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Examination of Selected Physicochemical Properties of Biodiesel after Electron Beam Sterilization in Flow System

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Abstract: The problem of microbial growth in fuels, especially biofuels, is a very important issue. Water is a necessity for microbial growth to occur. Unfortunately, it is very hard to exclude all water from fuel systems during daily operations, especially when biodiesel is added to the fuel mix. The aim of this work was to investigate the impact of ionizing radiation on selected properties of fatty acids methyl esters in order to evaluate the possibility of using irradiation techniques to sterilize biodiesel and its blends. Ionizing radiation influences the chemical composition of biodiesel samples containing especially unsaturated bonds. Irradiation was performed in a specially designed flow system. The tested parameters were compared with the normative values from the ISO 14214 standard. Density, kinematic viscosity, flash point, water content and cold filter plugging point as a macroscopic parameters do not change despite their irradiation with ionizing radiation. Change was observed in microscopic parameters as oxidation stability and esters content. During irradiation, Rapeseed Methyl Ester (RME) samples formed oxidation products, which lower the oxidative stability. It was observed that, for esters content, requirements of the standard could be met in the case of using very small doses. In the case of RME samples saturated with water, the changes in the ester concentration were smaller, but the resulting products decreased the oxidation stability. Sterilization fatty acids methyl esters (FAME) was observed with the use of e beam radiation is to apply very low doses (up to 2 kGy) in a flow system without adding water. Sterilization FAME saturated by water requires the use of higher doses of radiation, which adversely affects the oxidative stability.

Keywords: biodiesel; FAME; e-beam irradiation; flow system; sterilization; physicochemical properties

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1. Introduction

Because of the finite resources of fossil fuels and the concern for environment, research on alternative fuels for automotive engines has been taking place for several decades. An important group of these fuels are biofuels [1]. Among them one of the most significant is biodiesel, that is, fatty acids methyl esters (FAME).

Several advantages of biodiesel are worth mentioning, for example, the lack of sulfur, high flash point [2] and low CO, hydrocarbons (HC) and particulate matter (PM) emissions [3]. A significant drawback is unsatisfying oxidation stability [4], while biodegradability of FAME is an ambiguous issue. From the environmental point of view, high biodegradability (along with some other environmentally friendly properties of FAME [5]) is very desirable. Unfortunately, the biodegradability is associated with microbial growth and therefore may be the cause of many problems during the storage and use of biodiesel and its blends with diesel fuel. These are for example, fouling, filter plugging and increased steel corrosion of storage containers and engine fuel systems.

In the literature there are many papers presenting studies of microbial growth and its influence on the degradation of biodiesel-diesel blends. As was reported by Soriano et al., in biodiesel and biodiesel blend 18 microbial genera (8 fungal and 10 bacterial) were identified by DNA sequencing, which have been associated with, among others, fuel

microbial contamination and/or biodegradation, biofuel production or related enzymes' expression, biofilm formation and/or exopolysaccharide production [6]. Fosso-Kankeu et al. studied two types of bacteria, which were exposed to different concentrations of diesel to increase their adaptation to the fossil fuel and were used for the degradation of diesel-biodiesel blends [7]. Owsianiak et al. investigated the effect of surfactants on the microbial life of diesel/biodiesel blends [8]. Research by Schleicher et al. on the microbiological stability of biodiesel-diesel mixtures showed that microbial growth was the strongest in the B 20 samples [9]. The study of Sorensen et al. confirms that the introduction of biodiesel affects the types and activity of the microorganisms present in the fuel microcosms, which cause fuel degradation [10]. Pasqualino et al. shows the synergistic effect of biodiesel on the biodegradation of fossil diesel fuel [11]. Thompson et al. show the influence of water (that can be absorbed by biodiesel within the logistic line contained) on the development of microbial life in biodiesel and its mixtures [12]. Scientists are not unanimous on this matter. Experiments carried out by Owsianiak et al. showed that biodegradation of diesel was not remarkably influenced by the presence of biodiesel up to 50 vol% [8]. However, it is generally considered that adding biodiesel stimulates the activity of microorganisms [9,10] and therefore enhances the biodegradation of fossil-derived fuels [9]. Microbial growth in most cases occurs on the water-fuel interface. Biodiesel is therefore conducive to it because of its highly hygroscopic nature [12].

