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Presence of dithiocarbamates in vegetables sold fresh at open markets in the municipality of São Luís de Montes Belos, Goiás, Brazil

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Abstract
Pesticide residues pose a risk to consumers. Few publications address dithiocarbamates issue in Brazilian territory. Therefore, using the Keppel method, this study detected and quantified the presence of dithiocarbamates in fresh vegetables sold at two open markets in the municipality of São Luís de Montes Belos, Goiás, Brazil. From February to June 2023, 60 fresh samples were collected and analyzed, spectrophotometrically, at 435 nm. Pesticide levels above the limits allowed by national and international legislation were detected in tomatoes and bananas, with higher concentrations at market 1. Apples showed concentrations below the permitted limits, while lettuce and strawberries had low and consistent levels within the standards. Papaya had a higher concentration in the market 2 but still within the allowed limits All $p<0.05$. Findings highlight the need for strict monitoring and regulation of dithiocarbamate residues to ensure consumer safety in the region.

Keywords: Dithiocarbamates, fresh vegetables, open markets, São Luís de Montes Belos, Goiás, Brazil

Introduction
The production of vegetable foods plays a crucial role in food security, especially in local communities and regional markets [1]. However, this production faces significant challenges regarding pesticide use [2]. Small-scale producers, often with limited access to technology and information on sustainable agricultural practices, tend to use pesticides intensively to ensure productivity and combat pests, without a full understanding of the associated risks. This can result in the improper application of pesticides, such as dithiocarbamates, both in terms of quantity and frequency, leading to the contamination of agricultural products and the risk of exposure to toxic residues for consumers [3]. Additionally, the lack of rigorous oversight and educational programs directed at these producers contributes to the perpetuation of inadequate agricultural practices, exacerbating the issue of food safety [4].
Dithiocarbamates, a widely used class of fungicides in agriculture, play an important role in controlling fungal diseases in vegetables. However, their indiscriminate use in crops, especially by small producers who sell their products at open markets in Brazil, raises significant public health concerns [5]. The improper application of these compounds can result in residue levels that frequently exceed the Maximum Residue Limits (MRLs) established by national and international legislation. The lack of rigorous oversight at open markets, combined with producers' limited knowledge of safe pesticide application practices, aggravates the situation, exposing consumers to potential health risks, such as neurotoxic effects and endocrine disruptions [6].

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Studies have shown significant variations in dithiocarbamate concentrations in vegetables sold at free markets, with some products presenting alarming levels of contamination [7]. Thus, the issue of dithiocarbamate use in vegetables sold at open markets in Brazil requires a multifaceted approach, including the implementation of educational programs for producers, the strengthening of oversight practices, and the promotion of agroecological alternatives that can reduce reliance on these chemical compounds [8].

Considering aspects mentioned, there are few publications that describe the problem of dithiocarbamates and their residues in vegetables and fruits sold fresh in Brazil. Thus, this study aimed to detect and quantify this pesticide presence in vegetables sold fresh at open markets in the municipality of São Luís de Montes Belos, Goiás, Brazil.

Methodology

Between February and June 2023, samples of fresh apple (*Malus* x *domestica*), banana (*Musa sapientum*), lettuce (*Lactuca sativa* L.), papaya (*Carica papaya*), strawberry (*Fragaria* x *ananassa*) and tomato (*Solanum lycopersicum* L.) were directly acquired from vendors at randomly selected stalls at the two weekly open markets in the city of São Luís de Montes Belos, Goiás, Brazil. A minimum quantity of 1 kilogram (kg) of each crop was purchased, considering the whole head for lettuce samples and individual units for other vegetables, totaling 60 samples. The specimens were handled using new latex gloves, placed in clean low-density polyethylene bags, and sent to the Microbiology Laboratory at the Brasília University Center of Goiás for analysis according to the protocols and recommendations of Codex Alimentarius [9]. No washing of the samples was performed to accurately mimic what is passed on to customers and to avoid possible degradation of dithiocarbamates.

For monitoring analyses, the Keppel method was applied for dithiocarbamates determination, based on the acidic decomposition of these compounds to release carbon disulfide (CS_2), which subsequently reacts with a diethanolamine solution to form a yellow-colored complex. This complex was then quantified spectrophotometrically at 435 nm, allowing the determination of dithiocarbamate concentration in the samples.

For each plant specimen, the methodology was previously validated, obtaining specific Limits of Detection (LOD) and Limits of Quantification (LOQ). Thus, in a volumetric flask (a), 250 grams of the sample were added along with a 40% solution of tin(II) chloride ($SnCl_2$) and 10% HCl, heated. In trap (b), a 30% lead acetate $Pb(CH_3COO)_2$ solution was added, 2.5 mol L^{-1} NaOH in trap (c), and the color reagent in trap (d). After boiling, the mixture remained under a nitrogen (N_2) flow for one hour. Then, the absorbance of the formed complex was measured, obtaining the concentration in µg CS_2 mL^{-1}, which was later converted to mg CS_2 kg^{-1}.

