EPR study on sugar radicals utilized for detection of radiation treatment of food

**Grzegorz P. Guzik,
Wacław Stachowicz,
Jacek Michalik**

Abstract. Radicals produced by ionizing radiation in sugars, the components of dried and raw fruits give rise to stable multicomponent electron paramagnetic resonance (EPR) signals unidentified so far. The subject of the present EPR study is to identify the radicals stabilized in γ-irradiated crystalline sugars, D(+)mannose and L(-)sorbose extracted from cranberries (*Vaccininum oxycoccos*) and rowan berries (*Sorbus aucuparia*), respectively. EPR measurements and density functional theory (DFT) simulations were employed for that purpose.

Key words: density functional theory (DFT) • dried fruits • electron paramagnetic resonance (EPR) • irradiation • mannose • sorbose

G. P. Guzik, W. Stachowicz, J. Michalik✉
Institute of Nuclear Chemistry and Technology,
16 Dorodna Str., 03-195 Warsaw, Poland,
Tel.: +48 22 504 1205, Fax: +48 22 811 1532,
E-mail: j.michalik@ichtj.waw.pl

Introduction

The complex electron paramagnetic resonance (EPR) spectra of γ-irradiated sugars derived from irradiated food containing crystalline sugars [9] such as dried figs, mangos, papayas, raisins and vegetables are stable at room temperature [11] and for that reason can be used for the detection of radiation treatment of food [3, 10] and as dosimeters in nuclear accidents [2, 6, 8]. The multicomponent spectra are composed of overlapping signals of different radicals. Till now those individual signals are not identified and the dose values are calculated based on the intensity of complex spectrum. However, the decay rate of particular radicals depends on storage conditions such as temperature, humidity and others. For that reason, it is important to deconvolute the complex EPR spectra and assign the particular lines to individual radicals.

In the present study the EPR spectra of gamma-irradiated D(+)mannose and L(-)sorbose in polycrystalline form were investigated. The sugars were extracted from dried and fresh fruits: D(+)mannose from dried cranberries, while L(-)sorbose from crude rowan berries.

Cranberries (*Vaccinium oxycoccos*) are rich in A, C and group B vitamins. Drugs containing cranberry extracts prompt the action of pancreas preventing the penetration of microbes to urinary tracts and formation of nephroliths.

Rowan berries (*Sorbus aucuparia*) contain organic acids, carotenoids, tannic substances, vitamin C and provitamin A. Dried berries are the components of herbal drugs and antiphlogistic, laxative and diuretic agents.

D(+)Mannose is C-2 epimer of D-glucose distinguished by a crooked aliphatic chain forming an angular form (two D isomers have a six-element pyranose ring while remaining two-pentamerous furanose ring). Configuration of D(+)mannose is as that of D-glyceraldehyde at the penultimate atom of the carbon chain. D(+)mannose differs from D-glucose with the reverse localization of substituents at C-2 carbon forming the chiral centre of a molecule. A dominant isomer (67%) of D-mannose is α-D-mannopyranose with a six-element ring.

L(-)Sorbose, in turn, is ketose, a monosaccharide well solvable in water with sweetness equivalent to sucrose (table sugar). The sweetening ability of L-sorbose is 0.25, a little less than 0.3 for xylitol, commonly used as sweetener replacing table sugar [4]. At 27°C a dominant isomeric form in water is alpha-sorbopyranose (98%) [1, 7]. L-Sorbose has the configuration of naturally occurring sugar, for example, in *Sorbus aucuparia*. It is formed by biotransformation of L-sorbitol stimulated by *Acetobacter suboxydans* bacteria. Commercial production of vitamin C (ascorbic acid) often begins with sorbose used as reagent.

Experimental

Materials

Cranberries and rowan berries, available commercially in the market were crumbled to a pulp, then treated with different solvents – demineralized water, methanol or ethanol. Clear solutions containing mannose and sorbose were kept at room temperature in opened Petri dishes for several weeks to obtain sugars in the crystalline form. Then, white crystallites were separated mechanically from the saturated solutions and dried with a blotting-paper. The identity of both sugars was proven by refractometry measurements.

Methods

Sugar samples were irradiated at room temperature with a dose of 4 kGy with ^{60}Co gamma rays using a Gamma Chamber 5000 irradiator. After irradiation, the sugar samples with an average weight of 100 mg were placed in EPR tubes with a diameter of 5 mm and examined at ambient temperature with a Bruker ESP 300, X-band spectrometer. The EPR signals were recorded using modulation frequency of 100 kHz, modulation amplitude of 1.5 G and microwave power of 0.4 mW. The magnetic field was swept in the range of 200 G. Next, the samples were heated at temperatures close to sugar melting points – mannose 10 min at 95°C and sorbose 50 min at 140°C and measured again. The signals for unheated and heated samples were normalized and subtracted using a computer program.

