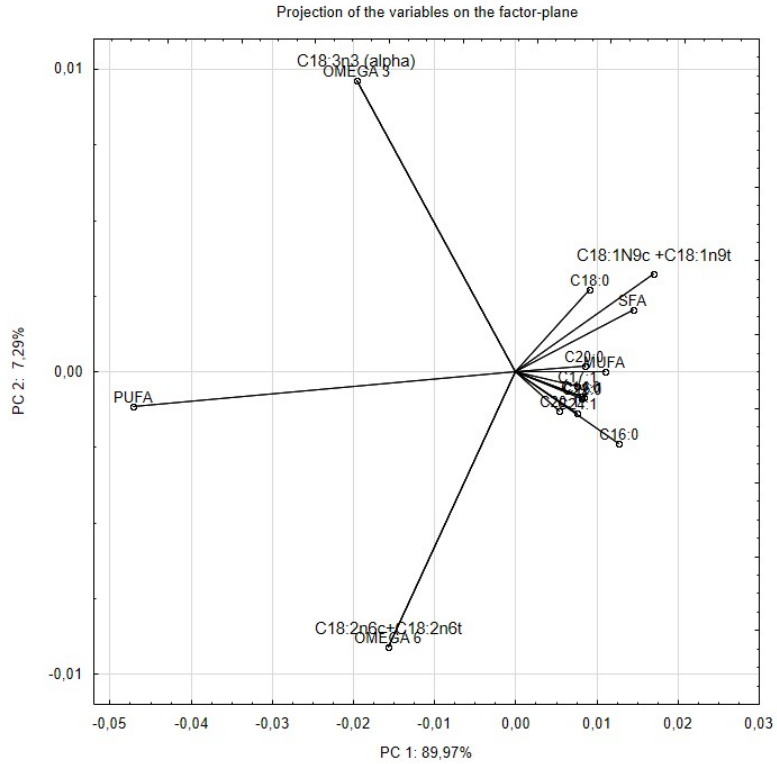

Figure 6. The plot of two first principal components after PCA analysis of fatty acid compositions. Projection of the variables on the PC 1 and PC 2 plane.

4. CONCLUSIONS

The obtained results reveal a correlation between the employed method of fertilisation, using digestate or a traditional NPK mixture, and the soil condition as well as the quality of the products, i.e. spring rapeseed and the oil cold-pressed therefrom.
Based on said results it can be concluded that statistically significant differences can be observed in terms of the content of nutrients available to plants between the control and fertilised plots. Statistically significant differences were also observed between the content of the analysed nutrients in the soil prior to sowing and after the harvest, with the exception of control plots related to the use of NPK fertiliser.
Analyses of the oil in terms of its oxidative stability revealed no direct correlation between the qualities of particular oils and the method of cultivating the seeds from which they were extracted, although the statistical analysis did reveal significant differences in terms of oil obtained from the 'Bios' cultivar, which was characterised by the lowest stability relative to digestate fertilisation. It is noteworthy that clear, although not particularly big, differences in terms of band intensity in the general cross-section of the FTIR spectrum were observed for samples fertilised with the mineral mixture, as compared to digestate. The same signify the possibility of a very efficient use of this method of field fertilisation. It is also noteworthy that FTIR spectroscopy techniques can be used for the purposes of rapid classification of oil samples without the need for sample preparation. FTIR spectroscopy techniques provide exquisite structural insights into the functional groups of oils, as required for discriminant analyses. FTIR spectroscopy has been found to be the most efficient method of oil discrimination and classification. To recapitulate, FTIR spectroscopy constitutes a simple, efficient and low-cost quantitative quality control tool available to the fat and oil industry.

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REFERENCES

Andruschkewitsch M., Wachendorf C. and Wachendorf M. 2013. Effects of digestates from different biogas production systems on above and belowground grass growth and the nitrogen status of the plant-soil-system. Jpn. Grassl. Sci. 183.

Atabani A., Silitonga A., Ong H., Mahlia T., Masjuki H., Badruddin I.A. and Fayaz H. 2013. Non-edible vegetable oils: a critical evaluation of oil extraction, fatty acid compositions, biodiesel production, characteristics, engine performance and emissions production, Renewable Sustainable Energy, reviews 18:211.

