

Radioinduced Products in Maize Starch: Glyceraldehyde, Dihydroxyacetone, and 2-Hydroxymalonaldehyde

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We measured the amounts of glyceraldehyde, dihydroxyacetone, and 2-hydroxymalonaldehyde (triose reductone or 2-hydroxy-1,3-propanedial) induced in maize starch by γ radiation. These three carbonyl derivatives were reduced by potassium borohydride or borodeuteride, silylated, and then studied by gas chromatography combined with mass spectrometry. We studied the influence of several parameters (dose, temperature, and atmosphere of irradiation; time of storage and water content of starch) and then proposed a mechanism for the formation of 2-hydroxymalonaldehyde which is here identified and measured for the first time in irradiated maize starch.

Among the radioinduced products in maize starch, the carbonyl derivatives require a careful study in order to do a toxicological evaluation of irradiated starch: we have measured a great number of these products (Berger et al., 1977; Michel et al., 1977) and tried to draw a balance of the whole carbonyl fraction (Raffi et al., 1978). However, we have not succeeded in this regard, until now, as we have only measured the sum of the two quantities of radioinduced glyceraldehyde (G) and dihydroxyacetone (DHA) (Michel et al., 1975).

Hereafter our purpose is to show how, through a reduction of these derivatives with borodeuteride followed by a silylation, we can draw a "total" titration by gas chromatography and a "qualitative analysis" of the different silylated labeled glycerols by mass spectrometry combined with gas chromatography. This method presents the advantage of allowing the titration of a new radioinduced compound which is the 2-hydroxymalonaldehyde (H2M) (triose reductone or 2-hydroxy-1,3-propanedial).

MATERIALS AND METHODS

Generalities. The maize starch was irradiated by a ^{60}Co source (dose rate: 1 kGy h^{-1}). The products were measured in the aqueous extract obtained as follows: X grams of irradiated starch was shaken for 1.5 h in $2X\text{ cm}^3$ of distilled water, and then the suspension was filtered.

The NMR and the mass spectra were obtained from commercial G and DHA (Sigma Chemical Co.) and from H2M synthesized according to the Schank and Wessling (1967) procedure. The NMR spectra were compared to those of previous works (Schank and Wessling, 1967; Obata et al., 1975), and the mass spectra were analyzed (before and after exchange with D_2O) in order to check the chemical formula of this last product.

"Total Titration" of the Carbonyl Derivatives. The reduction was directly performed on 120 cm^3 of the aqueous extract with 150 mg of KBH_4 for an irradiation dose of 10 kGy. The solution was shaken 16 h at room temperature and then put on a resin, H^+ AG50 W-X8 (Bio-Rad). The resin was washed by 200 cm^3 of water and the solution dried. The residue was reintroduced into 7 cm^3 of water and then precipitated by 100 cm^3 of ethanol. This filtrate was put again into 3 cm^3 of water, 2.5 cm^3 of which were dried, and added to 2 cm^3 of dimethylformamide and 2 cm^3 of BSTFA with 1% TMCS (Pierce).

The quantity of Me_3Si -glycerol, hence the total quantity of the three carbonyl derivatives, was determined by gas

chromatography (Perkin-Elmer 900) on SE30 or QF1 columns ($120\text{--}130\text{ }^\circ\text{C}$).

"Qualitative Analysis" of the Carbonyl Derivatives.

It was done by studying the mass spectra obtained on a LKB 9000 S combined with a gas chromatograph (same columns as before) of the mixture of the different Me_3Si deuterated glycerols. The deuteration was the same as the hydrogenation, with KBD_4 in place of KBH_4 .

