chemistry

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Mechanistic Insights into Sugar Racemization and Oxidative Degradation via Fenton and Alkaline Peroxide Systems

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Abstract: This study explores the oxidation and racemization of selected C5 and C6 sugars using hydrogen peroxide (H_2O_2) in alkaline and Fenton reaction conditions. The sugars studied include D-Glucose, D-Fructose, D-Mannose, D-Xylose, D-Lactose, D-Arabinose, D-Cellobiose, Sucrose, and D-Galactose. Oxidation reactions were conducted using both Fenton's reagent and NaOH/H_2O_2 to examine product formation, yield distribution, and stereochemical transformations. Under alkaline conditions, sugars primarily oxidized to yield sodium formate and hydrogen, with the minimal formation of intermediate sugar acids. Excess alkaline conditions further promoted the rapid degradation of sugars to sodium formate and hydrogen as primary products, indicating the strong influence of reaction conditions on oxidation pathways. A significant observation was that both alkaline and Fenton oxidation led to racemization, converting optically pure sugars into a racemic mixture of D- and L-enantiomers, thus producing products with zero optical rotation. The generation of L-enantiomers, metabolically inactive in biological systems, has implications for energy yield and biochemical efficiency.

Keywords: aldose; pentose; hydrogen peroxide; Fenton oxidation; NaOH; racemization; sodium formate

Academic Editor: Felix Plasser

Received: 8 November 2024
Revised: 14 December 2024
Accepted: 24 December 2024
Published: 26 December 2024

Citation: Köntös, Z.; Németh, Á. Mechanistic Insights into Sugar Racemization and Oxidative Degradation via Fenton and Alkaline Peroxide Systems. *Chemistry* **2025**, *7*, 2. https://doi.org/10.3390/chemistry7010002

1. Introduction

The oxidation of sugars and carbohydrates, such as D-Glucose, D-Fructose, D-Mannose, D-Xylose, D-Lactose, D-Arabinose, D-Cellobiose, Sucrose, and D-Galactose, by hydrogen peroxide (H_2O_2) is an interesting reaction in organic and carbohydrate chemistry [1]. As primary energy sources, they underpin a wide array of metabolic pathways crucial for cellular function, growth, and survival. Sugars are particularly indispensable in humans, where they serve as metabolic substrates for essential biochemical processes, including glycolysis, the pentose phosphate pathway, and the citric acid cycle. Among sugars, those with five (C5) or six (C6) carbon atoms hold a unique position due to their critical roles in energy production, the biosynthesis of macromolecules, and regulatory functions. However, the structural integrity and stereochemistry of these sugars are equally vital to their function. The chirality of sugars, defined by their specific three-dimensional arrangement around asymmetric carbon centers, dictates their recognition and utilization by enzymes, transporters, and other biomolecular machinery. In biological systems, a preference for D-enantiomers over L-enantiomers has evolved due to the homochiral nature of enzymes that metabolize these sugars.

Homochirality [2], which refers to the uniformity in the chirality (handedness) of molecules, is a fundamental characteristic of biochemical systems. This uniformity en-

sures that enzymes and other biomolecules interact specifically and effectively with their substrates. For instance, glycolytic enzymes such as hexokinase and glucose-6-phosphate isomerase are structured to recognize D-Glucose and cannot effectively interact with L-glucose or other L-sugars [3]. This evolutionary adaptation has streamlined energy production and biochemical efficiency by leveraging the structural specificity of D-sugars. Conversely, L-sugars, as stereochemical mirror images, are largely excluded from these pathways and are metabolically inactive in most organisms. This specificity, while advantageous under normal conditions, introduces a vulnerability: the disruption of sugar chirality through chemical or environmental factors can have profound biochemical and metabolic consequences.

The role of sugars as fundamental biomolecules [4] in living systems cannot be overstated. One of the significant biochemical phenomena capable of altering sugar chirality is oxidative stress because oxidative stress introduces a potential challenge to the chirality of sugars. Species such as hydrogen peroxide, superoxide anions, and hydroxyl radicals are highly reactive molecules capable of altering the structure and function of biomolecules. These radicals interact with a wide range of biomolecules, including sugars, proteins, lipids, and nucleic acids, often initiating a cascade of structural and functional disruptions. For sugars, this interaction can lead to racemization [5], which is a chemical process that converts a pure chiral molecule into a racemic mixture containing equal proportions of its enantiomers. In biological systems, the racemization of sugars primarily involves the conversion of D-sugars to a mixture of D- and L-sugars. Sugar oxidation is a complex chemical process influenced by several factors, including pH, the presence of oxidizing agents, and reaction conditions.

Fenton chemistry [6] introduces a more radical-driven oxidative pathway. In this reaction, H_2O_2 reacts with transition metals, such as Fe^{2+} or Fe^{3+}, to produce hydroxyl radicals [7], which exhibit high reactivity with organic substrates. For sugars, this reaction initiates a cascade of oxidative processes, including carbonyl formation, ring opening, and bond cleavage [8]. These reactions not only degrade sugars into smaller molecules but also facilitate racemization by altering the stereochemical configuration of chiral centers.

The interplay between oxidation and racemization in Fenton systems highlights the dual role of hydroxyl radicals in both structural degradation and stereochemical disruption. The non-selective nature of hydroxyl radicals makes Fenton oxidation a versatile method for degrading complex and persistent organic compounds [6]. Research by Köntös [9] and Neyens et al. [10] explored the conditions of optimal performance. Haber and Weiss [11], to improve their understanding of Fenton oxidation mechanisms, investigated the effect of reactant concentrations on the overall reaction kinetics.

Hydroxyl radicals are highly reactive entities that can be generated both endogenously and from environmental sources. Within the human body, these radicals [12] commonly arise as byproducts of metabolic activities [13].

Under alkaline conditions, H_2O_2 acts as a robust oxidizing agent, facilitating the degradation of sugars into smaller organic molecules, such as sodium formate, hydrogen gas, and intermediate sugar acids. At high pH levels, sugars rapidly degrade to yield sodium formate and hydrogen as primary products, reflecting the predominance of oxidative cleavage over intermediate transformations. Sodium formate (HCOONa) and hydrogen (H_2) are two critical substances in chemical and energy sciences due to their wide-ranging applications and potential to contribute to sustainable technologies. Sodium formate's versatility as a precursor to formic acid (HCOOH) [14] and hydrogen's role as a clean energy carrier underscore their relevance in addressing global challenges like energy storage, carbon management, and pollution reduction. The structural chemistry of transition metal peroxides such as iron peroxide (Fe_2O_2) is fundamentally different from

that of alkali metal peroxides like sodium peroxide (Na_2O_2). These differences are rooted in the distinct electronic and coordination properties of the metal ions involved. The ability of Fe^{2+} to form a stable, cyclic Fe_2O_2 structure involves a six-membered ring that significantly influences its chemical reactivity, particularly in its capacity to generate reactive species like ferroperoxyl radicals (FeO·). In contrast, the inability of Na^+ to form such cyclic structures limits the radical generation pathways available to sodium peroxide. The structure of iron peroxide, Fe_2O_2, is markedly different from that of sodium peroxide due to the nature of the metal ion involved.

Our work investigates the oxidative pathways of C5 and C6 sugars under Fenton and alkaline conditions, examining racemization, product distribution, and radical behavior through spectroscopic analysis. These findings provide insight into the role of reaction conditions, especially the influence of hydroxyl and ferroperoxyl radicals on sugar degradation and product formation. The results are presented across different reaction setups, focusing on the key findings that elucidate oxidation mechanisms and the impacts of radicals on the stereochemistry of sugars.

