EPR-Investigation of Irradiated Imported Foodstuffs and Parameters of EPR Signals

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Abstract

Study by EPR (electron paramagnetic resonance) technique of a wide range of food products imported to Kazakhstan and of analogous domestic foodstuffs has been carried out in order to develop a methodic that would make it possible to control the fact of radiation sterilization of food. Elaborated method of sample preparing for EPR-study included dividing into components, removing moisture by drying or lyophilizing then grinding till dimensional work fraction. Selection of optimal conditions for spectra recording and algorithm of spectra treatment enabled authors to allocate correctly initial EPR-signals and to obtain information on shape, intensities and parameters. Model gamma-irradiations of the initial whole products with subsequent study of prepared EPR-specimen have been carried out.

Influence of irradiation on the intensities of EPR-signals and possible changes of EPR-characteristics have been studied in dose range (0.1-25) kGy. EPR-signals of free radicals (FR) of radiation origin were obtained from the EPR-spectra of gamma-irradiated delicious fruits, citrus, vegetables and others. Nonmonotonous dependences of FR concentration on a dose, along with linear ones were observed. Intensities of initial signals and of radiation-induced ones are much higher in solid components, than in pulp, dependences on dose are more close to linearity, which can be used in some cases for retrospective EPR-dosimetry. The obtained results can be explained taking into account specificities of the studied objects, and probable impact of radiochemical reactions of the irradiated water products with radicals formed in substances of foodstuff at irradiation.

Introduction

Radiation technologies are widely used in world practice [1-3]. High irradiation doses up to kilograys are used to extend storage time of foodstuffs and to transport them at long distances. Radiation sterilization of products (RSP) causing destruction of microorganisms, fungi and viruses is applied to a wide range of foods – delicious and exotic fruits, vegetables, semi-finished meats, seafood, dried fruits, spices and other products [1-8]. Radiation treatment by lower irradiation doses is also used to delay ripening of vegetables and for other purposes [2, 3]. Powerful facilities for radiation sterilization work in Turkey, Europe, on Eurasian and African continents [1, 3, 6]. The USA and China share the first place in commercial irradiation of foodstuffs. South Africa and Latin America subject mango and papaya fruits to radiation treatment [1, 4] before transportation to European countries. Although application of RSP technologies for food storage has been approved by the IAEA and the WHO [3], they may cause problems related to nutrition safety. During irradiation treatment of vegetables, meat and other products free radicals can be produced [4, 6-9] in excess quantities, moreover, along with them unknown, and, probably, harmful products may be formed.

EPR analysis is recommended as an effective method controlling the process of radiation treatment; it has been widely used for detection of paramagnetic products – free radicals [1, 4, 6-11]. International standards [12-14] for the applied irradiation doses and for the quality of irradiated foods have been developed. However, additional irradiation by high dose rates and high doses may lead to sharp increase in the concentration of free radicals,

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and these radicals can differ from metabolic radicals, i.e. high-energy radiation can destroy molecules leading to unpredictable products.

Special methods and protocols exist in some countries to control doses of irradiated products by EPR or other techniques [6-14], the irradiated food must be specially marked, other countries have limited import of such products. At present, there is no control of imported foodstuffs in Kazakhstan. The first experience in the EPR analysis of irradiation influence on foods was gained at the Institute of nuclear physics, RK [15], and EPR showed promising results as a method of such control.

Therefore in order to develop such a technique of identification of the fact of food irradiation it is very important to study the direction of radiation impact on foods by doses used in practice of radiation sterilization using EPR-method, in particular, to study accumulation of paramagnetic centers and characteristics of irradiated products as free radicals.

Experimental

Materials and methods

The objects of investigation were samples of products imported to Kazakhstani markets, prepared for EPR analysis, and EPR-samples of similar domestic products.

