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Cadmium Contents in Biodegradable Films Made From Cassava

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Abstract

A Review of sample preparation and techniques used to determine Cadmium content in flexible films and biodegradable thermoformed products based on cassava. All determinations have been made using atomic absorption spectroscopy. The presence of this element in these matrices can be harmful if the maximum tolerated quantities are not complied by. The presence of Cadmium may be due, among other aspects, to the fact that it is present in the raw material, in industrial discharges or because it is found naturally. Its determination is an important parameter that needs to be considered as a good alternative for packaging.

Keywords: cadmium, thermoformed, flexible films, atomic absorption

1. Background and objectives

1.1. Introduction

The accelerated pace of industrialization, combined with rapid population growth, intensive agricultural techniques, and inappropriate waste management in developing countries have increased the levels of micropollutants such as heavy metals, considered harmful or toxic to living beings [1, 2]. These heavy metals can enter the human body—causing serious damage—via food, water, air, soil, skin absorption, polluting emissions, anthropogenic sources (treated sewage discharges, mining operations), contact with industrial and agricultural products such as pesticide formulations, urban traffic, contamination from chemical fertilizers, and irrigation with poor quality water [3–8].

The migration of heavy metals induced by substances in contact with food is given by the negative interaction between packaging and food. Another risk factor is constituted by the fact that heavy metals are nonbiodegradable and cannot be metabolized and, thus, persist and accumulate in the environment and in organisms over long periods of time [1–3, 9–11].

1.2. Biodegradable thermoformed and flexible films

Thermoformed and flexible films are very popular in the food packaging market given their low cost, large surface area by volume, and outstanding performance

across a wide temperature range [12]. Thermoforming is a generic term that encompasses several processing techniques, by which plastic articles can be obtained from flat sheets of different polymers. Thermoformed products are classified into two major categories: permanent or industrial products (shelves for medical or electronic equipment, decorative panels for cars, planes, motorcycles, bathtubs and bathroom fixtures, helmets and seats for boats, and skylights) and disposable products (packaging for medicines, bubble wrap, cups for hot and cold beverages, baking trays, food containers, and clear packaging that is shaped like the product) [13, 14].

The films are defined as thin flexible sheets of synthetic or natural origin, that reach a thickness of 0.01 inches or less. The polymeric matrix that forms the film can be defined as the random arrangement of the chains that make up the structure. Flexible films used in the food industry are currently divided into two groups: synthetic or non-biodegradable materials and biodegradable materials. The manufacture of these products is important, as they break down quickly and easily, without producing residues that cause unfavorable impacts on environmental ecosystems [14].

Biodegradable thermoformed and flexible films can be made of cassava flour (given its high starch content, making it suitable for use in the manufacture of various products in the food industry, as well as for the production of biopolymers) and cassava starch (as it can be converted into a thermoplastic material by interrupting the molecular interactions of the double helix chain, formed by hydrogen bridges between the hydroxyl groups, in the presence of a plasticizer aided by suitable temperature and shear stress [15, 16], and by adding matter such as fique fiber, gelatin, poly(butylene adipate-coterephthalate), polylactic acid, glycerol, plasticizer, green composites, cellulose, chitosan, clay, pullulan, natural extracts, poly(vinyl alcohol), and kaolin [7, 8, 17–35].

Industries are currently striving to improve their products by using natural and renewable sources to store, package, and wrap food products [7, 8, 29, 32, 36]. These include thermoformed and flexible films (biopolymers) obtained from agricultural sources (cassava), and constitute a new and environmentally friendly industrial alternative (composting) given their rapid and easy biodegrading processes. Their main purpose is the replacement of regularly used petroleum-derived plastic polymers and their associated waste [8, 18, 20, 22, 23, 26, 31, 37, 38]. A common production method for thermoforming is the compression molding technique (**Figure 1**), in which the material is placed into an open mold to which pressure and heat are applied.



Figure 1.
Compression molding machine used for thermoforming.

Single screw extrusion—a continuous process that forms the plastic material—is used to produce biodegradable flexible films, whereby turning the screw and applying heat pushes the material along while melting it [13, 21, 27, 32], turning it into a viscous material. Where the nozzle is positioned at the end of the cylinder, there is a hole shaped according to the use required (for films, the extrusion nozzle is round). From the extrusion hopper comes the plasticized mass, which is stretched, smoothed, and rolled by the rollers (**Figure 2**) [39].