2. Materials and Methods

All chemicals used were of analytical grade, obtained from Sigma Aldrich (Darmstadt, Germany), and utilized without further purification. All solutions were prepared with doubly distilled water. Optical rotation measurements were conducted on a Perkin Elmer 241 polarimeter (Shelton, CT, USA) (a Na lamp with a length of 10 cm cuvette) in distilled water, with a concentration (measured by weight) of 0.01 mol/L. Molecular modeling calculations were performed using AVOGADRO 1.2.0 on MacBook Air M2 (Apple, Cupertino, CA, USA).

2.1. General Procedure for Fenton Oxidation of C5 and C6 Sugars

The Fenton oxidation of C5 and C6 sugars was carried out following a modified protocol based on Köntös [9]. In a typical reaction, 0.100 mol of the target C5 or C6 sugar in 100 mL of water is combined with 1.00 g of ferrous sulfate ($FeSO_4 \cdot 7H_2O$), which has been pre-dissolved in 15 mL of water and 1 mL of concentrated H_2SO_4, in a 500 mL reaction flask. To initiate the oxidation process, 65 mL of 6% H_2O_2 solution (1.1 mol of hydrogen peroxide per mol of sugar) is added incrementally over a period of 5 h. Specifically, 13 mL of H_2O_2 is added each hour, with careful temperature control to prevent any rise exceeding 3 °C. After the addition of H_2O_2, the reaction mixture is neutralized at room temperature by adding an excess of powdered calcium carbonate ($CaCO_3$) and agitating. The calcium carbonate sediment is removed by vacuum filtration. The resulting filtrate is then concentrated into a syrup under vacuum distillation (bath temperature 45 °C), during which the substantial precipitation of calcium salts (mainly calcium sulfate) occurs. The residual syrup is mixed with two volumes of absolute ethanol. The mixture is filtered to remove precipitates and further concentrated to yield a thick, pale-yellow syrup, which is used directly for polarimetry measurements without further purification.

2.2. General Procedure for Equimolar H_2O_2/NaOH Oxidation of C5 and C6 Sugars

The crude sugar acids of C5 and C6 sugars are synthesized using equimolar H_2O_2/NaOH oxidation. To begin with, 0.100 mol of the selected C5 or C6 sugar is dissolved in 100 mL of water and then combined with 4.00 g (0.100 mol) of sodium hydroxide (NaOH) pre-dissolved in 50 mL of water in a 500 mL reaction flask. To sustain continuous hydrogen production, 65 mL of 6% H_2O_2 (1.1 mol H_2O_2 per mol of sugar) is gradually added over a period of 5 h at a rate of 13 mL per hour while controlling the temperature to limit increases to 5 °C. Once the hydrogen gas evolution ceases, the reaction mixture is neutralized at

room temperature using Amberlite IR120 (in the hydrogen form) to yield a crude product solution. Any remaining sediment is removed by filtration through a vacuum filter. The filtrate is then concentrated under vacuum distillation at 45 °C to obtain a pale-yellow syrup, which is subsequently used for polarimetry without additional purification.

2.3. General Procedure for Complete Oxidation of C5 and C6 Sugars with Excess H_2O_2/NaOH

For the preparation of crude sodium formate (HCOONa), 0.100 mol of the selected C5 or C6 sugar is dissolved in 100 mL of water and then combined with 4.00 g (0.100 mol) of NaOH, previously dissolved in 50 mL of water, in a 750 mL reaction flask. To maintain a steady release of hydrogen gas, either 325 mL or 390 mL of 6% H_2O_2 (5.6 or 6.6 mol of H_2O_2 per mol of sugar) is added over 5 h. During the reaction, the pH is monitored and maintained at 12 by adding additional NaOH if it drops below pH 9. After the cessation of hydrogen evolution, the reaction mixture is concentrated to a syrup under vacuum distillation at 45 °C. The concentrated syrup is then combined with three volumes of absolute ethanol, resulting in the precipitation of crude sodium formate. This precipitate is filtered, dried, and yields white HCOONa crystals as the final product. The sodium formate produced as the main product during the reactions is identified in all cases based on its ^{1}H NMR and ^{13}C NMR spectra, specifically using the signal at 8.4–8.5 ppm in the ^{1}H NMR spectrum and the signal at 171.1–171.3 ppm in the ^{13}C NMR spectrum.

In our study, the reaction was concurrently investigated using Electron Paramagnetic Resonance (EPR) spectroscopy, a powerful analytical tool for probing the complexities of chemical reactions, especially in advanced oxidation processes like the Fenton reaction [9]. EPR spectroscopy is specifically suited for studying materials with unpaired electrons, making it ideal for detecting and quantifying paramagnetic species such as the $Fe3^{+}$ ion. X-band EPR spectroscopy measurements were conducted at the Budapest University of Technology and Economics, utilizing a Bruker ELEXSYS E500 X-band EPR spectrometer (Billerica, MA, USA) equipped with a Wilmad-LabGlass WG-821-F-Q variable temperature insert and Wilmad-LabGlass 710-SQ-250M quartz sample tubes. Solution samples were prepared in quartz tubes (710-SQ-250M (Wilmad-LabGlass) 5 mm Thin Wall Quartz EPR Sample Tube 250 mm L) and measured at 150 K. The modulation amplitude was set to 1 G, with a microwave power of 2.074 mW and a frequency of approximately 9.3 GHz. Baseline measurements were taken using a quartz tube filled with distilled water, and this baseline was subtracted from the spectra prior to further analysis. Additionally, the magnetic field axis of all spectra was adjusted to a microwave frequency of 9.33 GHz. The measurements were performed on frozen solution samples (approximately 650 µL) at 150 K, except for Sample S6, which had a volume of 850 µL. The samples were loaded into 710-SQ-250M quartz tubes, and the WG-821-F-Q variable temperature insert allowed for precise temperature control via liquid nitrogen vapor flow. The samples became fully frozen about 5 min after the addition of Solution D. The microwave frequency was in the range of 9.28–9.29 GHz. To standardize the magnetic field (B) axis, the spectra were rescaled to a reference frequency of 9.28 GHz, where B-axis values were multiplied by the factor (f_0/f) for consistent analysis. EPR spectra were recorded in 2048 channels, covering a magnetic field range of 100–10,900 G. Baseline correction was applied to all spectra before final analysis and processing.

Measurements were performed with the following spectrometer settings:

Center field: 5500 G;
Sweep width: 10,800 G;
Sweep time: ~84 s;
Microwave power: ~2.07 mW;
Modulation amplitude: 2 G;

Modulation frequency: 100 kHz;
Time constant: 10.24 ms;
Conversion time: ~41 ms;
Number of scans: 2.

Measurement solutions (see Table 1):

Solution A: $FeSO_4 \cdot 7H_2O$ (0.15 g) dissolved in 10 mL H_2O;
Solution B: "Sucrose (Saccharose)" (0.1 g) dissolved in 10 mL H_2O;
Solution C: "Lactose" (0.05 g) dissolved in 10 mL H_2O;
Solution D: H_2O_2 solution (12.5%).

Table 1. Measurement solutions A-D.

	Solution A	Solution B	Solution C	Solution D
DD. Water	10 mL	10 mL	10 mL	87.5 mL
$FeSO_4 \cdot 7H_2O$	0.150 g			
Saccharose		0.100 g		
D Lactose			0.050 g	
H_2O_2				12.500 g

Measurement samples (see Table 2):

Solution B2: 400 µL (Solution B) + 400 µL distilled water.

Table 2. Measurement samples S2–S6.

	Sample S2	Sample S3	Sample S4	Sample S5	Sample S6
Solution A	50 µL	50 µL	50 µL	50 µL	50 µL
Solution B		400 µL	400 µL	200 µL	
Solution C	400 µL				400 µL
Solution D	200 µL	200 µL	200 µL	200 µL	200 µL

The samples were prepared in 2 steps: first, the solutions inside [ ] were mixed, then the third solution (this is mostly H_2O_2 solution) was added.