In order to prepare samples from the products chosen for EPR-analysis it was necessary to remove the maximal amount of moisture under temperature conditions not destroying existing free radicals. For this purpose the samples were first kept in the air medium, then in the oven at 45°C or lyophilized at 40°C in a special vacuum system [16]. The dried-up samples were grinded in the agate mortar till obtaining a work fraction with granules of (0.2-0.8) mm. Before recording EPR-spectra the samples were again subjected to 2h drying at 45°C in the drying oven.

EPR-spectra were registered by ESP300E spectrometer ("Bruker", Germany) in the X-range with the spherical cavity 9405sp330 in automatic mode with summation of obtained spectra, and the EPR-10mini spectrometer (SPIn, Russia). The samples of mass of 50-100 mg were placed into tubes from suprasyl ("Bruker") without initial EPR-signals.

In order to control reproducibility and to obtain reliable data, at least, three successive records of EPR-spectra of samples with intermediate shaking and subsequent tuning were made. In addition, the background spectra of the cavity with an empty measuring tube were recorded.

EPR spectra of prepared fractioned samples were first recorded under the same conditions, which enabled us to compare the amplitudes and to identify differences between the forms of initial EPR-signals. Then, for some types of samples, optimal parameters of spectrum recording were chosen and EPR-characteristics were measured; the procedure of selection of optimal parameters is described below.

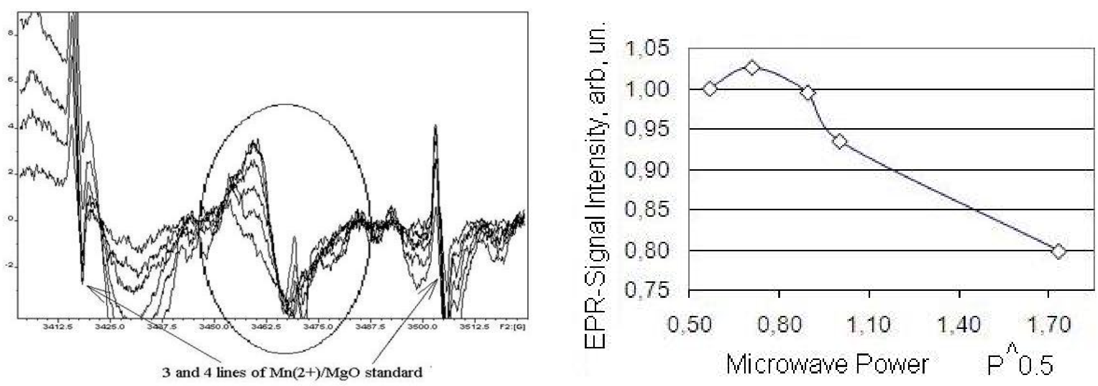
The irradiation experiments were carried out on the gamma ^{60}Co facility by a dose rate of 0.72 kGy per hour in the electron equilibrium conditions. After irradiation the samples were kept for over 24h before EPR-examination. The choice of doses was determined by a variety of aims pursued by the radiation processing. According to the WHO and the EC recommendations [1], in radiation sterilization of products doses up to kilograys can be used; irradiation of vegetables and fruits by a dose from 0.1 kGy to 1 kGy inhibits germination processes [1-3]. Therefore in this work food samples were irradiated not only by doses (5-25) kGy, used for disinfecting, but also by rather low doses of (0.1-0.5) kGy.

Results and Discussions

In order to choose optimal parameters we used the options provided by the software of ESP300E spectrometer, which makes it possible to record spectra with a tested parameter varied in the automatic mode within the predetermined limits. It was used for selection of microwave power and amplitude of modulation, considerably affecting the intensity of signals through different mechanisms. The stack of EPR-spectra shown in Figure 1a, illustrates that non-optimal parameters, in this case – microwave power – can essentially affect the true shape and sizes of informative signals; the figure shows EPR-spectra of a banana sample subjected to irradiation by microwave power ranging from 0.32 mW to 20 mW.

Based on the registered spectra the dependences of normalized amplitudes of EPR signals on variable parameters were obtained.

