Generation, structure, and nutritional hazard of thermally generated poly- and oligo-saccharide free radicals

W. Ciesielski

Institute of Chemistry and Environmental Protection, Jan Długosz University of Częstochowa, al. Armii Krajowej 13/15, 42-200 Częstochowa, Poland

Abstract. Processes generating free radicals in food are reviewed. Structure and properties of free radicals are presented with particular emphasis paid to saccharides, oligo- and polysaccharides. Potential nutritional hazard from such radicals is discussed. 

Introduction
Free radicals are known as products of specific decomposition of valence bonds. The latter split in a uniform manner into free radicals with unpaired electrons on their orbitals [1].

\[ X : Y \rightarrow X^+ + Y^- \] (1)

As a rule, species having unpaired electrons (free radicals) dispose with high energy causing their very high reactivity. They utilize each possibility of shearing this energy with environment. Simple, small free radicals such as \( H \), \( CH_3 \), \( HO \) contain their unpaired electrons on orbitals readily accessible for the partner with whom excessive energy might be sheared. Therefore, the lifetime of such radicals is of the order even of \( 10^{-15} \)s. Such short lifetime implies a very high, nonselective reactivity. Free radicals of a complex structure are much more stable because either the orbital carrying unpaired electron is well hidden inside the structure of such species or the electron is well delocalized (migrates) to several atoms. In such manner this electron is hard to encounter in any position [2-7].

There is a great concern about free radicals regardless their structure and properties. Example of molecular oxygen is a good example for a statement that not all free radicals are hazardous and some are even indispensable for our life processes. Molecular oxygen exists as biradical and this structure provides oxidative properties of this component of atmosphere

\[ O - O \] (2)

There are several other essential physiological processes in our organisms, which occur with involvement of free radicals [8].

Several methods of food treatment, particularly thermal treatment and also some methods of food preservation [nitrates (III) = nitrites] as well as foodstuff sterilization with \( \gamma \)-radiation can generate free radicals. Therefore, the concern about free radicals does not omit food producers, nutritionists, physicians, and consumers [9].

Structure of free radicals
Structure of free radicals obviously depends on the source of which they are generated. However, method of their generation (time, temperature, catalyst) also play a considerable role.
In this presentation effect of these parameters will be limited to these which might find application on food processing, storage and digestion. Also considerations will be focused mainly on saccharides and polysaccharides.

Free radicals generated from these sources thermally on one hand and either by ionizing radiation or mechanochemically on another are different in their structure and behavior (stability, biological hazard). Ionizing radiation and mechanical action on saccharides and polysaccharides causes the hydrogen (H) abstraction and homolytic cleavage of O-H, C-C, C-H, and C-O bonds [11].

Based on the shape of EPR spectra Henderson and Rudin [9] distinguished between four different types of radicals produced by radiation of D-glucose. Adamic [13] differentiated between three classes of radicals, and Yershow and Isakova [12] showed that these radicals were those with unpaired spin localized at C4, C1, and C5, respectively. Raffi et al. [11] distinguished two classes of saccharide radicals. In order to solve these discrepancies 12 D-glucosyl radicals were taken under consideration [4]. They were formally formed by abstraction of H from D-glucose.

Calculations:

- *ab initio* in the 6-311G basis involving Gaussian 98
- SCF LCAO MO AM1
- SCF LCAO MO MNDO with MOPAC program

were carried out for them. Heats of formation for them became available from these calculations (Table 1).

Maps of electron (Fig. 1) and spin (Fig. 2) density also confirmed that free electron fairly well migrated along the glucosyl radical ring and very randomly occupied position assumed for calculations. Table 2 presents discrepancies in assumed and calculated spin and electron localizations. For instance, unpaired spin in structure (1) partly diffused from the oxygen atom of the C1-OH group into the C2 atom, and unpaired spin in structure (4) spread from C4-O at both the C2 and C3 atoms. The spin density in structure (6) disperses between O1 and the C2-OH oxygen atom. Some residual spin densities were found on the oxygen atom of the 6-CH2OH group and C5 carbon atom. Some of the assumed carbon radicals, i. e. structure (7) and, to a certain extent, also (9), distributed their spin densities among other atoms. Spin density in the structure (7) and (9) migrated from the anomeric C2 and C4 carbon atoms to the ring O1 atom, respectively. This tendency of the spin density to delocalize in no manner corresponded to the binding energies and heats of formation reported in Table 1.

**Figure 1.** The AM1 calculated spin density maps

**Figure 2.** The AM1 calculated electron density maps

The maps demonstrate that the highest electron densities were frequently localized in other positions rather than those in which the unpaired spin was assumed to reside. In the oxa-radicals (1-6) the higher electron densities either moved from the assumed position into another oxygen atom (1 and 4) or separated among two oxygen atoms (2 and 3). In the radical (3) the pyranose ring oxygen atom also contributed to the electron density distribution. In the carbon radicals (7-12) electron densities spread more readily among other carbon and oxygen atoms.

The degree of diffusion of the electron density in no manner corresponded to heats of formation given in Table 1.