Sample S2 ("Lactose"): [50 µL (Solution A) + 400 µL (Solution C)] + 200 µL (Solution D);
Sample S3 ("Saccharose"): [50 µL (Solution A) + 400 µL (Solution B2)] + 200 µL (Solution D);
Sample S4 ("Without H_2O_2"): [50 µL (Solution A) + 400 µL (Solution B2)] + 200 µL (H_2O)'
Sample S5 ("Without sugars"): [50 µL (Solution A) + 400 µL (H_2O)] + 200 µL (Solution D);
Sample S6 ("Lactose" + double amount of H_2O_2): [50 µL (Solution A) + 400 µL (Solution C)] + 400 µL (Solution D).

3. Results

Fenton oxidation operates through a cyclical mechanism involving hydrogen peroxide (H_2O_2) and ferrous ions (Fe^{2+}), which generate hydroxyl radicals and facilitate oxidative degradation of sugars. The ongoing production of hydroxyl radicals creates a potent oxidative environment capable of degrading sugars and other organic molecules. The presence of hydroxyl radicals results in rapid racemization, as these radicals readily attack the chiral centers of C5 and C6 sugars. Sugars such as D-Glucose and D-Fructose, which normally exhibit optical activity due to their specific chiral configurations, are racemized under Fenton conditions, forming equal proportions of D- and L-enantiomers [15]. This racemization [depicted in Table 3] eliminates net optical rotation, confirming the structural

transformation of sugars. For both pentoses (e.g., D-Xylose, D-Arabinose) and hexoses (e.g., D-Glucose, D-Mannose), Fenton oxidation resulted in the rapid loss of optical activity, leading to racemic mixtures that are unsuitable for metabolic processes, as D-sugars alone are efficiently processed in biological systems. When C5 and C6 sugars were treated under alkaline oxidation with equimolar NaOH and H_2O_2, significant racemization was again observed, as shown in Table 3. Hydroxyl radicals generated from the H_2O_2 and NaOH reaction disrupt the chiral centers, converting D-sugars into a racemic mixture of D- and L-enantiomers with no optical activity. This non-selective and aggressive nature of the alkaline conditions underscores the versatility of oxidation in various pH settings, each inducing racemization and the subsequent metabolic inertness of sugars. In addition to racemization, the use of excess $NaOH/H_2O_2$ results in sodium formate (HCOONa) as a predominant product. With increased NaOH concentration, oxidation proceeds to completion, fully degrading sugars into sodium formate with minimal intermediate formation. Sodium formate's stability in alkaline conditions enables it to accumulate as a primary product, making this reaction pathway especially efficient for formate production. The formation of formate from D-Lactose, D-Cellobiose, D-Arabinose, and Sucrose highlights the consistency of these findings across various sugar types. The sodium formate produced as the main product during the reactions was identified in all cases based, as described above, on its ^{1}H NMR and ^{13}C NMR spectra.

Table 3. Optical rotation of oxidation products.

Sugar	Lit. $[\alpha_D^{25}]$ [°]	Fenton $[\alpha_D^{25}]$ [°]	Alkaline $[\alpha_D^{25}]$ [°]
D-Xylose (XYL)	+18.8	+0.001	+0.001
D-Arabinose (ARA)	+105	+0.001	+0.002
D-Glucose (GLU)	+52.7	+0.001	−0.001
D-Galactose (GAL)	+80.2	+0.001	+0.001
D-Mannose (MAN)	+14.2	+0.001	−0.001
D-Fructose (FRU)	−92.5	−0.001	−0.013
D-Cellobiose (CEL)	+135	+0.001	+0.001
D-Lactose (LAC)	+52.5	+0.001	+0.001
Sucrose (SUC)	+66.5	+0.001	+0.001

Electron Paramagnetic Resonance (EPR) spectroscopy provided key insights into the radical dynamics involved in the oxidation process. The analysis of samples S2 (D-Lactose), S3 (Saccharose), and S5 (blind sample, no sugars) through EPR revealed differences in radical generation across sugars. The spectra of samples S2 and S3 were notably similar, indicating that the specific nature of sugars has minimal impact on radical behavior under standard Fenton conditions (Figure 1).

The spectrum of sample S5 differs slightly from the spectra of samples S2 and S3, as shown in Figure 1, suggesting that the sugars influence the effect of H_2O_2. The spectrum of sample S4 includes minor contributions from solitary Fe^{3+} ions and Mn^{2+} ions [16]. These must be present in the original $FeSO_4 \cdot 7H_2O$ solution (Solution A) as contaminants. The spectra of samples S2 (black curve) and S3 (red curve) are very similar; the nature of sugar does not seem to have an appreciable influence on the EPR spectra, as seen in Figure 2.

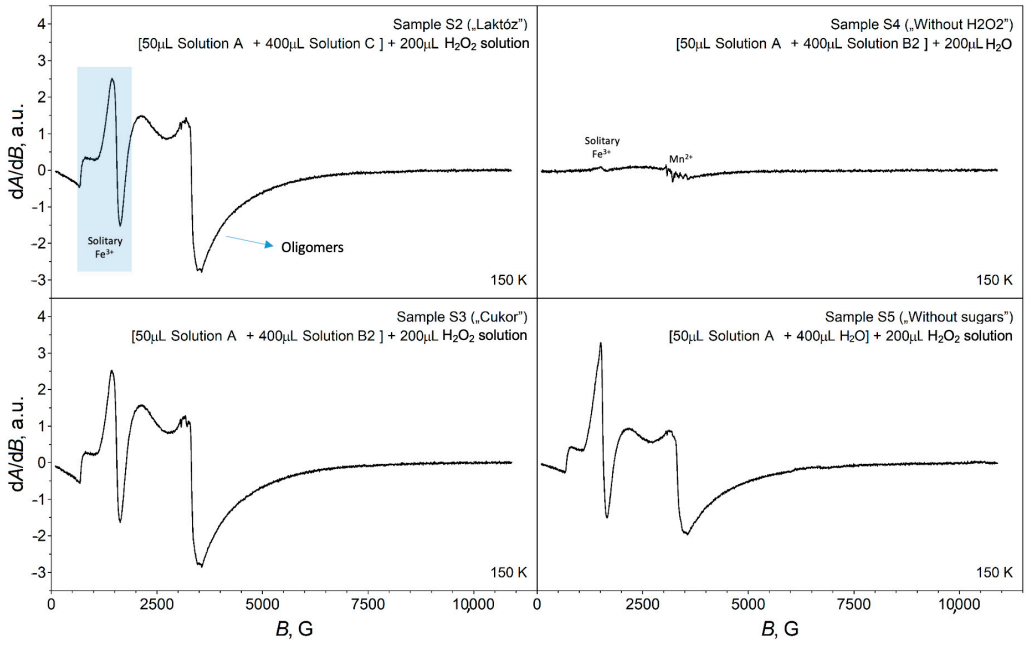

Figure 1. EPR spectra of Fenton oxidation of D-Lactose and Sucrose.