For DFT calculation, Gaussian 03W program with EPR-III, LANL2DZ and DGDZVP bases was used which made it possible to calculate the hydrogen coupling constants to selected carbon nuclei. Chemcraft program was applied for graphical presentation of radical structures.
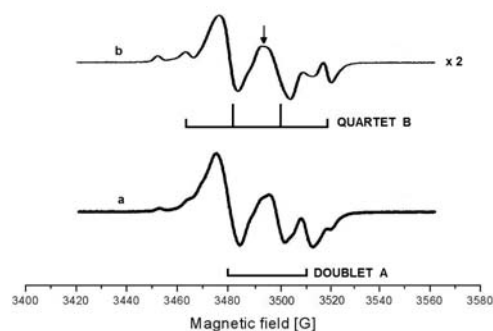

Fig. 1. Normalized EPR spectra of cranberries D(+)mannose γ-irradiated with a dose of 4 kGy. (a) Sample kept at room temperature, (b) sample heated at 95°C after irradiation. Markers indicate the positions of EPR doublet A (hfs = 30 G) and quartet B (hfs = 16 G) lines which compose experimental spectrum. The arrow marks the EPR line for which spectra were normalized.

Results

D-Mannose

The multicomponent EPR spectrum of D(+)mannose irradiated and measured at room temperature is composed of three broad major lines (Fig. 1a). The line intensity ratio and the separation between them suggest that this complex signal represents at least two radicals. However, the superposition of individual radical spectra is large which makes the distinction of them impossible. Nevertheless, it seems reasonable to postulate, that isotropic doublet with approximate hfs value equal to 30 G is one component of the complex spectrum (Fig. 1a).

When the D(+)mannose sample is heated at 95°C after irradiation, the EPR spectrum distinctly changed. The outer lines at low and high magnetic fields are clearly visible as the separate features indicating the presence of EPR quartet (Fig. 1b). The approximate hfs value calculated from the distance between the outer lines assuming interaction with three equivalent hydrogens is equal to 16 G. Owing to this spectral change, our assumption about the contribution of EPR doublet in complex spectrum was confirmed. The strongest line recorded at low field represents superposition of a doublet and a quartet. It can be stated in conclusion that the complex EPR spectrum of irradiated D(+)mannose contains doublet A and a quartet B as the main components.

A doublet dominates the EPR spectrum in the sample kept at room temperature after irradiation, whereas a quartet is a major spectral component when the sample was heated at 95°C after irradiation.

To identify radicals which might be responsible for the EPR quartet and doublet the DFT calculation has been performed for all molecular structures with unpaired electron at every carbon of the pyranose ring. The theoretical calculations indicate that the radical with unpaired electron at C-3 carbon interacting with hydrogen atom bonded to C-2 carbon gives rise to the EPR doublet with hyperfine splitting of 27 G with good agreement with the experimental value (Fig. 2a). In turn, the experimental quartet, according to theoretical calculations, derives from the radical formed by hydrogen detachment from C-5 carbon. Then, unpaired
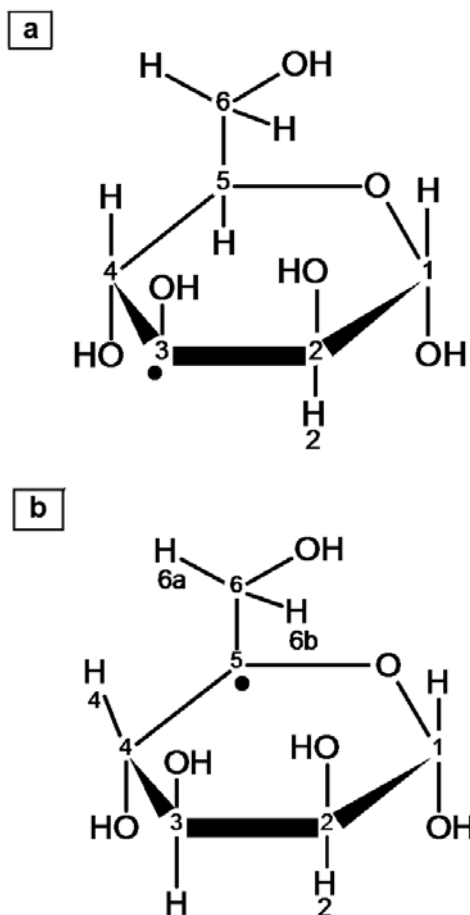
Fig. 2. The proposed structures of the radicals stabilized in γ-irradiated D(+)mannose based on EPR measurements and DFT calculations. (a) C-3 centred radical represented by EPR doublet A, (b) C-5 centred radical represented by EPR quartet B.