The casting technique, using native and ozonated cassava starch, glycerol as the plasticizer, and water as the solvent is used for the elaboration of biodegradable films from cassava starch modified by ozone at different levels [40].

1.3. A case study of heavy metal pollution: cadmium

Biodegradable polymers may lead to heavy metal contamination during their manufacture if the raw material used to process them is contaminated (cassava flour and starch, fique fiber, polylactic acid, glycerin, etc.). They may also be subject to cross-contamination during production, for example, via petroleum- and nonpetroleum-associated activities, from containers used for cooking and storage, during drying, and from contaminated utensils or water [4, 41, 42].

Thus, tracking the manufacturing process is essential in determining the presence of toxic heavy metals in the biopolymers, in turn, to safeguard public health by limiting exposure [1, 9].

Among these metals, cadmium (Cd) is considered toxic to plants and animals; it is widespread in the atmosphere, soils, and water, and is a serious health hazard, affecting the gastrointestinal, cardiovascular, musculoskeletal, nervous, renal, and reproductive systems. Long-term exposure may cause mitochondrial damage and possible death. Cd bioaccumulates in individual organisms [4, 5, 43–46] and is likely to continue to do so due to the future use of biodegradable polymers in the packing and packaging of dry foods and other degradable products, as it enters the food chain.

The US Department of Health and Human Services has insisted that an excessive Cd accumulation in humans may be the cause of cancer as Cd and Cd salts are considered a “possible human carcinogen” [4, 44]. Certain plants can also accumulate Cd in their tissues, and levels are even more significant in plants grown in peri-urban areas with soil contaminated by irrigation using wastewater and sewage sludge [10, 47–50].

There are currently no studies reporting Cd found in thermoformed and biodegradable flexible films; however, the literature does report the presence of Cd in cassava tubers’ cortex [42], cassava food crops [51], as well as Cd contaminated rice which causes the Itai-itai disease [52] by exposure to Cd-contaminated water



Figure 2.
Film extrusion machine.

used for irrigation or from farms affected by petroleum- and nonpetroleum-related activities. The safety of the materials that come into contact with food is assessed by the amount of substances that migrate into it from the biopolymer and whether or not these meet the conditions established in the legislation on foods.

The legislation limits the migration of toxic pollutants from reaching unacceptable levels and helps to maintain the integrity of foodstuffs, thereby preventing health hazards, contamination, and alternations in food composition and sensory properties [9]. The current Colombian regulation NTC 4096 [53], allows a maximum level of Cd of 1 mg/kg for plasticizers while the Agency for Toxic Substances and Disease Registry [44] estimated that average Cd intake in Americans is 30 mg/day and that only one-tenth of this amount is absorbed into the tissues. Given that thermoformed and flexible film can be used as food containers, their Cd levels must be strictly monitored.

2. Sample preparation and analytical techniques

2.1. Sample pre-treatment

The samples must be treated prior to analysis. The particle size of thermoformed samples are reduced via mechanical methods, starting with manual cracking; followed by maceration (**Figure 3**) to a particle size of less than 1,135 mm; and finally, the removal of water content by placing the particles in an oven at 70 °C for 3 hours [54].

For flexible film samples, all that is required is manual cutting as shown in **Figure 4**, and drying at 50 °C for 45 minutes [54–58].

The samples' moisture content can be determined in triplicate by calculating the weights obtained before and after drying (see Eq. (1)).

$$\text{Moisture (\%)} = \frac{\text{Moist sample weight} - \text{Dry sample weight}}{\text{Moist sample weight}} * 100 \quad (1)$$

Following drying in a furnace, the moisture values for thermoformed samples were between 3.71% and 5.80% (RSD lower than 3.4%). Biodegradable flexible films revealed higher values of moisture (following 45 mins of drying in a furnace) at 7.81–10.35%, with relative standard deviation (RSD) lower than 1.96% [54].

2.2. Optimization of acid digestion using the reflux system

When an analyte cannot be determined, it must be transformed to a state in which an appropriate identification and quantification technique can be applied.