Figure 1b shows the value of the EPR-signal intensity as a function of the square root of the microwave power in the range from 0.25 mW to more than 3 mW; such dependencies show signal saturation with increasing microwave power. It is seen that the best value of microwave power is close to 0.5 mW.



a b

(a) – EPR-spectra for various conditions obtained in the automatic mode (banana, Costa-Rica);
(b) – Signal intensity as a function of microwave power (kiwi, Greece)
Fig. 1 – EPR-signal intensity as a function of microwave power.

It should be noted that in processing of EPR-spectra and comparing intensities of signals the values of amplitudes of EPR-signals were always normalized to mass, usually 100 mg, and to chosen conditions of registration. EPR-spectra were recorded in the range of magnetic fields (10-12) mT, which enabled us to control the position of EPR-signals and their intensities with respect to the 3-rd and 4-th SFS lines (superfine structure) of Mn (2+) ion in the MgO reference sample.

The algorithm of spectra processing and options of the program of ESP300E spectrometer enabled us to allocate EPR-signals as initial ones and signals corresponding to radiation response, which were caused by formation of free radicals as a result of irradiation.

In order to obtain informative EPR-signals the subtracting the background spectra registered at the same conditions and in the same range of magnetic fields from the obtained experimental spectra has been done. To take into account fluctuations of sensitivity the experimental spectra were normalised to the 3-rd line of the 6-line SFS of the reference Mn (+2)/MgO sample, taking into account also the quality of the loaded cavity. Table 1 shows parameters of initial EPR-signals for some samples.

Tables 2 and 3 show EPR-characteristics of some foodstuffs irradiated by doses up to (5-25) kGy and by lower doses (0.1-0.5) kGy.

In most cases the initial EPR signals from different components of the same product were of the same type, but of different intensities.

The increase in the irradiation dose caused the change in signal intensity; in some cases it also caused changes in EPR-characteristics, which could be indicative of formation of the other type of radicals. Table 3 presents parameters of signals obtained after irradiation and initial signals.

Table 1
EPR- signals in the initial products

Product name	Country of origin	Component	Shape of signal	Parameters	
				Width of signal ΔH, mT	g-factor ±0.0005
Lime	China	Peel	Singlet	0.77	1.9990
Orange	Morocco	Peel	Singlet	0.86	2.0057
Kiwi	Greece	Peel	Singlet	0.64	2.0045
Banana	Costa Rica	Peel	Singlet	0.72	2.0043
Banana	Costa Rica	Pulp	Singlet	0.82	2.0041
Banana	Brazil	Peel	Singlet*	0.86	2.0044
Banana	Brazil	Pulp	Singlet*	0.86	2.0045
Avocado	South Africa	Peel	Singlet	0.84	2.0042
Avocado	Israel	Peel	Singlet	0.64	2.0041

* - A singlet in banana components is actually a sum of two singlets, whose parameters are not essentially differed.

Table 2

Types and characteristics of EPR-signals in the spectra of irradiated fractions of products

Product name	Country	Dose of irradiation, kGy	Signal type	Width of signal ΔH, mT	g-factor
Lime	China	0.5	Singlet	0.77	2.0090
Orange	Morocco	10	Asimm. dublet	Centr. 0.48 max. 0.85	2.0056
Avocado	South Africa	10	Singlet	0.77	2.0040
Avocado	The same	20	Singlet	0.63	2.0040
Avocado	-“-	25	Singlet	0.65	2.0041