electron interacts with 3 hydrogen atoms, one bonded to C-4 and two bonded to C-6 carbons with hyperfine splittings 19 G (H-4); 15 G (H-6a) and 19 G (H-6b), respectively (Fig. 2b). The experimental linewidths are too large to observe the splitting for particular hydrogens H-6a; H-6b and H-4, but the average value of calculated hfs equal to 18 G does not differ much from the value experimentally.

L-Sorbose

The EPR spectrum of sorbose irradiated at room temperature looks like a quartet. However, both central lines are much wider showing clearly deflection points which indicates that two different spectra overlap (Fig. 3c). The spectrum of irradiated sorbose sample heated at 140°C differs distinctly in the central part from the spectrum presented in Fig. 3d.

Spectrum (c) denotes unheated sample while spectrum (d) sample heated at 140°C. Markers in the centre indicate position and structure of EPR quartet D representing C-2 centred radical. Arrow marks the EPR line for which spectra were normalized.

The outer lines remain nearly unchanged, but the broad central features become better resolved and two distinct lines appear with a linewidth of 5 G, the same
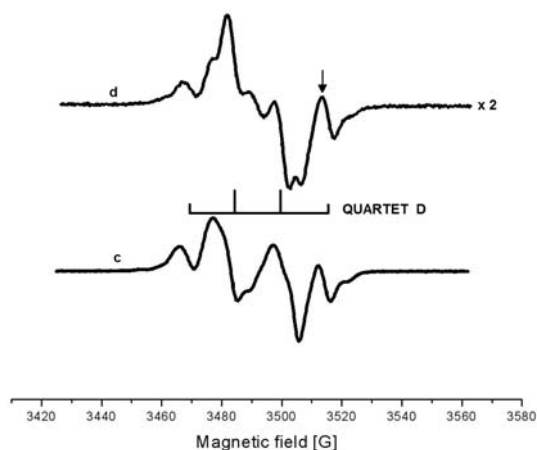

Fig. 3. Normalized EPR spectra of rowan berries L(-)sorbose γ-irradiated with a dose of 4 kGy. Spectrum (c) denotes unheated sample while spectrum (d) sample heated at 140°C. Markers in the centre indicate position and structure of EPR quartet D representing C-2 centred radical. Arrow marks the EPR line for which spectra were normalized.

as linewidths of outer lines. The splittings between lines are equal, to 16 G which proves that the quartet is one of the components of complex EPR spectrum observed in irradiated L(-)sorbose. To find another spectrum, which overlaps with quartet D, we subtracted the spectra presented in Fig. 3. Before substraction the spectra were normalized for the last line of quartet D in the spectrum of sample heated at 140°C. We chose this line because it did not change much, alike the first line at low magnetic field, during sample heating. The result is shown in Fig. 4.

The main component of differential spectrum is doublet E with a hyperfine splitting of 23 G. The intensities of doublet lines differ by ca. 20% and the presence of additional features indicates that the third EPR signal of unknown origin might enter into the composition of experimental spectrum.

Knowing values of hfs for two major EPR signals in irradiated L(-)sorbose, we run DFT calculation for all C-centred radicals in pyranose ring in order to select the radicals with hfs values closest to the experimental ones, as shown in Fig. 5.

For quartet with a splitting of 16 G, the radical with unpaired electron centred at C-2 carbon and interacting with two hydrogen atoms bonded to C-1 carbon and one hydrogen bonded to C-3 carbon atom gave hfs values closest to the experimental one. The
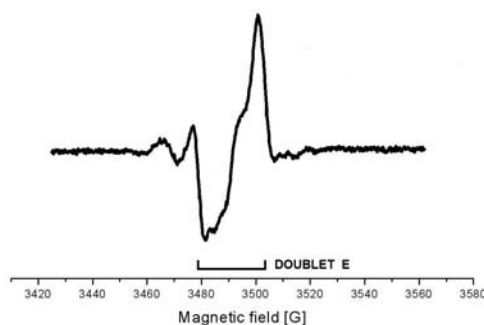

