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STUDY ON ORGANIC RADICALS GIVING RISE TO MULTICOMPONENT EMR SPECTRA IN DRIED FRUITS EXPOSED TO IONIZING RADIATION

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The study is described on time and temperature induced transformation of EMR signals recorded with crystalline sugars i.e. fructose, glucose and sorbose, all extracted from resins and rowan berries. Supposition as to identity and specificity of radical responsible for temperature induced transformation of EMR spectrum of irradiated fructose is given.

INTRODUCTION

The candied, dried and dehydrated fruits like dates, figs, resins, berries etc, contain typically crystalline sugars which originate in fruit pulp as crystalline domains by successive decrease of water content during the treatments. Dried fruits imported from oriental countries happen technologically irradiated to eliminate fruit insects in there. It has been observed, however, that exposition of dried fruits to the action of ionizing radiation (gamma rays or accelerated electrons) give rise in EMR to a specific, multicomponent signal which is relatively strong (Stachowicz et al. 1995) and stable at room temperature. It is postulated that the signal is derived from sugar born radicals localised in crystalline sugar domains mentioned (Raffi & Angel, 1989; Raffi et al. 1991). The signal recorded in irradiated dried fruits is easily distinguished from a weak, non specific native signal observed in any non irradiated fruit and for that reason was proposed to be used as a suitable indicator of radiation treatment in all foodstuffs containing crystalline sugar (EN standard 13708:2003). Similar signal appears in pure sugar samples of glucose, fructose etc. However, multicomponent signal in sugar and sugar containing food represents in fact an envelope of some spectra derived from several radical species and both remain not identified experimentally so far. It has been observed by us for the first time in earlier study (Guzik et al. 2008) that multicomponent EMR signals in crystalline decay slowly in time being slightly changed during a long time observation (360 days). Similar effect but much faster (several minutes) has been observed by heating of sugar containing samples to higher temperature than below sugar melting point. The change of the shape of the EMR signals in the course of prolonged storage and after heating are of a very similar character. It is believed, therefore, that a very similar pathway of radical transformation occurs in both cases. This experimental approach seems to open a new way for the investigation of complex EMR spectra in irradiated sugars and sugar containing foodstuffs. The aim of present study was to test whether the observation of heat dependant transformation of EMR spectra in crystalline sugars could be helpful in identifying of at least some of radiation induced radicals giving rise to non identified multicomponent signal observed in dried fruits and in simple sugars extracted from them.

MATERIALS AND METHODS

The subject of investigation were crystalline sugar fractions extracted from resins and from dried rowan berries both purchased in the market. Whole fruits were grinded with a mixer to a pulp and by applying manifold rinsing with water and/or methanol, solutions containing extracted sugars were collected. The extraction of resin pulp with water results in obtaining solution containing practically fructose only, while that extracted with methanol glucose. The extraction of the pulp of rowan berries with water, in turn, resulted in obtaining water solution reach in sorbose (Table 1) Subsequently, solutions were purified with charcoal, while pure, colourless solutions undergo a slow, room temperature crystallization. The beginning of crystallization from saturated solutions was typically observed after at least one month of storage with air access. Crystalline sediments were carefully separated from saturated

solution, dried an d afterwards irradiated with the dose of 4 kGy in a 60Co gamma source Issledovatel (dose rate ca 0.9 kGy). The EMR spectra were obtained with BRUKER ESP 300 spectrometer in X band. Refraction measurements to prove the identity of sugars extracted from fruit pulps were done with Rudolf Refractometer (model J 357).

