In addition, PPSL measurements were also done with intestinal tracts removed from shellfish and with intestines cut off from the shells (Table 3).

Validation of the described PPSL test study rendered possible to include shelled oysters and Roman snails to the list of samples suitable for PPSL examination whether irradiated. The detection of irradiation is also successful if intestines separated from blue mussels, and shrimps are examined.

EPR STUDY ON STABLE RADICALS PRODUCED BY IONIZING RADIATION IN CRYSTALLINE SUGARS, COMPONENTS OF FRUITS

Grzegorz P. Guzik, Wacław Stachowicz

Ionizing radiation produces in crystalline sugars, components of dried fruits, stable radicals giving rise in EPR to multicomponent signals unidentified so far. In our earlier studies [1] it was observed that radiation-induced multicomponent EPR signals observed in sugars (fructose and glucose) isolated from dried fruits decay slowly and undergo some transformation by prolonged storage at room temperature (360 days). Similar effect occurs by heating of a sample at elevated temperatures below, but near to its melting point [2]. Some of spectral lines decay faster and some slower. Thus, by applying a computer controlled subtraction of normalized spectra before and after prolonged storage or programmed heating the spectra of less stable radicals could be recorded. By applying this technique, the supposed spectral structure of less stable radicals produced in fructose has been proposed. In the present study the same methodology enriched with DFT simulation processing has been adapted to study stable radicals produced be radiation in sorbose separated from service berries.

Crystalline L-sorbose was separated from fresh service (*sorb*) berries.

Separation procedure of sugar fraction:
- mechanical crumbling of berries into small pieces;
- subsequent extraction of sugars from fruit pulp with the use of demieralized water and/or ethanol to a clear solution (Table);
- slow crystallization of sugars from saturated solutions in opened air;
- mechanical separation and drying of sugar crystals from solution. The identity of separated sugar was controlled by refraction measurements with a Rudolf J357 refractometer.

Irradiation of sugar samples with a ^{60}Co gamma rays from Gamma Chamber 5000 with a dose of 4 kGy.

EPR examination of irradiated samples – both kept at room temperature and heated – with a Bruker ESP 300 spectrometer.

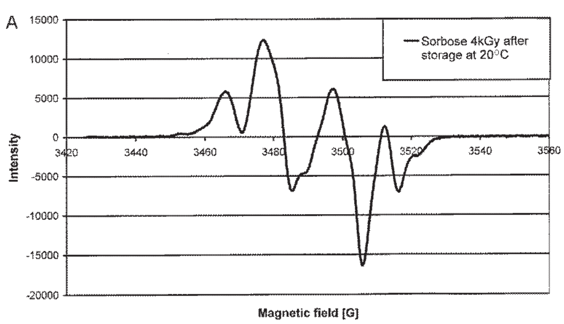
Heating of sugar samples at selected temperature of 140°C was done in the oil thermostate.

DFT simulation and relevant quantum-mechanical calculations were done with the use of Gaussian program 03W.

In Figure 1 the EPR spectrum of irradiated sugar sample taken at room temperature two days after the treatment (A) is compared with normalized spectrum of the same sample but after the heating for 50 min at 140°C, *i.e.* at a temperature below "the softening" temperature of sorbose crystals (B).

Table. Characteristics of crystalline sorbose separated from service berries.

Characteristics of separated sorbose crystallites	Single orthorhombic needles *ca.* 0.05 x 0.05 x 0.9 mm	
Solvents used for room temperature crystallization of sorbose	distilled and subsequently demineralized water	ethanol, chemical purity
Refraction coefficient [n] of separated crystallites, 20°C	1.35458 ± 0.00136	1.35795 ± 0.00061
Refraction coefficient [n] of crystalline sorbose, literature data	1.35484 ± 0.00001	1.35484 ± 0.00001