Fig. 4. The differential EPR spectrum obtained by subtraction of normalized signals of L(-)sorbose kept at room temperature and heated at 140°C. Markers indicate the line position of a doublet E representing C-6 centred radical.
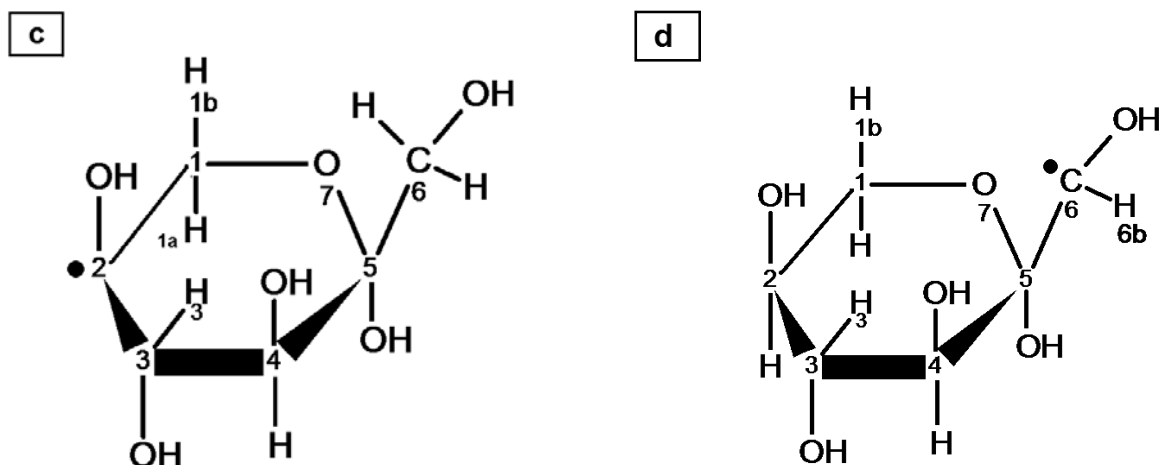
Fig. 5. The proposed structures of the radicals stabilized in γ-irradiated L(-)sorbose based on EPR measurements and DFT calculations. (c) C-2 centred radical represented by quartet D, (d) C-6 centred radical represented by doublet E.

theoretical hyperfine splittings are equal to 16 G for H-1a, 15 G for H-1b and 14 G for H-3. Such small differences in hfs values are hidden in the linewidths and the experimental spectrum appears as a quartet arising as a result of unpaired electron interaction with three equivalent H atoms.

Concerning doublet with a splitting of 23 G, the DFT calculations showed unequivocally that only the radical with unpaired electron centred on C-6 carbon outside pyranose ring and interacting with hydrogen bonded to the same carbon can be responsible for this spectrum. The theoretical value of hyperfine splitting is 24 G in very good agreement with the experimental one.

Discussion

Despite the fact that the EPR spectra of sugar radical are used to identification of irradiated food containing sugars, such as dried figs, mangos, papayas, raisins and also for radiation accidental dosimetry very little is known about molecular structure of radicals which are formed radiolytically and remain stable at room temperature. The reason is that the EPR spectra of γ-irradiated polycrystalline sugars are complex because signals of different radicals overlap making interpretation difficult. In some cases the EPR measurements with different microwave powers are helpful to assign particular EPR lines to the specific radical. For EPR spectra of irradiated mannose and sorbose, this did not work because the spectral changes with microwave power were too small for reasonable spectral analysis.

The other approach is to keep recording the spectral changes during storage of irradiated samples at room temperature. If one radical decays faster than the other one, then it is possible to assign the EPR lines to the spectra of particular radicals. However, the spectral changes in polycrystalline sugars at room temperature are usually very slow and quite often even half a year of storage is too short to make conclusive assignment of EPR lines [5]. The novelty of our experimental approach lies in the heating of irradiated sugar samples at elevated temperature, lower only 20–30°C from their melting points (161–163°C for sorbose and 132–134°C for mannose).

We put first time this idea to the practice studying radicals in irradiated D-fructose. The EPR spectra after controlled heating showed significant changes that made it possible to assign the EPR lines to the spectra of individual radicals and measure hyperfine splittings. The identification of the major radicals stabilized in γ-irradiated D-fructose was confirmed by density functional theory (DFT) calculations. We assigned it to C-1 centred radical [5] although C-5 radical had been proposed earlier [13].

