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Investigation of the effect of pre-sowing electrical stimulation of winter rapeseed on its spectral-luminescent properties

Abstract. The samples of rapeseed oil obtained by dissolving ground rapeseed in hexane (chemical extraction method) were studied. Portion of sample seeds was left unthreatened as the reference, another was treated by an electrostatic field at intensity of $E = (0,5 \div 2,5)$ kV/cm and $t = 30c$. The behavior of fluorophores of solutions of rapeseed oil in hexane, namely, tocopherol, phenol, fatty acids, vitamins and pigments provided information on the chemical composition and cell structure of the studied seeds. It was established that the fluorophores of solutions of rapeseed oil in hexane prepared from unthreatened seeds and seeds treated at an electrostatic field intensity of $E = (0,5 \div 2,5)$ kV/cm and $t = 30c$ are characterized by identical spectral-luminescent properties. The spectral-luminescent properties of phenols, tocopherols, fatty acids, vitamins and pigments of rapeseed oil obtained from the untreated and treated seed, were identical, regardless of the modes of its pre-sowing treatment. Pre-sowing electric treatment of rapeseed by an electrostatic field in this mode does not cause changes in the chemical composition and structure of the cell, its fatty acid composition and the content of vitamins and pigments.

Streszczenie. Badano próbki oleju rzepakowego otrzymanego przez rozpuszczenie zmielonych nasion rzepaku w heksanie (metoda ekstrakcji chemicznej). Część próbek nasion pozostawiono bez naprężeń jako referencyjną, inną poddano działaniu pola elektrostatycznego o natężeniu $E = (0,5 \div 2,5)$ kV/cm i $t = 30c$. Zachowanie się fluoroforów roztworów oleju rzepakowego w heksanie, tj. tokoferolu, fenolu, kwasów tłuszczowych, witamin i pigmentów dostarczyło informacji o składzie chemicznym i budowie komórkowej badanych nasion. Ustalono, że fluorofory roztworów oleju rzepakowego w heksanie sporządzonych z nasion niezagrażonych i nasion traktowanych polem elektrostatycznym o natężeniu $E = (0,5 \div 2,5)$ kV/cm i $t = 30c$ charakteryzują się identycznymi właściwościami spektralno-luminescencyjnymi. Właściwości spektralno-luminescencyjne fenoli, tokoferoli, kwasów tłuszczowych, witamin i barwników oleju rzepakowego uzyskanego z nasion nietraktowanych i traktowanych elektrycznie były identyczne, niezależnie od sposobu ich przedsięwziętej obróbki. Przedsięwzięta obróbka elektryczna nasion rzepaku polem elektrostatycznym w tym trybie nie powoduje zmian w składzie chemicznym i strukturze komórki, składzie kwasów tłuszczowych oraz zawartości witamin i barwników. (Wpływ przedsięwziętej stymulacji elektrycznej rzepaku ozimego na jego właściwości spektralno-luminescencyjne)

Keywords: electrical stimulation, spectral-luminescent properties, rapeseed

Słowa kluczowe: stymulacja elektryczna, właściwości spektralno-luminescencyjne, rzepak

Introduction

Winter rape is the most common oil crop of the cabbage family. The main purpose of growing this crop is to obtain rapeseed oil, the content of which in the seeds is 38-50%. Rapeseed oil is widely used as a food product as well as in the confectionery, canning and food industries. The presence of harmful substances (erucic acid, glucosinates) in rapeseed determines the use of rapeseed processing products for industrial use in the textile, metallurgical, paint and other industries. Rapeseed oil is used to produce fuel - biodiesel, which is more environmentally friendly than petroleum products. Rapeseed varieties with a low content of erucic acid and glucosinates, the so-called "0"- zero-varieties, were bred for food needs [1]. In order to increase the yield of the sowing material of existing rape varieties and improve the quality of seeds, various methods of pre-sowing seed treatment are used, in particular methods of physical impact. The seed treatment by the alternating electromagnetic [2, 3, 4, 5], pulsed electric [6, 7, 8], weak magnetic field [9], acoustic cavitation [10], laser irradiation [11] or electrostatic field [12, 13, 14, 15] was the most common one among them. Pre-sowing treatment of rapeseed by the electrostatic field has become more widespread. In most cases, this treatment is carried out simultaneously with the electroseparation of rapeseed, which ensures the separation of various injured seeds and weed seeds. To select the most effective treatment mode, it is necessary to study the mechanism of influence of electromagnetic fields on the biological processes that occur in the treated seed and seedlings obtained from it. Some researchers have found that pre-sowing seed treatment by an electrostatic field increases the efficiency of

