ESR Study of Irradiated Polysaccharides
ESR investigation of gamma irradiated pectin

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Polysaccharides irradiation is usually used for their decontamination of fungi, pathogenic bacteria, toxigenic molds, parasitic organisms. ESR studies are performed just to evidence their irradiation. Therefore the accent is put on radical stability in time and ESR signal intensity dependence on irradiation dose. Less attention is given to radical nature and their dependence on plant nature. In this report a study of gamma irradiated pectin is given. Dose dependence of double integrated ESR signal is analyzed in terms of a recombination reaction during the radiation period. Radical stability dependence on water content is put in evidence.

Keywords: free radicals, gamma irradiation, pectin, ESR

1. INTRODUCTION

Polysaccharides, like pectin, corn and potato starch and cellulose are widely used in food industry. The pectins are heteropolysaccharides, having a heterogeneous chemical structure depending function on the plant origin and extraction method [1]. The main structure is a backbone chain made of uronic acid (galacturonic acid) residues linked through α-1-4 glicosidic bonds. Several side chains containing sugars like arabinose, glucose, fucose, mannose, xylose are linked to the backbone chain. Pectins are soluble vegetable fibers, having the role of emulsifier, stabilizer and thickening agent in the food industry. It is extracted from the peel of some fruit. The richest fruits in pectin are citrus fruits (up to 30%) and apples (1-3%). Galacturonic acid has the structure:

Chemical structure of galacturonic acid:

\[ \text{CO}_2\text{H} \]

Carboxylic groups have poor acidic properties, so that:

\[ \text{C}=\text{O} \]

\[ \text{O}^- \]

\[ <-\text{C}=\text{O}-> \]

\[ <-\text{C}=\text{O} \]

\[ \text{OH} \]

The pectins contain about 200 molecules of galacturonic acid, the acid units being linked to (1 → 4) α-D-galacturonic acid linkages, linked to α-L-rhamnose residues (simple saccharide structures). In the natural state most of the carboxyl groups are methylated (about 80%) with the structure:

\[ \text{C} \]

\[ \text{O}^- \]

\[ \text{O-CH}_3 \]
The pectins are extensively studied [2, 3]. The apple pectins contain more sugars and have a molecule larger than the citrus pectins. Many studies have demonstrated that food irradiation with γ, X rays or high electrons (with energies below 10 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, 5]. 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 is accepted as a standard detection method for a large variety of irradiated solid food (meat with bones, sugars, spices and herbs, fruits, vegetables, fish and shellfish) [6]. This is confirmed by the European Standards emitted by European Committee for Standardization (CEN)[7]. Irradiated polysaccharides have been widely studied by ESR [8, 9, 13, 15]. 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, on temperature, humidity, the presence of oxygen and other factors [5, 7, 8, 11]. In irradiated fruits pectin radicals are significant, but are associated with radicals in other major constituents like cellulose, pulp, lignin [16]. Therefore it is important to identify the contribution of pectin free radicals to the whole ESR spectrum. This is one of the purposes of this paper. In the same time dose dependence of free radical concentration and their time stability will be investigated.

2. MATERIALS AND METHODS

The samples used in this paper are apple and citrus pectin, imported by Hofigal S.A., the main Romanian manufacturer of the natural products. All investigated specimens were presumed not be irradiated. Irradiation was performed in air, at room temperature, at a dose rate of 6.1 kGy/h, using a Co60 Gamma Chamber 5000 (BRIT, India) irradiation facility of Horia Hulubei National Institute of Physics and Nuclear Engineering Bucharest. 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 3, 7, 9 kGy, respectively, with an average uncertainty of 0.1-0.2 kGy. EPR measurements were performed at room temperature by using an X-band (9.5 GHz) Adani CMS 8400 spectrometer with the following characteristics: frequency range: 9.1-9.6 GHz; temperature: 83 K < T < 480 K; magnetic field: 0.01 T < B < 0.7 T; sensitivity: 2.5x1010 spins/Gauss. Irradiated and non-irradiated samples were introduced into quartz tubes (1 cm sample length, 3-4 mm inner tube diameter) and placed in the resonant cavity always in the same position. All measurements were performed at the same microwave power. Finally, the spectra were scaled at the same amplification and a standard sample weight. The numerical analysis of ESR spectra was performed with the Win-ESR Simpfonia, ESR spectra simulation program from Bruker.
3. RESULTS AND DISCUSSION
Examples of irradiated apple pectin are given in fig.1

![EPR spectra of apple pectin samples irradiated](image)

Fig. 1. EPR spectra of apple pectin samples irradiated

Irradiation increases their intensity, but does not induce noticeable changes in their shapes. The double integrated spectra, corresponding to the area below the absorption curve, show a monotonous increase with irradiation dose. These values are proportional to radical concentration in sample, therefore its dose dependence corresponds to radical concentration dose dependence. Their dependence is given in Figure 2.

