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Study on organic radicals giving rise to multicomponent EMR spectra in dried fruits expose to ionizing radiation II. D-Glucose

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Abstract. Crystalline D-glucose extracted from dried resins was irradiated with ^{60}Co gamma rays. The multicomponent EMR spectra of the samples kept at ambient temperature were compared with those heated at 105°C – the temperature close to the melting point of glucose. Normalized EMR spectra recorded with both samples were subtracted, resulting in a differential EMR spectrum. Spectral analysis of all three spectra has been done resulting in the identification of isotropic doublet of doublets assigned to less stable radical derived from parent glucose molecule.

Key words: dried fruits • electron magnetic resonance (EMR) • irradiation • glucose • organic radicals

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Introduction

Glucose is one of the important components of food, occurring typically in fruits employed in medicine and dietetics. Fresh fruits contain 10–20% of dry mass with 5–18% of hydrocarbons. Drying or dehydration of fruits increases markedly dry mass resulting in the increase of the percentage of hydrocarbons to 60–75% [8] while sugar fraction undergoes crystallization with the formation of crystalline domains in the pulp of fruit. It is the reason why commercially available dried or candied fruits like resins, dates, mango, papaya, pineapple, contain crystalline domains of glucose, fructose and/or mannose, sorbose or maltose (Figs. 1 and 2). In order to avoid the spoilage of dried fruits done by moulds or insects during transportation and storage the ionizing radiation is applied as an effective conservation agent [1].

It has been observed earlier [10, 11] that in dried fruits exposed to ionizing radiation stable EMR signals are produced. It is believed that they are derived from radicals trapped in rigid crystal lattice of sugars occurring in dried fruits. High stability [12] and specificity (broad multicomponent spectrum) [13] of these signals resulted in their use as sensitive markers of radiation treatment of dried fruits [3]. However, identity and origin of these complex signals remain unknown and this is a serious weakness of the standard method. Having in mind both practical and scientific aspect, we undertook studies on EMR signals produced by ionizing radiation

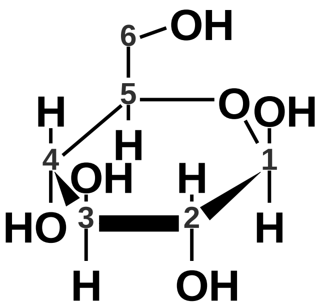

Fig. 1. Structure of β-D-glucopyranose, the most often (65% of general occurrence) appearing in nature the tautomeric form of glucose.

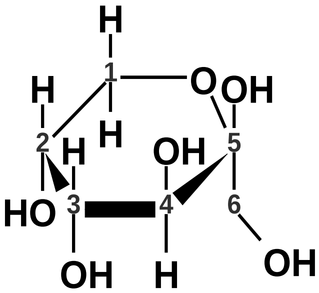

Fig. 2. Structure of β-D-fructopyranose the most often appearing in nature (73% of general occurrence) the tautomeric form fructose, given for comparison.

in sugars extracted from dried fruits. In a previous study we focused our attention on the stability of EMR signals produced by radiation in crystalline fructose obtained by slow crystallization from solutions extracted from fruits. It has been observed for the first time that after prolonged storage (360 days) or following isothermal heating of sugars at the temperatures below their melting points some noticeable changes in the shape of EMR signals occur [4]. The most reasonable explanation of this effect is different stability of some of radicals giving rise to multicomponent EMR signal observed. Some of these radicals are more and some less stable resulting in the changes of recorded EMR signals. In a subsequent work [5] we recorded the EMR spectra of fructose, glucose and sorbose stored at ambient temperature (ca. 20°C) comparing them with those taken after isothermal heating of samples at temperatures closed to the melting points of investigated sugars (105, 97 and 140°C, respectively). The differential spectra obtained by computer subtraction of both types of normalized spectra were also given. In view of our supposition the differential signals correspond to less stable paramagnetic species decaying in time or by heating faster than the others. We focused our attention on fructose differential EMR signal which exhibits highly symmetric, well resolved spectroscopic structure. Basing on precise graphic analysis of the spectrum, it has been concluded that the dominating two pairs of isotropic lines may be attributed to a doublet of doublets originating from α (hfs = 3.6 mT) and β (hfs = 1.4 mT) splitting. The proposed structural formula of radical giving rise to this signal based on the literature data [14] has been also given. In the present study our efforts are focused on spectral analysis of differential signal obtained by computer subtraction of EMR signal recorded with heated and unheated glucose in order to throw some more light on the origin of multicomponent signal produced by radiation in this sugar occurring in fruit and widely used in dietary and medicine. It is known from a private source that these products are preserved with ionizing radiation.