Biodegradation of any fuel can seriously affect its quality, which is why the problem of microbial growth in biodiesel remains an important issue. It is worth considering irradiation sterilization as a possible solution.

Sterilization is a process of elimination of microbial life, for example, bacteria, fungi and viruses [13]. Ionizing radiation is a widely described method of sterilization with many applications. The most popular are food preservation and decontamination of healthcare products such as syringes, sutures and tissue allografts [13–16]. There are three main types of irradiation technologies applied for sterilization, which are electron beam (e-beam, by using ionizing energy emitted from electron beam), X-ray (by using ionizing energy emitted from a Rhodotron electron beam), and gamma-ray (by using photons from a radioisotope) [14]. Generally, the maximum energy of radiation is limited to 10 MeV and the overall maximum dose allowed is 50 kGy. The value of the sterilization dose used depends on the application to the specific material [17,18].

The lethal impact of irradiation on microorganisms occurs due to either direct or indirect effects. The former are the results of non-specific collisions of high-energy radiation with atoms in bio-molecules, for example, DNA. The latter involve the formation of free radicals generated during the radiolysis of water, which is present in cells in high amounts. Free radicals and further created oxidizing agents compromise the cell membrane, leading to cellular leakage and then complete cell lysis [19].

Irradiation sterilization has many benefits. It is highly effective, efficient and eco-friendly because it does not need chemicals to be added to the process. The deactivation of microorganisms occurs non-thermally, that is why it may be used to decontaminate fresh food (e.g., dairy produce), which can be distributed immediately after treatment. In the case of e-beam radiation, an additional advantage is lack of the radioactive residue (compared to ^{60}Co, as a radiation source), as well as the possibility of switching the accelerator on and off [14,19,20].

Ionizing radiation influences the chemical composition of samples containing especially unsaturated bonds. Depending on the irradiation conditions, chain breaking or cross-linking reactions may take place. Cross-linking reactions dominate under vacuum conditions. During sterilization with ionizing radiation with FAME fuels, mainly chain breaking and oxidation reactions may take place [21].

Water is a necessity for microbial growth to occur. Unfortunately, it is very hard to exclude all water from fuel systems during daily operations, especially when biodiesel is added to the fuel mix. The reason for this is that biodiesel by nature is more hygroscopic than fossil fuels and the ability to contain water depends largely on the temperature [12].

This allows free water to accumulate by precipitation in the storage tanks as a result of temperature changes [10].

Research on biodiesel seems to be particularly important due to its newer applications, for example as a solubilizer in the mixture of ethanol and diesel fuel in self-ignition engines [22]. This allows the change of combustion technologies that have been tested to improve CO_2 emissions and improve NO_x-Soot tradeoffs such as specific fuel design and a hollow cone fuel injection system [23,24].

The aforementioned positive qualities of e-beam sterilization make it a really promising means of solving the problem of microbial growth in biodiesel. However, because FAME must meet the quality requirements of EN 14214 [25], it is important to check the influence of irradiation on the properties specified in the standard. The purpose of this work was to investigate the impact of e-beam irradiation on selected properties of FAME in order to evaluate the possibility of using irradiation to sterilize this biofuel.

2. Experimental Section

Research described in the previous work of the authors has shown that e-beam radiation has quite a distinct influence on fatty acid methyl esters [26,27]. However, this influence was smaller in the case of FAME samples saturated with water. In this study the irradiated samples were large enough to determine a number of properties such as density, kinematic viscosity, flashpoint, water content, cold filter plugging point (CFPP) and ester content.

2.1. Samples

Rapeseed Methyl Esters (RME) (B100 without additives) used in the study had been produced by the ORLEN Południe Group. Eight samples of RME (about 500 mL each) were poured into glass bottles. Additionally, because preliminary studies [26,27] suggested that water was weakening the destructive potential of the e-beam, it was decided to investigate water-saturated RME as well. Saturation was performed by adding 45 mL of water to 3.75 l of RME and constantly stirring for 30 min. Then the mixture was left for 24 h so the excessive amount of water could have separated at the bottom of the vessel. After that, eight 500 mL samples of RME saturated with water were prepared from the upper phase.

