



Lead (II) Ions Strongly Inhibit the Maillard Reaction in a Glucose–Lysine Model System: Implications for Flour Safety

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Abstract

Background: The Maillard reaction is responsible for color and flavor development in baked goods and has been proposed as a potential mechanism for heavy metal sequestration due to the chelating capacity of melanoidins. Therefore, this study aimed to investigate the influence of lead (II) ions on the Maillard reaction under simulated bread-making conditions. **Methods:** A glucose–lysine model system (pH 6.5) was heated and maintained at 100 °C for 60 minutes in the presence or absence of 0.5 mg/L Pb²⁺. Browning development was monitored by measuring absorbance at 450 nm. Data were analyzed statistically using two-way analysis of variance (ANOVA) followed by Tukey's post hoc test. **Results:** Lead ions significantly inhibited browning intensity at all time points, reducing absorbance by 66–76% relative to controls ($p < 0.01$). Time-dependent browning was observed only in control samples, whereas Pb²⁺-treated systems showed no significant progression. **Conclusion:** Pb²⁺ strongly suppresses the Maillard reaction, likely by sequestering lysine or masking reactive carbonyl groups, thereby preventing early-stage Schiff base formation. Although partial metal binding may occur, the substantial loss of sensory attributes limits the feasibility of Maillard-based lead decontamination in flour systems.

Keywords: Maillard reaction; lead poisoning; flour; food safety; browning

1. Introduction

Food safety concerns regarding heavy metal contamination have increased substantially worldwide, with lead (Pb) representing a particularly hazardous contaminant due to its persistence in agricultural soils and bioaccumulation potential [1,2]. Heavy metals are significant contaminants in the food system, particularly in urban environments, where industrial emissions, historical use of leaded gasoline, and atmospheric deposition have resulted in persistent soil contamination [3]. Lead (Pb), as a potent neurotoxin with no established safe exposure threshold, poses severe health risks even at trace concentrations, especially to children, where it impairs cognitive development and causes behavioral disorders [4]. Urban agricultural systems face particular challenges, as crops absorb Pb through their roots, with flour serving as a primary vector of human exposure [5].

Recent studies in Northeast Iran have revealed that 70% of Pb in bread originates from flour, with urban centers showing concentrations exceeding international standards [5]. In the Mashhad region, bread samples contained several metals (Al, As, Cr, Fe) exceeding World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO) limits. At the same time, historical data from Tehran indicated Pb contamination in bread ranging from 0.27 to 0.52 mg/kg on a dry-weight basis [6]. This is particularly concerning given evidence linking consumption of bread contaminated with Cd and As to increased cardiovascular disease risk (11.97% and 11.9%, respectively) [7].

The Maillard reaction is a complex sequence of non-enzymatic reactions between reducing carbonyl compounds and amino-containing compounds. It plays a central role in the development of color, flavor, and aroma in baked goods and other thermally processed foods [8].

The Maillard reaction has previously been studied as a potential method for decontamination. It has been shown that Maillard reaction products (MRPs) from milky products inhibit the growth of bacteria and yeast [9]. This is achieved through alterations in pH and humidity caused by melanoidins. Also, certain MRPs have shown that, through their antioxidant activities, they can reduce the oxidant stress required for microbial development. Certain MRPs have also been shown to reduce the oxidant stress required for microbial development through their antioxidant activities [10].

The reaction proceeds through three main stages, each characterized by distinct intermediates and products. The early stage involves the formation of Schiff bases and their rearrangement into Amadori compounds. During the intermediate stage, these compounds undergo degradation to yield reactive α -dicarbonyl compounds and Strecker aldehydes, which are key contributors to flavor development. The final stage is characterized by condensation and polymerization reactions, resulting in the formation of high-molecular-weight brown polymers known as melanoidins [11].

During the early and intermediate stages of the Maillard reaction, flavor-active compounds are formed that significantly contribute to the characteristic taste and aroma of



baked products. These include products formed by enolization of Amadori compounds, which leads to the formation of specific flavor compounds, such as pyrazines, commonly associated with roasted and nutty aromas [12]. At the same time, the Maillard reaction can yield aldehydes, ketones, and furfurals, which impart fruity, caramel, and sweet notes to baked foods [13]. In contrast, melanoidins are primarily formed during the final stage of the Maillard reaction and are responsible for the brown coloration and certain functional properties of baked products, more specifically, the brown color that can be observed.