![Dose dependence of double integrated EPR spectra of apple pectin samples](image)

Fig. 2. Dose dependence of double integrated EPR spectra of apple pectin samples.

It can be fitted with the equation

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

(1)

Where $A_0$ is the initial value, $A_{sat}$ and $D_{1/2}$ parameters related to radical generation and recombination rate. As you can see from Fig. 2, saturation effect appears at doses:
\[ D_{1/2} = 6.193 \text{ kGy} \pm 1.169 \text{ kGy}; \ A_{\text{sat}} = 437 \pm 23, \ A_0 = 0, \ \text{Adj.R-Square}=0.99428. \]

Such a dependence is quite common for irradiated food and was reported for many materials [17]. It corresponds to a differential equation for time dependent radical concentration \( N(t) \),

\[
\frac{dN}{dt} = Q - kN \quad (2)
\]

Where \( Q \) – rate of free radicals induced by irradiation in the sample (constant), \( k \) – reaction rate for a monomolecular reaction. The time dependence is changed in dose dependence

\[
t = \frac{D}{D_0}
\]

where \( D_0 \) is the dose rate. The solution for \( N(D) \) (with initial condition \( N(0) = 0 \)) is:

\[
N(D) = N_{\text{sat}} \left[1 - \exp\left(-\frac{\ln 2}{D_{1/2}} \cdot D\right)\right] \quad (3)
\]

Where \( N_{\text{sat}} = \frac{Q}{k} \), \( D_{1/2} = \frac{\ln 2}{kD_0} \) is the dose at which \( N = \left(\frac{1}{2}\right) N_{\text{sat}} \). Relation (3) is equivalent to (1). The dose dependence for citrus pectin is similar, with \( D_{1/2} = (5.47 \pm 2.37) \text{ kGy} \)

The complex spectrum of irradiated pectins is composed of at least three different signals, a single line in the center, a doublet component on each side of the single and a broad line whose structure can not be resolved in components. The parameters of these components are summarized in table1

| Sample          | Irradiation Dose(kGy) | g-value | Hyperfine A-value(mT) | Line width (mT) | Component identification               |
|-----------------|-----------------------|---------|-----------------------|-----------------|----------------------------------------|
| Apple pectin    | 12                    | 2.003   | ---                   | 8               | Single line                             |
|                 | 2.001                 | 13.5    | 9.5                   |                 | Doublet component                       |
|                 | 2.005                 | ?       | 30-35                 |                 | Broad line, unresolved structure        |

A single line of such width was identified in other polycarbonates containing uronic acids [16, 17]. It can be associated to a \( \text{C-O-O} \) type radical, very likely to be produced in any uronic acid and monosaccharide sugars. The doublet component can be associated to a radical of the type

\[-C_{\text{O-O}}^/-\]

The hyperfine interaction is due to the hydrogen nucleus. The third line, very probably a superposition of different lines of a triple-quadruplet structure, is quite common in various irradiated monosaccharides [17], but in such complex material it is impossible to resolve its structure using only ESR spectroscopy (ENDOR investigations could solve this problem).
Concerning the radical time stability it is evident that for samples with a higher water content, like citrus pectin, the stability is much lower. Such water effect on free radical stability was frequently reported in literature [18].

The following picture (Fig.3) shows the amplitude of the EPR signal of citrus pectin irradiated 7kGy after 2h, 4h respectively after irradiation. After 30 hours of irradiation, the signal disappears. In apple pectin, less wet, the time dependence is much slower. For citrus pectin free radical concentration decreases exponentially, with a half time $T_{1/2} = 3.20 \pm 0.75$

**Fig.3-** EPR signal of citrus pectin samples irradiated

4. CONCLUSIONS
- The gamma-irradiation induces stable radicals in apple and citrus pectin. They can be observed up to 30 hours after irradiation for wet samples and for much longer time periods for dry samples.
- The dose dependence of the double integrated area, i.e. proportional to free radical concentration, follows a generation with saturation mechanism.
- The gamma irradiated citrus pectin has a similar shape as apple pectin, but much less stable at room temperature, due to its higher water content.
- EPR spectrum is composed at least of three components. A qualitative analysis made possible assignments to most probably free radicals types.

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