In sorbose we identified two C-centred radicals, the first one with unpaired electron at C-2 carbon in pyranose ring represented by EPR doublet and the second one with unpaired electron at C-6 carbon out of pyranose ring. The C-2 centred radical starts decaying at 140°C and its concentration after heating is nearly two times lower than at room temperature, while concentration of C-6 radicals remains unchanged even after a few hours of storage of this temperature. The only literature data on the structure of radicals formed in irradiated L(-)sorbose are presented in the paper of Vanhaelewyn et al. [12]. The authors recorded the complex EPR spectra of single crystal of L(-)sorbose for magnetic field parallel to a, b and c crystal axes and in order to identify them, they analysed theoretically the radical structures formed by hydrogen abstraction from C-2, C-3, and C-4 carbon atoms as well as abstraction of hydroxyl and hydroxymethyl groups from C-5 carbon. They assigned the EPR spectrum of irradiated L(-) sorbose to two conformations of C-4 centred radical. This assignment is different than ours which might be related to the fact that in polycrystalline sugars the arrangement of molecules is much less ordered facilitating different radical reactions than in the single crystal. As a result, different radicals would be stabilized in both matrices.

As to our knowledge, there are no literature data on radicals stabilized in γ-irradiated D(+)mannose. Our analysis of EPR spectra supported by DFT calculations resulted in the identification of two major radicals with unpaired electron at C-3 and C-5 carbon atoms. The radical centred at C-3 carbon decays slower at temperature close to the melting point of mannose than C-5 centred radical.

Conclusions

The heating of γ-irradiated crystalline ʟ(-)sorbose and ᴅ(+)mannose made it possible discrimination of single spectra in multicomponent EPR spectra and measurements of experimental hyperfine splittings. Having them, we were able to run the DFT calculations in order to identify the radicals stabilized in both sugars. Owing to that novel approach to the analysis of complex EPR spectra, we found that in γ-irradiated ʟ(-)sorbose the C-2 and C-6 centred radicals were stabilized at room temperature. The C-6 centred radical remains stable even at 140°C. In γ-irradiated ᴅ(+)mannose the C-3 and C-5 centred radicals were identified at room temperature. The C-3 radical is stable at 95°C in contrast to the C-5 radical.

The results presented for both sugars show that heating of irradiated sugar samples at temperatures close to their melting points together with DFT calculation might be very useful methodology which makes possible discrimination of complex EPR spectra and identification of individual radicals.

References

1. Collins PM (ed) (2005) Dictionary of carbohydrates. CRC Press, Hoboken
2. Da Costa ZM, Pontuschka WM, Campos LL (2005) A comparative study based on dosimetric properties of different sugars. Appl Radiat Isot 62:331–336
3. European Committee for Standardisation (2001) EN-13708:2001E: Foodstuffs-detection of irradiated food containing crystalline sugar by ESR spectroscopy. CEN, Brussels
4. Glaser D (2002) Specialization and phyletic trends of sweetness reception in animals. Pure Appl Chem 74;7:1153–1158
5. Guzik GP, Stachowicz W, Michalik J (2008) Study on stable radicals produced by ionizing radiation in dried fruits and related sugars by electron paramagnetic resonance spectrometry and photostimulated luminescence method – I. D-fructose. Nukleonika 53;Suppl 2:S89–S94
6. Karakirova Y, Yordanov ND, De Cooman H, Vrielinck H, Callens F (2010) Dosimetric characteristics of different types of saccharides: An EPR and UV spectrometric study. Radiat Phys Chem 79:654–659
7. Merck Index (1999) 12th Edition, 8874
8. Nakajima T, Otsuki T (1990) Dosimetry for radiation emergencies: radiation-induced free radicals in sugar of various countries and the effect of pulverizing on the ESR signal. Appl Radiat Isot 41:359–365
9. Raffi J, Angel J-P (1989) Electron spin resonance identification of irradiated fruits. Radiat Phys Chem 34;6:891–894
10. Raffi J, Stachowicz W, Migdał W et al. (1998) Establishment of an eastern network of laboratories for identification of irradiated foodstuffs. Final Report of Copernicus Concerted Action, CIPA-CT94-0134, CCE
11. Stachowicz W, Strzelczak G, Michalik J, Wojtowicz A, Dziedzic-Gocławska A, Ostrowski K (1992) Application of EPR spectroscopy for control of irradiated food. J Sci Food Agric 58:407–415
12. Vanhaelewyn G, Jansen B, Pauwels E, Sagstuen E, Waroquier M, Callens F (2004) Experimental and theoretical electron magnetic resonance study on radiation-induced radicals in L-sorbose single crystals. J Phys Chem A 108:3308–3314
13. Vanhaelewyn G, Lahorte P, Proft F, Mondelaers W, Geerlings P, Callens F (2001) Electron magnetic resonance study of stable radicals in irradiated D-fructose single crystals. J Phys Chem Chem Phys 3;9:1709–1735