Data were expressed as absolute and relative frequencies. Statistical analysis was conducted using GraphPad Prism version 5.01 for Windows. One-way ANOVA tests followed by Bonferroni post-tests were used, considering statistical significance at $p<0.05$.

Results and Discussion

Dithiocarbamates are a class of chemicals primarily used as fungicides and represent one of the oldest classes of broad-spectrum drugs used globally to control fungal diseases in various crops. Their synthesis is relatively simple, and production costs are low, making them widely used pesticides in modern agriculture [10]. Dithiocarbamates are also employed as precipitating agents applied in wastewater treatment, chelation of heavy metals, preservative agents to protect wood against fungi and insects, among others [11].

Despite their rapid degradation in the environment (by photolysis and/or hydrolysis), dithiocarbamates are still frequently detected in food analyses in Brazil, with a high frequency of exceeding MRLs [8]. These compounds can bind to metals (Such as Cu, Fe, Co, Mn, Ni, and Pb), acting as enzyme inhibitors. They affect catalytic and regulatory thiol groups of cytoplasmic constituents through organic electrophiles generated by the metabolism of parent molecules or coordinated metal ions (Such as Zn and Mn) [12].

In this study, the analysis of dithiocarbamate levels in vegetable samples sold at two weekly open markets in the city of São Luís de Montes Belos, Goiás, Brazil, in 2023, revealed statistically significant variations ($p<0.05$) between the evaluated markets (Table 1). It was found that the Limits of Detection (LOD) and Limits of Quantification (LOQ) of dithiocarbamates varied, particularly in tomato and banana samples. The LOD for tomatoes was 3.1 mg CS_2 kg^{-1} at market 1, compared to 2.0 mg CS_2 kg^{-1} at market 2, and the LOQ was 3.7 mg CS_2 kg^{-1} and 2.1 mg CS_2 kg^{-1}, respectively. These concentrations exceeded the MRLs allowed by national legislation (2 mg CS_2 kg^{-1}) and international regulations (3 mg CS_2 kg^{-1}). In the case of bananas, higher concentrations were observed in the first market (2.5 mg CS_2 kg^{-1}) compared to the second (2.2 mg CS_2 kg^{-1}), with LOQ values also higher in the first market (2.4 mg CS_2 kg^{-1}) compared to the second (1.6 mg CS_2 kg^{-1}); these values are above the national and international MRL of 2 mg CS_2 kg^{-1}.

Table 1. Dithiocarbamate concentration in vegetable samples sold at two weekly open markets in the city of São Luís de Montes Belos, Goiás, Brazil, 2023. *$p<0.05$. One-way ANOVA and Bonferroni post-tests.

Vegetables	LOD (mg CS_2 kg^{-1}) *		LOQ (mg CS_2 kg^{-1}) *		MRL (mg CS2 kg-1)	
	Open market 1	Open market 2	Open market 1	Open market 2	National	International
Apple	1	0.7	1.18	0.9	5	Prohibited
Banana	2.5	2.2	2.4	1.6	2	2
Lettuce	0.1	0.2	0.1	0.1	2	2
Papaya	2.2	2.8	2.14	4.2	5	5
Strawberry	0.1	0.1	0.2	0.1	3	Prohibited
Tomato	3.1	2	3.7	2.1	2	3

LOD: Limits of Detection
LOQ: Limits of Quantification
MRL: Maximum Residue Limits established by Brazilian and international legislation

For apples, the concentration of dithiocarbamates was lower in the second market (0.7 mg CS_2 kg^{-1}) compared to the first (1 mg CS_2 kg^{-1}), with a LOQ also lower in the second market (0.9 mg CS_2 kg^{-1}) compared to the first (1.18 mg CS_2 kg^{-1}), both concentrations below the national and international MRL. Lettuce presented low and consistent LOD and LOQ values between the markets, both in compliance with the national and international MRL of 2 mg CS_2 kg^{-1}. Papaya showed a marked difference between markets, with a higher concentration in the second one (2.8 mg CS_2 kg^{-1}) compared to the first (2.2 mg CS_2 kg^{-1}) and a significantly higher LOQ in the second market (4.2 mg CS_2 kg^{-1}) compared to the first (2.14 mg CS_2 kg^{-1}), but still below the MRL of 5 mg CS_2 kg^{-1}. For strawberries, values were homogeneous between markets, with low and consistent LOD and LOQ values, within the national limit of 3 mg CS_2 kg^{-1}, and prohibited internationally (p<0.05, Table 1). These findings indicate that consumers may be exposed to potentially hazardous levels of dithiocarbamates, especially in markets where inspection is less rigorous, as observed in the municipality of São Luís de Montes Belos.

The Codex Alimentarius Pesticide Index [9], developed by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), is an international reference that establishes guidelines and standards for the evaluation and control of pesticide residues in food. This index provides a set of technical parameters aimed at protecting consumer health and facilitating international food trade by harmonizing MRLs for pesticides accepted globally. For each active substance, including dithiocarbamates, the Codex defines specific MRLs for different food categories, based on extensive toxicological evaluations and dietary exposure studies. Dithiocarbamates receive special attention due to the risk of forming toxic by-products during the degradation process. The Codex establishes strict guidelines for monitoring these residues and recommends agricultural practices that minimize the presence of such by-products in food. Additionally, the index promotes the continuous reassessment of MRLs considering new scientific findings and changes in agricultural practices, ensuring that international standards remain up-to-date and effective in protecting public health.