Table 1. Sugars extracted from dried fruits

Product: dried fruit	Eluent/solution for crystallization	Product of crystallization	
		Sugar identified (refractometry)	Efficiency (%)*
Sultan resins	Water, demineralised	Fructose	0.58
Sultan resins	Methanol, for analysis	Glucose	0.14
Rowan berries	Water, demineralised	Sorbose	0.14

*) efficiency refers to the initial mass of fruit

Molecular structure of three investigated sugars have the same molecular formulae - $C_6H_{12}O_6$ and differ in spatial position of OH groups. Thus glucose is defined as α-D-glucopiranose, fructose as β-D-fructofuranose, while sorbose as α-L- sorbopyranose. All three sugars show similar crystallographic structures and are growing in the form of orthorhombic crystals in the course of slow crystallization. However, some difference do appear when the shape of single crystals is compared (see Table 2).

Table 2. Characteristics of sugars extracted from resins and rowan berries

D-FRUCTOSE	L-SORBOSE	D-GLUKOSE
Single orthorhombic crystals dimensions up to 1 x 1 x 2 mm	Single orthorhombic needles dimensions up to 0.5x 0.5x 9 mm	Aggregates of small orthorhombic crystals (dimensions < 0.01 mm)
Crystallization from EtOH 95% analytical grade	Crystallization from distilled and demineralised water**	Crystallization from methanol 99% analytical grade
Refraction coefficient [n]* measured at 20° C	Refraction coefficient [n]* measured at 20° C	Refraction coefficient [n]* measured at 20° C
1.34494 ± 0.00005	1.35484 ± 0.00001	1.34645 ± 0.00003

*Refraction coefficient are consistent with those of pure crystalline sugars (Hand-book of physical chemistry (1974) WNT Warszawa 1974)

** G.Vanhaelewyn, F.Callens et al. 2004

RESULTS AND DISCUSSION

The EMR spectra of crystalline sugars extracted from resins and rowan berries irradiated with 4 kGy of gamma rays are comprehended in the left side of Figure 1. Blue colored lines draw the EMR signals recorded with samples irradiated and stored at 20°C, while red colored lines show the signals recorded with samples which were irradiated with the same dose but subsequently heated to the temperatures shown in the windows (glucose 107°C, fructose 97°C, sorbose 140°C). It is clearly seen that heat treatment provokes considerable changes in the shape of all three spectra implying concentration changes or transformation of some radicals contributing to multicomponent EMR signal registered at 20°C. In the right side of the figure there are shown three EMR differential spectra obtained by computer operated subtraction of "red" spectra from "blue" ones. The obtained differential spectra could be assigned to the group of less stable radicals which decay faster than the others by programmed heating of samples. Another words heating enables to divide the original multicomponent spectrum in two no doubt less complex groupings. Identification of individual radicals by the development of this method seems certainly perspective indeed.

Radical reactions or transformation of radicals are less probable to occur inside sugar crystals . It is because the major part of radicals (the exception are H atoms, for example) remain immobilized in crystal matrix. The reaction of radicals with neighbouring molecules or with other more distanced radicals may occur in liquid state only. Numerous molecular products of radical reactions like 2-deoxytetrose, arabonic acid, ribonic acid, glyceraldehyde etc, were identified by Von Sonntag in crystalline sugars but after dissolution of irradiated crystalline sugars in water. It has to be remembered, however, that these molecular products are produced in very fast radiolytic processes of unstable oxyl radicals produced by deprotonation of parent radical cations (Von Sonntag & Schuchmann 2001) i.e. follow the pathway of reactions that occurs in liquid state only. It seems rational to believe that the most of stable radicals contributing to the formation of multicomponet signals in rigid sugar matrix are originated from parent radicals of sugars and are produced by their structural transformations.