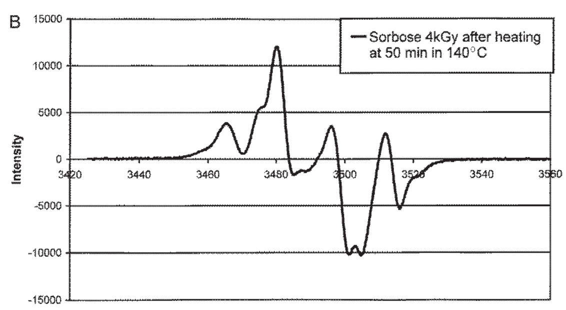
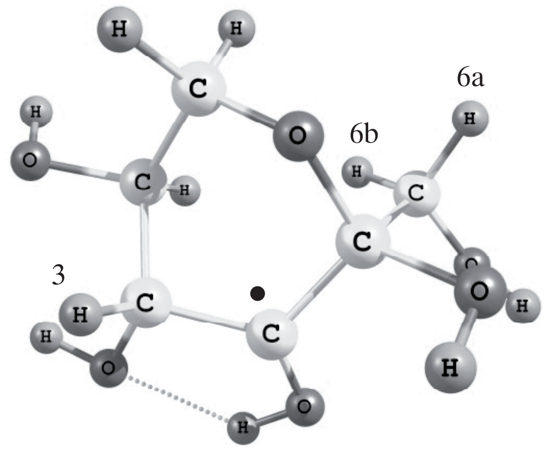


Fig.1. The EPR signals recorded with irradiated sorbose separated from service berries: A – EPR signal recoded with a sample kept at room temperature, B – EPR signal recorded at room temperature after heating of the sample at 140°C/50 min.

From the EPR spectrum of sorbose kept for a few days at room temperature (Fig.1A) it is deduced that the dominating lines in the multicomponent signal belong probably to a doublet of the splitting $A_{iso} \approx 2.6$ mT. The signal recorded after heating of the sample at 140°C (Fig.1B) is markedly changed. The doublet ($A_{iso} = 2.6$ mT) is decreased, while a quartet with the splitting A_{iso} of about 1.6 mT is more pronounced. The doublet observed in unheated sample can be still recognized in the form of shoulders in the central lines of the quartet.



The electron near C-4 with an atom	The constant of the coupling A
H-3	32.08 G
H-6a	5.43 G
H-6b	0.39 G

Fig.2. Computer simulation of the postulated sorbose radical produced by radiation in irradiated service berries. Unpaired electron (black circle) is localized at C-4 atom in the pyranose ring. Unpaired electron interacts with H-3 nuclear spin positioned above C-3 ring atom giving rise in EPR to a doublet of $A_{iso} = 3.208$ mT and weaker with H-6b nuclear spin localized below C-6 atom; $A_{iso} = 0.543$ mT.

The structural analysis of α-L-sorbose molecule and the knowledge of the earlier assignments of radiation-induced radicals involved [3] allowed to propose a very probable localization of unpaired electron in the sorbose radical produced by radiation. This localization is at carbon C-4 of the pyranose ring. The structure of the corresponding radical obtained by the DFT method is shown in Fig.2.

Unpaired electron interacts strongly with the H atom at C-3 carbon atom which is at relatively short distance from C-4, being positioned above the plane of pyranose ring. The EPR splitting A_{iso} corresponding to the interaction of unpaired electron with this proton calculated by the DFT method was evaluated for 3.208 mT. In addition, a much weaker interaction of unpaired electron with 6a hydrogen atom at C-6 carbon atom was demonstrated, localized at the rotating group outside the pyranose ring. The calculated A_{iso} splitting corresponding to this interaction was equal to 0.543 mT. No spectral lines in the irradiated sorbose signal was assigned to this individual so far.

The localization of radiation-induced radicals in crystalline lattice of sugars and their relatively high stability guarantee the resistance of these species against the action of oxygen and minimize the probability of intra molecular and intra radical reactions. It is postulated, therefore, that only radicals produced by the detachment of protons from parent molecules can be identified in irradiated sugar crystals [2]. This is in contrast to sugar solutions in which a high variety of radicals and molecular products are produced [4]. Thus, by the identification of stable radicals in irradiated sugars the parent radicals varying in the positioning of unpaired electron are considered.

In the present study the doublet identified in an unheated sample was assigned on the basis of DFT fitting to parent sorbose radical with unpaired electron at C-4 carbon of the pyranose ring. The supposed quartet of the average splitting about 1.6 mT remains still unidentified.

References

[1]. Guzik G.P., Stachowicz W., Michalik J.: Nukleonika, 53, S89-94 (2008).

[2]. Guzik G.P., Stachowicz W., Michalik J.: Curr. Top. Biophys., 33, Suppl. A, 81-85 (2010).

[3]. Tarpan M., Sagstuen E., Waroquier M., Callens F.: J. Phys. Chem. A, 112 (2004).

[4]. von Sonntag C., Schuchmann H.-P.: Studies in physical and theoretical chemistry: carbohydrates. In: Radiation chemistry: present status and future trends. Elsevier, Amsterdam 2001, pp. 481-509.