Melanoidins are anionic, high-molecular-weight polymers with the ability to chelate metal ions such as Fe^{2+} , Cu^{2+} , and Zn^{2+} [14]. This metal-binding capacity has attracted interest for food safety and decontamination applications, as studies have shown that melanoidins can reduce free metal ion concentrations in model systems [15]. In order to better understand how the melanoidins can be used to reduce the free metal ions, they were observed in the variations of time and how this changes the concentration over time. This was measured by the measuring absorbance at 450 nm (A_{450}) by a Cary 50 UV spectrophotometer (Varian Inc., Victoria, Australia), in which a correlation was observed with the presence of free ion metals with an increase in absorbance, and therefore melanoidins (Table 1).

Table 1. Absorbance at 450 nm (A_{450}) during Maillard reaction progression in control and Cu^{2+} -treated, Zn^{2+} -treated, and Fe^{2+} -treated systems.

Treatment	Time (h)	<i>n</i>	Mean $\pm$ SD	CV (%)
Control	3	6	0.080 $\pm$ 0.005	6
Control	4	6	0.110 $\pm$ 0.007	6
Control	5	6	0.150 $\pm$ 0.009	6
Cu^{2+} (1 mg/L)	3	6	0.180 $\pm$ 0.011	6
Cu^{2+} (1 mg/L)	4	6	0.300 $\pm$ 0.018	6
Cu^{2+} (1 mg/L)	5	6	0.420 $\pm$ 0.025	6
Zn^{2+} (5 mg/L)	3	6	0.190 $\pm$ 0.011	6
Zn^{2+} (5 mg/L)	4	6	0.340 $\pm$ 0.020	6
Zn^{2+} (5 mg/L)	5	6	0.500 $\pm$ 0.030	6
Fe^{2+} (50 mg/L)	3	6	0.140 $\pm$ 0.008	6
Fe^{2+} (50 mg/L)	4	6	0.220 $\pm$ 0.013	6
Fe^{2+} (50 mg/L)	5	6	0.300 $\pm$ 0.018	6

SD, Standard Deviation; CV, Coefficient of Variation.

Conversely, transition metals such as Fe^{2+} , Cu^{2+} , and Zn^{2+} can catalyze Maillard browning reactions, and their suppression has been associated with a reduced formation of Maillard reaction products [16].

Pb^{2+} chemistry differs significantly from that of transition metals—it lacks accessible redox states and mainly acts as a Lewis acid [17]. Pb^{2+} strongly binds to amino acids—particularly those with thiol or carboxyl groups—and can catalyze carbohydrate reactions [17,18]. Recent research demonstrated that post-transition metals (Sn, Mg) can reduce browning in Maillard systems by donating elec-

trons to dehydroreductones, reversing key intermediates [19]. However, this mechanism requires reducing conditions and neutral-to-alkaline pH, which may not apply to flour systems.

This study bridges this knowledge gap by evaluating Pb^{2+} interference under simulated bread-making conditions (glucose-lysine, pH 6.5, 100 °C). In our study, we seek to understand how the presence of Pb^{2+} , and more specifically, whether this method could be used as an example to salvage contaminated flour by the Maillard reaction. As previous experiences have shown us that certain heavy metals can be both catalysts for the Maillard reaction, the aim of the study was therefore to observe how one of the most common contaminants in urban areas, Pb^{2+} , could affect the process, and if it could be used as a decontamination method for flour. We hypothesized that Pb^{2+} would be sequestered into melanoidins, allowing normal browning progression. Instead, we observed profound inhibition—a finding with implications for both food safety and sensory quality.

2. Materials and Methods

2.1 Chemical Reagents and Equipment

D-glucose ($\geq 99.5\%$) and L-lysine ($\geq 98\%$) were obtained from certified suppliers. Lead nitrate ($\text{Pb}(\text{NO}_3)_2$), sodium phosphate salts, and all reagents were analytical grade.

Experimental equipment included a Hot Plate Magnetic Stirrer (AGIMATIC-ED-C, US-1500S), a 500-mL Erlenmeyer flask with standard taper joints fitted with reflux condensers, a Shimadzu UV-1800 UV-Visible spectrophotometer, an A&D GF-600 analytical balance (600×0.001 , ± 0.1 mg accuracy), and a pH Meter (150MI).

