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Identification of foreign matter and histological elements in plant-based beverages marketed in Brazil

Maria Isabel Andrekowisk Fioravanti [a,c,d,*], Elaine Cristina de Mattos [b], Flávia de Carvalho [b], Beatriz Fernandes Lopes [b], Marcelo Antônio Morgano [c], Adriana Pavesi Arisseto Bragotto [d]

[a] Adolfo Lutz Institute, Rua São Carlos, 720, Vila Industrial, Campinas, SP, 13035-420, Brazil
[b] Adolfo Lutz Institute, Av. Ramiro Colleoni, 240, Vila Dora, Santo André, SP, 09040-160, Brazil
[c] Institute of Food Technology, Av. Brasil 2880, Jd. Chapadão, P.O. Box 139, Campinas, SP, 13070-178, Brazil
[d] School of Food Engineering, University of Campinas (UNICAMP), Rua Monteiro Lobato 80, 13083-862, Campinas, SP, Brazil

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ABSTRACT

The use of plant-based proteins for the production of plant-based foods is already a reality in the food industry, and an increasing number of successful applications has been reported. However, these products still present bottlenecks linked with the lack of systematic information about functional and health claims, including food safety assessment. Analysis involving food microscopy have been used for a long time to identify product authenticity and potential fraudulent products. Therefore, this study evaluated the presence of foreign matter and histological elements in 30 plant-based beverages sold in Brazil using microscopic techniques. Microscopic foreign matter was found in 23 % of the samples and 60 % of assessed brands, so monitoring by authorities and manufacturers is recommended to determine whether or not a foreign matter limit should be established for this class of food. Foreign matter of larger diameter such as whole insects, textile fibers, metal and plastic particles were not found in the samples, indicating good plant hygiene and sanitation. In the analysis of histological elements, the main ingredients listed on the product labels were found, and no fraud was observed in relation to the addition of inferior raw materials. Identification of histological elements using microscopic techniques proved to be feasible, but required knowledge and experience of analysts. This study demonstrates that plant-based beverages are a safe option for consumers seeking alternative beverages to milk or allergens. Despite not being a simple technique, the histological analysis of food remains an effective method while screening techniques, such as real-time PCR and chemometrics, which are more precise, are more used in food fraud. These results have not been reported before and may support future regulations for plant-based products.

1. Introduction

The burgeoning plant-based food industry has emerged as a highly promising market in Brazil. This growth can be attributed to the increasing demand from a range of consumers, including individuals with dietary restrictions, such as allergies and intolerances, as well as vegans, vegetarians, and those who prioritize their health. This market segment seeks options that not only cater to their specific dietary needs but also provide a satisfying and varied eating experience [1–3]. Today, among the various alternative sources of protein, plant-based protein is preferred by potential consumers, as this source of protein tends to be widely available in local markets [1].

The use of plant-based proteins is already a reality in the food industry, and an increasing number of successful applications has been reported either in new applications or replacing conventional protein systems. Innovative processing methods have enhanced these proteins, but challenges persist due to a lack of systematic data on their functionality, health claims, and food safety assessments [4]. For example, consumer confidence can be negatively affected after a food fraud scandal. Fraud can also represent a health risk, and it is often difficult for consumers to measure its occurrence when consuming an industrialized product [5].

Food fraud refers to intentional adulteration, i.e. economically motivated adulteration (EMA) that occurs when someone intentionally

Abbreviations: IF, insect fragments; FM, foreign matter; PB, plant-based beverage; IFP, insect fragment patterns; MH, mammalian hair.

* Corresponding author.
E-mail address: maria.fioravanti@ial.sp.gov.br (M.I.A. Fioravanti).

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takes out, or substitutes a valuable ingredient or part of a food, or when someone adds a substance to a food to make it appear better or of greater value [6]. Adulteration can also be unintentional, when unwanted substances are added due to poor knowledge, inattention or lack of proper facilities and hygiene during food processing [7].

According to a study conducted by Johnson [8], the main types of fraudulent foods reported were fish and seafood (31 %), oils and fats (11 %), alcoholic beverages (8 %), meat and meat products (7 %), dairy products (6 %), grains and grain derivatives (about 5 %), and honey and other natural sweeteners (5 %).

An adulteration is assessed or determined from several measurements of analytical data compared with reference or control information [9]. Different techniques can be used to detect food adulterations, such as vibrational spectroscopy (visible to near infrared (Vis-NIR)), Fourier transform infrared (FT-IR) spectroscopy, enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), microscopic methods, among others [10,11].