the seed cell functioning due to improved permeability of water through the membrane into the cell [16]. The cell water permeability, in turn, is a trigger for the start of hydrolytic enzymes, in particular amylase. This allows the seed to realize the genetic potential more effectively, to be more resistant to the effects of adverse environmental factors (lack of moisture, temperature differences, etc.) [14, 15, 16]. However, the reaction of the seed to the above methods of pre-sowing treatment is ambiguous. It depends on the parameters of a particular type of stimulation and the type of the seed. Therefore, despite numerous theoretical and experimental studies to identify the mechanisms of biological processes and the results of various pre-sowing seed treatments, the final answer to the questions is not obtained [17, 18, 19]. Despite the stimulating effect of various physical methods of pre-sowing seed treatment [13]. Kovalyshyn and Shvets [14] their inhibitory effect on the course of biological processes in treated seeds and their seedlings has also been registered. Data given in the work Molotkov [20] indicate the mutations in the seeds of winter wheat due to the effect of an electric field. Due to the fact that currently there are insufficient theoretical and experimental data that would reveal the causal relationship between pre-sowing electroprocessing of seeds and the resulting crop, the lack of thorough results in studying the mechanism of influence of electromagnetic fields on structural changes in cells of treated seeds and their seedlings, it is necessary to conduct in-depth studies, the results of which would expand knowledge about the mechanism of biological processes in treated seeds. The purpose of our research was to study the possible effect of pre-sowing treatment by an electrostatic field at intensity of

$E = (0,5 \div 2,5)$ kV/cm and $t = 30$ s on the biological activity of rapeseed, structure and chemical composition of its cell. The spectral-luminescent analysis of the rapeseed product (oil) was chosen as a provider of information on the structure and chemical composition of the seed cell. The spectral-luminescence analysis is characterized by high sensitivity, which allows to obtain additional knowledge about the causal relationship of pre-sowing electric treatment with chemical and biological processes occurring in the treated seeds. The absence of changes in the photoluminescence spectra and photoluminescence excitation spectra of the components of the oil obtained from the electrically treated seed relative to the oil obtained from the untreated seed may indicate no effect of electrostatic treatment of these parameters on the structure of its cell. If changes in these spectra are detected, it can be asserted that pre-sowing treatment of rapeseed by the electrostatic field leads to a change in the structure and chemical composition of the cell. This may indicate the presence of mutations in the seeds, which is undesirable taking into consideration the possible content of genetically modified organisms in products obtained from the electrically treated rapeseed.

Materials and methods

For research, the samples of rapeseed oil obtained by dissolving ground rapeseed in hexane (chemical extraction method) were used. The seeds were treated by an electrostatic field at intensity of $E = (0,5 \div 2,5)$ kV/cm and $t = 30$ s. In addition, the spectral-luminescent characteristics of samples of rapeseed oil, similarly prepared from control rapeseed that had not been treated by an electrostatic field, were registered as a benchmark. The photoluminescence spectra and photoluminescence excitation spectra of the above mentioned oil were recorded using the unit presented in figure 1. In the case of registration of photoluminescence spectra, the required region of exciting light was released from the radiated continuum of the deuterium lamp with the help of the monochromator MDR-12. Measurement of the spectra was carried out in the geometry of a direct angular. The investigated samples were in a quartz cuvette (10 mm x 10 mm x 45 mm). The registration of a light fluorescent signal was carried out in the mode of single photons calculation using a monochromator MDR-2 and a photoelectron multiplier FEP-100. The signal from the photomultiplier is converted to a digital code by a channel converter and processed by a personal computer. The measurement results were displayed in graphic and digital forms..