One might see that all three approaches produced different results. They pointed to different atoms (bold figures) in the glucosyl radicals to be sites of localization of unpaired electrons. However, more
thorough inspection of the data leads to the conclusion that energy differences between several radicals are of the order of energy portions available from the intermolecular collisions. Thus, the differences in several magnitudes in Table 1 might be not very essential. In a consequence one might deduce that unpaired electron was fairly well delocalized in the whole structure of D-glucosyl radical what should be interpreted as stabilizing factor increasing their lifetime and, simultaneously, decreasing they reactivity and risk on contact with them. Delocalisation is better documented in Table 2.

Mono- and di-saccharides develop free radicals on melting as shown in Table 3 (Barabasz et al., 1990). Their concentration (free radical count – FRC) was determined by means of quantitative EPR spectroscopy. Usually such additives as Na₂CO₃, NaOH, citric acid, FRC glycine, lactic acid are present on thermal treatment of saccharides. In industrial processes of caramelization they act as catalyst. In the case of cooking they are simply present in foodstuffs. In our studies they either behaved neutrally or increased FRC even by two orders.

The thermal treatment of saccharides is assisted by deep chemical changes in the saccharide structure. They form caramelan, caramelene, and caramel in by intensive dehydration (Tomasik et al., 1989):

\[
\begin{align*}
6 \text{C}_{12}\text{H}_{22}\text{O}_{11} - 12 \text{H}_2\text{O} &= 6 \text{C}_{12}\text{H}_{12}\text{O}_9 \quad \text{caramelan} \\
6 \text{C}_{12}\text{H}_{22}\text{O}_{11} - 18 \text{H}_2\text{O} &= 2 \text{C}_{36}\text{H}_{18}\text{O}_{24} \quad \text{caramelene} \\
6 \text{C}_{12}\text{H}_{22}\text{O}_{11} - 27 \text{H}_2\text{O} &= 3 \text{C}_{24}\text{H}_{26}\text{O}_{13} \quad \text{caramel}
\end{align*}
\]

Around 200°C the following reaction occurs:

\[
11 \text{C}_{12}\text{H}_{22}\text{O}_{11} = 7 \text{CO}_2 + 27 \text{H}_2\text{O} + \text{C}_{125}\text{H}_{188}\text{O}_{80}
\]

Generally an extended chromophore system is formed (a deep brown color of caramels and dextrins).

Original polysaccharide structure in thermally generated free radicals is highly demolished. First free radicals appeared at temperature at which fast decomposition of polysaccharides occurred. It was proved by thermogravimetric studies of starches (Ciesielski et al., 1998). The thermal stability of starches depended on their botanical origin (Ciesielski and Tomasik, 1996) (Table 4).

Polysaccharide free radicals generated from starch appeared to be very stable towards air, water and alcohol. They survived almost one-year contact with the air. Attempts of cross-linking of starch and starch – cellulose blends with free radicals generated thermally in situ from D-fructose, D-glucose and sucrose failed. Only a residual cross-linking of polysaccharides was observed.

Inspection of Table 4 reveals that first free radicals appeared at temperatures above these, which are usually utilized in food preparation. However, processed foodstuffs are combinations of polysaccharides, lipids, proteins and mineral salts. Their presence certainly should modify thermal properties of all of them and one might assume that decrease in thermal stability and FRC could be met. They may decrease temperature at which free radicals would be generated and reduce time necessary for their generation. Moreover, we do not really know whether the same free radicals would be generated.

**Hazard of saccharide and polysaccharide free radicals**

As all free radicals also saccharide and polysaccharide free radicals were charged for their mutagenicity. Method of generation of such free radicals is essential for a verdict. It was demonstrated that there was a low mutagenicity against *Escherichia coli* from free radicals generated by γ-radiation. However this mutagenicity ceased in tests with *Bacillus subtilis* and *Saccharomyces cerevisiae* (Vakil et al., 1973; Truhaut et al., 1976). In view of delocalization of
unpaired electrons in glucosyl radicals such finding is not surprised. However, certain toxicity can be anticipated from products of leading to stabilization decomposition of such radicals. Among those compounds is formaldehyde. Saccharide free radicals are generated in a certain, usually aqueous medium. γ-Irradiation affects all components of medium. Water is a source of hydrogen peroxide, which decomposes into hydroxyl radical responsible, among others, for oxidative stress of cells.

Free radicals generated thermally from saccharides should be even less hazardous because their stability exceeds that of glucosyl radicals. Unpaired electrons may delocalize through extended chromophore system of π-electrons. There are more than 25 reports on mutagenicity of caramels tested on Salmonella typhimurium. Half of them claimed mutagenicity of caramels and another half reports present lack of any symptoms of mutagenicity (Tomasik et al., 1989). Test for mutagenicity with Escherichia coli confirmed lack of mutagenicity of such caramels. Thus, in spite of free radical character caramels are non-mutagenic (Barabasz et al., 1990).