Figure 3.
Sample: Thermoforming and maceration.

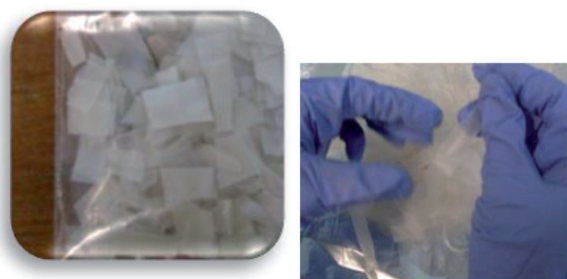


Figure 4.
Sample: Flexible films and cutting.

These transformations are usually dissolutions or digestions, which involve the sample passing from a solid state to a liquid one, using the correct solvent (acids or bases of different strength, oxidizing agents, or enzymes, etc.); in this energetic process, heating and agitation increase the speed of mass transfer, unifying the decomposition and elimination of organic matter, and leaving the trace components of interest (metallic ions) in solution [59, 60].

In this procedure, the organic matter is destroyed by a wet process and the sample is digested (with an acid/oxidant mixture), combining sulfuric, nitric, perchloric acid or hydrogen peroxide, in a system that can be open or refluxed [61];

This technique is generally preferred to dry oxidation (where the sample is heated to 450–500 °C), as the presence of large volumes of acids produces less loss of trace elements through evaporation. However, there is also the danger of elements being lost by evaporation (antimony, arsenic, boron, chromium, tin, germanium, mercury, osmium, rhenium and selenium), although these losses can be prevented by adjusting the conditions (mounting of reflux, temperature control and time) [59, 60].

A wide range of acid digestion procedures are reported in the literature, in which various mixtures of inorganic acids and, in some cases, hydrogen peroxide have been used (HNO_3 , $\text{HNO}_3\text{-H}_2\text{SO}_4$, $\text{HNO}_3\text{-HF}$, $\text{HNO}_3\text{-H}_2\text{O}_2$, $\text{HNO}_3\text{-HF-H}_2\text{O}_2$, $\text{HNO}_3\text{-HClO}_4$, y $\text{HNO}_3\text{-HClO}_4\text{-H}_2\text{O}_2$) [11, 62–64].

Of the mineral acids, nitric acid offers the best digestion result for all types of samples; however, there is no consensus on the addition of other substances, such as perchloric acid or hydrogen peroxide, to accelerate the process and reduce the volume of nitric acid used [60].

This study employed acid digestion in its sample preparation [51, 54]. Optimizing acid digestion using the reflux system involved the determination of cadmium in a thermoformed sample (HMC-1) and flexible film (SM 707–17 hydrolyzed), taking four absorbance readings and considering the following variables: sample weight, temperature, time, and acid ratio. Each test should be performed in triplicate.

2.2.1 Acid ratio optimization

The optimal acid ratio for the digestion of thermoformed samples is determined experimentally, by varying the amount of mineral acids: HNO_3 at 65% (20 to 5 mL) and HClO_4 at 48% (5 to 20 mL), as shown in **Table 1**. Each test should be performed in triplicate.

For flexible films, the optimization was only possible with an acid ratio of 3:1 as this was the best response to the digestion procedure for thermoforming.

Digestion was performed using the sample dissolved in 20 mL of a mixture containing HNO_3 (65%, Merck): perchloric acid (48%, Merck) at a 3:1 ratio [54].

HNO ₃ :HClO ₄ Ratio	HNO ₃ (mL)	HClO ₄ (mL)
1:0	20.0	—
3:1	15.0	5.0
1:1	10.0	10.0
1:3	5.0	15.0
0:1	—	20.0

Table 1. Acid ratio (HNO₃ and HClO₄) for acid digestion using the reflux system.

2.2.2 Sample weight optimization

This parameter is optimized by varying the sample quantity, which for our study, has been: 0.5000 (\pm 0.0001) g, 1.0000 (\pm 0.0001) g and 2.0000 (\pm 0.0001) g, for thermoforming, and 0.5000 (\pm 0.0001) g and 1.0000 (\pm 0.0001) for flexible films, on a dry base.