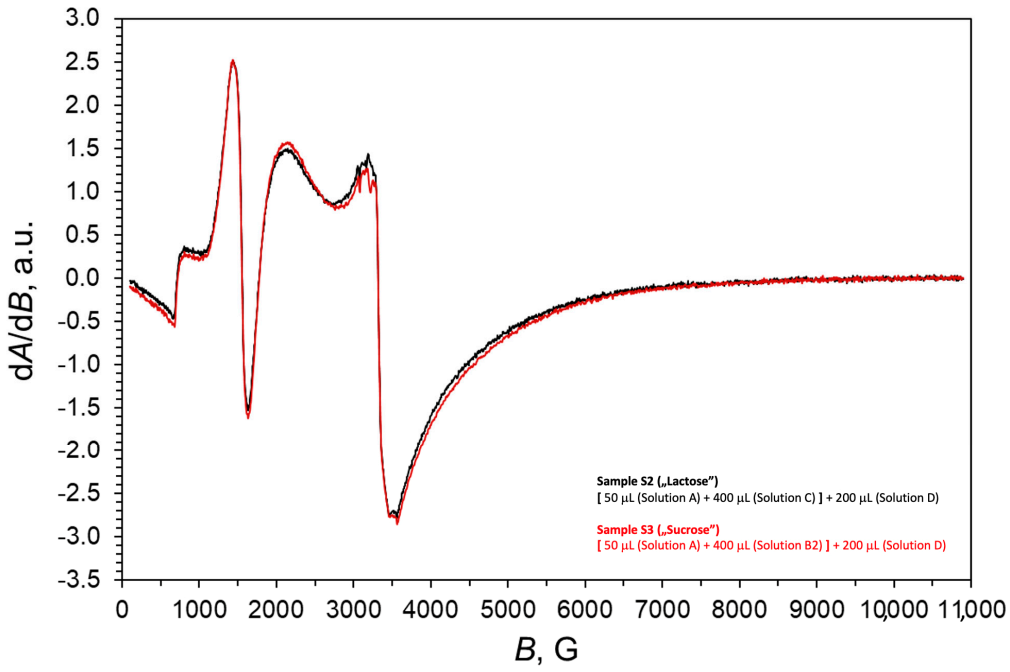

Figure 2. EPR spectra of D-Lactose and Sucrose.

However, sample S5 displayed a slightly altered spectrum, suggesting that certain sugars may subtly influence the effectiveness of H_2O_2 in radical formation (Figure 3).

Sample S4's EPR spectrum revealed contributions from solitary Fe^{3+} ions and minor Mn^{2+} ions, which are attributed to contaminants in the $FeSO_4 \cdot 7H_2O$ reagent. These contaminants likely influence oxidation efficiency by altering radical production dynamics. Doubling the concentration of H_2O_2, as in sample S6, reduced signal intensity rather than enhancing Fe^{3+} ion formation, suggesting that additional H_2O_2 contributes to dilution rather than accelerating radical production (Figure 4).

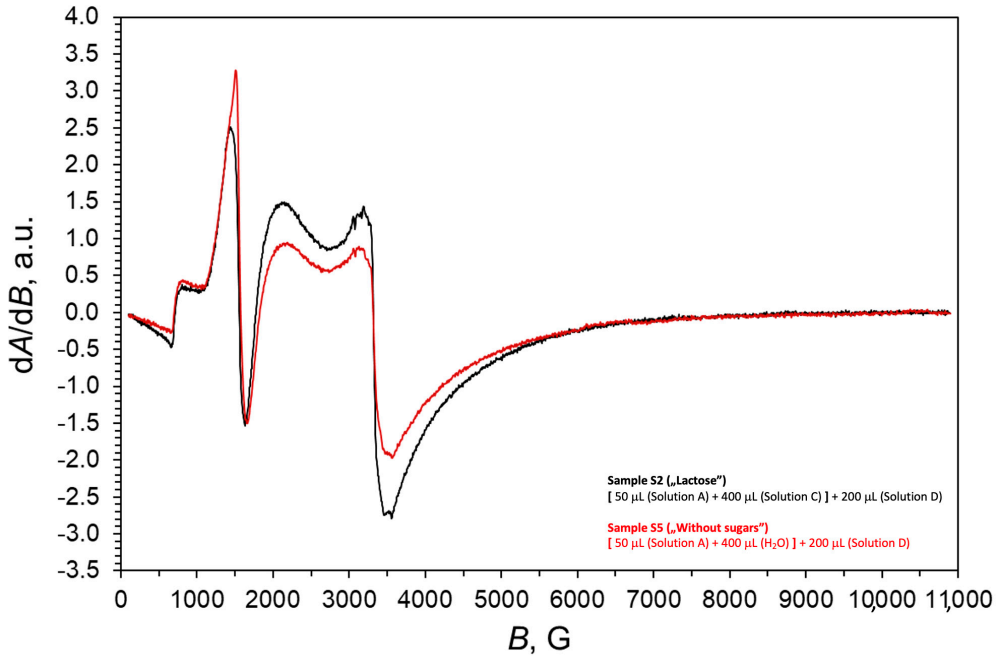

Figure 3. EPR spectra of D-Lactose and the sugar-free sample.

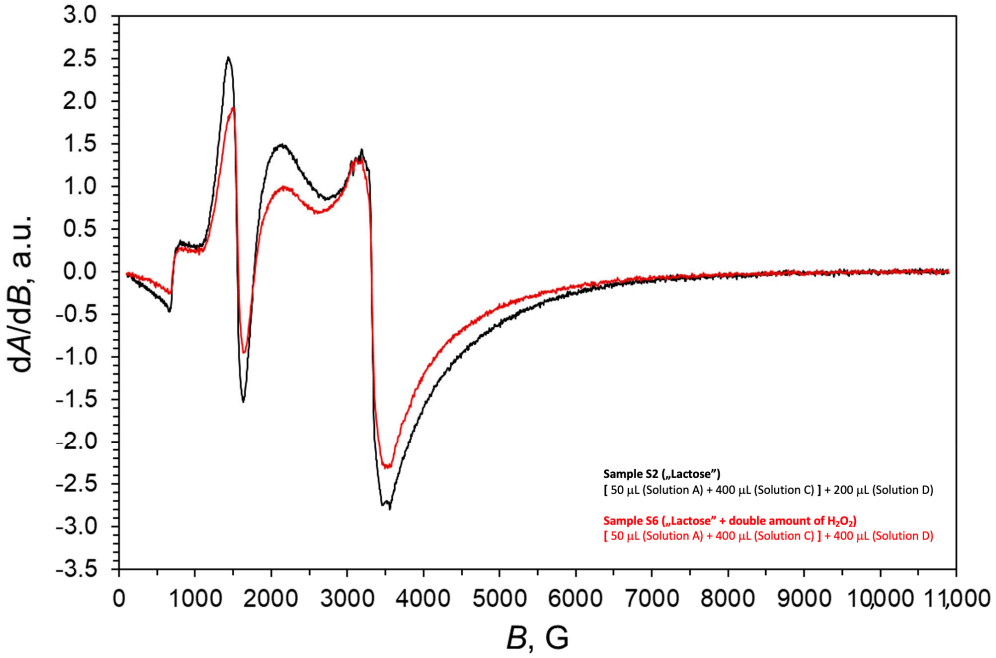

Figure 4. EPR spectra of D-lactose oxidation using different amounts of H_2O_2.

EPR spectroscopy also captured time-dependent changes in radical concentrations. Samples S2 and S3, held at room temperature after the initial measurement, exhibited minor spectral variations over approximately 140 and 130 min, respectively. This observation indicates a gradual release of solitary Fe^{3+} ions from oligomeric complexes over time, contributing to a dynamic oxidative environment (Figures 5 and 6).