2.2. Irradiation Treatment

Electron-beam irradiation was conducted at the Institute of Applied Radiation Chemistry, Lodz University of Technology, Poland. Irradiation was performed using an Elektronika ELU-6e linear electron accelerator of 6 MeV of energy with a pulse duration of 2 ns to 4 µs.

The irradiation was carried out in a flow system, therefore it was possible to irradiate large volumes of biofuel in a relatively short time. The system consisted of an e-beam accelerator, a peristaltic pump and two bottles behind an aluminum cover, a glass tube at a distance of 40 cm in front of the waveguide of an accelerator and polytetrafluoroethylene (PTFE) tubes connecting all the elements.

Seven samples of each series (RME and RME with water) were pumped through the system, each sample in its own specific time calculated using the calibration curve of absorbed dose versus rounds per minute (RPM) of pump (Figure 1a.) and the auxiliary curve of flow rate versus RPM (Figure 1b.). To plot the main calibration curve, six portions of Fricke dosimeter, each at different RPMs, were pumped through the system and then their absorbed doses were calculated based on the absorbance at 304 nm. PerkinElmer LAMBDA™ 35 was used to record the spectra.

The determined doses absorbed by RME samples and particular times of flow are presented in Table 1.

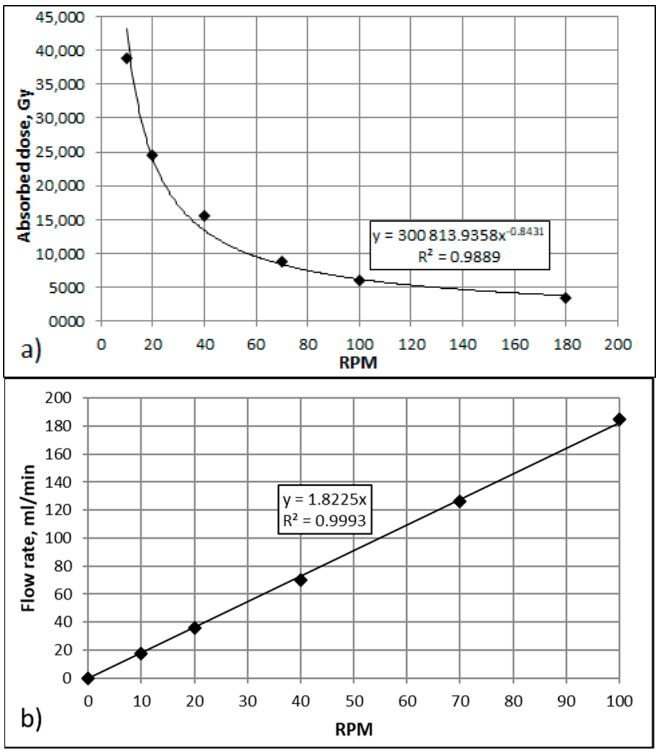


Figure 1. Calibration curves: (**a**) Absorbed dose versus round per minute (RPM) of pomp, (**b**) Flow rate versus RPM.

Table 1. Absorbed dose and flow time of particular samples.

Sample No in Series	Assumed Dose, kGy	Calculated RPM	Used RPM	Determined Absorbed Dose, kGy	Flow Rate, ml/min	Flow Time, s
1	1.00	842.12	600 *	1.34	1093.5	27
2	2.00	373.48	373	2.00	679.8	44
3	4.00	165.64	166	3.99	302.5	99
4	6.00	102.94	103	6,00	187.7	160
5	8.00	73.46	73	8.04	133.0	225
6	10.00	56.54	57	9.93	103.9	289
7	20.00	25.08	25	20.05	45.6	658

*—maximum RPM available in the used pomp.

2.3. Determination of Selected Physicochemical Properties

Density at 15 °C was determined according to EN ISO 3675. A thermohydrometer and a 500 mL glass cylinder were used.

Viscosity at 40 °C was determined with the use of a glass Ubbelohde viscometer, in accordance with EN ISO 3104. A water bath was used to thermostat the samples.

The determination of a flash point was performed in a Pensky-Martens apparatus Herzog HFP 380, according to EN ISO 2719.

Water content was measured by the coulometric Karl Fischer titration method, as described in EN ISO 12937. The used apparatus was Metrohm 701 KF Titrino.