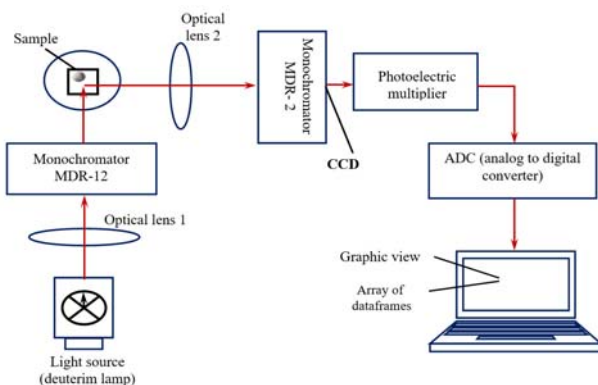


Figure 1. Block diagram of the unit for registration of spectral-luminescent characteristics of rapeseed oil obtained by the method of chemical extraction

To register the photoluminescence excitation spectra of the above mentioned oils in the same geometric configuration, the required region of the photoluminescence spectrum was distinguished using the monochromator MDR-2. As in the case of registration of photoluminescence spectra, the photosignal registration was carried out using the photoelectron multiplier of FEP-100.

Results

The first step towards determining the effect of pre-sowing treatment of rapeseed by an electrostatic field is to study the chemical composition of the seed cell. The behavior of fluorophores of solutions of rapeseed oil in hexane, namely, tocopherol, phenol, fatty acids, vitamins and pigments that are part of it, provided information on the chemical composition and cell structure of the studied seeds. We expected that in case of changes in the specificity of the interaction of fluorophores of solutions of rapeseed oil in hexane, the nature of relaxation channels of fluorophores can alter. As a result, the process of relaxation of electronic excitations, which are formed owing to UV radiation absorption, should be sensitive to the specificity of the interaction of the above mentioned fluorophores with the nearest neighbours. Figure 2 shows the spectra of luminescence (curves 1-4) and the luminescence excitation spectra (curves 1'-4') of solutions of rapeseed oil in hexane, provided that the solutions are excited with light at $\lambda_{excit} = 280$ nm.

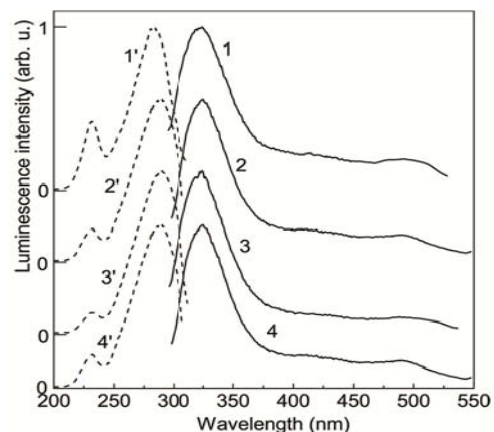


Figure 2. Photoluminescence spectra of 5% rape flour solutions prepared from rape seeds which were not subjected to (1) or subjected to different modes of pre-treatment by an electrostatic field in the case of solution excitation with light at $\lambda_{excit} = 280$ nm (curves 2-4), and the spectra of excitation of the photoluminescence band at $\lambda_{max} = 325$ nm of these solutions (curves 1' and 2'-4', respectively). 1, 1' - solutions of rape flour prepared from rape seeds which were not subjected to pre-sowing treatment by an electrostatic field; 2, 2' - solutions of rape flour prepared from rape seeds which were subjected to pre-sowing treatment by an electrostatic field at intensity of $E = 0,5$ kV / cm and $t = 30$ s; 3, 3' - solutions of rape flour prepared from rape seeds which were subjected to pre-sowing treatment by an electrostatic field at intensity of $E = 1,5$ kV / cm and $t = 30$ s; 4, 4' - solutions of rape flour prepared from rape seeds which were subjected to pre-sowing treatment by an electrostatic field at intensity of $E = 2,5$ kV / cm and $t = 30$ s.

