Gamma irradiated dill and parsley, two very used aromatic herbs, were studied by electron spin resonance (ESR) spectroscopy. Stable radicals are put in evidence and their concentration versus irradiation dose, following a saturated exponential dependence, was determined. ESR spectra structure was simulated with an orthorhombic spin Hamiltonian. The spectra are quite stable in time, contrary to previously reported data. ESR spectroscopy can be used for irradiation identification of such very dry plant samples.

Key words: free radicals, aromatic herbs, ESR.

1. INTRODUCTION

Aromatic herbs and spices are widely used in food, pharmaceutical and cosmetic industry. Grown, processed and stored often in rather inadequately controlled conditions with respect to food hygiene, the herbs and spices can be exposed to high level of natural contamination by pathogenic bacteria, parasitic organisms, fungi, toxigenic moulds, or pathogens like *Salmonella*, *Escherichia coli* or *Bacillus cereus*. The extra decontamination is still necessary [1], even when good practices during harvest and processing are used. Three types of decontamination techniques have been used – heat decontamination, fumigation with ethylene oxide and methyl bromide, and ionizing radiation decontamination. Heat decontamination, although very simple, can cause thermally induced changes, like thermal decomposition, loss of volatile components, and production of thermally induced radicals. The use of above mentioned substances for fumigation is also not recommended, as they are toxic compounds. Therefore, they are banned in many countries, including Europe. Food irradiation is, by far, less harmful as it does not leave chemical residues in the food product [2, 3]. Notwithstanding its use in food decontamination progressed rather slowly. One reason was the lack of enough irradiation units; a significant number of them became operating in the last
years. On the other hand the irradiation concept is slowly accepted by the public community and society, as it is associated with the harmful effect of nuclear radiation. Many toxicological studies have demonstrated that food irradiation with \( \gamma \), X rays or high energy electrons (with energies below 5 MeV) and doses up to 10 kGy does not effect food quality, evidently does not induce radioactivity in it, being absolutely safe for consumers [4]. Among measures taken to increase the public acceptance of irradiated food, its identification and labeling is of a major importance. Together with other methods like thermo- and stimulated photoluminescence, viscosity measurements, chromatographic analysis of hydrocarbons, DNA comet assay screening, microbiological screening DEFT/APL, electron spin resonance (ESR) spectroscopy is accepted as a standard detection method for a large variety of solid food (meat with bones, sugars, spices and herbs, fruits, vegetables, fish and shellfish) [5]. This is confirmed by the European standards emitted by European Committee for Standardization (CEN) [6].

Irradiated spices and herbs have been widely studied by ESR [7-10]. Irradiation induces free radicals, stable for various time periods, which give measurable ESR spectra. The type and time stability of these signals are strongly dependent on the material, as well on temperature, humidity, the presence of oxygen and other factors [9, 11, 12]. Usually, typical three-line ESR spectrum (a central line having a left and right smaller but wider satellites separated by 6.05±0.05 mT) is observed. Such a “cellulosic-like” radical is stable over a period of 70-90 days [3, 7], but different thermal behaviors were also reported [13]. Although this type of signal is recommended in EN 1787 (2000) for determination of irradiated food containing cellulose, it is not observed in all cases of cellulosic containing plants. Narrow, single line shape spectra are often reported [7, 8, 12]. Such type of ESR spectra are associated to “quinone – molecule radicals”, quite similar to radicals existing in degraded complex organic materials [14]. Therefore, an ESR study of each type of irradiated plant is worthwhile. This paper is dealing with irradiated parsley and dill, two aromatic herbs largely used in Romanian cuisine.

2. MATERIALS AND METHODS

Fresh exemplars of parsley and dill bought from local market in Bucharest, were carefully cleaned up and dried without taking any special conditions. The investigated specimens were presumed not be irradiated. All samples were ground using an agate mortar, then sieved and weighed. Irradiation was performed in air, at room temperature, at a dose rate of 6.1 kGy/h, using a \(^{60}\)Co Gamma Chamber 5000 (BRIT, India) irradiation facility of Horia Hulubei - National Institute of Physics and Nuclear Engineering, Bucharest. The absorbed doses were evaluated by means
of an ethanol-chlorobenzene dosimetry system with oscillometric readout method, and expressed as absorbed dose in water. The target doses applied to samples were of 1, 3, 5, 7, 9, and 15 kGy, respectively, with an average uncertainty of 0.1–0.2 kGy. EPR measurements were performed at room temperature by using a X-band (9.5 GHz) Adani CMS 8400 spectrometer, in National Institute of Materials Physics, with the following characteristics: frequency range in between 9.1–9.6 GHz; temperature range 83 K < T < 480 K; magnetic field 0.01 T < B < 0.7 T, and sensitivity of 2.5 x 10^{10} spins/Gauss. Irradiated and non-irradiated samples were introduced into quartz tubes placed invariably in the same position in the resonant cavity. All measurements were performed at the same microwave power. Finally, the spectra were scaled at the same amplification and a standard sample weight. The simulation of ESR spectra was performed with Bruker Win-EPR soft.

3. RESULTS AND DISCUSSION

Examples of ESR spectra for non- and irradiated parsley and dill samples, are given in Fig. 1 and Fig. 2. The native samples present weak signals with asymmetric shape, centered around \( g = 2.000 \). The \( \gamma \)-irradiation increases their intensity, but does not induce noticeable changes in their shapes (except the 15 kGy irradiated parsley).