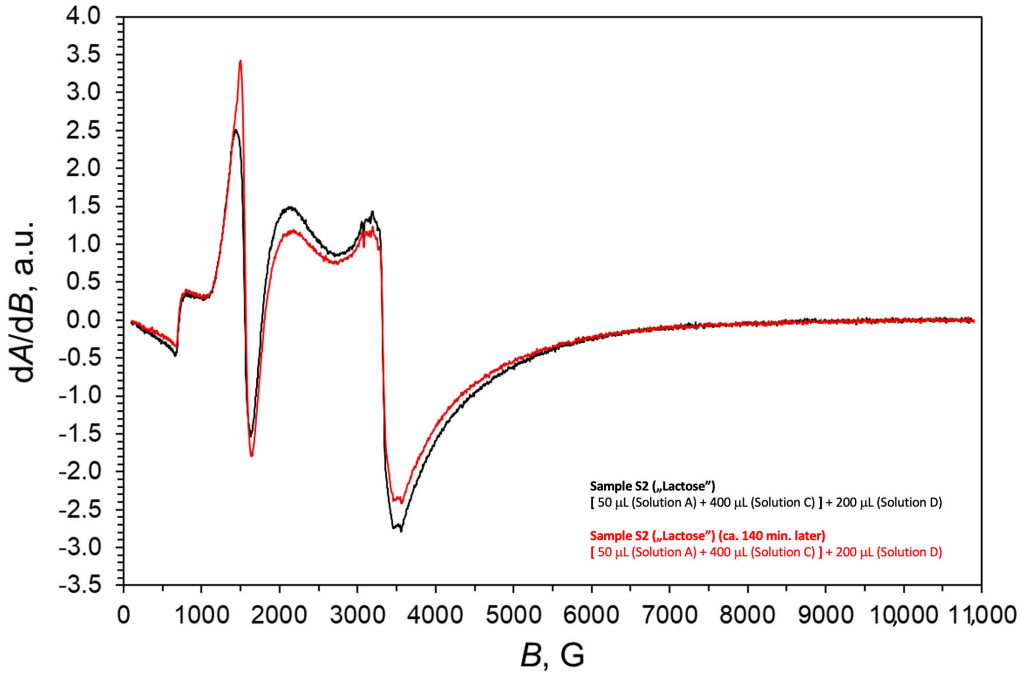

Figure 5. EPR spectra time dependency of D-Lactose oxidation.

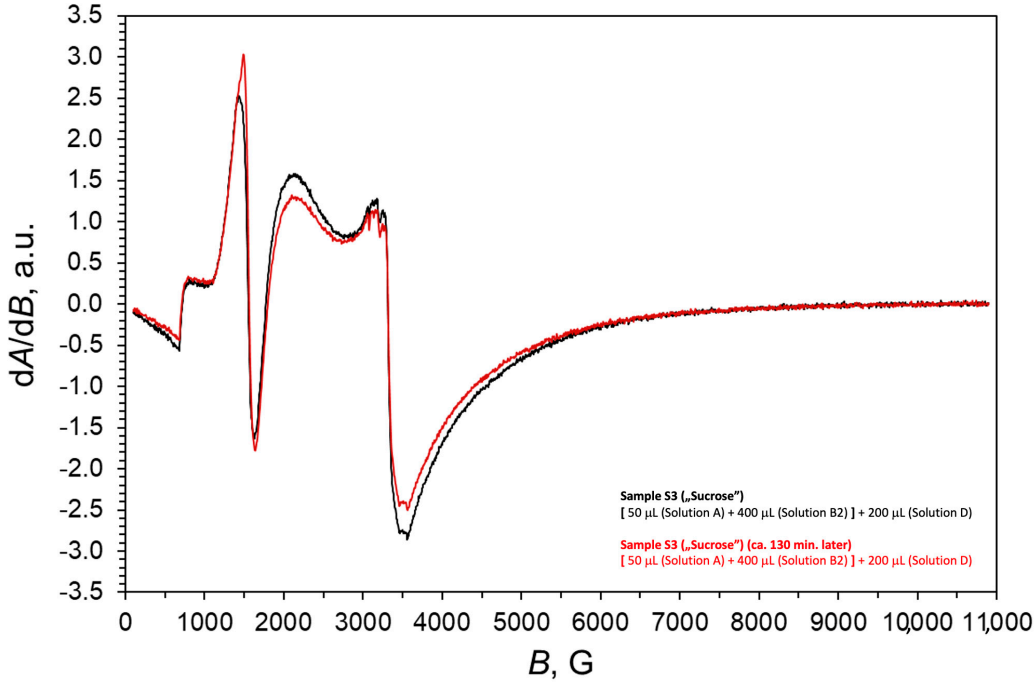

Figure 6. EPR spectra time dependency of Sucrose oxidation.

The broad EPR component in sample S2's spectrum suggests the significant presence of oligomeric species, supporting the hypothesis that iron oligomers contribute to sustained radical generation (Figure 7).

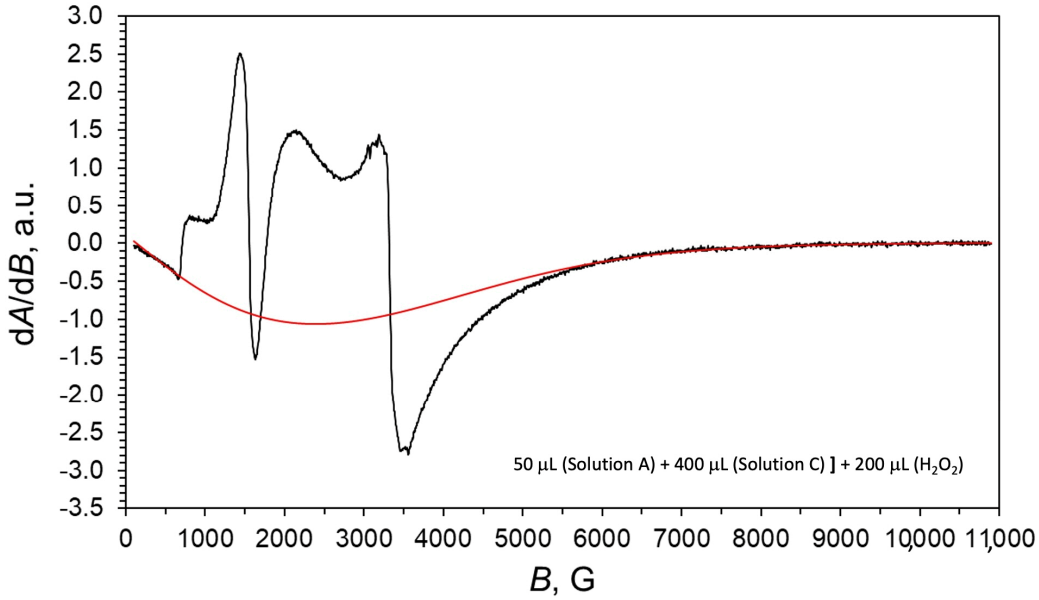

Figure 7. EPR spectra of oligomers during oxidation.

4. Discussion

Through Fenton and $NaOH/H_2O_2$ oxidation, we observe that radical interactions play a significant role in degrading sugars, impacting their stereochemistry and metabolic potential. The study's findings provide key insights into oxidative mechanisms and implications for biochemical pathways, especially regarding the formation and regeneration of reactive intermediates like ferroperoxyl radicals (FeO·). Fenton oxidation relies on a reaction between Fe^{2+} and H_2O_2, which generates hydroxyl radicals (OH·) through a series of redox reactions. This process initiates with the formation of an iron peroxide complex, represented as Fe_2O_2, which can then decompose to produce FeO· radicals. These radicals play a crucial role in oxidizing organic substrates, such as sugars, through hydrogen abstraction at the chiral centers. The sustained oxidation in Fenton's system is attributed to the cycle of Fe^{2+} oxidation to Fe^{3+}, followed by its reduction back to Fe^{2+}, which ensures the continued availability of Fe^{2+} for radical formation.

Our findings reveal that in the presence of FeO· radicals, both C5 and C6 sugars, including D-Glucose, D-Fructose, D-Xylose, and D-Arabinose, undergo racemization, converting optically pure D-enantiomers into racemic mixtures of D and L-forms. This racemization arises from radical-induced stereochemical disruption, which removes the sugars' chiral specificity. Notably, sugars that initially exhibit specific optical rotation lose their chirality in the reaction, yielding racemic mixtures with zero net optical rotation. This loss of chirality is a significant finding because it illustrates how Fenton oxidation can modify the stereochemical integrity of sugars, impacting their metabolic efficiency, as D-sugars are essential in biological pathways.