Table 3

EPR-signals and parameters in the spectra of initial and irradiated products

Product	Country of origin	Applied dose	Shape of signal	Width of signal ΔH, mT	g-factor ±0.0005
Grapes, peel	Spain	Initial	“-“	0.621	2.00405
Grapes, peel	-“-	5 kGy	“-“	0.620	2.00403
Grapes, peel	-“-	10 кГр	“-“	0.666	2.00486
Grapes, seed	Spain	Initial	“-“	0.704	2.00470
Grapes, seed	-“-	5 kGy	“-“	0.704	2.00471
Grapes, seed	-“-	10 кГр	“-“	0.727	2.00471
Grapes, seed	-“-	20 кГр	“-“	0.760	2.00480
Peach, peel	Uzbekistan	Initial	Singlet	0.771	2.00486
Peach, peel	The same	5 kGy	Singlet	0.789	2.00456
Peach, pulp	-“-	Initial	The same	0.908	2.00490
Peach pulp	-“-	5 kGy	“-“	0.804	2.00462
Peach pulp	-“-	10 kGy	“-“	0.804	2.00459
Peach, stone	-“-	5 kGy	“-“	0.841	2.00460
Peach, stone	-“-	10 kGy	“-“	0.806	2.00454
Peach, stone	-“-	20 kGy	“-“	0.634	2.00447

In order to estimate the integrated radiation dose absorbed by the studied object, the EPR-dosimetry uses the method of additional irradiation, in which correct dose estimations are obtained by back extrapolation of linear dose dependence of signal intensity. Such a method takes into account individual radiation sensitivity of a given object [17].

In this work we used additional gamma-irradiation of initial whole samples. Some part of the whole product, subjected to the first dose of γ-radiation, was used for preparation of EPR-samples of components in the form of dimensional granules. The remaining part was irradiated by the second dose, and so on. This enabled us to obtain a function of concentrations of free radicals vs irradiation dose in range (0.2-25) kGy. For such experiments samples of exotic fruits, citrus and other products were used.

In some cases the dependence of the EPR-signal on the absorbed dose corresponded to the “standard” type of the curve of accumulation of radiation defects [18]. Similar dose dependence is shown in Fig. 2a. In the initial dose interval the EPR-signal intensity, corresponding to the concentration of free radicals, increases linearly, and then the rate of FR accumulation slows down.

In many cases dose dependences were not monotonous. The accumulation stage was followed by the “saturation” stage, where the rate of formation of free radicals decreased and their concentration remained almost at the same level, and in some products PMC concentration decreased.

In Fig. 2b the curve of FR concentration in grapes from Spain showed a weak growth in grape seeds (upper curve) and peel (lower curve) in the range of irradiation doses up to 5 kGy and a decrease in both components with subsequent increase in the irradiation intensity up to 20 kGy. The initial EPR-signal had the width ΔH_{pp} 0.62 and 0.70 mT for peel and seed, after irradiation the growth of wider ΔHpp signals, 0.67 mT and 0.76 mT, was observed.

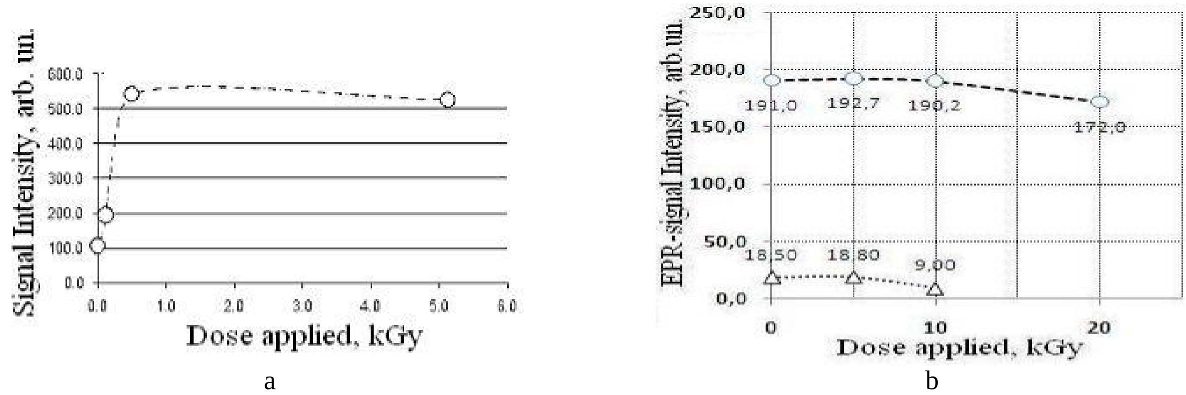


Fig. 2. Dose dependence of EPR- signals in the spectra of irradiated lime (peel) and grapes (seed and peel).