The photoluminescence spectra of solutions of rapeseed oil in hexane prepared from rapeseed flour, which seeds went through various modes of pre-sowing treatment by the electrostatic field, are represented by curves 2-4. In all cases, the bands at $\lambda_{max} = 325$ nm with a long-wave continuum in the spectral region of 375-525 nm, are registered in photoluminescence spectra. Spectral position and structure of the photoluminescence band at $\lambda_{max} = 325$

nm (curves 2-4) are identical with the properties of solutions of rapeseed oil in hexane prepared from untreated (control) seeds, which have not been treated by the electrostatic field of the indicated parameters (curve 1). Identical for the above mentioned spectra of photoluminescence are their excitation spectra (curves 2', 3', 4' and 1' respectively), which are a duplet band at $\lambda_{excit.1} = 285$ and $\lambda_{excit.2} = 300$ nm with a short-wave satellite in the spectral region of 265 nm. These spectral-luminescent characteristics of the solutions of rapeseed oil in hexane, prepared from the rapeseed treated by the electrostatic field of the indicated parameters, are coincided with the spectral-luminescent characteristics of α -tocopherol [21, 22]. Proceeding from the identity of the spectral-luminescent characteristics of solutions of rapeseed oil in hexane prepared from rapeseed, which had not been subjected to the treatment or had been treated by the electrostatic field at intensity of $E = (0.5-2.5)$ kV/cm and time $t = 30$ s., it can be affirmed that treatment of sowing rape seeds by an electrostatic field with the above parameters did not lead to visible changes in the nature of the interaction of the tocopherol component of the solutions of rapeseed oil in hexane prepared from the above mentioned rape seeds, in the case of excitation of solutions with light at $\lambda_{excit.} = 280$ nm. The identical nature of the relaxation channel of solutions of rapeseed oil in hexane prepared from the treated and untreated rape seeds indicates the immutability of the nature of the interaction of a tocopherol component with other fluorophores (the presence of which is confirmed by the presence of a long-wave continuum in the spectral region of 375-525 nm. Actually high sensitivity of the spectral position of the maximum luminescence of the tocopherol component in vegetable oils, which may vary under the influence of external factors and the chemical composition of oil, makes it possible to investigate the specificity of the interaction between fluorophores of solutions of rapeseed oil in hexane as a result of the effect on the cell of the rapeseed of a constant electrostatic field at intensity of $E = (0.5-2.5)$ kV/cm and time $t = 30$ s. As noted above, the mechanism of electrophysical influence on seeds is reduced to a change in the penetration of water and various nutrients through the membrane [17]. That is, regardless of the permeability of the membrane, the structure and chemical composition of the cell have not changed. This is confirmed by the results of the studies conducted by means of pre-sowing treatment by an electrostatic field with the above parameters for the cereal seeds [23]. The long-wave part of the luminescence spectra (curves 1-4) in the spectral region of $\lambda > 370$ nm indicates the existence of other centres of luminescence of solutions of rapeseed oil in hexane. Indeed, in the case of luminescence excitation of solutions of rapeseed oil in hexane with the light at $\lambda_{excit.} = 300-325$ nm, in the luminescence spectrum the intensive luminescence bands are registered in the spectral region at $\lambda \geq 400$ nm (Figure 3, curves 1-4). The spectra of excitation of the luminescence band at $\lambda_{max} = 400$ nm are represented by curves 1'-4'. The intensive bands in the region of 300, 315, 325 nm in the spectrum of excitation of the luminescence band at $\lambda_{max} = 400$ nm are identical to the structure of the spectra of excitation of polyunsaturated fatty acids (linoleum, linolenic, arachidone) and monounsaturated one (olein) [24, 25]. Such a coincidence indicates that the above polyunsaturated fatty acids are included in the composition of the solutions of rapeseed oil prepared by the method of chemical extraction. It should be noted that the intensive band in the region of 250 nm in the spectrum of the luminescence band at $\lambda_{max} = 400$ nm is characteristic of the solvent used by us for the preparation of solutions of rapeseed oil.