A unique aspect of this research is the proposed mechanism involving FeO· radicals generated from the decomposition of the intermediate complex. These complexes consist of Fe atoms bridged by one or two peroxide groups, which stabilize the structure before decomposing into highly reactive FeO· and oxygen radicals (O·), as seen in Figure 8.

The formation of FeO· radicals offers a more controlled mechanism for oxidation compared to traditional hydroxyl radical-driven reactions. This reduction is crucial, as it regenerates Fe^{2+} ions, allowing for a continuous cycle of oxidation and radical production. The cause of the phenomenon appears to be that the auto-oxidizer (Fe^{2+}) takes up oxygen to form a sort of peroxide, which is then destroyed in the oxidation of the associated substance.

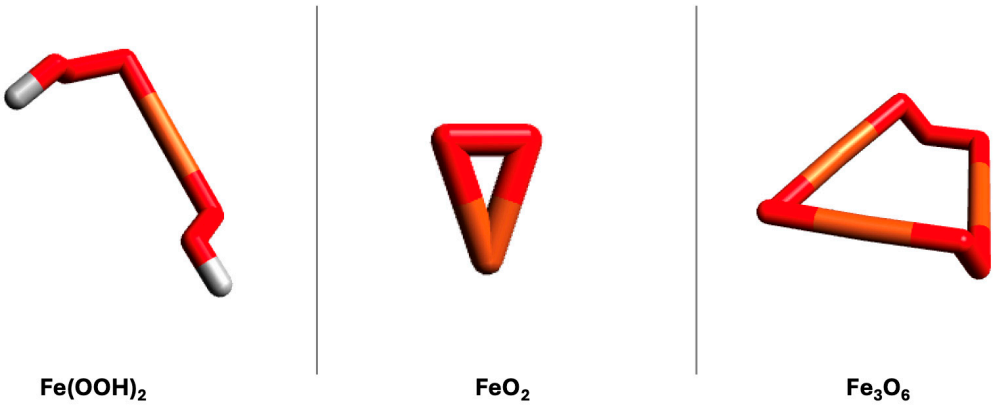

Figure 8. The proposed structures of iron peroxides.

Briefly, the auto-oxidizer, A (Fe^{2+}), gives temporary salts, as seen in Figure 8. In the reaction of A + O-O = A(O-O), and then in contact with the oxidizable substance, B (C5 or C6 sugar), the reaction moves forward as follows: A(O-O) +B = AO + BO.

In the absence of B (C5 or C6 sugar), the second reaction takes place with the aid of a second molecule of A; thus, A(O-O) + A = 2 AO.

The auto-oxidizer, A, is not a catalyst since it oxidizes in proportion to its own mass, and it does not emerge unchanged from the reaction it has caused. Let us suppose that in the case of the auto-oxidizer, A, opposed by the oxidizable substance, B, the latter can be oxidized not only at the expense of the unstable peroxide but also by reducing the stable oxide, A:O; we then have the following succession of reactions:

$$H_2O_2 = 2H^+ + O\text{-}O^{2-}$$

$$Fe^{2+} + O\text{-}O^{2-} = Fe(O\text{-}O)$$

$$Fe(O\text{-}O) + B = FeO\cdot + BO$$

$$FeO\cdot + B = BO + Fe^{2+},$$

where catalyst A (Fe^{2+}) is regenerated.

Thus, the auto-oxidizer would be entirely regenerated and could again be derived as a carrier of free oxygen to the oxidizable substance. A limited amount of A could serve to oxidize an unlimited amount of B; A would then be an oxidation catalyst.

Under alkaline conditions using NaOH and H_2O_2, sugars also undergo oxidation and racemization, like Fenton oxidation, but with distinct outcomes in terms of product stability and distribution. The hydroxyl radicals generated in alkaline conditions attack the chiral centers of sugars, converting D-sugars into racemic mixtures. The aggressive, non-selective nature of oxidation under alkaline conditions allows for a more extensive degradation of sugars, facilitating the formation of simpler oxidation products.

The racemization observed in both Fenton and alkaline oxidation conditions has profound implications for the metabolic usability of sugars. Biological systems depend on D-sugars for metabolic efficiency, with enzymes specifically designed to recognize and process D-enantiomers. The conversion of D-sugars to racemic mixtures introduces non-metabolizable L-sugars, which may disrupt energy production pathways in biological systems. In the context of oxidative stress, this racemization could represent a potential risk, as the transformation of usable sugars into non-functional enantiomers may impair cellular energy processes.

The study highlights sodium formate (HCOONa) as the primary product under high NaOH/H_2O_2 concentrations. This finding demonstrates that alkaline oxidation,

particularly under excess conditions, pushes sugar degradation towards the complete breakdown of carbon skeletons, leading to stable formate salts. The accumulation of sodium formate suggests that the oxidative environment favors not only racemization but also extensive degradation pathways. For industrial applications, the controlled production of sodium formate represents a potential pathway for the sustainable production of formate salts, which have applications in fields such as sustainable energy and chemical synthesis.

The conversion of sugar byproducts into formic acid (HCOOH) via their sodium salt intermediates presents a promising route for efficient hydrogen storage and production. This pathway is particularly important because formic acid acts as a hydrogen carrier, with hydrogen stored in its chemical bonds and readily accessible through simple decomposition. Formic acid is a stable molecule with hydrogen embedded in its molecular structure. Unlike molecular hydrogen gas (H_2), which requires high-pressure tanks or cryogenic storage, formic acid can be stored safely in liquid form at ambient conditions, minimizing the risks and costs associated with hydrogen storage. Converting sugars into sodium formate and subsequently into formic acid offers a practical way to "store" hydrogen as a liquid, making it accessible and manageable for a range of applications. Using sugar byproducts for formic acid production is both efficient and sustainable, providing a valuable way to repurpose biomass waste. The first step involves converting sugar byproducts into their sodium salts, such as sodium formate (HCOONa), which are more chemically stable and easier to isolate. Following this, sodium formate can be processed through a cation exchange resin, which replaces sodium ions with hydrogen ions, yielding formic acid. This process is relatively straightforward and leverages widely available materials, making it both scalable and cost-effective. By transforming sugar-derived sodium salts into formic acid, this approach opens new avenues for utilizing agricultural and industrial waste in the energy sector. One of formic acid's most significant advantages is its ease of decomposition into hydrogen (H_2) and carbon dioxide (CO_2) under mild conditions, often in the presence of a simple catalyst or through thermal decomposition. This reaction is highly efficient and can be conducted at relatively low temperatures compared to other hydrogen-releasing reactions. As a result, formic acid provides an immediate and controllable source of hydrogen without the need for high energy input. This capability makes formic acid especially suitable for on-demand hydrogen production, facilitating its use in fuel cells and other hydrogen-powered systems where continuous hydrogen generation is required. Moreover, since sugar byproducts originate from renewable biomass, this pathway supports a circular economy. Converting sugar byproducts into sodium formate and then into formic acid represents a highly efficient, safe, and sustainable method for hydrogen storage and production. Formic acid's properties as a hydrogen carrier and its straightforward decomposition into H_2 make it an appealing solution in the pursuit of cleaner energy sources. By utilizing abundant, renewable sugar byproducts, this approach not only maximizes resource efficiency but also provides a pathway toward a more sustainable and resilient energy infrastructure.

EPR spectroscopy played a critical role in elucidating the dynamics of radical species and iron complexes throughout the oxidation process. The spectra of samples S2 and S3 were remarkably similar, suggesting that the specific type of sugar does not significantly influence radical formation under standard Fenton conditions. However, the spectrum of sample S5 displayed subtle differences, hinting that certain sugars might slightly affect the interaction with H_2O_2, possibly due to structural variations in sugar reactivity (Figure 8).