Normalized EPR-signals for solid components – seeds and stones – are higher than those for components of pulp and peel. This is seen for the components of grape, Spain, (Fig. 2b) and peach, Uzbekistan, (Fig. 3a). The intensity of the EPR-signal in Fig. 3a is higher for stone (upper curve) than for pulp (lower curve).

In this case EPR-signal in all components was probably formed by one type of free radicals, EPR-characteristics of which are presented in Table 3 – width ΔH_{pp} is about (0.8-0.9) mT, g-factor is (2.0046-2.0048); it changes after irradiation only insignificantly in the spectra of these components.

The width of the EPR-signal observed in the solid component decreased with radiation, at a dose of 20 kGy it became more narrow (0.63 mT). This fact indicates a change of the dipole width of the EPR-signal connected with the increase in the local concentration of radicals [18]. This effect was observed under identical registration conditions in different series of measurements.

In the other samples the dose dependence of the EPR-signal had a different character, for example, in the lemon (Spain) and orange peels (Morocco – see Fig. 3b) the signal intensity decreased at the beginning of irradiation, and then increased again.

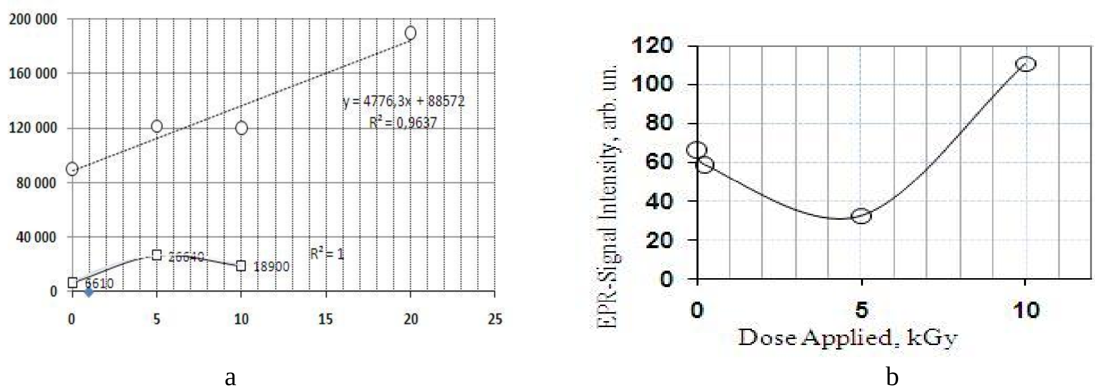


Fig. 3. Dose dependence of EPR-signals for components of irradiated peach (a) and orange, peel (b)

Such facts cannot be explained without taking into account specificities of the objects under study. It is possible to suppose that free radicals could be formed in product samples as a result of other influences. It is quite probable that in peels of fruits they could be formed as a result of preliminary radiation – ultraviolet part of the solar spectrum.

Similar nonmonotonous changes in FR concentration were observed in the apple subjected to successive irradiation by (2-10) kGy doses. This dependence is shown in Fig. 4 together with two approximating curves and corresponding correlation coefficients.

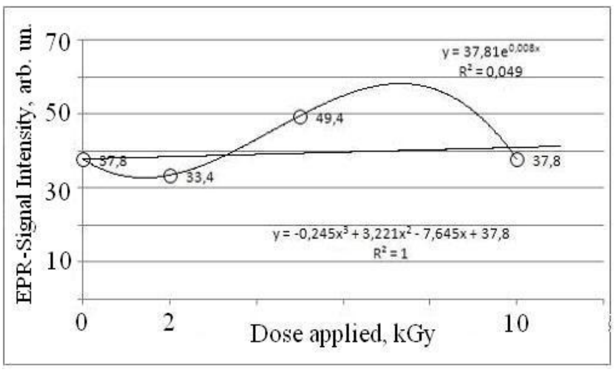


Fig. 4. Dose dependence of EPR-signals in the apple spectra

The obtained data show that irradiation of the whole products gives nonlinear and nonmonotonous dependences of FR concentration on the radiation dose. It is observed more often for the components containing more moisture where biochemical processes can be more active. In solid parts of fruits the dose dependences were close to linearity.