The cold filter plugging point was determined in the GAS-COOP GT apparatus, according to EN 116. Oxidation stability index was determined according to EN 14112.

For the determination of microorganisms in RME after irradiation, 100 mL of each sample was stored at room temperature without light over 2 weeks. In these same conditions,

samples were stored without irradiation. The experiment was conducted in conformity with the standard IP 385/99 for aerobic microbial content and IP 385/96 for the occurrence of nonaerobic microbial.

Ester content in samples was measured by gas chromatography with the use of an internal standard, according to EN 14103. Chromatograms were recorded using Agilent Technologies 7820A gas chromatograph with a flame-ionization detector (FID). The content of esters was calculated based on the surface of their peaks and the surface of a methyl heptadecanoate peak (internal standard).

Each of the measured parameters was measured three times and the results presented in the charts are the average of these measurements. Based on the obtained results, a statistical analysis was carried out. The research results are discussed, taking into account the statistical analysis of the results.

Obtained results of the studies were compared with the specifications of EN 14214, and are presented in Table 2.

Table 2. Specifications of EN 14214 concerning examined properties.

Property	Unit	Lower limit	Upper limit
Density at 15 °C	kg/m^3	860.0	900.0
Kinematic viscosity at 40 °C	mm^2/s	3.50	5.00
Flash point	°C	101	-
Water content	mg/kg	-	500
Cold filter plugging point *			
- Type B (16 April–30 September)	°C	-	0
- Type F (16 November–28 February)		-	−20
- Type D (Rest of year)		-	−10
Oxidation Stability Index	hours	8.0	-
Ester content	wt%	96.5	-

*—values for Poland (PN-EN 14214).

3. Results and Discussion

3.1. Density

Density of both series of samples are shown in Figure 2. For pure RME, there was a decrease in the density of samples irradiated with doses from 1.335 kGy to 8.043 kGy in comparison with non-irradiated samples. However, this difference was only 1 kg/m^3. Samples of the two highest doses (9.932 kGy and 20.052 kGy) had a density equal to the density of the non-irradiated sample. In the case of RME saturated with water, all irradiated samples had the same density and a value that was 2 kg/m^3 lower than that of the non-irradiated sample.

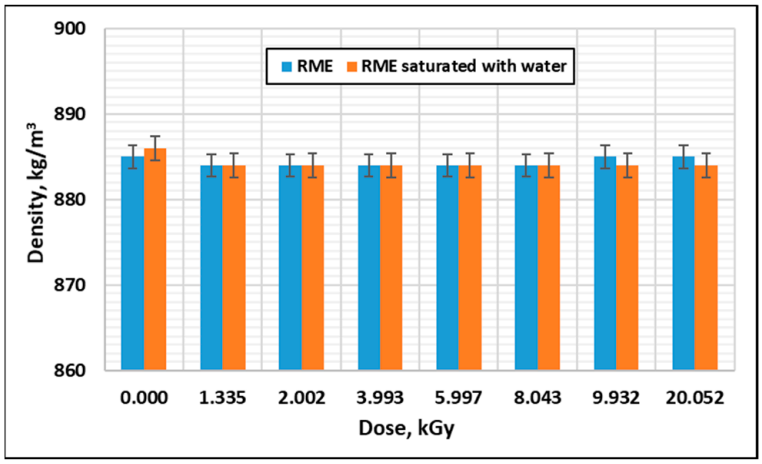


Figure 2. Density of all samples.

The changes observed in all samples were very small and showed no upward or downward trend. The reason for this is the accuracy of the method used, which is 2 kg/m^3, and not the effect of irradiation.

All tested RME samples met the requirements of EN 14214 for density.

3.2. Kinematic Viscosity

Comparisons of the kinematic viscosity of both series of samples are shown in Figure 3. In the case of pure RME, the changes in kinematic viscosity of the irradiated samples were very small and did not show any trends. The viscosity of the non-irradiated sample was 4.45 mm^2/s. The lowest viscosity was observed for the 1.335 kGy sample, while the highest was observed for the sample of 9.932 kGy. The values were 4.42 mm^2/s and 4.47 mm^2/s, respectively.