Digestion was performed using a 1.0 g of the sample dissolved in acid [54].

2.2.3 Temperature and digestion time optimization

The optimal digestion temperature is determined experimentally at three heating temperatures (35, 50 and 70 °C) for both thermoformed samples and flexible films.

For digestion time, 60, 120 and 180 minutes were tested for the thermoformed samples, for the acid ratios shown in **Table 1**, and for flexible films 15, 30, 45, 60 and 120 minutes, for the acid ratio 3:1. After cooling to room temperature, all digested solutions were filtered using a filter crucible, and then stored in polyethylene containers at 4 °C for further analysis by atomic absorption spectrometry.

Thermoformed samples were dried at 70 °C in a furnace for 3 h. Biodegradable flexible films were dried at 50 °C in a furnace for 45 min [54].

Numerous techniques have been used to determine the concentration of heavy metals in different samples, such as X-ray fluorescence spectrometry (XRFS), atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS). XRFS makes it possible to analyze solid materials without sample pretreatment; however, this advantage is limited by the need to use appropriate certified reference materials for calibration, making it a very expensive technique. In ICP-MS and EAA, dissolved liquid samples are usually required, so the samples have to be previously digested. This procedure can be tedious, time consuming and results in systematic errors due to incomplete extraction or solubility of the analyte [65].

When compared to the flame atomization method, the graphite furnace atomic absorption spectrometry is the most suitable technique to determine elements such as Cd, in trace level concentrations in polymer samples. The latter has many advantages such as its high sensitivity, its limits of detection in the order of micrograms per liter $\mu\text{g/L}$ to ng/L , its tolerance to complex matrices, the fact that it minimizes analyte loss, that it uses few sample volumes, and that it reduces analyst contamination risk [65–69]. With this technique, samples are often introduced in solution form, however, the EAA-HG technique with solid samples has been reported as a simple and fast method for the determination of lead and cadmium in polymer samples [65].

2.3. Statistical treatment

The following statistical tests have to be applied in order to analyze the degree of agreement between the individual data obtained for the standardization of the analytical method:

The Shapiro–Wilk and Levene tests are used to evaluate the normality and homogeneity of the data obtained for repeatability and intermediate precision, with a confidence interval greater than 95%. It is also necessary to determine the standard deviation and the coefficient of variation to determine whether the method is precise.

For linearity, the Pearson correlation coefficient and the coefficient of determination (R^2) must be determined, a one-way ANOVA applied, and the t-student test performed to evaluate whether the slope differs significantly from zero.

For samples of thermoformed, flexible films, flour and starch samples, one-way ANOVA needs to be applied in order to determine whether there are any significant differences between them.

For statistical analysis, programs such as: Microsoft Office Excel 2007 and SPSS Statistical Software version 11.5 are used.

3. Fine-tuning of the analytical technique

The analytical technique has to be fine-tuned prior to analysis:

3.1. Determine the stability of the cadmium hollow cathode lamp

To determine the stability of the lamp, the absorbance value of at least three element standards of different concentrations 0.2, 0.6 and 1.0 $\mu\text{g Cd/L}$ must be monitored for at least 30 minutes throughout three hours, as shown in **Figure 5**.

Subsequently, the normality and homogeneity of the data must be analyzed by applying the Shapiro–Wilk test with $N-1$ degrees of freedom and Levene test with degrees of freedom 1 and 2, determined as $K-1$ and $(k-N)-K$ ($N = \text{absorbance}$