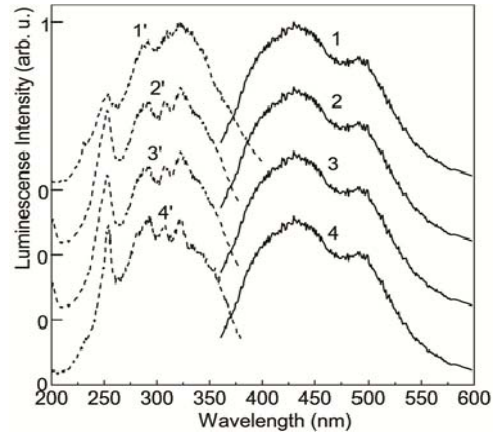


Figure 3. Photoluminescence spectra of 5% rape flour solutions prepared from rape seeds which were not subjected to (1) or subjected to different modes of pre-treatment by the electrostatic field in the case of solution excitation with light at $\lambda_{excit.} = 325$ nm (curves 1-4), and the spectra of the excitation of the photoluminescence band at $\lambda_{max} = 400$ nm of these solutions (curves 1' and 2'-4', respectively). 1, 1' - solutions of rape flour prepared from rape seeds which were not subjected to pre-sowing treatment by the electrostatic field; 2, 2' - solutions of rape flour prepared from rape seeds which were subjected to pre-sowing treatment by the electrostatic field at intensity of $E = 0,5$ kV / cm and $t = 30$ s; 3, 3' - solutions of rape flour prepared from rape seeds which were subjected to pre-sowing treatment by the electrostatic field at intensity of $E = 1,5$ kV / cm and $t = 30$ s; 4, 4' - solutions of rape flour prepared from rape seeds which were subjected to pre-sowing treatment by the electrostatic field at intensity of $E = 2,5$ kV/cm and $t = 30$ s.

As a result of excitation of the solvent in the region of 250 nm, it luminesces effectively in the region of 320 nm, which leads to an effective reabsorption of the radiation of the solvent with fatty acids, which are included in the composition of the solutions of rapeseed oil. Analyzing the luminescence spectra (curves 1 and 2-4) of the solutions of rapeseed oil prepared from the seed that was not subjected to (curve 1) or subjected to treatment by an electrostatic field at intensity of $E = 0,5 \div 2,5$ kV/cm and $t = 30$ s (curves 2-4), it can be noted that treatment of rapeseed by an electrostatic field (with the indicated parameters) did not lead to a change in its fatty acid composition of polyunsaturated fatty acids (linoleum, linolenic, arachidone) and monounsaturated one (olein). The non-elementary structure of the luminescence spectrum of solutions of rapeseed oil in the spectral region of $\lambda \geq 400 \div 525$ nm in figure 4 (curves 1-4) indicates the existence of other centres of luminescence, except already described - a tocopherol component and a fatty acid component of rape oil solutions. Namely, an increase in the wavelength of exciting UV radiation to $\lambda_{excit.} = 350$ nm leads to the appearance of new luminescence bands with $\lambda_{max} = 400, 425, 465, 490$ and a shoulder in the spectral region of 520 nm (Figure 4, curves 1-4). The spectra of excitation of each luminescence band are presented in figure 5 (curves 1', 2', 3', 4', respectively). The spectral position of the indicated luminescence bands and the structure of the spectra of the indicated bands coincide with the spectral-luminescent characteristics of vitamins B6 (pyridoxine) ($\lambda_{max} = 425$ and 465 nm), vitamin D ($\lambda_{max} = 490$ nm). The spectral position of the shoulder in the region of 520 nm coincides with the spectral position of the luminescence bands of vitamins B2 (riboflavin) and E (tocopherol) [25, 26].