Conclusions
We should not worry about free radicals formed from saccharides and polysaccharides on food manufacture and processing. These free radicals are stable, they are not mutagenic and usually they are formed at temperatures above these usually applied for food processing. However, it should be mentioned that thus far we know nothing about chemical and biological properties of free radicals generated from saccharides and polysaccharides and lipids and/or amino acids. Moreover, attention should be paid to the toxicity of by-products of the thermolysis of saccharides and polysaccharides.

References
[1] Abagyan G V Apresyan A S 1979 Arm Khim Zhurn 32 850
[2] Achremowicz Z Ciesielski W Korus J Tomasik P 1997 Carbohydr Polym 34 303
[3] Barabasz W Brzozka L Krzeczek J Tomasik P 1990 Starch/Staerke 42 69
[4] Ciesielski W Koziol J J Tomasik P 2001 Pol J Food Nutr Sci 10/2 212
[5] Ciesielski W Tomasik P 1996 Carbohydr Polym 31 205
[6] Ciesielski W Tomasik P 1998 Z Lebensm Untersuch Forsch A 207 292
[7] Ciesielski W Tomasik P Baczkowicz M 1998 Z Lebensm Untersuch Forsch A 207 299
[8] Hashiwagi H Enomoto S 1981 Chem Pharm Bull 29 913
[9] Henderson A M Rudin A 1981 J Polym Sci Polym Chem Ed 19 1721
[10] Hiramatsu M Yoshikawa T Inoue M (eds.) 1997 Food and free radicals Plenum Publ Corp New York
[11] Raffi J Agnel J P Thierry C J Frejaville C M Saint-Lebe L R 1981 J Agric Food Chem 29 1232
[12] Yershow B G Isakova O V 1987 Izv Akad Nauk SSSR Ser Khim 10 2337
[13] Adamie K Ceve P Korotchenko K A 1967 Starch/Staerke 19 336
Figure 1. The AM1 calculated spin density maps
Figure 2. The AM1 calculated electron density maps
Table 1. Heats of formation of α-D-glucose and α-D-glucosyl radicals (1-12)

| Radical | AM1 [kcal/mol] | MNDO [kcal/mol] | GAUSSIAN [kcal/mol] |
|---------|----------------|-----------------|---------------------|
| α-D-Glucose | -327.64 | -221.15 | -231.49 |
| C-1 | -241.64 | -224.52 | -231.52 |
| C-2 | -235.31 | -265.58 | -231.52 |
| C-3 | -239.94 | -214.42 | -231.65 |
| C-4 | -224.74 | -189.58 | -234.18 |
| C-5 | -245.83 | -145.47 | -233.01 |
| O-1 | -268.19 | -200.56 | -231.10 |
| O-2 | -273.67 | -189.58 | -234.54 |
| O-3 | -275.11 | -145.47 | -233.75 |
| O-4 | -267.23 | -206.53 | -234.18 |
| O-5 | -269.30 | -212.53 | -229.98 |

*The number of the given oxygen atom is the same as the number of the carbon atom to whom it is bound.

Table 2. Assumed and AM1 calculated positions of the highest spin and electron densities in α-D-glucose radicals (1-12)

| Accepted | Positions | Calculated Densities* |
|----------|-----------|-----------------------|
|         | Spin      | Electron              |
| C1-O    | C1-O, C2  | C2-O                  |
| C2-O    | C2-O      | C1-O, C2-O            |
| C3-O    | C3-O      | C3-O, C4-O, O_in   |
| C4-O    | C4, C5    | C2-O                  |
| C3-O & C6-O | C6-O | O_in, C6-O, C4-O |
| O_in   | O_in, C2-O, C4, C6-O | C6-O, O_in, C2-O |
| C1     | O_in, C1, C2-O | O_in, C6-O |
| C2     | C2        | C2, C6-O, C5         |
| C3     | O_in     | O_in, C6-OH           |
| C4     | C4        | O_in, C1              |
| C5     | O_in     | O_in, C6-O, C1-O, C3 |
| C6     | C6-O     | C6-O, C4-O, C2-O     |

*If there are multiple positions carrying unusually high densities, they are listed in order of decreasing density. The atoms carrying the highest density are given in bold characters.
Table 3. Free radical count (FRC) in molten saccharides (200°C, 1 hour, in the air exposure)

|       | FRC (x10^15/g of saccharide) |
|-------|-------------------------------|
| D-Fructose | 5 x 10^16/ g of saccharide |
| D-Glucose  | 1 x 10^17/ g of saccharide  |
| Sucrose   | 2 x 10^17/ g of saccharide   |

Table 4. Number of spins (FRC) in thermolyzed starches of various botanical origin.

| Temp. [°C] | Time [min] | FRC (x10^15/g) |
|------------|------------|----------------|
|            |            | Potato | Triticale\(^a\) | Oat | Cassava\(^b\) |
| 285        | 90         | 0      | 0     | 10  | 0  |
|            | 120        | 0      | 5     | 20  | 0  |
| 300        | 90         | 5      | 2     | 10  | 6  |
|            | 120        | 6      | 3     | 30  | 6  |

\(^a\)Also wheat, waxy maize, rye, and corn starches  
\(^b\)Also amaranthus starch.