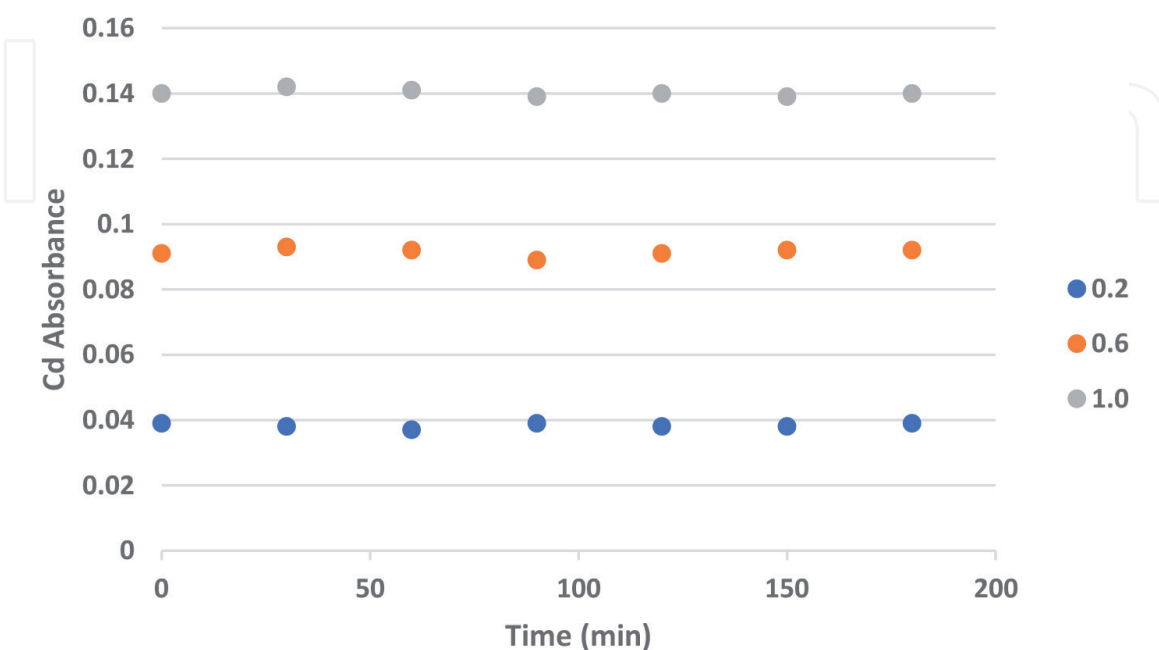


Figure 5.
Cadmium hollow cathode lamp stability [70].

readings and K = number of times). One-way ANOVA statistical treatment is applied next to determine whether there are any significant differences between the absorbances obtained for each standard during the three hours of analysis.

In the Shapiro–Wilk test, the H_0 was accepted, since the calculated W was lower than the tabulated W (0.999) and the homogeneity of variances with calculated F was lower than the tabulated F (5.100), indicating that the means obtained are representative values of the absorbances of each one of the standards, with good variation coefficients (<3.5%) [70].

The one-way ANOVA used to measure the absorbance of each of the standards for the metal, showed a constant value for the sum of squares, as for the root mean square (0.000). We can also see that the calculated F is lower than the tabulated F at a 95% confidence level, indicating that there were no significant differences between the values of the absorbances at different times when the analysis was performed [70].

3.2. Optimizing the calcination and atomization temperature to determine cadmium by graphite furnace

In order to obtain a higher sensitivity for cadmium determination, the calcination and atomization temperature in the graphite furnace must be optimized. To do so, a standard of 1 $\mu\text{g/L}$ of cadmium must be prepared and the atomization temperature set by varying the calcination temperature from 500 to 800 $^{\circ}\text{C}$ in 50 $^{\circ}\text{C}$ intervals. Once this is done, the highest absorbance is observed (which in our study was between 500 and 550 $^{\circ}\text{C}$) and another variation of the calcination temperature in this range is made, but this time by modifying it in 10 $^{\circ}\text{C}$ intervals. Once the calcination temperature has been optimized, a standard of 0.8 $\mu\text{g/L}$ of Cd is prepared and read on the atomic absorption equipment, setting the calcination temperature at 800 $^{\circ}\text{C}$ and varying the atomization temperature from 900 to 1800 $^{\circ}\text{C}$. This variation is made at intervals of 100 $^{\circ}\text{C}$. **Table 2** shows the optimum temperatures at 530 and 1750 $^{\circ}\text{C}$ [54, 70].

3.3. Quantification method

Cd quantitative analysis was performed using the calibration curve for which standard solutions of the metal were prepared as follows:

The calibration curve was constructed based on the stock solution of 1000 $\mu\text{g Cd/L}$ by preparing 5 mL of six cadmium standards in a range of 0.1 to 1.0 $\mu\text{g Cd/L}$ in 0.2% HNO_3 solution. For each Cd standard, four absorbance readings are taken in the AAS-HG.