Organic substances of fruit and other studied objects consist of amino acids, biopolymeric chains, and sugar. One can suppose that all these components can give contribution to radiation-chemical processes in a whole “alive” product.

For products containing more sugar, cellulose and oils, we carried out experiments on their extraction by various types of solvents in order to determine contribution of these substances to EPR-signal intensity and to reveal possible difference in its characteristics.

It was shown that EPR signals corresponding to the concentration of free radicals increased after extraction by water and tested organic solvents. The obtained results are ambiguous and need further more detailed study.

Irradiation causes ionization of molecules including the remaining molecules of water [1].

$$H_2O + \gamma = H_2O^{+*} + e^- \quad (1)$$

The cation-radical formed in this reaction can be transformed thermally with formation of HO* radical and a proton or with formation of an anion-radical HO^{*-} and an atom of hydrogen.

$$H_2O^+ = HO^* + H^+ \quad (2)$$

$$H_2O^+ = HO^{*-} + H^* \quad (3)$$

These particles are small and extremely active. They recombine through interaction with inactive macroradicals which already exist in the sample.

Calculations made by the semi-empirical PM6 [19] method according to the MOPAC2012 program, showed that the process (3) prevailed in a vacuum or in the environment with a low dielectric constant ε. With growth of dielectric constant it becomes more favorable (2).

Hence, formation of products of radiation from different substances in high concentrations leads to interaction between them with recombination.

Thus, formation of peroxides in lipids affects food quality as it initiates autoxidation in unsaturated fats [9].

Figure 5 shows the energy of separation as a function of distance.

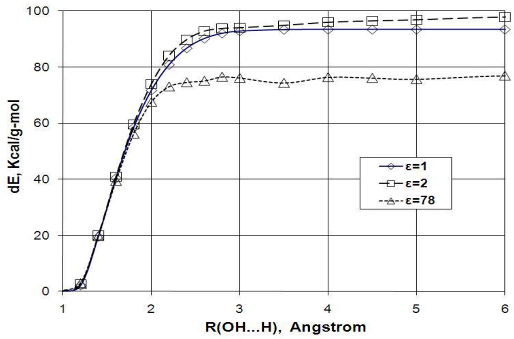


Fig. 5. The energy of separation as a function of distance for various ε values.

When part of substances is removed, we register selected type of radicals; this fact can be helpful for increasing sensitivity of the developed effective express methodics of EPR-identification of radiation- sterilized foodstuffs.

The dependence of the EPR-signal intensity on the irradiation dose can be used for control of preliminary irradiation in two cases. If the dose dependence of concentration is linear in a certain dose interval, it is possible to suppose that it indicates a possibility of application of extrapolation.

The curve of FR accumulation in the lime peel was close to linearity in the interval up to 0.5 kGy. A retrospective approach to this case gives a rather low dose, which can be most likely explained by peel irradiation by UV-component of the solar spectrum.

Nonmonotonous dependences indicate the effect of radiation exposure, in this case it is more convenient to use solid parts of fruits (stones, seeds), which can contain much higher concentration of free radicals than, for example, pulp.

The thin skin, peel also contains higher concentration of radicals after radiation than pulp, but this component could be exposed to sunlight. In such a case we have to distinguish the effect of sterilization irradiation from the effect caused by natural ultraviolet solar radiation.

The new radiation effects are mainly in agreement with our previous data and conclusions of other authors, and need further more detailed investigation.

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