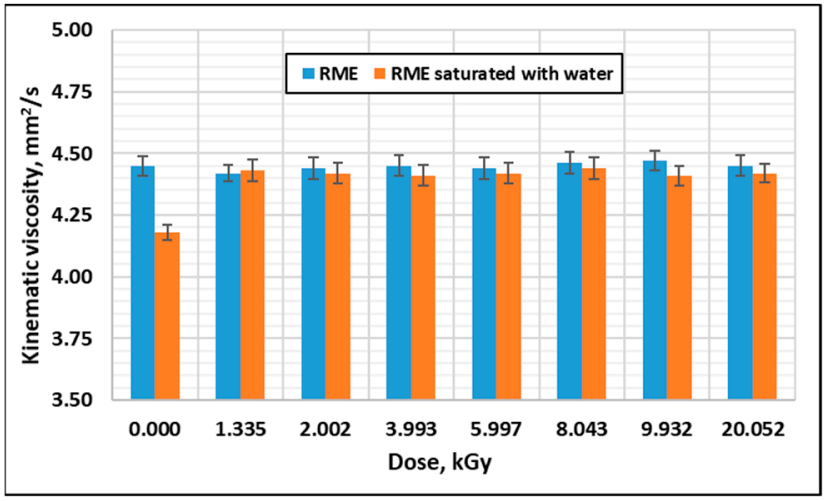


Figure 3. Kinematic viscosity of all samples.

Another trend was observed for RME samples saturated with water. The non-irradiated sample had a significantly lower kinematic viscosity (4.18 mm^2/s) than its not-saturated equivalent. The viscosity of all other samples was significantly higher. It was in the range of 4.41–4.44 mm^2/s, showing no upward or downward trend with increasing absorbed dose.

Based on the analysis of kinematic viscosity determination results, it can be concluded that adding water to RME brought about quite a significant decrease in the value of this parameter. However, even the minimal dose of ionizing radiation caused the viscosity to reach values close to the samples not saturated with water. It may be related to the radiolysis of water—created free radicals could have initialized the polymerization of unsaturated esters; created compounds increased the viscosity.

All tested RME samples met the requirements of EN 14214 for kinematic viscosity.

3.3. Flash Point

The process of flash point determination was unusual for most of the RME samples. Instead of the typical subtle ignition of the source, a burst of green flame was observed over the whole surface of the biofuel. Only one (1.335 kGy) pure RME sample and four (2.002 kGy, 8.043 kGy, 9.932 kGy, 20.052 kGy) saturated with water behaved normally. All results are shown in Figure 4.

The tested RME samples had very different values of flash points and no trend can be observed. Radiolysis is a radical process characterized by a certain degree of randomness, so it is possible that products with greater volatility than FAME are produced during the irradiation of RME, therefore it is difficult to unambiguously determine the influence of ionizing radiation on the flash point of RME. What is important is that, in every case, the

measured flash point was above 101 °C, so all tested samples met the requirements of EN 14214 concerning this property.

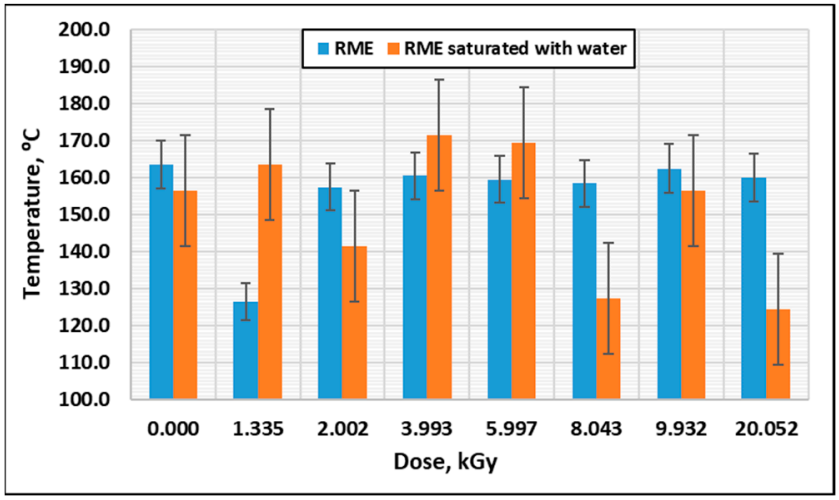


Figure 4. Flash point of all samples.

3.4. Water Content

Water content was determined in RME saturated with water, as well as in not-saturated samples. Average results are shown in Table 3.