2.2 Preparation for Model System Solutions

A 0.2 M phosphate buffer (pH 6.5) was prepared to maintain the pH of white flour [20]. The buffer was made by dissolving 2.00 g NaH_2PO_4 and 0.47 g Na_2HPO_4 in 80 mL of distilled water. The pH was carefully adjusted to 6.50 ± 0.05 using micro-additions of either salt solution, and the solution was diluted to 100 mL. The pH was measured before and after the reaction in order to observe how the reaction could affect the pH.

Reaction mixtures were prepared as follows: 3.60 g of D-glucose and 2.92 g of L-lysine were dissolved in 90 mL of phosphate buffer. The solution was divided into two equal portions. To one portion, 5.0 mL of Pb^{2+} working solution (10 mg/L) was added to achieve a final concentration of 0.5 mg/L Pb^{2+} . The other portion received 5.0 mL of buffer as a control. Both solutions were then diluted to a final volume of 100 mL with buffer. These concentrations were selected to model the primary Maillard precursors in wheat flour systems, where lysine is among the most reactive amino acids for browning reactions and glucose is the principal reducing sugar involved in early reaction stages [8,21,22].

2.3 Thermal Treatment Procedure

Reaction solutions were transferred to 500-mL Erlenmeyer fitted with reflux condensers and placed in a water bath maintained at 100 °C using the Hot Plate Magnetic Stirrer (AGIMATIC-ED-C, US-1500S) set to 300 rpm. This temperature and agitation profile simulates conditions during the early stages of bread baking when the Maillard reaction initiates [8]. Aliquots (2–3 mL) were withdrawn at 30, 45, and 60 minutes.

2.4 Browning Assessment and Statistical Analysis

Browning intensity was quantified by measuring absorbance at 450 nm (A_{450}) using the Shimadzu UV-1800 spectrophotometer. Unheated reaction mixture served as the blank reference. All measurements were performed in triplicate samples using 1 cm pathlength quartz cuvettes. Data were analyzed using two-way analysis of variance (ANOVA) with treatment (lead vs. control) and time (30, 45, 60 min) as independent factors. Tukey's honest significant difference (HSD) test was used for post hoc comparisons when main effects were significant. Within-group time effects were examined using a one-way ANOVA with a Bonferroni correction for multiple comparisons. Statistical significance was defined as $p < 0.05$. All statistical procedures were conducted using GraphPad Prism software version 9.0 (San Diego, CA, USA).

3. Results and Discussion

3.1 Pb^{2+} Causes Profound and Consistent Browning Suppression

The chosen treatment for the experiment was an adaptation of the previous method used [15]. However due to the different system that was used we adapted the times and the reactive. The previous experiment was based on a lactose-glycine, a model for lactic products, but this was adapted to the Maillard reaction for flour-based products, in this case as it was observed by the medium of glucose-lysine, as this are the first sugars and amino acids to react in the Maillard reaction based on flour, and also adapting the time due to the fact that the reaction is quicker with these components, therefore there was a reduction on the periods of observation from 3, 4 and 5 hrs to 30, 45 and 60 mins.

Pb^{2+} treatment markedly reduced A_{450} across all time points (Table 2; Fig. 1). Mean absorbance in Pb-treated samples was 66–76% lower than controls, with the greatest suppression at 30 min (75.6% reduction).

This contrasts with the previous experience, in which in all the cases there was a notorious increase of the absorbance and therefore of the concentration of melanoidins (Table 1), in which the presence of metal ions (Cu^{2+} , Zn^{2+} , and Fe^{2+}) has contributed to the increase of melanoidins. Therefore, it's possible to conclude that ions of Pb are not useful as a catalyst for the production of MRPs.

Table 2. Absorbance at 450 nm (A_{450}) during Maillard reaction progression in control and Pb^{2+} -treated systems.

Treatment	Time (min)	n	Mean $\pm$ SD	CV (%)
Control	30	4	0.460 $\pm$ 0.045	9.8
Control	45	4	0.538 $\pm$ 0.130	24.2
Control	60	4	0.657 $\pm$ 0.072	11.0
Pb (0.5 mg/L)	30	4	0.112 $\pm$ 0.075	67.0
Pb (0.5 mg/L)	45	4	0.146 $\pm$ 0.155	106.2
Pb (0.5 mg/L)	60	4	0.223 $\pm$ 0.170	76.2

Data are presented as Mean $\pm$ SD (n = 4), with Coefficient of Variation.

In order to confirm if the reaction of Maillard was affected by the difference in pH between control and Pb-treated, it was attempted to regulate the pH by using a buffer solution, however the pH was affected by the two different treatments. At the end of the reaction the Pb-treated solution dropped its pH to an average of 6.13 and the control solution to 6.23. However the final pH between the two was rather similar.