The Pearson's Correlation Coefficient showed that the calibration curve for cadmium has a high r value, above 0.9950, demonstrating that there is a positive correlation between the absorbance of the metal and its concentration [54, 70].

Stage	Temperature($^{\circ}\text{C}$)	Time(s)	Argon Gas Flow (L/min)
Drying	100	30	0.2
Calcination	530	20	0.2
Atomization	1750	3	Off
Cleaning	2500	3	0.2

Table 2.
Optimal graphite furnace temperature programming for Cd determination.

3.4. Standardization of the analytical method for cadmium determination

Statistical quality parameters must be evaluated in order to standardize the analytical method, as described below.

3.4.1 Linearity

To examine linearity, a calibration curve needs to be prepared in a concentration range including at least six different cadmium concentrations between 0.1 and 1.0 $\mu\text{g Cd/L}$. This should be analyzed using the atomic absorption equipment, taking four absorbance readings for each cadmium standard. The calibration curve should be plotted (Absorbance vs. concentration) and the correlation coefficient and slope should be statistically evaluated, through statistical treatment of the parametric data obtained for each concentration level [54, 70].

A regression analysis for the model $Y = \beta_0 + \beta_1 X$ is required to confirm whether the degree of relationship is significantly linear between the two variables (absorbance and concentration). **Table 3** illustrates the coefficient that defines the slope of the regression line and the t-statistics for cadmium, making it possible to contrast the null hypothesis (H_0) that the slope has a value of zero. According to the results, the calculated statistics are greater than the tabulated ones, confirming that the slopes differ significantly from zero and therefore, the absorbance is significantly correlated to the concentration [54, 70].

The one-way ANOVA was applied to confirm the association between the two variables (absorbance and concentration) by means of a linear regression for cadmium. The results showed that the calculated F (6119.282) was higher than the tabulated F (4.080) and therefore, the null hypothesis raised is rejected ($H_0 =$ There is no significant linear relationship between absorbance and concentration) [54, 70].

3.4.2 Precision

Standard deviation (s) and the coefficient of variation (CV) must be determined in order to analyze the degree of agreement between the individual data obtained when the method is repeatedly applied to multiple aliquots of a homogeneous sample.

Accuracy is evaluated at two levels: Repeatability and Intermediate accuracy as described below.

3.4.2.1 Repeatability

We analyzed the precision obtained after performing five calibration curves, in a concentration range of 0.1 to 1.0 $\mu\text{g/L}$ for cadmium, where absorbance is measured four times per standard, under the same operating conditions in a short time interval (same day) by the same analyst and using the same equipment, materials, and reagents. This value corresponds to 0.136 [54, 70].

Metal	Slope	Calculated T	Tabulated T
Cadmium	0.1324	78.226	2.048

Table 3.
Regression analysis for model $Y = \beta_0 + \beta_1 x$.

3.4.2.2 Intermediate precision

We analyzed the precision obtained after performing seven calibration curves, in the same concentration range as for repeatability, where absorbance is measured four times per standard under the same operating conditions, in different time intervals (7 different days), by the same analyst and using the same equipment, materials, and reagents. This value corresponds to 0.135 [54, 70].

This method increases sensitivity when the analysis is conducted on the same day, indicating that the proposed method for sample preparation is appropriate. The sensitivity measured for the method was 0.136.

For the two previous procedures (Repeatability and intermediate precision), the Shapiro–Wilk test was applied with $N-1$ degrees of freedom (N = readings of absorbance) and Homogeneity of variances with degrees of freedom 1 and 2 calculated as $K-1$ and $(k-N)-K$ respectively (where N = readings of absorbance and K = number of calibration curves), posing the corresponding null and alternative hypotheses at a 95% confidence level. In addition, for each of the (standard) concentration levels, the respective standard deviations and variation coefficients were obtained to determine whether the method is accurate.