Czekala W., Pilarski K., Dach J., Janczak D. and Szymanska M. 2012. T he analysis of possibilities of management of post ferment substances obtained from biogas plant. The agriculture, garden and forest technique. Agricultural Horticultural Technique 4:1.

Cetin M. and Sevik H. 2016. Measuring the Impact of Selected Plants on Indoor CO. Pol. J. Environ. Stud. 25(3):973.

Codex Alimentarius. 2001. Joint FAO/WHO food standards programme. Fats, oils and related products, vol. 8. 2nd ed. Rome: Codex Alimentarius.

Comparetti A., Febo P., Greco C. and Orlando S. 2013. Current state and future of biogas and digestate production, Bulgarian J. Agricultural Science 19:1.

Cichosz G. and Czeczot H. 2011. Oxidative stability of edible fats – consequences to human health, Bromat. Chem. Toksykology 44:50.

Ciemniewska-Żytkiewicz H., Ratusz K., Bryś J., Reder M. and Koczoń. P. 2014. Determination of the oxidative stability of hazelnut oils by PDSC and Rancimat methods. J. Therm Anal Calorim 118:875.

Cheema M.A., Malik M.A., Hussain A., Shah S.H. and Basra S.M.A. 2001. Effects of time and rate of nitrogen and phosphorus application on the growth and the seed and oil yields of canola (*Brassica napus* L.). J. Agron. Crop Sci. 186:103.

Damerau A. 2015. Oxidative stability of solid foods with dispersed lipids. ISBN 978-951-51-0873-9.

Dennehy C., Lawlor P.G., Croize T., Jiang Y., Morrison L., Gardiner G.E. and Zhan X. 2016. Synergism and effect of high initial volatile fatty acid concentrations during food waste and pig manure anaerobic co-digestion, Waste Management 56:173.

De Leonardis A. and Macciola V. 2012. Heat oxidation stability of palm oil blended with extra virgin olive oil. Food Chemistry 135:1769.

Eickenscheidt T., Freibauer A., Heinichen J., Augustin J. and Drösler M. 2014. Short-term effects of biogas digestate and cattle slurry application on greenhouse gas emissions affected by N availability from grasslands on drained fen peatlands and associated organic soils, Biogeosciences 11:6187.

Farnworth C.R., Stirling C., Sapkota T.B., Jat M. L., Misiko M. and Attwood S. 2017. Gender and inorganic nitrogen: what are the implications of moving towards a more balanced use of nitrogen fertilizer in the tropics? International J. Agricultural Sustainability 15(2):136.

García-Sanchez M., Siles J.A., Cajthaml T., García-Romera I., Tlustos P. and Szakova, Z. 2015. Effect of digestate and fly ash applications on soil functional properties and microbial communities. Euro. J. Soil Biol. 1.

Gawrysiak-Witulska M., Rudzińska M., Wawrzyniak J. and Siger A. 2012**.** The effect of temperature and moisture content of stored rapeseed on the phytosterol degradation rate, Journal of the American Oil Chemists' Society 89:1673.

Grant C.A., Derksen D.A., McLaren D.L. and Irvine R.B. 2011. Nitrogen fertilizer and urease inhibitor effects on canola seed quality in a one-pass seeding and fertilizing system. Field Crops Research 121:201.

Grant C.A., Mahli S.S. and Karamanos R.E. 2012. Sulfur management for rapeseed. Field Crops Research. 128:119.

Guillen M.D. and Cabo N. 1997. Infrared spectroscopy in the study of edible oils and fats, Journal of the Science of Food and Agriculture 75:1.

Gurdeniz G. and Ozen B. 2009. Detection of adulteration of extra-virgin olive oil by chemometric analysis of mid-infrared spectral data, Food Chemistry 116:519.

Harel Z., Gascon G., Riggs S., Vaz R., Brown W. and Exil G. 2002. Supplementation with omega-3 polyunsaturated fatty acids in the management of recurrent migraines in adolescents. Journal of Adolesc Health 31(2):154.

Hong Y., Irudayaraj J. and Paradkar M.M. 2005. Discriminant analysis of edible oils and fats by FTIR, FT-NIR and FT-Raman spectroscopy. Food Chem. 25.

Inon F.A., Garrigues S., and De La Guardia M. 2004. Nutritional parameters of commercially available milk samples by FTIR and chemometric techniques. Analytica Chimica Acta 513:401.