Table 3. Water content in Rapeseed Methyl Esters (RME) samples.

Samples Series	Water Content, mg/kg
RME without water	288 ± 35
RME saturated in water	1118 ± 75

The water content in not-saturated samples was from 224 mg/kg (parts per million, ppm) for a dose of 5.997 kGy to 388 ppm in a 1.335 kGy sample. No trend was observed in this series.

In the case of water-saturated samples, water content in the irradiated ones was slightly higher than in the non-irradiated sample. It ranged from 1153 mg/kg in a sample of 1.335 kGy to 1338 mg/kg in a sample of 3.993 kGy. The increase in water content in the irradiated samples could be explained by the presence of stable radiolysis products, for example, aldehydes or ketones formed during oxidation, which reacted with methanol (which is the reaction environment in the Karl-Fischer method).

The water content of pure RME samples did not exceed 500 mg/kg and thus met the EN 14214 requirements for this parameter. In the case of samples saturated with water, it does not make sense to assess the compliance of the water content with the standard. This is due to the verification of these samples in terms of sterilization in real conditions prevailing in the logistics chain of this type of fuel.

3.5. Cold filter Plugging Point

The obtained results of the cold filter plugging point test are shown in Figure 5. CFPP of samples not saturated with water was generally maintained at −17 °C. In only two cases (non-irradiated sample and 3.993 kGy sample), the value was −16 °C. Among the watered samples the dominant CFPP was also −17 °C, but there were two lower values. These were −18 °C for a sample of 2.002 kGy and −19 °C for a sample of 9.932 kGy.

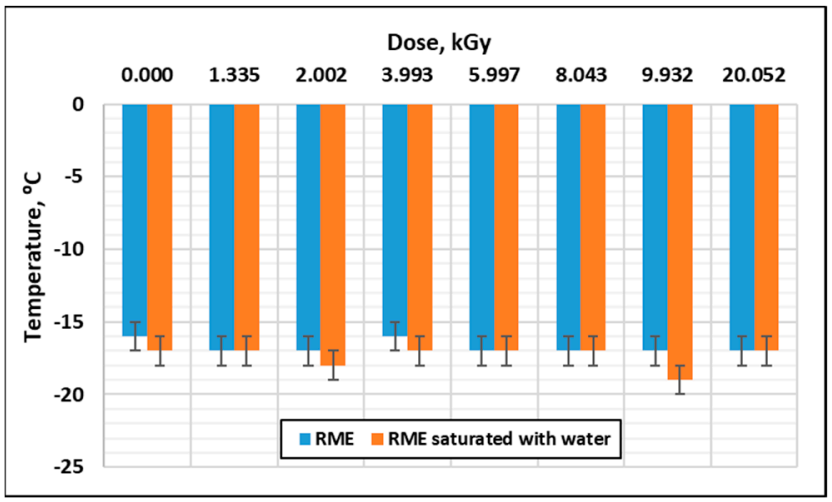


Figure 5. Cold filter plugging point of all samples.

Changes in the cold filter plugging point were too small to state the unambiguous impact of radiation on this property. However, a slightly bigger decrease in two water-saturated samples suggested that irradiation could have led to forming compounds that behaved like flow improver additives. They were probably products of the polymerization of esters initiated by radicals derived from water radiolysis. All tested samples met the requirements of PN-EN 14214 for type D (max. −10 °C).

3.6. Oxidation Stability Index

The oxidation stability index (OSI) of both series of samples are shown in Figure 6. As can be seen, the samples after irradiation in the presence of water show lower oxidative stability. The oxidation stability up to a dose of 2 kGy is above 8 h. Above this dose, the oxidative stability drops drastically, both in the presence and absence of water. The observed changes result from the by-products produced during FAME irradiation. During the measurement, hot air is passed through the test samples. The air flow causes the sample components to oxidize, which is much easier in the case of samples exposed to ionizing radiation. The primary and secondary oxidation products formed during the OSI measurement have a much higher volatility (they are usually short-chain organic acids), which increases the electrical conductivity of water much faster, on the basis of which the final OSI result is read.