![ESR spectra of parsley sample.](image1)

![ESR spectra of dill sample.](image2)

The double integration of these spectra, corresponding to the area below the absorption curve, shows a monotonous increase with irradiation dose for both herbs. This dependence, given in Figure 3, is often observed for irradiated herbs, seeds, etc. [12, 15, 16], being considered a common dependence (activation curve with saturation).
The equation used for fit is

$$A(D) = A_0 + A_{\text{sat}} \left(1 - e^{-\frac{D}{D_{1/2}}}\right)$$

(1)

where $A_0$ is the initial value, $A_{\text{sat}}$ and $D_{1/2}$ are the parameters related to radical generation and recombination rate, respectively.

The double integrated area (A) of the ESR signal is proportional to the number of radicals in the sample, N. Its dose dependence reflects the kinetics of reactions leading to the observed radical concentration. Usually, the mechanism leading to the final stable radical is very complex, even in the case of one component sample. Such generation curve with saturation implies a step with a monomolecular recombination reaction. Formally, if one presumes

$$\frac{dN}{dt} = K_d - kN$$

(2)

where $K_d$ – radical generation rate, $kN$ – radical recombination rate, $t = \frac{D}{d}$, $d$ – dose debit, one gets $k = \ln 2 - \frac{d}{D_{1/2}}$. The $D_{1/2}$ and k parameters are given in Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$D_{1/2}$ (kGy)</th>
<th>k (h$^{-1}$)</th>
<th>$\chi^2$ coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>parsley</td>
<td>1.95 ± 0.60</td>
<td>0.46 ± 0.13</td>
<td>0.9515</td>
</tr>
<tr>
<td>dill</td>
<td>5.83 ± 0.87</td>
<td>1.38 ± 0.21</td>
<td>0.9942</td>
</tr>
</tbody>
</table>
The samples kept at room temperature show slow recombination kinetics of the free radicals. In figures 4 and 5 are shown the ESR spectra of parsley and dill samples irradiated with 15 kGy, recorded immediately (a) and after 50 days (b).

These results do not agree with the literature data [7] for parsley, neither concerning the shape of the radical, nor its time stability. Therefore, a careful analysis of the ESR spectra was attempted. A spin-Hamiltonian for $S = 1/2$, $I = 0$, in orthorhombic symmetry was used

$$H = \beta \vec{B} \cdot \vec{g} \cdot \vec{S} = \beta \vec{B} \left( g_1 \alpha_1 S_x + g_2 \alpha_2 S_y + g_3 \alpha_3 S_z \right)$$  \hspace{1cm} (4)$$

$\vec{n}(\alpha_1, \alpha_2, \alpha_3)$ is the orientation of the magnetic field $\left( \vec{n} = \frac{\vec{B}}{B} \right)$ in the $g$ tensor principal axes reference system. The spectra have been simulated with Win-EPR soft. These are shown in Figs. 6 and 7, and the ESR parameters obtained are given in Table 2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose (kGy)</th>
<th>g tensor component</th>
<th>Line widths (mT)</th>
<th>Line shape</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$g_x$  $g_y$  $g_z$</td>
<td>$\Delta B_x$  $\Delta B_y$  $\Delta B_z$</td>
<td></td>
</tr>
<tr>
<td>Parsley</td>
<td>0</td>
<td>1.997  2.001  2.001</td>
<td>0.35  0.3  1.5</td>
<td>Gaussian</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.997  2.008  2.007</td>
<td>0.3  0.3  1.3</td>
<td>Gaussian</td>
</tr>
<tr>
<td>Dill</td>
<td>0</td>
<td>2.008  1.997  1.993</td>
<td>0.4  0.55  1.4</td>
<td>Lorentzian</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>2.008  1.997  1.990</td>
<td>0.3  0.55  0.6</td>
<td>Lorentzian</td>
</tr>
</tbody>
</table>
The simulated ESR spectra are quite acceptable. It suggests that, in spite of the heterogeneous composition of these plant samples, there is one major free radical, the same in both non-irradiated and irradiated samples. This is just a possible conclusion that must be confirmed by more experimental data.

4. CONCLUSIONS

The main results of this study are the following:
- The gamma-irradiation induces stable radicals in dry parsley and dill. They can be observed even after 50 days after irradiation.
The structures of the ESR spectra are quite complex. Simulation with orthorhombic spin-Hamiltonian for a paramagnetic center with \( S=\frac{1}{2}, I=0 \), give reasonable agreement. It does suggest the existence of at least a dominant radical type.

The dose dependence of the double integrated area, proportional to free radical concentration, follows a generation with saturation mechanism. The two plants have quite different behavior, suggesting that the reaction mechanisms during irradiation are different.

The results do not agree with published data in irradiated parsley, concerning both the shape of the ESR line and its time stability. Having in view the sensitivity of ESR spectrum to sample preparation, the differences obtained may be explained by different preparation techniques.

Acknowledgments. We are grateful to Dr. M.N. Grecu for ESR measurements and fruitful discussions. RMS would also acknowledge the Doctoral School of Physics Faculty and the Naval Academy “Mircea cel Batran” Constantza for all support during her studies.

REFERENCES

15. Polat, M., Korkmaz, M. *The ESR spectroscopic features and kinetics of the radiation-induced free radicals in maize (Zea mays)*, Food Res.Int., 37, 293–300, 2004