A microscopic analysis assesses structural, cellular, and internal tissue characteristics of the food matrix and generally uses powdered or processed samples, where macroscopic characteristics are ineffective or indistinguishable. Furthermore, microscopy serves as a tool for monitoring and identifying the presence of physical contaminants. This includes detection of insects, insect fragments (IF), and inanimate substances such as pieces of wire, nylon, stones, or sand. What sets microscopy apart is its ability to uncover these non-plant materials, which might remain unnoticed in other procedures. In spite of the good results of the detection of adulterant and food component based on their microscopic characteristics, this analysis does not ensure quantitative detection and requires high expertise [5,7,9,10,12].

Microscopic analyses are frequently used to detect the presence of foreign matter (FM) in foods. In Brazilian legislation RDC 623/22 [13] and the Food Defect Levels Handbook [14], some food groups present allowed levels of FM that do not represent any danger to health. These limits are established due to inevitable FM that occurs in food even with the adoption of best practices [13]. For example, insects in wheat grains (primary infestation) enter the milling process and are reduced to small fragments or parts of insects, such as elytra, head capsules, mouthparts or legs, and contaminate the processed food [15].

Food microscopy has been used for a long time to identify products because of its advantages such as reliability and low cost. Therefore, this study evaluated the presence of FM (insects, hair, among others) and histological elements in plant-based beverages sold in Brazil using microscopic techniques. In addition to identifying the histological elements based on the list of ingredients on the packaging, analysis was also conducted for possible fraud, such as the addition of some raw material not declared on the labels.

2. Method

2.1. Samples

A total of 30 plant-based beverages (PBs) of 10 different brands and seven plants bases – rice (*Oryza sativa* L.), almond (*Prunus dulcis*), oat (*Avena sativa* L.), cashew nuts (*Anacardium occidentale* L.), peanuts (*Arachis hypogaea* L.), coconut (*Cocos nucifera* L.), and soybeans (*Glycine* ssp) – were purchased in supermarkets in Campinas (São Paulo, Brazil). Table 1 shows the samples with their commercial name (base), brand, number of total ingredients described on the labels, and the ingredients with possible histological identification.

2.2. Preparation of insect fragment pattern

Due to the difficult detection of insect fragment patterns (IFPs) on the market, microanalytical patterns for insect fragments were prepared in the laboratory using the technique described by Brickey et al. [16]. The IFPs were prepared with the forewings of cockroaches (order: Blattodea; family: Blattidae) as they are easy to obtain and identify. On graph paper and with the help of a scalpel blade, the anterior and posterior (rounded) ends of the wings were discarded. Longitudinal cuts were made to obtain 0.5 mm squares, as illustrated in Fig. 1 The IFPs were immersed in transparent gelatin and stored frozen. The samples were contaminated by adding gelatin containing IFPs in the desired amount.

Table 1
Commercial identification and main ingredients listed on sample labels ($n = 30$).

Samples	Commercial name	Brand	Ingredients* (n)	Ingredients with histological identification
S1	Rice	A	8	Rice
S2		B	5	Rice
S3		C	9	Rice
S4		C	10	Rice and coconut
S5		C	11	Rice and cocoa
S6	Almond	C	7	Almond
S7		D	9	Almond
S8		E	10	Almond and cocoa
S9		F	9	Almond, coconut, and pea
S10		F	10	Almond, coconut, and pea
S11	Oat	G	4	Oat
S12		G	5	Oat
S13		G	5	Oat and cocoa
S14		B	5	Oat
S15	Cashew nut	H	2	Cashew nuts
S16		H	3	Cashew nuts and Brazil nuts
S17		H	4	Cashew nuts and cocoa
S18		C	7	Cashew nuts
S19		E	8	Cashew nuts
S20		F	8	Cashew nuts
S21	Peanut	I	4	Peanuts and coconut
S22	Coconut	E	8	Coconut
S23		F	9	Coconut and pea
S24		D	10	Coconut
S25	Soybean	D	10	Soybean
S26		D	10	Soybean
S27		J	15	Chicory, soybean, pineapple, pea, and cabbage
S28		J	17	Chicory, soybean, pineapple, pea, and cabbage
S29		J	16	Soybean, pea, chicory, cocoa, pineapple, and cabbage
S30		J	16	Soybean, pea, chicory, cocoa, pineapple, and cabbage

* Estimated number of ingredients due to variation in the form described on product labels.