Jeleń H.H., Obuchowska M., Zawirska-Wojtasiak R. and Wąsowicz E. 2000. Headspace Solid-Phase Microextraction Use for the Characterization of Volatile Compounds in Vegetable Oils of Different Sensory Quality. J. Agric. Food Chem. 48:2360.

Kondratowicz-Pietruszka E. 2014. Assessment of oxidative stability of rapeseed and sunflower oils, Quality Food 77.

Kouřimská L., Poustková I. and Babička L. 2012. The use of digestate as a replacement of mineral fertilizers for vegetables growing, Scientia Agriculturae Bohemica (Czech Republic). 43(4):121.

Krasucki W., Tys J., Grela E. R. and Szafran K. 2001. Chemical composition of rapeseeds stored under conditions simulating the industrial silos. Oilseed Crops, Tom XXII: 247.

Krygier K. 1997. The contemporary vegetable fats edible, the food industry. Food Industry 4:11. KQ/PB-17-76-77 rev. 04 of 02.07.12 - Physical, physicochemical and chemical analysis of sewage sludge and sewage.

Lemke R.L., Mooleki S.P., Malhi S.S., Lafond G., Brandt S., Schoenau J.J., Wan H., Thavarajah D., Hultgreen G. and May W.E. 2009. Effect of fertilizer nitrogen management and phosphorus placement on canola production under varied conditions in Saskatchewan. Can. J. Plant Sci. 89:29.

Li Y., De-xin K. and Hong W. 2013. Analysis and evaluation of essential oil components of cinnamon barks using GC–MS and FTIR spectroscopy. Ind Crops Prod. 269.

Malheiro R., Rodrigues N., Manzke G., Bento A., Pereira J.A. and Casal S. 2013. The use of olive leaves and tea extracts as effective antioxidants against the oxidation of soybean oil under microwave heating, Industrial Crops and Products 44:37.

Malhi S.S. and Gill K.S. 2007. Interactive effects of N and S fertilizers on canola yield and seed quality on S-deficient Gray Luvisol soils in northeastern Saskatchewan. Can. J. Plant Sci. 87:211.

Mason M.G. and Brennan R.F. 1998. Comparison of growth response and nitrogen uptake by canola and wheat following application of nitrogen fertilizer. J. Plant Nutr. 21(7):1483.

Möller K. and Müller T. 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review, Engineering in Life Sciences 12:242.

Mensink R.P. and Katan M.B. 1990. Effect of dietary trans-fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. New England Journal of Medicine 323(7):439.

Murawa D. and Warminski K. 2005. The impact of diverse plant protection on the chemical composition of the spring rapeseed. Acta Scientiarum Polonorum. Agricultura 4:77.

Murkovic M., Hillebrand A., Winkler J., Leitner E. and Pfannhauser W. 1996. Variability of fatty acid content in pumpkin seeds (*Cucurbita pepo* L.), Zeitschrift für Lebensmittel-Untersuchung und Forschung 203:216.

Odlare M., Pell M. and Svensson K. 2008. Changes in soil chemical and microbiological properties during 4 years of application of various organic residues, Waste Management 28:1246.

Parry J., Su L., Luther M., Zhou K., Yurawecz M.P., Whittaker P. and Yu L. 2005. Fatty acid composition and antioxidant properties of cold-pressed marionberry, boysenberry, red raspberry, and blueberry seed oils, Journal of Agricultural and Food Chemistry 53:566.

PM-EN ISO 5509:2001. "Vegetable and animal fats and oils"- The analysis of fatty acid methyl esters.

PN – A – 04018/A z 3:2002 Agricultural food products. Determination of nitrogen by the Kjeldahl method and expressing as protein.

PN- EN 1163:1999 Test methods. Determination of the oil and fat content.

PN-ISO 10390:1997 - Soil quality. Determination of pH.

PN-R-04024:1997 - Determination of the available phosphorus, potassium, magnesium, and manganese in organic soil.

Poyato C., Ansorena D., Navarro-Blasco I. and Astiasarán I. 2014. A novel approach to monitor the oxidation process of different types of heated oils by using chemometric tools. Food Res. Int. 57:152.

Ramadan M.F. 2013. Healthy blends of high linoleic sunflower oil with selected cold pressed oils: Functionality, stability and antioxidative characteristics, Industrial Crops and Products 43:65.

Ramirez K.S., Craine J.M. and Fierer N. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes, Global Change Biology 18:1918.