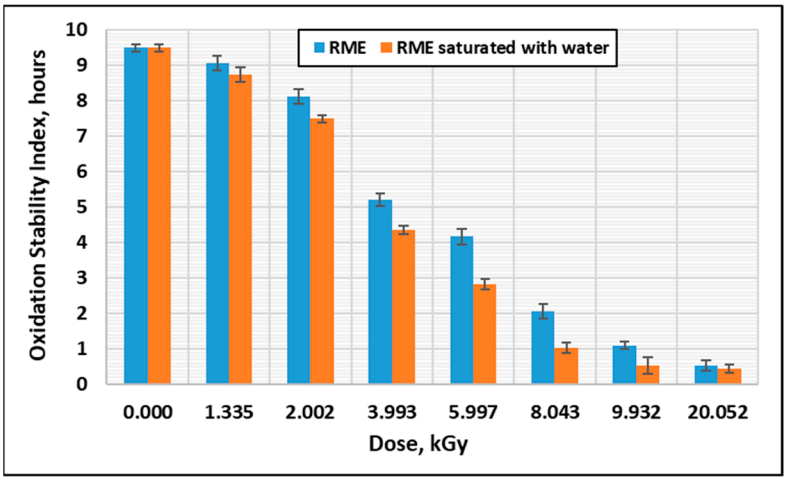


Figure 6. Oxidation stability index point of all samples.

OSI changes show how severe the changes in FAME samples are when irradiated with ionizing radiation. According to the EN 14214 standard, the oxidation stability index should be above 8 h. Only samples irradiated with a dose below 2 kGy meet this stringent parameter. Additionally, the presence of water in RME lowers OSI. The radicals formed during water radiolysis, reacting with fatty acid esters, have a direct impact on the observed phenomenon. The resulting stable products oxidize faster and increase the conductivity of the water much faster.

3.7. Microbial Content

Aerobic microbial content and nonaerobic microbial occurrence was determined in RME saturated with water, as well as in not saturated samples. Average results are shown in Table 4. As can be seen from Table 4, both in unsaturated RME samples and water-saturated samples, no anaerobic microorganisms were observed. Only aerobic microorganisms were observed in the samples. According to the tests performed, the highest content of microorganisms, after incubating all samples in the same conditions, was observed in non-irradiated samples. The lowest dose (1.34 kGy) causes an almost threefold reduction in the content of aerobic microorganisms. These results are directly correlated with the results presented in Table 3 regarding the water content of the tested samples. Microorganisms thrive at the water/fuel interface [28,29]. In the samples saturated with water, the content of aerobic microorganisms was about 40% higher than in the samples not saturated with water.

Table 4. Aerobic and nonaerobic microorganisms in RME samples.

Dose, kGy	RME		RME Saturated with Water	
	Number of Aerobic Microorganisms, col/cm^3	Presence of Anaerobic Microorganisms	Number of Aerobic Microorganisms, col/cm^3	Presence of Anaerobic Microorganisms
0	48.7 ± 3.9	not observed	87,7 ± 2.1	not observed
1.34	15.0 ± 2.0	not observed	25.0 ± 2.4	not observed
2	1.3 ± 0.5	not observed	13.0 ± 1.6	not observed
3.99	0.3 ± 0.5	not observed	7.3 ± 1.2	not observed
6	0	not observed	0.7 ± 0.5	not observed
8.04	0	not observed	0	not observed
9.93	0	not observed	0	not observed
20.5	0	not observed	0	not observed

The content of microorganisms is not a standard parameter mentioned among the parameters to be measured in accordance with the EN 14214 standard [25]. However, as can be seen in the case of RME, the use of a dose of about 4 kGy completely eliminates microbiological life from the tested samples without any water content other than that resulting from the industrial process. In the case of RME samples saturated with water, the sterilization dose is twice as high, which results from the possibility of the development of microbiological life in the water layer. This effect is obtained without the use of chemical biocides.

3.8. Ester Content

Total ester content in all samples has been compared with the results of the authors' previous research [26,27]. The e-beam treatment had been performed differently—a small amount (about 5 mL) of each sample had been exposed to constant irradiation, so the impact on RME was quite significant. In this study, biofuel was irradiated while flowing through a glass pipe in greater amounts. Limited time of exposure, as well as constant

mixing caused by the flow, weakened the influence of the radiation. The compared results are shown in Figure 7.