To examine how the two factors, time and treatment, affect the Maillard reaction, a two-way ANOVA was conducted with these factors as independent variables. The Treatment and time variables are more determinative of differences among the groups.

The results of the Two-way ANOVA model revealed highly significant main effects for both treatment ($F(1,18) = 115.2$, $p < 0.0001$) and time ($F(2,18) = 8.95$, $p = 0.002$), but no significant interaction between these factors ($p = 0.115$). This absence of interaction indicates that the lead's inhibitory effect was consistent throughout the 60-minute reaction period, rather than intensifying or diminishing over time. Tukey's post hoc analysis confirmed significant differences between control and Pb^{2+} -treated samples at all time points ($p = 0.0072$, $p = 0.0024$ and $p = 0.0008$; Fig. 1).

3.2 Pb^{2+} Halts Normal Reaction Kinetics

In the control group, A_{450} increased significantly over time ($p = 0.039$), with 30 vs. 60 min being the only significant pairwise difference ($p = 0.0135$). In contrast, the Pb-treated group showed no significant time effect ($p = 0.553$), indicating no reaction progress (Fig. 2).

This notorious reduction in the absorbance, however, still remains consistent with the one observed in Table 1, in which the higher absorbance (and therefore the highest concentration of melanoidins) is directly proportional to the time of the reaction. This remains consistent with the previous experience of Ramonaitytė et al. [15]. However, as shown in Fig. 2, this concentration difference is not statistically significant; therefore, it suggests that the disruption in the production of melanoidins could be significant.

Visually, Pb-treated solutions remained pale yellow with no characteristic aroma that is distinctive from non-treated solutions.