2.3. Analytical quality control for FM assays

For quality control, the FM analysis method was validated according to the INMETRO [17] and AOAC [18] guidelines for figures of merit: limit of detection (LOD) and selectivity. The LOD is defined as the minimum level at which the analyte can be reliably detected, and was determined by performing 10 analytical blank assays contaminated with 1 IFP. Recovery of 10 readings was 100 %, indicating the LOD of the method is 1 IF.

Selectivity was performed to assess the presence of a matrix effect. A PB sample (S1) was contaminated with IFP at four different levels (1, 3, 5, and 10). All assays were performed in triplicate, independently with both samples and blanks. The results obtained were 100 % IFP recovery at all levels, demonstrating no matrix interference at the contamination levels studied.

2.4. Analysis and identification of FM

The technique suggested by AOAC [19] – Method 972.35 (16.5.18 -

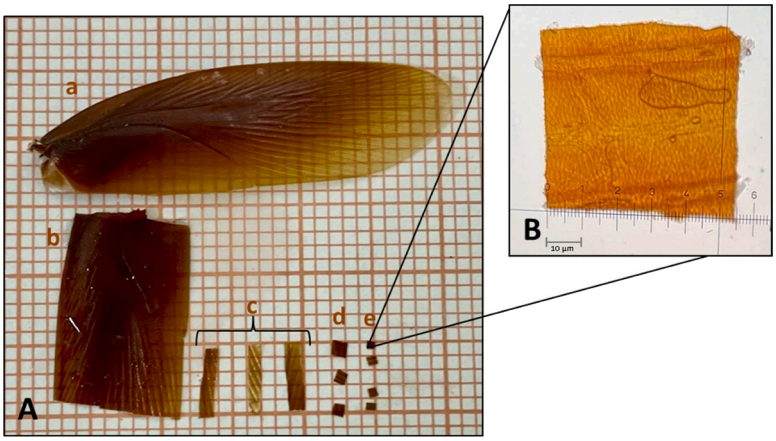

Fig. 1. A - Sequence of cuts of cockroach wings: (a) whole wing; (b) wing without rounded ends; (c) 1 mm strips; (d) 1 mm squares; (e) squares measuring around 0.5 mm (IFP). B – Detail of IFP measuring around 0.5 mm under an optical microscope (400x magnification; 1 mm ruler).

Light filth in starch) – was used in this analysis, in which 225 g of the homogenized sample and 1200 mL of filtered water were added to a 2000 mL beaker. This mixture was quantitatively transferred to Tamis sieve n° 230 and washed until the wash water became clear. The sample that remained in the sieve was then transferred and filtered on pre-pleated qualitative filter paper (Whatman 40), using a vacuum pump (CA, famem Ltda). The material collected on the filter paper was observed under a stereoscope (SZ-III-BR-SIT, Micronal®) (30x magnification) and, when necessary, the impurities were removed, placed on a slide and observed under an optical microscope (CBA -K, Olympus®) for identification (400x magnification). All analyses were performed in duplicate and with an analytical blank. If the number of IFs between the repetitions of each sample showed a difference above 10 units, the analysis was repeated [15].

2.5. Analysis and identification of histological elements

The isolation method for the identification of histological elements was based on the method described by Rodrigues et al. [20]. The sample (10 g) was dissolved in filtered water (250 mL) and the solution was vacuum filtered using qualitative filter paper. The material collected on the filter paper was placed on a slide and observed under an optical microscope for identification (400x magnification). When necessary, a Lugol's solution (Laborclin) was used to stain the slide. Due to the scarcity of histological elements, some samples had to be centrifuged (Eppendorf centrifuge, Hamburg, Germany), 10 mL at 3500 g for 5 min, or the residues obtained in the FM analysis (item 2.4) were used in slide preparation.

The characteristic histological elements of each sample and possible fraud were identified by comparing the patterns available in the Adolfo Lutz Institute microscopy laboratory and the structural characteristics described by Winton and Moeller [21] and Menezes Junior [22,23].

3. Results and discussion

3.1. Analysis and identification of FM

Larger diameter FMs such as whole insects, textile fibers, metal and plastic particles, were not found in the samples. The absence of these FMs in the samples was expected since the beverage manufacturing process includes filtration stages [2,24] that retain physical contaminants, among other elements. The absence of FM also indicates that manufacturing facilities have good hygiene and sanitation conditions. Table 2 and Fig. 2 present the smaller diameter FMs that were observed in seven samples: presence of IF (S6, S11, S20), larva (S30), and mammalian hair (MH) (S1, S8, S9).

Table 2
Samples with foreign matters.