For repeatability, the results show that at a 95% confidence level, the calculated W is lower than the tabulated W (0.999) for all levels of metal concentration, with 3 degrees of freedom for cadmium. Thus, the null hypothesis is accepted and it is concluded that the data come from a normal distribution. Applying Levene's test for cadmium data, we can see that for 4 and 15 degrees of freedom, the calculated statistic is lower than the tabulated statistic for all levels of concentration. Thus, we conclude that the variances are homogeneous. The standards present a low standard deviation (less than 0.004) and a coefficient of variation that is lower or equal to 5%, thus indicating that the method used presents a good repeatability [54, 70].

For the intermediate precision, the calculated Shapiro–Wilk statistic was lower than the tabulated statistic (0.999), therefore concluding that the data come from a normal distribution at a 95% confidence level. Taking into account Levene's statistic, the calculated statistic was lower than the tabulated one, thus concluding that the variances are homogeneous [54, 70].

The metal standards, show a small deviation (less than 0.005) and a coefficient of variation that is lower than or equal to 4.0%, leading to the conclusion that the method is of good accuracy [54, 70].

3.4.3 Sensitivity

Sensitivity is assessed as analytical sensitivity and calibration sensitivity, as described below.

3.4.3.1 Analytical sensitivity

The parameters used to determine analytical sensitivity are the limit of detection and quantification. Fifteen absorbance readings were taken from the metal target and the standard deviation calculated along with the detection and quantification limits, following the method suggested by IUPAC (1995) [70, 71].

The LOD of Cd was 0.02 mg/L (0.4 mg/kg) and corresponds to the minimum amount of Cd derived from the lowest analytical signal that can be detected with reasonable certainty. The LOQ of Cd was 0.07 mg/L (1.4 mg/kg) and represents the minimum concentration that can be measured with precision and accuracy. The LOD and LOQ are adequate for the quality control of biopolymers [54, 70].

3.4.3.2 Calibration sensitivity

This parameter is determined by comparing the slopes of the Cd calibration curves obtained with the precision of the method (repeatability and intermediate accuracy). The slopes obtained for repeatability (0.1358 ± 0.004) and intermediate precision (0.1351 ± 0.004), show that there is a greater response to concentration changes in repeatability [70].

3.4.4 Accuracy assessed as percent recovery

The accuracy of the method is determined in terms of percent recovery by adding in triplicate, known amounts (4, 8 and 12 μL) of a 1000 $\mu\text{g/L}$ standard of cadmium to a thermoformed or flexible film sample prior to the digestion process. Following this, the respective readings (4 absorbance readings for cadmium) are taken using the atomic absorption equipment of the doped sample and of the sample with the standard added.

To calculate the percentage recovery, the cadmium concentrations in the samples are determined previously, as indicated in Eq. (2).

$$\text{Recovery}(\%) = \frac{\text{Concentration with stock added} - \text{Concentration with no stock added}}{\text{Concentration with stock added}} * 100 \quad (2)$$

The percent recovery of Cd ranged between 96.23% and 97.31% for the MPER 183 thermoformed material, which makes it possible to conclude that the extraction method used is suitable, and that the determinations are therefore reliable [54, 70].

A statistical t-student test should be applied to determine whether there are significant differences between the value obtained from recovery and the 100% level. The experimental t-values are compared with the tabulated values ($t(0.05, 8) = 1.860$) for 8 degrees of freedom in our case. The results show that for all the recoveries of the thermoformed samples, there are significant differences between the average values and 100% since $t_{\text{calculated}} > t_{\text{tabulated}}$ ($11.489 > 1.860$) [54, 70].

3.5. Stability of the metals

To establish the testing time for a standard and a sample in a laboratory, the stability of the samples over time must be determined as follows.

3.5.1 Standard stability

Cadmium standards are prepared (0.8 $\mu\text{g/L}$) and kept refrigerated while they are analyzed for ten consecutive days. A one-way ANOVA analysis (calculated $F < \text{tabulated } F$, $1,675 < 2,420$) indicates that there are no significant differences between the average absorbances during the days analyzed, and therefore this standard can be prepared and stored at a temperature of 4 $^{\circ}\text{C}$ for analysis for up to 10 days [54, 70].

3.5.2 Stability of metals in digested samples

To evaluate the stability of cadmium, a thermoformed sample is subjected to digestion via a dilution of 5 to 100 mL, which is refrigerated and read on the equipment for ten consecutive days. The samples for flexible film were not read, as in our case, they were not detectable.