Rathke G.-W., Christen O. and Diepenbrock W. 2005. Effects of nitrogen source and rate on productivity and quality of winter oilseed rape (*Brassica napus* L.) grown in different crop rotations. Field Crops Research 94:103.

Rohman A. and Che Mana Y.B. 2010. Fourier transform infrared (FTIR) spectroscopy for analysis of extra virgin olive oil adulterated with palm oil. Food Research International 43:886.

Rohman A., Sismindary, Erwanto Y. and Che Man Y.B. 2011. Analysis of pork adulteration in beef meatball using Fourier transform infrared (FTIR) spectroscopy. Meat Science 88:91.

Rohman A. and Che Man Y.B. 2010. Fourier transform infrared (FTIR) spectroscopy for analysis of extra virgin olive oil adulterated with palm oil. Food Res Int. pp. 886.

Shahidi F. 2005. Edible oil and fat products: specialty oils and oil products, Hoboken: Wiley-Interscience, Vol. 3: 6th ed.

Shin H.-Y., Lim S.-M., Kang S.C. and Bae S.-Y. 2012. Statistical optimization for biodiesel production from rapeseed oil via transesterificaion in supercritical methanol. Fuel Processing Technology 98:1.

Scholze B. and Meier D. 2001. Characterization of the water-insoluble fraction from pyrolysis oil (pyrolytic lignin). Part I. PY–GC/MS, FTIR, and functional groups. J Anal Appl Pyrolysis 41.

Szydłowska-Czerniak A., Trokowski K., Karlovits G.R. and Szłyk E. 2013. Spectroscopic determination of metals in palm oils from different stages of the technological process, Journal of Agricultural and Food Chemistry 61:2276.

Terhoeven-Urselmans T., Scheller E., Raubuch M., Ludwig B. and Joergensen R.G. 2009. CO_2 evolution and N mineralization after biogas slurry application in the field and its yield effects on spring barley. Appl. Soil Ecol. 297.

Tsaknis J., Lalas S. and Lazos E.S. 1997. Characterization of crude and purified pumpkin seed oil, Grasas y aceites 48:267.

Tys J., Jackowska I. and Rybacki R. 2006. The value of the technological seeds of different varieties of rape which were intended to be used for biofuels production. Acta Agrophysica 8:1017.

USDA, USDA Foreign Agricultural Service. EU -28 Oilseeds and Products Annual, in 2016.

Qian Y., Zuo C., Tan J. and He J. 2007. Structural analysis of bio-oils from sub-and supercritical water liquefaction of woody biomass. Energy 196.

Warminski K., Murawa D., Adomas B. and Pykalo I. 2001. The oil and protein seed of the spring rape varieties a population pharmacokinetic and mieszancowej complex project Margo grown in 1999 year depending on the used funds to protect plants, oil plants. Oilseed Crops 22:265.

Wroniak M. and Łukasik D. 2007. Evaluation of oxidative stability of selected cold pressed edible oils. Oilseed Crops Tom XXVIII: 302.

Wu X. and Leung D.Y. 2011. Optimization of biodiesel production from camelina oil using orthogonal experiment, Applied Energy 88:3615.

Van de Voort F.R. 1992. Fourier transform infrared spectroscopy applied to food analysis. Food Res Int. 397.

Van Duren I., Voinov A., Arodudu O. and Firrisa M.T. 2015. Where to produce rapeseed biodiesel and why? Mapping European rapeseed energy efficiency, Renewable Energy 74:49.

Vaneeckhaute C., Meers E., Ghekiere, G., Accoe F. and Tack F.M.G. 2013. Closing the nutrient cycle by using bio-digestion waste derivatives as synthetic fertilizer substitutes: a field experiment. Biomass Bioenergy 175.

Vázquez-Rowe I., Golkowska K., Lebuf V., Vaneeckhaute C., Michels E., Meers E., Benetto E. and Koster D. 2015. Environmental assessment of digestate treatment technologies using LCA methodology. Waste Management 43:442.

Yang H., Irudayaraj J. and Paradkar M.M. 2005. Discriminant analysis of edible oils and fats by FTIR, FT-NIR and FT-Raman spectroscopy. Food Chemistry 93:25.

Ziemlański S. and Budzyńska-Topolowska J. 1991. Food fats and body lipids. PWN, Warsaw, 171.

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