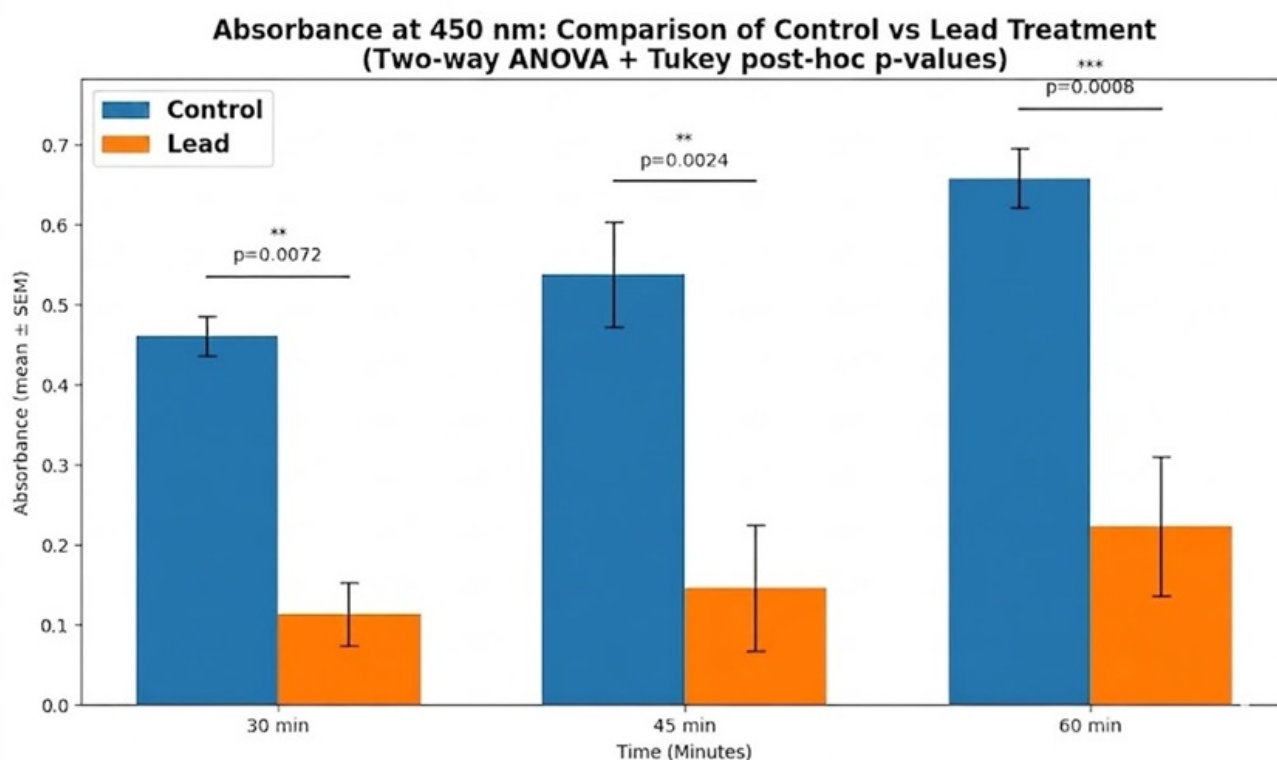


Fig. 1. Effects on absorbance by the treatment of Pb across all the time stamps. Mean absorbance at 450 nm ($A_{450} \pm \text{SEM}$) for control and Pb^{2+} -treated glucose–lysine systems at 30, 45, and 60 min. Pb^{2+} significantly inhibited Maillard browning at all time points compared with controls (two-way ANOVA followed by Tukey post-hoc test, $**p < 0.01$, $***p < 0.001$). Indicates that Pb^{2+} could halt normal reaction kinetics. ANOVA, analyzed using two-way analysis of variance followed by a Tukey's range test.

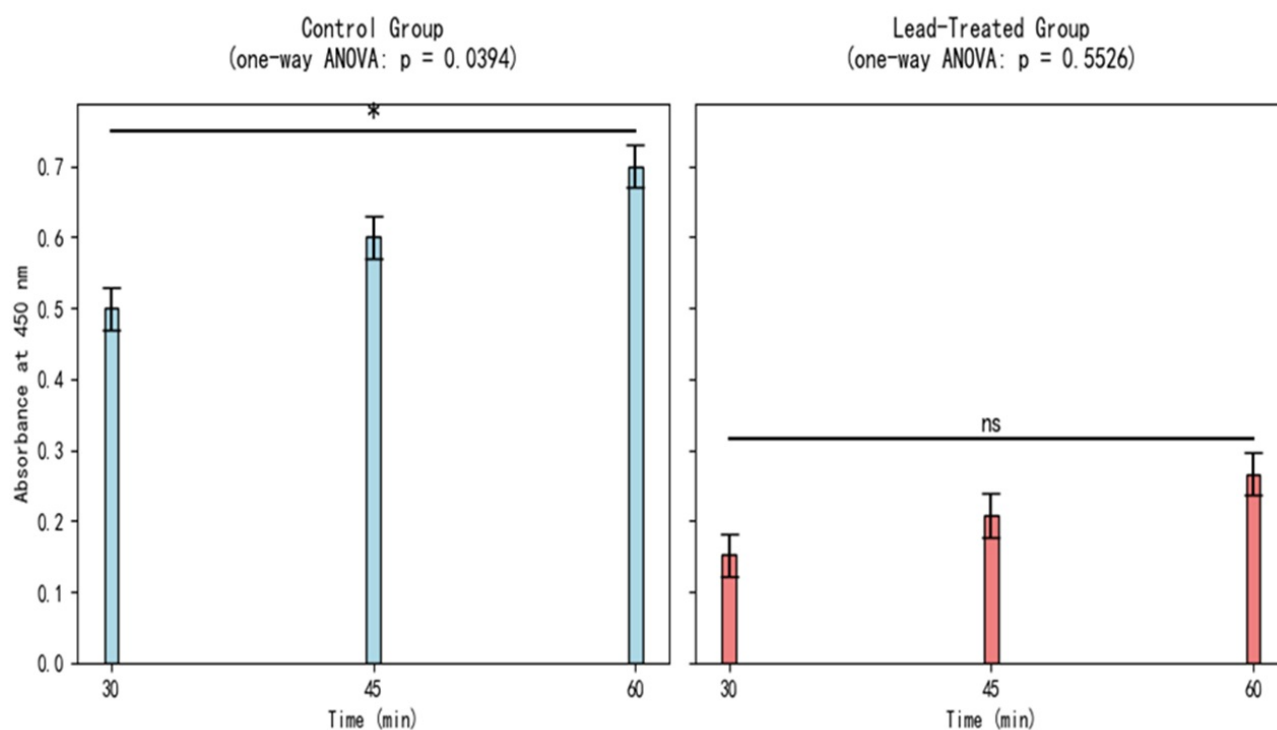


Fig. 2. Effects of time in the absorbance in both control and Pb-treatment. Within-group kinetics: (blue) Control shows significant browning progression (* = significant) ($p = 0.039$); (red) Pb-treated shows no change (ns = not significant).