Further analysis revealed minor contributions of Fe^{3+} and Mn^{2+} ions in sample S4, suggesting the presence of contaminants within the $FeSO_4 \cdot 7H_2O$ solution. This finding implies that trace metal impurities could alter the oxidation dynamics in practical applications, particularly in environments sensitive to minor compositional changes. The contaminants' influence on reaction kinetics could provide insight into the impact of reagent purity on

Fenton oxidation outcomes, supporting the need for rigorous control in experimental setups involving redox-active species. The effect of H_2O_2 concentration on radical behavior was also assessed in sample S6, where doubling the H_2O_2 concentration led to reduced EPR signal intensity, which was attributed to dilution effects. This finding underscores how increasing the H_2O_2 concentration does not necessarily correlate with higher Fe^{3+} production, suggesting that further radical formation may be constrained by dilution. This effect has implications for scaling up oxidation reactions, where adjusting H_2O_2 levels can modulate oxidation intensity without necessarily increasing radical production.

Time-dependent EPR observations revealed that holding samples S2 and S3 at room temperature for extended periods resulted in gradual Fe^{3+} formation, likely due to the slow dissociation of oligomeric iron complexes. Figures 6 and 7 indicate that over time, these complexes release Fe^{3+} ions, contributing to a dynamic oxidative environment. The broad spectral component observed in sample S2 further supports the hypothesis of oligomeric species formation, showing that iron clusters contribute significantly to sustained oxidation. Understanding these time-dependent dynamics may inform controlled oxidation protocols where prolonged radical presence is required. Expanding the application of EPR spectroscopy in real-time radical analysis may offer new perspectives on intermediate species formation and radical dynamics. By refining this analytical approach, future studies could obtain a more precise understanding of radical transformations and how they interact with different substrates. This would be particularly valuable for applications in chemical synthesis, where controlled radical presence is necessary. In summary, the oxidative mechanisms in Fenton and alkaline systems highlight how reaction conditions, radical formation, and sugar structure influence oxidation outcomes. EPR spectroscopy has proven invaluable in providing real-time insights into Fe-species dynamics, emphasizing its role in fine-tuning oxidation processes for industrial and biochemical applications.

Additionally, the Fenton reaction was simulated using a simple Python 3.9.6 script (Figure 9) to model the temporal concentration changes in the involved species. The Python model included key components of the Fenton reaction, such as the interaction between ferrous ions (Fe^{2+}), hydrogen peroxide (H_2O_2), and glucose. By incorporating reaction kinetics, the script simulated the behavior of these species over time, providing insight into how the concentrations of reactive intermediates evolve under typical Fenton conditions. This approach enables the visualization of complex, dynamic changes in real time, aiding in understanding the reaction's progression and its effects on substrate oxidation. Figure 9 illustrates the modeled concentration profiles of the main species involved in the Fenton reaction on both linear and logarithmic scales. The linear scale graph provides an intuitive view of initial rapid changes, where ferrous ions and hydrogen peroxide concentrations decrease. The log scale graph, meanwhile, highlights concentration trends over extended reaction times, capturing the gradual decline in intermediates and revealing details about reaction kinetics that may be less apparent on a linear scale; see Figure 9.

These modeled concentration profiles [initial concentrations based on the General Procedure of Fenton Oxidation in mol/L: cFeSO4 = 0.036, cH2O2 = 0.409, cH = 0.367, cGlucose = 1.000,] support our experimental findings, showing that the rapid generation of Fe^{3+} occurs within the early stages of the Fenton reaction, driving the oxidation of sugar degradation. By simulating these concentration dynamics, the model provides insights into how changes in reactant concentration, reaction rate constants, and time impact the overall efficiency and outcomes of the Fenton reaction. Additionally, the Python-based model offers a flexible framework that can be adapted to explore different reaction conditions or substrate types, making it a valuable tool for further investigations. This integration of computational modeling with experimental work underscores the importance of predictive models in designing and optimizing oxidative processes in chemical and biochemical applications.

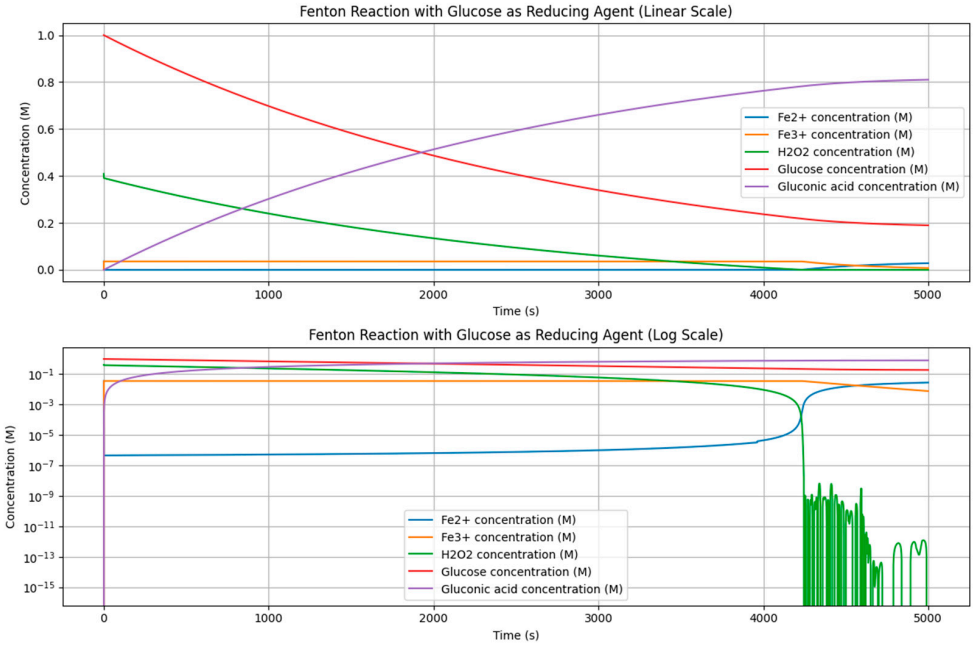

Figure 9. Python simulation of Fenton oxidation of D-Glucose.

5. Conclusions

This study offers an in-depth examination of the oxidation of C5 and C6 sugars under Fenton and alkaline conditions, providing valuable insights into radical-driven racemization, product formation, and the underlying mechanisms that govern sugar degradation. By comparing oxidation behaviors in different environments, we identified the key factors that influence sugar racemization, radical dynamics, and product stability, shedding light on potential applications in both biochemical and industrial fields.

Our findings highlight the significant role of hydroxyl and ferroperoxyl radicals in the oxidation of sugars. Under Fenton conditions, hydroxyl radicals initiate oxidation, targeting the chiral centers of sugars and causing rapid racemization. The racemization observed in both Fenton and alkaline oxidation conditions has substantial metabolic implications. The conversion of optically active D-sugars to racemic mixtures of D- and L-enantiomers disrupts chiral integrity, reducing the metabolic usability of these sugars. Biological systems are highly specific to D-enantiomers, and the formation of non-metabolizable L-forms results in the loss of energy yield, impairing efficiency in metabolic processes. These findings highlight the potential impact of oxidative stress on sugar metabolism in biological systems, suggesting that chronic exposure to radical-rich environments could lead to gradual metabolic inefficiencies due to racemization.