Materials and methods

The subject of investigation was glucose crystallite obtained from sultan resins by slow, room temperature crystallization from methanol. Fruits were grinded with a bender to pulp. After adding of methanol and vigorous stirring by manifold repetition of this procedure glucose was extracted from the pulp. Methanol solution of glucose was cleared with charcoal and subjected to slow room temperature crystallization taking about one month. Thereafter, glucose crystallite was separated from saturated methanol solution and dried in the openair, dust free. Dried product was controlled by refraction measurement. Refraction coefficient *n* equal to 1.34645 ± 0.00003 as found is consistent with the literature data [6].

Glucose crystallite in a glass vessel was irradiated with 4 kGy in a ^{60}Co gamma source Issledovatel (dose rate 0.9 kGy/h). The irradiated product was stored for a week at room temperature with access of air to eliminate short-lived radiation induced EMR signals [12]. Sugar samples weighing ca. 100 mg were placed in EMR signal free ampoules 5 mm in diameter and examined with a Bruker ESP 300 spectrometer in X-band. The operating parameters of EMR measurement were as follows – microwave power 0.4 mW, modulation frequency 100 kHz, modulation amplitude 0.15 mT. The magnetic field sweep for recording full EMR signal was 20 mT. Next, the ampoules with samples were isothermally heated for 120 min at 105°C, the temperature close to glucose melting point, and measured again. The recorded signals taken with unheated and heated samples were normalized against signal amplitudes and subsequently subtracted using a computer program.

Results and discussion

The differential spectrum obtained by subtraction of EMR signals recorded with unheated and heated samples of glucose shown in Fig. 3 is simpler and better resolved than the two previously mentioned. Despite the fact that it represents still not one but more paramagnetic species including anisotropic ones, some of the well resolved lines allow to judge that isotropic radical spectra are involved. From graphical analysis of differential spectrum it was clear that only four lines marked A, B, C, D in Fig. 3 can be taken into consideration for finding the best fitting to isotropic radical spectrum. The distance between lines A and B and that one between lines C and D of the spectrum are almost identical equal to hfs (hyperfine splitting) = 1.25mT (12.5 G). However, the distance between lines B and C was found markedly higher and counts to 1.87 mT. In addition, the intensities of lines A and B are similar. Such position makes fitting to isotropic quartet less probable. However, it is possible to consider another

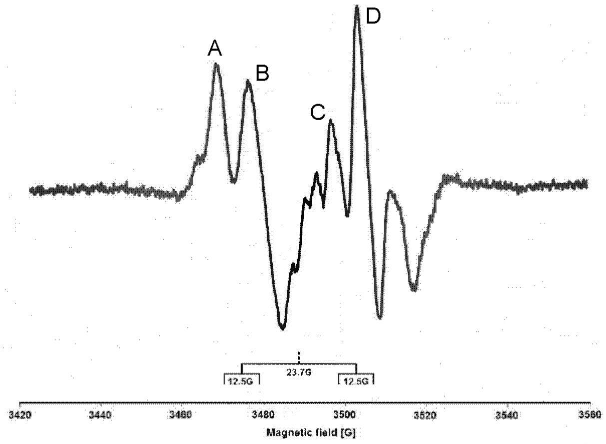

Fig. 3. Differential spectrum of D-glucose extracted from resins obtained by subtraction of normalized spectra recorded with samples heated at 105°C during 120 min from those kept at room temperature (ca. 20°C).