The study of the different spectra (see Table I), after the peaks heights normalization with the convention $h_{103} + h_{104} = 100$, is a means to measure the unknown concentrations. Let X , Y , and Z be the different concentrations of G, DHA, and H2M. Here, $X + Y + Z = 1$, so, from Table I

$$h_{103} = 50X + 100Y$$

$$h_{104} = 50X + 100Z$$

$$h_{117} = 116X + 63.3Y$$

$$h_{118} = \dots$$

The precision obtained on the different values is $\sim 10\%$ on X and Z and 20% on Y . The $M - 15$ peak, too weak, could not be used.

The preliminary calculation was done on synthetic mixtures of G, DHA, and H2M, and we checked to make sure that glyceric, mesoxalic, tartronic, and 3-hydroxypropionic acids did not give appreciable quantities of Me_3Si -glycerol.

RESULTS

We determined the coefficients of extraction of these three derivatives from irradiated starch and found, each time, $47 \pm 2\%$ for the first extraction and respectively 79 and 93% after two and three extractions. Thus, when we determine the concentrations ($\mu\text{g mL}^{-1}$) of the studied products in the aqueous extract, it will be easy to calculate the quantities by gram of dry starch.

However, in the following study of the influence of the different parameters of the radiolysis, we only consider in the curves the radioinduced quantities per milliliter of aqueous extract.

Irradiation Dose. The concentrations of the radioinduced products increase in a linear manner (Figure 1) with the dose up to 50 kGy (5 Mrad). They are 0.49 (G) 0.12 (DHA), and 0.62 (H2M) $\mu\text{g g}^{-1}\text{ kGy}^{-1}$.

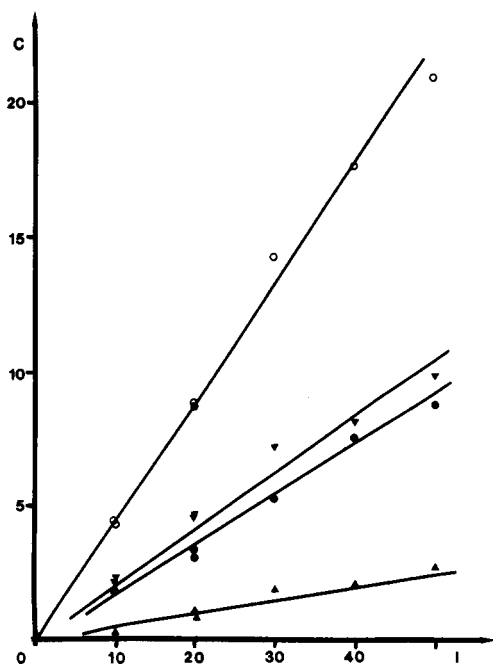
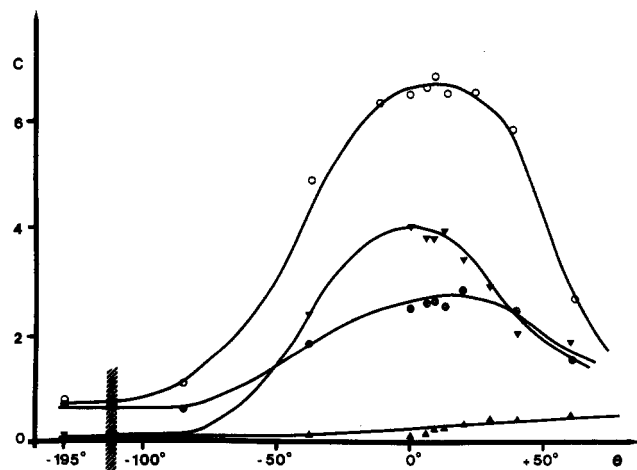
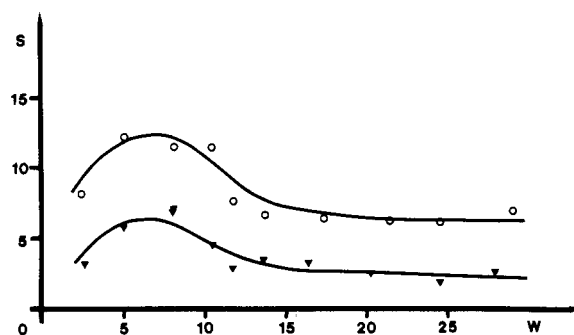
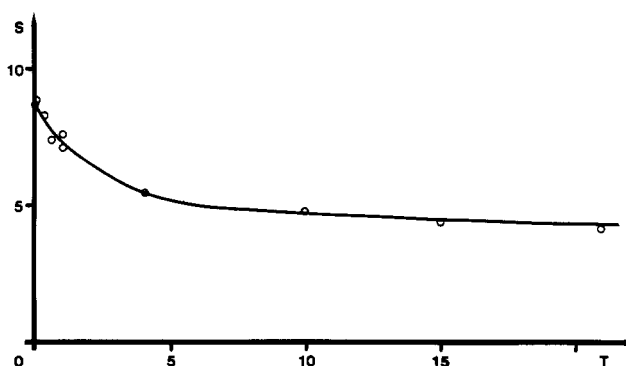
Irradiation Temperature. The quantity of DHA slightly increases with the temperature of the irradiation (Figure 2), while the quantities of G and H2M are maximum in the range $0\text{--}20\text{ }^\circ\text{C}$.