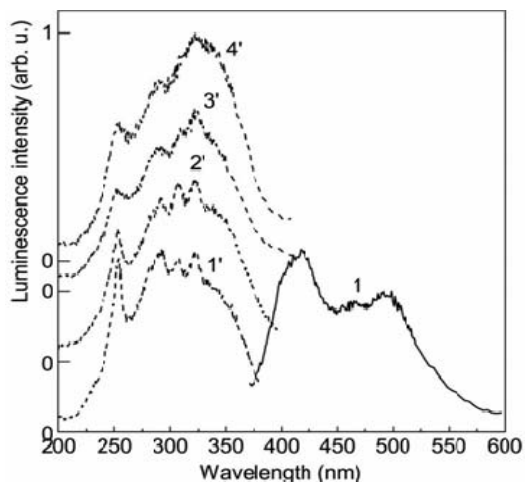


Figure 4. Photoluminescence spectra of 5% rape flour solution prepared from rape seeds which were not subjected to (1) or subjected to different modes of pre-treatment by an electrostatic field in the case of a solution excitation with light at $\lambda_{excit} = 350$ nm (curves 2-4), and spectra of the excitation of the photoluminescence band at $\lambda_{max} = 490$ nm of the indicated solutions (curves 1' and 2'-4', respectively). 1, 1' - solutions of rape flour prepared from rape seeds which were not subjected to pre-sowing treatment by the electrostatic field; 2, 2' - solutions of rape flour prepared from rape seeds which were subjected to pre-sowing treatment by the electrostatic field at intensity of $E = 0,5$ kV/cm and $t = 30$ s; 3, 3' - solutions of rape flour prepared from rape seeds which were subjected to pre-sowing treatment by the electrostatic field at intensity of $E = 1,5$ kV/cm and $t = 30$ s; 4, 4' - solutions of rape flour prepared from rape seeds which were subjected to pre-sowing treatment by the electrostatic field at intensity of $E = 2,5$ kV/cm and $t = 30$ s.

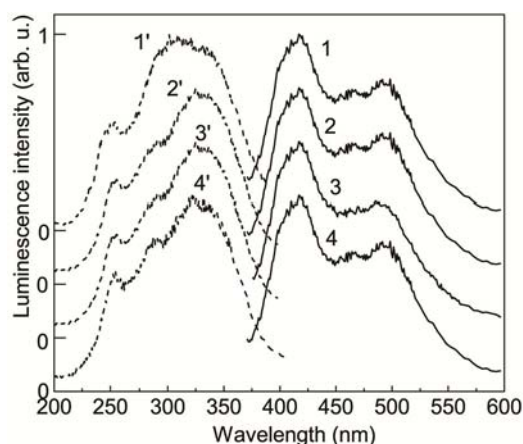


Figure 5. Photoluminescence spectra of 5% rape flour solutions prepared from rape seeds which were subjected to pre-sowing treatment by an electrostatic field at intensity of $E = 2,5$ kV / cm for 30 s (curve 1) and spectra of excitation of the luminescence bands of this solution at $\lambda_{max} = 400$ nm (curve 1'), $\lambda_{max} = 420$ nm (curve 2'), $\lambda_{max} = 460$ nm (curve 3'), $\lambda_{max} = 490$ nm (curve 4')

Thus, excitation of solutions of rapeseed oil in the spectral region $\lambda_{excit} = 350$ nm leads to luminescence of vitamins B6, D, E, B2 (Figure 5, curve 1), which are characterized by excitation spectra in the region of $250 \div 400$ nm (curves 1'-4'). Identity of spectral-luminescent properties of solutions of rapeseed oil prepared from rapeseed, which was treated by an electrostatic field at intensity of $E = 0,5 \div 2,5$ kV/cm and $t = 30$ s, indicates that the above seed treatment does not lead to changes in the chemical composition and nature of the interaction of fluorophores of solutions of rapeseed oil - tocopherol, polyunsaturated fatty acids and vitamins

Conclusion

1. Solutions of rapeseed oil contain three types of fluorophores: tocopherol (variety of vitamin E), polyunsaturated fatty acids (linolenic, linoleum, arachidone) and vitamins B12, B6, D, B2.
2. Treatment of rape seeds by an electrostatic field at intensity of $E = 0,5 \div 2,5$ kV/cm and $t = 30$ s does not lead to changes in the chemical composition of fluorophores of solutions of rapeseed oil - tocopherol (a variety of vitamin E), polyunsaturated fatty acids (linolenic, linoleum, arachidone) and vitamins B12, B6, D, B2. Confirmation of this is lack of changes in photoluminescence spectra and luminescence excitation spectra of fluorophores, which are part of the solutions of rapeseed oil.
3. Stability of the chemical composition of the solutions of rapeseed oil prepared from the seeds, which went through various pre-sowing treatment by the electrostatic field and more active vegetation of the treated seed confirms the assumption that pre-sowing treatment by the electrostatic field contributes to more efficient water penetration into the seed cell.
4. The obtained results of the studies testify that the electric treatment of rape seeds does not lead to any changes in the structure of the cell. On the basis of this, it can be affirmed the expediency of pre-sowing electric treatment by the electrostatic field at intensity of $E = 0,5 \div 2,5$ kV/cm and $t = 30$ s during seed preparation.

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