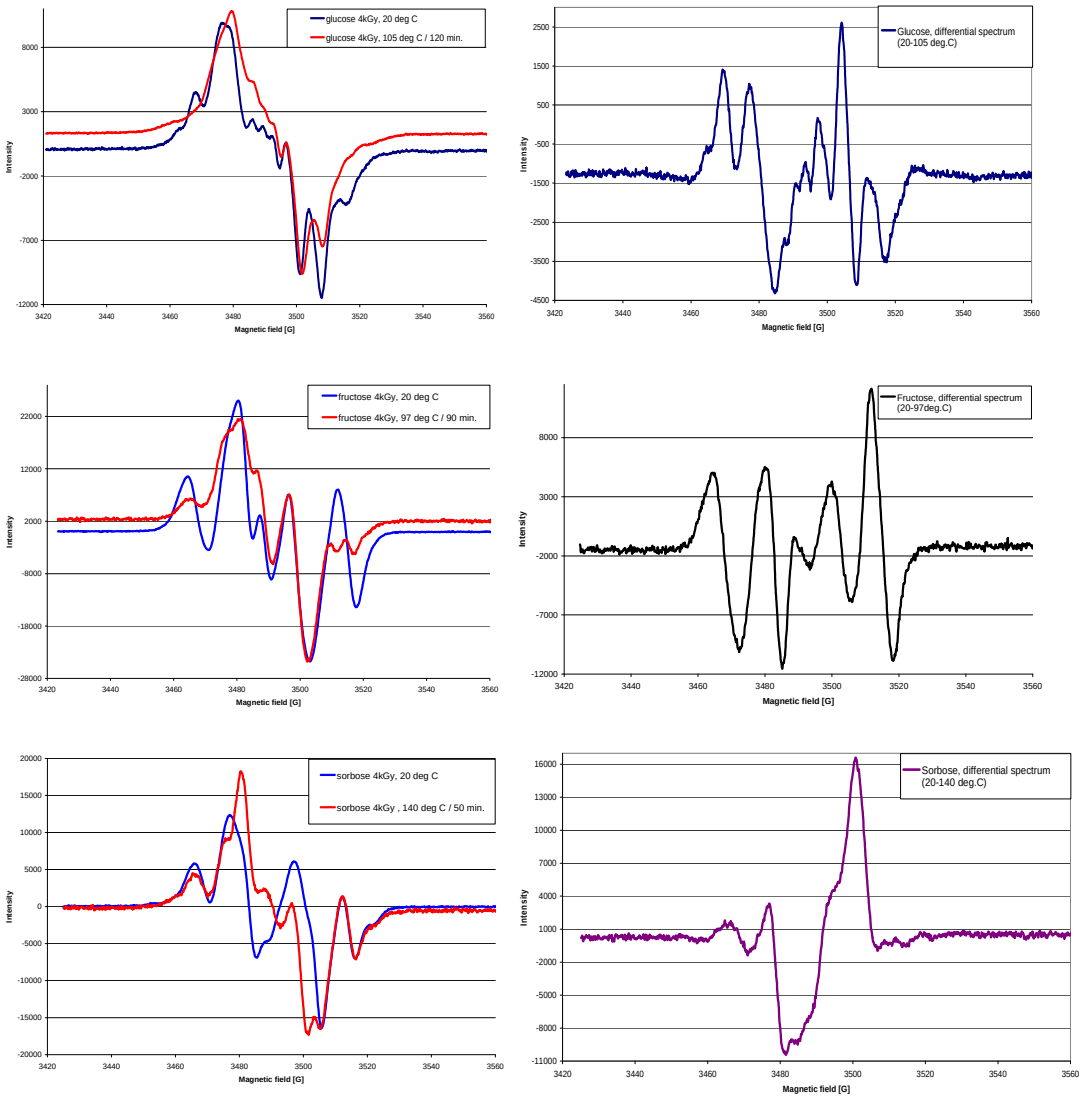

Fig.1. The EMR spectra of three sugars (glucose, fructose, sorbose) extracted from resins and rowan berries. The spectra in the left side correspond to irradiated sugars (4 kGy) kept at 20°C (blue lines) and to heated sample – red lines (temperatures of heating given in the windows). The spectra in the right side of the figure represent the differential spectra obtained be subtraction of the spectra of heated samples (red) from those kept in 20°C.