Samples	Brand	Ingredients with histological identification	Number and type of FM	
S1	A	Rice	1	MH not identified
S6	C	Almond	1	IF
S8	E	Almond and cocoa	4	MH not identified
S9	F	Almond, coconut, and pea	1	MH not identified
S11	G	Oat	1	IF
S20	F	Cashew nut	1	IF
S30	J	Soybean, pea, chicory, cocoa, pineapple, and cabbage	1	Larva

FM = foreign matter; IF = insect fragment; MH = mammalian hair.

The PB food group is not included in the legislation and standards that allow FM levels, mainly because it is a new food class, without related studies available or specific legislation. Considering the raw materials present in the ingredient lists of the samples, the Brazilian legislation [13] allows FM (IF and rodent hair) in cocoa products. The FDA [14], in addition to cocoa products, also allows FM in peanuts, peas, and nuts (only macroscopic FM in nuts). Except for sample S11, the samples in which FM was found show at least one ingredient with an acceptable level of FM in cited references.

Fig. 2 shows the main FMs found in the samples: 3 samples with IF (Fig. 2- A, B, and C) identified by its color, shape, pores, and bristles. The insect larva (Fig. 2D) is mainly characterized by the presence of the mandible. The technique used to isolate foreign matters from the samples was efficient because it did not use any acid in the process, which could destroy weakly sclerotized parts, such as larvae and pupae [15].

All six fragments of MH found in S1, S8, and S9 had similar characteristics: medulla with a single layer of cells and fine hair, suggestive of rodent hair (fur hair). However, it is not possible to confirm that as other animals have hair with a similar structure, and the main characteristic of rodent hair is the presence of a medulla with multiple layers of cells and thick hair (guard hair) [25]. The detection of four unidentifiable mammalian hairs in sample S8, which almond and cocoa, is a warning of potential issues within the product's supply chain and production process. It raises questions about the quality of the raw materials used and the overall hygiene and sanitation conditions during manufacturing. This finding is particularly significant when compared to Brazilian legislation [13], which allows only 1 rodent hair fragment in cocoa products. This stark contrast highlights the need for a thorough review of the quality control measures and safety standards employed in the production of this particular item to ensure compliance with

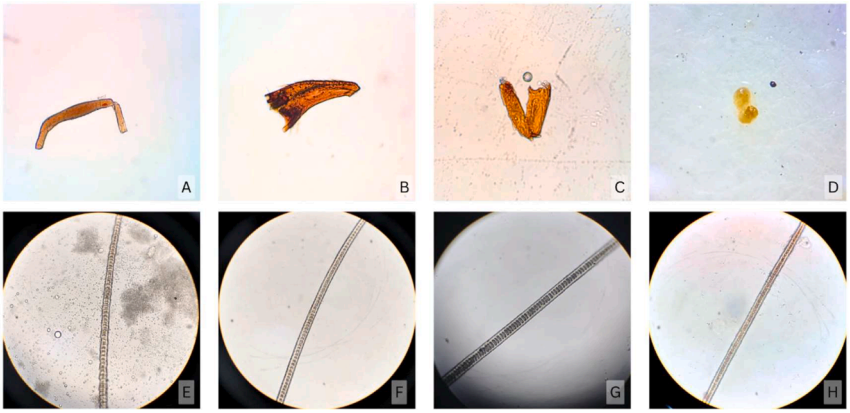

Fig. 2. FMs isolated from PB samples. (A) insect fragment isolated from S6; (B) insect fragment isolated from S11; (C) insect fragment isolated from S20; (D) larva isolated from S30; E, F, G, and H) fragments of mammalian hair isolated from S1, S8, and S9 (400x magnification).

relevant regulations and consumer safety.

Čapla et al. [26] evaluated the presence of FMs in different European regions and, with regarding food commodities, bakery and confectionery products, fruits, vegetables, and convenience foods were those most frequently reported. The authors concluded that about 4 % FM was found in the analyses, which is a relatively significant rate, according to them. The study by Campolo et al. [15] evaluated ground wheat flour and found that half (50 %) of the products analyzed by the light filth method were infested by insects, and that such contamination is probably a consequence of the hygiene and sanitation conditions of the plant.

In our study, samples with FM represented 23 % of the total samples evaluated and 60 % of the brands showed at least one product with FM. No study assessing the presence of FM in plant-based drinks was found in the literature for comparison.