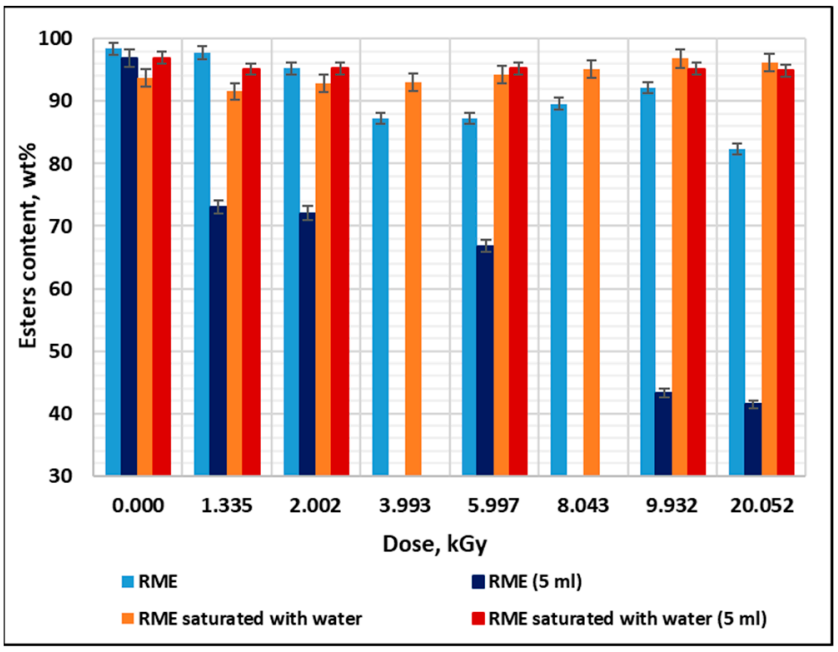


Figure 7. Ester content in all samples in comparison with preliminary research [26,27].

There are different trends for both series of samples. In the case of RME not saturated with water, the ester content decreased significantly with increasing absorbed dose of radiation. A slight increase for doses of 8.043 kGy and 9.932 kGy was most likely due to the unsatisfactory accuracy of the method used. The observed decrease in FAME content was not as high as in the case of samples from preliminary research. The reason was the aforementioned difference in irradiation procedure.

Analysis of the results of the water-saturated samples showed that the radiation did not cause changes of ester content in them. This corresponds to the results described in previous work of the authors [26,27]. Therefore, it is logical to assume that the water acts in a protective manner during irradiation of RME.

According to EN 14214, biodiesel should contain a minimum of 96.5 wt% of FAME. Maintaining this ester content could be possible with the addition of water prior to irradiation or performing the process in a flow system, applying minimum doses (up to about 2 kGy). Changes in the concentration of methyl esters of higher fatty acids are the same as the change in the oxidative stability index. This should be explained by the resulting stable products that reduce the content of methyl esters measured in accordance with EN 14103.

4. Conclusions

The applied flow system facilitated the effective irradiation of RME samples. Collected research material concerning the studied properties of FAME allowed us to draw the conclusions presented above.

The tested parameters were compared with the normative values from the ISO 14214 standard. Density, kinematic viscosity, flash point, water content and cold filter plugging point as a macroscopic parameters do not change despite their irradiation with ionizing radiation. Change was observed in microscopic parameters such as oxidation stability and esters content. During irradiation in RME, the samples formed oxidation products that lower the oxidative stability. It was observed that, for esters content, the requirements of the standard could be met in the case of using very small doses. In the case of RME samples saturated with water, the changes in the ester concentration were

smaller, but the resulting products decreased the oxidation stability. Sterilization FAME was observed with the use of e beam radiation to apply very low doses (up to 2 kGy) in the flow system without adding water. Sterilization FAME saturated by water requires the use of higher doses of radiation, which adversely affects the oxidative stability. The results presented in this paper directly indicate the possibility of using ionizing radiation for FAME sterilization without affecting the fuel performance parameters. The results indicate that sterilization should be carried out immediately after production without access water. The conclusions of the research can be transferred to materials type FAME. However, in the case of FAME with a significantly different chemical composition, tests should be performed to check the effects of radiation. In the case of fatty acids containing a greater amount of unsaturated bonds, the changes will most likely have an even greater impact on the oxidative stability or low-temperature parameters.

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