By applying a Shapiro–Wilk statistical analysis, we were able to conclude that the data come from a normal distribution, since the calculated W is smaller than the tabulated W and the data maintain a homogeneous distribution, since the calculated Levene statistic is smaller than the tabulated one [70].

A one-way ANOVA was applied to determine any significant differences in the average absorbances between the different days analyzed. The results show that cadmium was stable in the thermoformed sample analyzed in our study (CM 4574–7) as the calculated $F <$ tabulated F ($1.87 < 2,420$) [70].

According to the results obtained, cadmium is stable in the thermoformed digested sample. Thus, these samples can be stored at 4 °C and analyzed on consecutive days [70], allowing the laboratory to establish its analysis times and organize the relevant protocols.

3.6. Determination of cadmium by atomic absorption spectrometry

3.6.1 Thermoformed and flexible films

To quantify cadmium in the samples, the graphite furnace atomic absorption spectrometer was used under the instrumental conditions shown in **Table 4**. The metal was measured in triplicate in the thermoformed and flexible film samples, as well as in the source materials (cassava flour, cassava starch and fique fiber), in order to determine whether the metal in the samples derives from the raw material used for manufacture.

Cd concentrations in the thermoformed products typically ranged between 4.2 mg/kg and 17.9 mg/kg, which could be the result of the quality of the raw materials used to process the biopolymers. Sample concentrations were significantly different from each other, given that when applying one-way ANOVA, the calculated F value was greater than tabulated F ($1507.861 > 3.501$) at 95% confidence.

Cd was not detected in flexible films, meaning that no contamination was present.

The cadmium concentrations found in the thermoformed products were lower than those established by NTC-4096¹ (1 mg Cd/kg). Thus, these biopolymers can be used for these purposes, but care needs to be taken as this metal can bioaccumulate, causing serious environmental problems in the long term.

Method	Graphite Furnace-AAS
Atomization	Electrothermal
Element	Cd
Lamp	Hollow cathode Cd
Background correction	D2 Quadline
Wave length (nm)	228.8
Injection volume	20
Slit (nm)	0.5

Table 4.
Instrumental conditions in the GF-AAS for cadmium determination.

¹ The standard that regulates the maximum level of heavy metals in samples that are going to be in contact with food.

3.6.2 Raw material

Once the Cd concentration in the samples of thermoformed products had been determined, we proceeded to analyze the main raw material—cassava flour and fique fiber—in order to identify whether the metal found in the thermoformed products originated in the processing materials.

The results show that the flours presented Cd concentrations of between 3.5–18.2 mg/kg, while Cd concentration in the fique fiber was 7.2 mg/kg, meaning that it contributes the most Cd to the thermoformed film.

Given that the fique fiber and flour are agricultural products, the presence of cadmium in these samples may be due to the fact that this metal is used in herbicides used to control weeds such as Linuron or Dinuron. It can also be present in soil from municipal waste and the incineration of plastic materials, in fertilizers (phosphorous and nitrogenous fertilizers), pesticides and fungicides such as copper oxychloride and carbofurans among others, used to cultivate cassava [4, 10, 42, 72].

4. Conclusions

The GF-AAS method developed was efficient (highly sensitive and acceptable in terms of accuracy and reliability) to quantify Cd in thermoformed and biodegradable flexible films and cassava flour samples. The method is therefore reliable, with low variation coefficients, and limits of detection and quantification that indicate that the standardized method is optimal.

The concentrations that were found in the samples of thermoformed and flexible films, are below the amounts allowed for products that come into contact with food (1 mg/Kg), but, as mentioned above care needs to be taken as Cd bioaccumulation can lead to grave environmental problems.

Cd content found in thermoformed films is associated with prior contamination of the raw material (during cultivation, pretreatment, and/or transportation) or contamination derived from old machinery used for manufacturing.

This study also provides new data for food safety authorities and which broaden the existing knowledge of the contribution of raw materials in terms of Cd concentrations in biopolymers.

There is great potential in using biopolymers in packaging and food conservation, in terms of the value these materials add to agricultural activity and in helping to reduce nonbiodegradable plastics in the environment.

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Conflict of interest

The authors declare no conflict of interest.

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