3.2. Analysis and identification of histological elements

In all samples, some typical plant structures of the raw materials listed on the product labels were identified, except for the cabbage in the samples of J brand. When conducting a search for histological elements to identify possible fraud, such as replacement of the main raw material with another element, the result was negative for fraud. It demonstrated that, in general, these beverages actually present the ingredients described on their labels and are safe alternatives for consumers who have food allergies and dietary restrictions.

Because most samples are formulated with a mixture of raw materials and involve manufacturing steps that use heat, many typical cell structures (such as starch grains) are altered, which makes it difficult to recognize these cells and conduct the analysis [2]. Also, this analysis required analysts with deep knowledge and experience in food microscopy, since the recognition of different cell structures from those commonly found for matrices was critical for this study. A previous study was also required, with individual patterns of each plant matrix analyzed for familiarization with plant structures.

One strategy adopted in this study was the preparation of slides with centrifuged material or residues from the prepleated paper used in the FM analysis. The analysis and identification of histological elements of the PB required considerable time, since the analysis of slides with residues from the FM analysis should only be used as a complement to the histological analysis considering that some histological elements (such as starch) are eliminated during the experimental procedure.

Another challenge during the analyses was the similarity between the histological elements of plants in the same sample, such as cashew nuts and Brazil nuts (S16) or soybeans and peas (S27, S28, S29, and S30). Then, the microscopic analysis to identify fraud or sample composition is viable, but takes a long time and requires analysts with deep knowledge. All identifications of the histological elements of plants described below were based on the studies conducted by Winton and Moeller [21], and Menezes Junior [22,23]. Fig. 3 shows examples of plant structures found in some samples.

The analysis of rice-based beverages revealed altered starch substances (which turned blue when Lugol's solution was added), lacking typical grain characteristics. Out of the five samples studied, S1, S2, and S3 showed exclusively displayed rice pericarp cells, while S4 contained both rice and coconut endosperm cell structures. In S5 the presence of cocoa fragments was also identified. Due to its hypoallergenicity, rice-based PB is an alternative for consumers who are allergic to cow's milk or nut and soybean protein [2]. Therefore, assessing and ensuring that these beverages have no fraud is extremely important.

Almond-based beverages, across all samples, exhibited typical almond histological elements, notably large truncated sperm cells, discernible after centrifugation. Samples S6 and S7 lacked characteristic elements of other plants. The presence of cocoa in S8 was easily detected, while S9 and S10 showcased histological elements of almond, coconut, and pea. Pea identification was attributed to palisade cells of spermoderm. The popularity of nut products, like almonds and hazelnuts, owing to their healthful image, has surged in the market. Almond-based PB emerges not only as an alternative to dairy but also to soybean-based beverages [27].

Oat-based beverages, all samples exhibited characteristic histological elements like elongated cells and unicellular, conical hairs of the pericarp. Sample S13 (oat and cocoa), presented a significant proportion of cocoa cotyledonary parenchyma fragments. Cashew nut-based beverages exhibited distinctive histological features, showcasing small and slightly elongated epidermal cells, and well-defined brown cells, validating their authenticity. Among the six samples analyzed, four displayed solely cashew nut elements, while one sample (S16) containing two types of nuts required specific knowledge to differentiate their elements. Identification of Brazil nut elements could only be identified after analyzing the filter paper residue analysis due to similarities with cashew nuts, particularly in brown cells with sclerenchymatized walls. Brazil nuts were distinguished by the presence of large polygonal thick-walled cells, along with globoids and crystalloids. Sample S17 exhibited both cocoa and cashew nut elements.

The peanut-based sample (S21) displayed histological elements of both peanuts and coconuts. Distinctive peanut cells, characterized by polygonal, thick-walled subepidermis cells of the spermoderm, were identifiable, alongside spongy parenchyma cells. However, typical globular starch grains found in peanuts were absent. Samples S22 and S24 isolated coconut histological elements via filter paper residues from FM analysis. Sample S23, listing pea among its ingredients, required centrifugation to identify its characteristic histological elements. According to the study by Tulashie et al. [24], coconut milk, known for its low cholesterol and lactose content, is deemed suitable for vegetarians,