Starch Water Content. The amounts of G and DHA and the sum of the three carbonyl compounds have a

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Table I. Average Heights of the Main Peaks of the Mass Spectra of the Three Deuterated and Then Silylated Aldehydes and Silylated Glycerol

original product	peak	glycerol (HOCH ₂ CHOHCH ₂ OH)	G (OCHCHOHCH ₂ OH)	DHA (HOCH ₂ COCH ₂ OH)	H2M (OCHCHOHCHO)
cleavage of one extremity, -CH ₂ OSiMe ₃	103	100	50	100	
	104		50		100
CH ₂ (OSiMe ₃)CH ₂ , deriving from the migration of one "proton" of the extremity	117	116	63.3		
	118		52.7	116	
	119				102
	120				14
Me ₃ SiOCH ₂ CHOSiMe ₃	205	238	119		
	206		119	238	
	207				238
Me ₃ SiOCH ₂ CH(OSiMe ₃)CH ₂ , by elimination of HOSiMe ₃	218	34	11.5		
	219		22.5	34	
	220				11.5
	221				22.5
(M - 15) ⁺ , by elimination of one CH ₃	293	ε			
	294		ε	ε	
	295				
	296				ε


 Figure 1. Variations of the concentrations C ($\mu\text{g mL}^{-1}$) of G (○), DHA (▲), H2M (▼), and their sum (○) with the irradiation dose I (kGy).

 Figure 2. Variations of the concentrations C ($\mu\text{g mL}^{-1}$) of G (○), DHA (▲), H2M (▼), and their sum (○) with the irradiation temperature θ (°C). Irradiation dose: 20 kGy.

 Figure 3. Variations of the sum S ($\mu\text{g mL}^{-1}$) of the three carbonyl compounds with the water content W (in percentage) of the starch. Irradiation dose: 20 kGy, under oxygen (○) or nitrogen (▼).

 Figure 4. Variation of the sum S ($\mu\text{g mL}^{-1}$) of the three carbonyl compounds with the time of storage T (weeks). Irradiation dose: 20 kGy, under oxygen. Water content of the starch: 12%.

maximal value for a water content of $\sim 6-7\%$ under either oxygen or nitrogen. But the water content has a negligible effect on the formation of H2M (Figure 3).

Irradiation Atmosphere. If the irradiation is carried out under nitrogen instead of oxygen, the total quantity and the units of each compound sharply decrease (Figure 3). This oxygen effect is more important in the moist state for G and in the dry state for DHA but is independent of the water content for H2M.