In Brazil, Resolution RDC No. 4, of February 10, 2012 [13], established by the National Health Surveillance Agency (ANVISA), in the same manner, defines the MRLs of pesticides allowed in marketed foods, including specific compounds such as dithiocarbamates. These limits are safety parameters aimed at protecting consumer health, ensuring that the daily intake of chemical residues from pesticides does not exceed levels considered safe, based on rigorous toxicological assessments. In the case of dithiocarbamates, the RDC specifies different MRLs for each type of food, considering these compounds potential toxicity and the frequency of consumption of the foods to which they are applied. The definition of MRLs also considers the cumulative impact of these residues over time, integrating data on the chronic exposure of the population to these substances.

Another more recent national regulation is Joint Normative Instruction No. 2, of February 7, 2017 [14], developed by ANVISA, the Ministry of Agriculture, Livestock and Supply (MAPA), and the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), which establishes the technical and administrative procedures for the evaluation and reevaluation of pesticide residues, including dithiocarbamates. This regulation details the criteria and methodologies to be adopted for analyzing MRLs, considering both the acute and chronic effects these compounds may cause to human health. The evaluation involves the review of toxicological studies, environmental monitoring data, and dietary risk assessments, ensuring that pesticide residues present in food remain within acceptable levels and do not pose a health risk. Specifically, for dithiocarbamates, the regulation calls for a careful evaluation due to the potential of these compounds to degrade into toxic by-products, such as ethylene thiourea (ETU), a known carcinogen.

The presence of dithiocarbamates in vegetables sold at open markets has become a growing concern in scientific studies, particularly due to the potential impact on public health. These compounds, widely used as fungicides in agriculture, have been detected in varying concentrations in several types of vegetables, often exceeding MRLs set by national and international regulations [7, 11, 15]. In Brazil, studies conducted by Araujo *et al.* (2022) [8] show that vegetables like tomatoes, lettuce, and pumpkins, often purchased at open-air markets, present levels of dithiocarbamates that exceed the permitted limits, indicating a gap in oversight and the application of good agricultural practices in small local markets.

The situation is concerning, considering that open markets are an important source of food supply for the Brazilian population, especially in small towns, villages, and the outskirts of large cities. The research by Liu *et al.* (2023) [16] reveals that prolonged exposure to dithiocarbamates, even at low levels, may be associated with neurotoxic and endocrine effects, as well as other health complications. This risk is amplified when considering the lack of regulation and rigorous monitoring in informal markets, where products may not undergo adequate quality controls. A comparison with international studies, such as those by Atuhaire *et al.* (2017) [17] and Skovgaard *et al.* (2017) [18], shows that the issue of pesticide residues in vegetables is a global concern, but the severity of the problem can vary significantly depending on the strictness of control policies.

The analysis of international markets also highlights differences in the enforcement of food safety standards. In the European Union, for example, the policies for monitoring pesticide residues are stricter, with more restrictive limits for dithiocarbamates in vegetables [19]. However, even in countries with more stringent regulations, challenges remain in the implementation and enforcement of these standards, particularly in local markets where oversight may be less effective. This scenario is similar to that found in Asian countries, where research by Li *et al.* (2022) [20] identified concerning levels of dithiocarbamates in vegetables sold in traditional markets, underscoring the need for a global and coordinated approach to addressing the issue of pesticide residues.

Furthermore, the variability in dithiocarbamate levels found in vegetables from different markets in Brazil suggests that agricultural practices and pesticide application can vary significantly between regions. This variability may be attributed to factors such as unequal access to agricultural technologies, limited knowledge about the safe use of pesticides, and the lack of uniform enforcement [21]. This highlights the importance of education and awareness

programs for farmers and vendors to promote safer agricultural practices and reduce the public health risks associated with the consumption of contaminated vegetables.

In summary, the presence of dithiocarbamates in vegetables purchased at open markets is an issue that requires continuous attention both in Brazil and abroad. The harmonization of food safety policies and the implementation of safer agricultural practices are essential to minimize the risks associated with the consumption of these compounds [22]. The scientific literature points to the need for a joint effort between governments, producers, and consumers to ensure that the food available in the market is safe for consumption, thus protecting public health and promoting sustainability in agricultural production.

Conclusion

Dithiocarbamate levels above the limits allowed by national and international legislation were detected in tomatoes and bananas, with higher concentrations in the market 1. Apples showed concentrations below the permitted limits, while lettuce and strawberries had low and consistent levels within the standards. Papaya had a higher concentration in the market 2 but still within the allowed limits. Data suggest that consumers may be exposed to potentially hazardous levels of dithiocarbamates, especially in markets with less stringent oversight. Therefore, monitoring and regulating dithiocarbamate residues is essential to ensure the food safety for vegetables consumers sold at weekly open markets in São Luís de Montes Belos, Goiás, Brazil.

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Declaration of interest statement

Authors report no declarations of interest.

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