3.3 Pb^{2+} Disrupts the Early Stage Via Reactant Sequestration

The Maillard reaction proceeds through three principal stages: an early stage involving Schiff base formation and Amadori rearrangement, an intermediate stage characterized by the formation of α -dicarbonyl compounds and Strecker aldehydes, and a final stage leading to the production of melanoidins [11]. The absence of time-dependent browning in the Pb-treated group ($p = 0.553$), together with the lack of a significant interaction effect ($p = 0.115$), suggests that the reaction was arrested at the earliest stage under the experimental conditions. This interpretation is further supported by the absence of detectable aroma in the Pb-treated solution, as key flavor- and aroma-active compounds are generated during the early and intermediate stages of the Maillard reaction, prior to melanoidin formation, and originate largely from the degradation of Amadori compounds [12,13].

At pH 6.5, the ϵ -amino group of lysine is partially protonated, thereby reducing nucleophilicity and reactivity toward carbonyl compounds [8]. The presence of Pb^{2+} is likely to exacerbate this effect through the formation of stable complexes with lysine's amino and carboxyl groups. This mechanism is supported by adsorption studies demonstrating a high affinity of Pb^{2+} for lysine at near-neutral pH [18], as well as spectroscopic and computational analyses showing that Pb^{2+} coordinates strongly with aliphatic amino acids via nitrogen- and oxygen-donor atoms [23].

Alternatively, Pb^{2+} may act as a Lewis acid to catalyze the benzoylation of glucose, thereby masking the reactive carbonyl group and preventing nucleophilic attack by amino groups [17]. Either mechanism would inhibit Schiff base formation and effectively halt the Maillard reaction cascade at its initial step. This behavior contrasts with that of transition metals such as Fe^{2+} , Cu^{2+} , and Zn^{2+} , which are known to accelerate Maillard browning by catalyzing redox-dependent reaction steps [15,16]. Pb^{2+} , possessing a filled 6s orbital and lacking redox activity, instead functions as a static Lewis acid, providing a plausible explanation for its inhibitory rather than catalytic effect on the Maillard reaction [23].

However due to the limitations of equipment in the laboratory, there should be future studies that should observe the presence of lysine and glucose in order to confirm these speculations.

3.4 Implications for Food Safety and Quality

While Pb^{2+} may partially bind to residual Maillard products, the complete loss of sensory attributes (color, aroma) renders this approach impractical for decontamination. Consumers reject pale, odorless bread. However, the marked visual signal (lack of browning) could serve as a low-cost indicator of Pb contamination in artisanal settings—a novel application of reaction inhibition. However this should be contemplated in future studies.

3.5 Limitations

The experiments were carried out in ITMO and there were certain limitations in equipment that forced the experiment to go through several redesigns. It was originally contemplated that the different solutions should be measured after the Maillard reaction as way to directly correlate the concentration of browning elements with the concentrations of ions of lead. As well as this experiments was used specifically to contemplate in a model based in glucose-lysine, in order to simulate the wheat flour based food systems. Therefore this study is limited to only this reactives. However due to the lack of equipment to measure inorganic agents, this was not possible. It was also contemplated to make use of chromatography as a second possible way to measure the browning agents, however the available chromatography in the institution were not readily available, and the high volatility of the agents demanded a quick analysis alongside the spectrophotometry measurements. It should be noted that the experiment was at the beginning limited to a rather short time frame. Therefore, other variations of the same experiments weren't possible and should be recommended to make in future expansions of this research.

4. Conclusion

Under simulated bread-making conditions, Pb^{2+} strongly inhibits the absorbance which indicates that the Maillard reaction could be inhibited by disrupting early-stage intermediates and preventing melanoidin formation in a glucose-lysine model system. This inhibition compromises sensory quality and precludes the use of Maillard-based lead decontamination strategies. The marked reduction in browning may nevertheless provide a rapid visual indicator of lead contamination. Future studies should validate these findings in complex flour matrices and explore mitigation strategies that preserve sensory attributes, and it is important for future experiences to observe the effects with other metals that can be contaminants in urban centers.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

The single author prepared the figure or conducted the literature search for the manuscript, and wrote the paper. RGH has participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflicts of Interest

The author declares no conflicts of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During manuscript preparation, the authors used an artificial intelligence-based language model (e.g., ChatGPT-4o) to assist with editing for clarity, grammar, and academic style. The authors reviewed and revised all AI-assisted text and take full responsibility for the scientific content and conclusions presented.

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