Storage. During storage (Figure 4) under oxygen, at room temperature, the sum of the three compound concentrations undergoes a decrease which is rapid during the first week (20%) and slower after that (20 weeks to get a 50% decrease). But this average speed hides the fact that G and DHA decay slowly (in 20 weeks, 35-40%) and that

Table II. Radioinduced Carbonyl Compounds^d

after reaction of 2,4-dinitrophenylhydrazine and	absorbance of the complex
the whole carbonyl fraction ^a	1.47 ± 0.22
the identified carbonyl compounds not linked to the radiodextrins ^b	0.66 ± 0.10
the carbonyl compounds linked to the radiodextrins insoluble in ethanol ^a	0.43 ± 0.07
soluble in ethanol ^c	0.14 ± 0.03
	1.23 ± 0.20

^a Values derived from the first table of this type proposed by Michel et al. (1977). ^b New value derived from the conclusions of this datum. ^c Value derived from the theoretical study of the radiodepolymerization of starch (Raffi et al., 1980). ^d Dose: 10 kGy; water content: 13%.

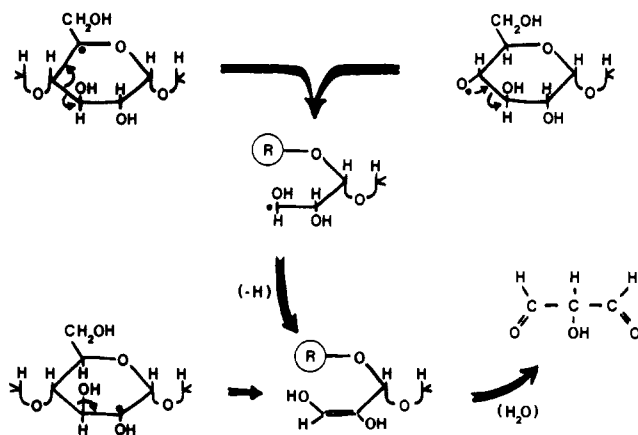


Figure 5. Mechanistic proposals for the formation of the radioinduced 2-hydroxymalonaldehyde.

H2M decays rapidly (70% in the same time). This fact could be expected since hydrogen peroxide is also radioinduced in starch (Berger and Saint-Lébe, 1971).

Comparison with Heating. The difference between the amounts induced in irradiated (10 kGy) and heated (1 h; 125 °C) starch is lower than the experimental error. But heating irradiated starch allows a decrease in the quantity of G by a factor 2 and the ones of DHA and H2M by a factor 5.

DISCUSSION

Balance of the Radioinduced Carbonyl Derivatives.

These data allow for a slight correction of the balance of the whole carbonyl fraction of the irradiated starch (Table II). However, it is difficult to assert that this point is definitive because the error (± 0.22) is of the same order than the "theoretical" unknown fraction (0.24).

Mechanisms. Mechanisms of the radiolysis of carbohydrates in aqueous solution or in solid state have already been proposed by several authors (Baugh et al., 1976; Von Sonntag et al., 1976; Dizdaroglu et al., 1977). Specific mechanistic models for G and DHA (Von Scherz, 1971; Berger et al., 1977) have been proposed to explain the formation of these products from the primary free radicals deriving from hydrogen abstraction on C₂ or C₅ atoms or from breaking of the glucosidic linkage and, then, rearrangement leading to the break of the C₃-C₄ linkage.

We can now put forward a mechanistic proposal for the formation of H2M (Figure 5). The experimental activator effect of oxygen might derive from the capture of molecular oxygen by the radioinduced radicals with decomposition of the peroxide radicals and formation of carbonyl derivatives [see Von Sonntag and Dizdaroglu (1977)]. But, in each case, those proposals have to be checked by mechanistic studies, for instance, by electron spin resonance.

Even if further studies remain to be done, we can now conclude that the radioinduced quantities of glyceraldehyde, dihydroxyacetone, and 2-hydroxymalonaldehyde are too low to involve health hazards.

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