The racemization of sugars presents a potential threat to human metabolism by disrupting the body's delicate balance of chiral compounds, which is essential for proper biochemical function. In biological systems, chirality, or the "handedness" of molecules, is crucial because enzymes, transporters, and receptors are highly specific to the three-dimensional arrangement of molecules. The human body predominantly uses D-sugars, such as D-Glucose, in metabolic pathways like glycolysis, the citric acid cycle, and the pentose phosphate pathway. Racemization, which is the process of converting a D-sugar into a 1:1 mixture of its D- and L-enantiomers, disrupts this chiral purity and can lead to several issues that ultimately impair metabolism and cellular function. Enzymes are highly selective, generally recognizing only one chiral form of a substrate due to the specific orientation of amino acids in their active sites. If D-Glucose undergoes racemization and forms L-glucose, this enantiomer is not readily processed in the glycolytic pathway, leading to inefficient glucose utilization. The body would then experience a reduction in

ATP production because the racemized sugars are not broken down efficiently, potentially resulting in cellular energy deficits and weakened physiological function. When racemized sugars, particularly L-enantiomers, accumulate in the bloodstream or tissues, they may interfere with normal metabolic processes. L-sugars are not compatible with most human enzymes, meaning they cannot be broken down or used effectively for energy production. As a result, these L-sugars may build up, potentially leading to metabolic imbalances and osmotic stress within cells. Osmotic imbalances can cause cells to absorb excessive water, potentially leading to cell swelling, dysfunction, or even death, which can impair tissue and organ function over time. Chirality is also critical in cellular communication and signaling pathways. Chiral molecules, including sugars, interact with membrane receptors and transporters, which often recognize only one specific enantiomer. For instance, glucose transporters (GLUTs) are specific for D-Glucose. If racemized sugars produce L-forms that cannot be transported efficiently, this limits the glucose uptake by cells, causing reduced energy availability and impairing cellular function. Additionally, L-sugars could occupy or block receptor sites without eliciting an appropriate response, disrupting normal signaling and leading to metabolic confusion at the cellular level. Prolonged exposure to oxidative conditions not only promotes racemization but also damages proteins, lipids, and nucleic acids within cells. Racemization can, therefore, exacerbate oxidative damage, leading to further cellular dysfunction. Over time, this oxidative stress may contribute to the development of age-related diseases, such as diabetes, neurodegenerative disorders, and cardiovascular diseases, where the body's ability to manage glucose metabolism and cellular health is compromised. The racemization of sugars poses a threat to human metabolism by generating L-sugars that are incompatible with the body's chiral-specific biochemical systems. This disruption can lead to reduced energy efficiency, the accumulation of non-functional molecules, impaired cellular communication, and increased susceptibility to oxidative stress, all of which undermine cellular and systemic health.

EPR spectroscopy plays a critical role in elucidating radical behavior and the transformation of iron species during oxidation. By analyzing radical formation and Fe-speciation in real time, EPR provides detailed insights into the radical generation kinetics and reveals the specific influence of sugar types on H_2O_2 efficiency. The presence of Fe^{3+} and Mn^{2+} ions in sample S4, attributable to contaminants, underscores the need for reagent purity in achieving consistent oxidation outcomes. These findings indicate that doubling H_2O_2 concentration does not necessarily increase Fe^{3+} formation due to dilution effects. Additionally, time-dependent EPR observations suggest a gradual evolution of solitary Fe^{3+} ions from oligomeric complexes, supporting the presence of a dynamic oxidative environment that adapts over time.

Future research could explore the effects of alternative metal catalysts or various oxidative agents to further understand radical-driven oxidation in carbohydrate chemistry. Potential applications include the controlled degradation of sugars for biofuel production, selective oxidation for chemical synthesis, and the development of antioxidant strategies to counteract the adverse effects of racemization in biological contexts. The application of Electron Paramagnetic Resonance (EPR) spectroscopy provides real-time insights into radical behavior, specifically allowing for the detailed characterization of Fe^{3+} species and dynamic changes in radical populations. EPR confirmed that sugar type has minimal influence on radical formation under standard conditions, yet it revealed subtle variations under specific conditions, adding depth to our understanding of sugar–radical interactions and oxidation processes. The insights into sugar oxidation and racemization mechanisms could inform approaches in biochemical research, particularly in understanding oxidative stress and its effects on sugar metabolism and in industrial settings where chiral or achiral product formation is required. These findings collectively offer novel insights into oxidation

chemistry and can establish a foundation for future applications in both biochemical and industrial fields, emphasizing the importance of reaction conditions in controlling product distribution and stereochemistry.

This research advances our understanding of Fenton and alkaline oxidation mechanisms for sugars, demonstrating how radical formation, product distribution, and reaction conditions collectively influence sugar degradation pathways. These insights provide a foundation for optimizing sugar oxidation processes in both metabolic and industrial applications, reinforcing the importance of radical control in carbohydrate chemistry. Our work reveals a detailed pathway for the racemization of optically active C5 and C6 sugars (such as D-Glucose, D-Fructose, D-Xylose, etc.) under both Fenton and alkaline oxidative conditions. The transformation of D-sugars into racemic mixtures of D- and L-enantiomers demonstrates how oxidative environments can disrupt chirality, impacting the sugars' biochemical roles. This finding is especially relevant in understanding how oxidative stress could affect sugar metabolism. A key novelty is the identification and role of ferroperoxyl radicals (FeO·), formed through the decomposition of Fe_2O_2 intermediates, in driving the oxidation and racemization of sugars. This mechanism goes beyond traditional hydroxyl radical-based oxidation, showing that FeO· can specifically target sugar aldehyde and ketone groups, thus presenting a more controlled oxidative process that could be exploited in synthetic and industrial applications.

Under high $NaOH/H_2O_2$ concentrations, sodium formate (HCOONa) becomes the predominant oxidation product, with minimal intermediate formation. This controlled degradation to formate represents a practical approach for producing stable end products from carbohydrate sources, with potential industrial applications in sustainable energy and chemical synthesis. The conversion of sugar byproducts into formic acid (HCOOH) via their sodium salt intermediates presents a promising route for efficient hydrogen storage and production. This pathway is particularly important because formic acid acts as a hydrogen carrier, with hydrogen stored in its chemical bonds and readily accessible through simple decomposition. Formic acid is a stable molecule with hydrogen embedded in its molecular structure. Unlike molecular hydrogen gas (H_2), which requires high-pressure tanks or cryogenic storage, formic acid can be stored safely in liquid form at ambient conditions, minimizing the risks and costs associated with hydrogen storage. This is particularly valuable in industries where stable, liquid hydrogen carriers are essential for safe transport and handling. Using sugar byproducts for formic acid production is both efficient and sustainable, providing a valuable way to repurpose biomass waste. The first step involves converting sugar byproducts into sodium salts, such as sodium formate (HCOONa), which are more chemically stable and easier to isolate. Following this, sodium formate can be processed through a cation exchange resin, which replaces sodium ions with hydrogen ions, yielding formic acid. This process is relatively straightforward and leverages widely available materials, making it both scalable and cost-effective. By transforming sugar-derived sodium salts into formic acid, this approach opens new avenues for utilizing agricultural and industrial waste in the energy sector.

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with water as the primary byproduct when used in fuel cells. This reduces reliance on fossil fuels and contributes to lowering greenhouse gas emissions. Formic acid's properties as a hydrogen carrier and its straightforward decomposition into H_2 make it an appealing solution in the pursuit of cleaner energy sources. By utilizing abundant, renewable sugar byproducts, this approach not only maximizes resource efficiency but also provides a pathway toward a more sustainable and resilient energy infrastructure.

Author Contributions: Conceptualization, Z.K.; methodology, Z.K.; validation, Z.K. and Á.N.; formal analysis, Z.K.; investigation, Z.K.; resources, Z.K.; data curation, Á.N.; writing—original draft preparation, Z.K.; writing—review and editing, Z.K.; visualization, Á.N.; supervision, Z.K.; project administration, Z.K.; funding acquisition, Z.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: All relevant data are contained within the manuscript.

Acknowledgments: The authors express their thanks to Ferenc Simon (Institute of Physics, Budapest University of Technology and Economics, Budapest, Hungary) for making available the applied spectrometer to record the EPR spectra. The authors express their special thanks to Zoltán Klencsár.

Conflicts of Interest: The authors declare no conflicts of interest. Z.K. is a paid employee of the IOI Investment Zrt. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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