four-line model representing a doublet of doublets, the configuration of radical with unpaired electron interacting more strongly with one nuclear spin (α interaction = 23.7 G) and weaker with a second one (β interaction = 12.5 G) although the intensities of lines C and D are different. This is caused by the overlapping of both line C and D by undefined, probably anisotropic strong signal whose one of lines is resolved and seen at the high field side of the discussed signal. A similar model has been proposed earlier by us for fructose [5] a sugar of molecular structure resembling that of glucose (Figs. 1 and 2).

Considering the localization of unpaired electron at different carbon atoms of glucose molecule, the very probable radical structure giving rise to the identified doublet of doublets seems that with unpaired electron at carbon C-6 (Figs. 4a and 4b) outside the pyranose ring [9]. According to the model proposed, the unpaired electron at C-6 site interacts with two non equivalent hydrogen atoms. The stronger coupling occurs with hydrogen atom bonded at C-6a site (α interaction, hyperfine splitting, hfs = 23.7). The weaker one, in turn, with hydrogen atom bonded with C-5 atom of pyranose ring (β interaction, hfs = 12.5). Such localization of unpaired electron in glucose born radical has been also proposed by other authors in their ENDOR and DFT studies on radiation induced stable radicals observed in glucose-1-phosphate [2, 9]. It could be predicted that (C-6)-H_2-OH group bonded with carbon C-5 of pyranose ring rotates freely around its axis in outside pyranose ring. However, the DFT study showed that bonds between C-6 atom and its two hydrogen atoms are not equally strong. Thus, free rotation of the group is less probable.

The molecular structure of identified radical resembles that of parent molecule of glucose. The only difference lies in the lack of one hydrogen atom at C-6 bond of basic chain structure of glucose. It is very probable, therefore, that less stable radiation induced radicals in sugars are primarily irradiation products which did not undergo any further transformation. The literature does not deliver any suggestion on this subject.

EMR signals of sugar born radical are used for detection of radiation treatment in food articles containing

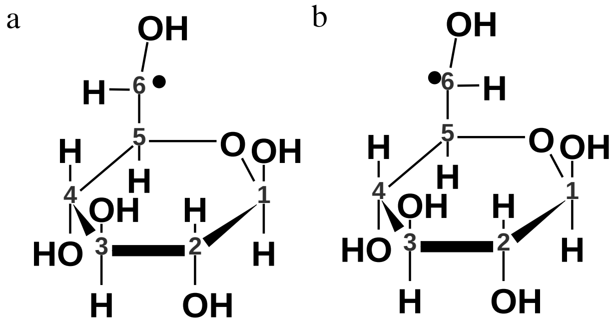

Fig. 4. The proposed molecular structure of glucose born radical giving rise to a doublet of doublets as marked in Fig. 3 with unpaired electron at C-6 carbon atom. In fact two structures can be considered. Structure a (left scheme) and structure b (right scheme) depending which of two hydrogen atoms is detached from C-6 atom during irradiation process.

crystalline sugars as dried fruits (figs, mangos, papayas, pineapples, raisins, etc.) as well as in diet supplements and medicines, for example. The same sugar born signal serves as a sensitive indicator of radiation exposure in accidental dosimetry [7]. However, very little is known about radicals responsible for this EMR signal remaining stable for a long time at room temperature. The reason is in the complexity and anisotropy of these signals followed by their mutual overlapping making interpretation of this multicomponent signal extremely difficult. In view of the obtained results a new approach proposed by our team based on the recording and interpreting of spectral changes occurring during prolonged storage or heating of irradiated sugar samples seems promising and perspective. The novelty of our present experimental approach lies in the heating of irradiated sugar containing samples at temperatures lower by ca. 20°C than sugars melting point the procedure accelerating radical decay process.

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