The localization of unpaired electrons in spherically "deformed" radicals may be different for a given configuration giving rise consequently to different EMR spectra. Such "deformed" radicals remain immobile in crystalline lattice of sugar and should be very stable indeed. The dissolution of sugar releases immediately the cascades of fast radical reactions including those postulated by von Sonntag. In view of the above discussion it is expected that stable EMR spectra produced by radiation in crystalline sugars represent first of all radical species of the formula $C_6H_{12}O_6$. However, anisotropic single lines typical for many crystalline structures make difficult their identification.

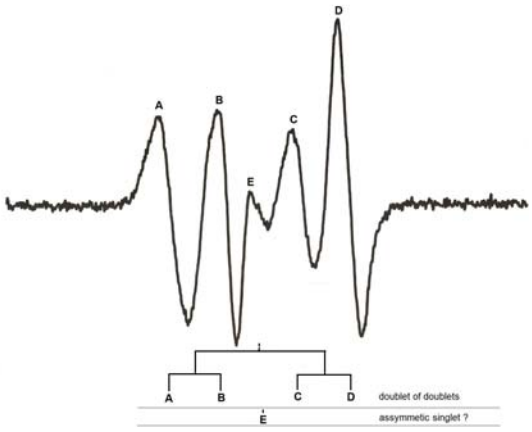

Fig. 2 Differential EPR spectrum of irradiated D-fructose recorded after 60 minutes of heating at 97°C and 20°C.

Computer subtractions of the EMR spectra of fructose recorded at 97°C from that taken at 20°C resulted in a differential signal composed of five well distinguished lines marked A,B,C,D and E (Fig.2). The signal is derived from less stable radicals decaying faster than the latter in the course of heating. Spectroscopic analysis of the signal shows conclusively that the distance between A-B and D-E lines is the same and equals ΔA=1.4 mT while that between B and D line is markedly different equaling ΔA=1.7 mT. It is suggested, therefore, that four A,B,C,D lines do not represent a quartet but doublet of doublets originating from α (36 gauss) and β (14 gauss) couplings. The weaker, asymmetric signal E which is seen between B and C lines belongs probably to another anisotropic signal overlapping partly the right doublet C-D.

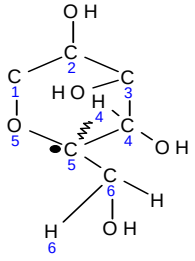

Fig.3. The hypothetical structure of the radical of irradiated D-fructose based on the structure proposed by Callens et al. [M.Tarpan, E.Sagstuen, E.Pauwels, H.Vrielinck, M.Waroquier and F.Callens (2008): Combined Electron Magnetic Resonance and Density Functional Theory Study of 10 K X-Irradiated β-D-Fructose Single Crystals. In: J.Phys.Chem. A 112, 3898-3905] with the use of MLCFA analyses.

Taking advantage of the above supposition (see text and Fig 2) concerning the structure of less stable radical originated from fructose it is possible to propose now the structural formula of this radical species as shown in Fig.3: unpaired electron localized at carbon C-5 of pyranose ring of D-fructose interacts with H atom 4 (H-bond) giving rise to a doublet with a splitting of ΔA=3.6 mT while two doublets A-B and C-D originate from weaker β interaction between unpaired electron at C-5 and H-6 atom.

Thus, the differential spectrum obtained by subtraction of experimental spectra recorded at 97°C and 20°C is supposed to be composed of 4 equivalent lines of the same intensity derived from isotropic doublet of doublets overlapped partly (lines C and D) by a weaker anisotropic signal with a peak marked with E (Fig.2).

In addition to the experiments described above two irradiated samples of fructose were heated at 97°C during 60 minutes in oxygen and in nitrogen atmospheres. The differential spectrum obtained by subtraction of EPR signals recorded with samples kept under air oxygen and nitrogen was very weak and did not show any specific signal. The remaining signal was negligible and rather accidental It is concluded, therefore, that slow decay of radiation induced radicals in sugars is not followed during the heating by any interaction with oxygen, an active radical scavenger. This observation supports the view that radical decay in crystalline sugars is not effected by external factors.

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