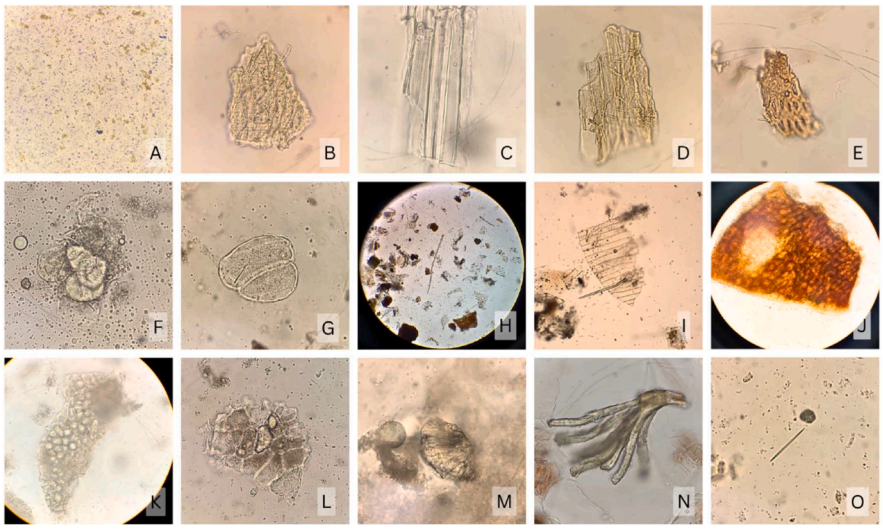

Fig. 3. (A) Altered starch substance in rice. (B) Rice tubular cells. (C-D) Coconut endosperm. (E) Fragment of cocoa cotyledonary parenchyma. (F) Almond endosperm. (G) Pea external parenchyma. (H-I) Plant hair and epicarp cells of oat. (J) Mesocarp with oil cavity in cashew nut. (K) Brazil nut procambium tissue. (L) Peanut subepidermis. (M) Soybean palisade cells. (N) Fragments of the cotyledonary parenchyma of chicory. (O) Pineapple raphide (400x magnification).

lactose-intolerant individuals, and those with heart diseases, thus considered a highly sustainable milk alternative.

Among the six samples assessed soybean-based, two from brand D (S25 and S26) explicitly listed soybeans in their ingredients. In contrast, four samples from brand J (S27, S28, S29, and S30) specifically listed soybean protein in their ingredient lists.

This difference between the main ingredients influences the histological analysis since, during processing, the structure of soybean can change, not allowing histological identification [28].

In all samples, the histological elements of soybean were scarce. When examining the slides, S25 and S26 showed the histological elements palisade and subepidermal cells. In brand J samples, histological elements characteristic of soybean protein isolate, and not soybean protein, were identified [28], showing that attention should be dedicated to the product labeling. The challenges of finding the characteristic elements during the experiment can be explained by the fact that soybean-based PBs listed the highest number of ingredients in their composition, from 8 to 17 ingredients (Table 1).

The largest number of ingredients with possible identification of histological elements was from brand J (5 or 6 different plants) and, all these samples showed, in small quantities in the centrifuged sample, typical histological elements of chicory (elongated epidermal straight-walled cells), pineapple raffia, and palisade cells of pea. In samples S29 and S30, in addition to the elements mentioned above, fragments of cocoa cotyledonary parenchyma were found.

Although concentrate cabbage juice was listed among the ingredients in J brand PB, its histological elements were not identified, probably because of its low concentration (the 12th or 13th item in the ingredient lists) or because filtered cabbage juice was added to the sample.

4. Conclusion

This study shows that microscopic analysis is an important tool for evaluating the hygiene and sanitation conditions of plant-based beverages. As FM was found in 23 % of the samples and in 60 % of the brands analyzed in this study, so monitoring by authorities and manufacturers is recommended to determine whether or not a foreign matter limit should be established for this class of food.

The results of the analysis of histological elements showed that, in general, the list of ingredients of PBs are reliable and safe for consumers seeking drinks with alternative ingredients to milk or any allergen. Identification of histological elements using microscopic techniques proved to be feasible for laboratories with qualified technicians for this type of analysis.

Despite not being a simple technique, with limitations for the identification of mixtures or very diluted ingredients, the histological analysis of food remains an effective method while more precise screening techniques, such as real-time PCR or chemometrics, are more used in food fraud. These results have not been reported before and may support future regulations for plant-based products.

CRediT authorship contribution statement

Maria Isabel Andrekowisk Fioravanti: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Validation, Writing – review & editing. **Elaine Cristina de Mattos:** Data curation, Formal analysis, Methodology, Validation, Writing – review & editing. **Flávia de Carvalho:** Formal analysis, Methodology, Validation. **Beatriz Fernandes Lopes:** Data curation, Formal analysis. **Marcelo Antônio Morgano:** Project administration, Supervision, Writing – review & editing. **Adriana Pavesi Arisseto Bragotto:** Conceptualization, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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