Food Safety and Risk

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Food integrity in plant-based meat marketed in Brazil: Identification of physical contaminants and histological elements

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Abstract

The increasing availability of plant-based (PB) food products has led to a critical need for safety and authentication, and it is essential to understand and assess the risks associated with the rapid expansion of this market. Therefore, this study used microscopic techniques to evaluate the presence of foreign matter and histological elements in 27 PB food products marketed in Brazil as meat substitutes and derivatives. Microscopic foreign matter was found in 100% of the samples. This finding requires further research to distinguish between unavoidable foreign matter, from the field, and foreign matter that can be avoided by improving good manufacturing practices. The results show the importance of monitoring the occurrence of this type of contamination, evaluating the need to establish specific limits for this food category. The study of histological elements provided confirmation of the presence of the main ingredients declared on the labels of most samples. However, six samples (22%) - two PB Ground Beef and four PB Burger samples - showed structures compatible with soy, an ingredient not included on the respective labels. Considering that soy is a potential allergen, this finding raises concerns as to product labeling and consumer safety. Microscopic analysis proved an effective tool for monitoring the integrity of PB food products. This study is disruptive in the research of this food type in Brazil and underscores the need for further research and regulatory initiatives in order to ensure the safety and reliability of this food category.

Keywords Food adulteration, Physical contaminants, Plant-based, Optical microscopy, Food safety

Introduction

The research and development of plant-based meat analog food products have been supported by two pillars: sustainability and healthfulness (Miller et al. 2024). And, in recent years, the growing market demand for foods with plant-based protein (PB) has been driven by rapid technological advances in innovations, leading the food industry to speed up the introduction of these products into the market (Bogueva and Marinova 2024; Tan et al. 2024; Lin et al. 2023).

The rapid expansion of commercially available PB food products has outpaced the development of effective safety and authentication measures. However, it is

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necessary to understand the potential risk factors and assess their safety levels. Despite substantial progress in identifying chemical and biological contaminants, there are still significant gaps, particularly as to cross-contamination and processing-related issues. (Lin et al. 2023; Tan et al. 2024; Karimi et al. 2025).

Physical contaminants are substances unintentionally added to foods that may compromise food safety or suitability (FAO/WHO, 2023). Although they do not always alter the taste or appearance of products, they can pose serious health risks to consumers (Payne et al. 2023). The term "foreign matter" refers to any nonconstituent of a product, associated with inadequate production, handling, storage or distribution conditions or practices, with potential to cause disease or injury. These contaminants can include undesirable raw material parts, such as lumps and bones, or external elements, such as fragments of stones, plastic, insects, sand, rodent feces or even pieces of equipment (ANVISA, 2022a; Duan et al. 2025). In the last 20 years, the presence of foreign matter has accounted for approximately 10% of food recalls, with plastic fragments being the most frequently reported cause (Payne et al. 2023).

According to Brazilian legislation (ANVISA, 2022a), foreign matter can be classified as indicative of failures in Good Practices, when they are associated with inadequate production conditions or practices (e.g.: arthropods considered proper to the crop and storage), and foreign matter that poses risks to human health due to the ability to carry pathogens to food or to harm consumers (example: rodent hair, flies, cockroaches and sharp objects).

Foreign matter tolerance limits for specific food groups are adopted in Brazil and USA due to available technology and production factors (FDA, 2018; ANVISA, 2022a). Although the European Union lacks a specific regulation for physical contaminant limits, the food safety system integrates these aspects within a broader consumer protection framework, such as regulation (EC) 178/2002 (EU, 2002), which includes physical contaminants as food risk, and the Rapid Alert System for Food and Feed (RASFF) includes "foreign bodies" as a monitored risk category and reports this item in its annual reports (EU, 2025).

All food products, including PB ones, are subject to containing numerous and varied contaminants that can cause serious harm to human health. In addition, intentional adulteration or cross-contamination with plant ingredients and possibly animal ingredients that are not declared in the ingredient list may also result in allergic reactions that pose a health risk to consumers. Food fraud is a particular concern in the food supply chain and fraudulent practices of any kind are illegal and undermine consumer trust (Galvin-King et al. 2018; Karimi et al. 2025).

With the increased globalization, in order to ensure quality control and food safety for all consumers, it is important to determine adulteration, authenticity and traceability in the food industry (Feltes et al. 2023). In this context, food microscopy represents a low-cost method that enables identifying histological elements, foreign matter and possible fraud or adulteration by means of detailed visualization of food structures and components (Pauli-Yamada et al. 2021). The study of Fioravanti et al. (2024), with plant-based beverages, demonstrates that microscopic food analysis is an important tool for evaluating hygiene and sanitation conditions, in addition to enabling the identification of histological elements and possible fraud in ready-to-eat foods.

Data on foreign matter presence and ingredients list compliance in PB foods are scarce, and no studies were found involving microscopic analyses of plant-based foods proposed as substitutes for meat and derivatives. Therefore, the objective of this study was to evaluate the presence of foreign matter and histological elements in 27 plant-based food products marketed in Brazil as PB substitutes for meat and derivatives. The results support the evaluation of the adequacy of label information and possible fraud/contamination.

Materials and methods

Samples

A total of 27 PB foods proposed as substitutes to meat and derivatives from five different brands (a, b, c, d, e): plant-based chicken (PBC, $n = 4$); plant-based ground beef (PBGB, $n = 4$); plant-based kibbeh (PBK, $n = 2$); plant-based penne type ready-to-eat pasta (PBP, $n = 2$); plant-based burger (PBB, $n = 9$); plant-based breaded fish strip or fillet (PBF, $n = 4$); plant-based cod fish cake (PBFC, $n = 2$). Sampling comprised plant-based products commercially available in Campinas, São Paulo State (Brazil), that were explicitly marketed as alternatives to meat products. Product selection was based on commercial availability, and at least two different batches of each product were analyzed. The samples were purchased frozen and kept in a freezer in their original packaging until the time of analysis. Sample identifications and label data are shown in Table 1.

Analysis and identification of foreign matter

Due to the differences in sample composition, the study for identification of foreign matter employed two analytical techniques described by AOAC: method No. 993.28 (16.13.02 – Light filth in bean paste), applied to 15 samples (PBC a1, a2, e1 and e2; PBGB a1, a2, e1 and e2; PBB a1, a2 and a3; PBB c1, c2, c3 and c4), and method No. 969.41 (16.6.06 – Light filth in alimentary pastes), applied to 12 samples (PBK b1 and b2; PBP b1 and b2; PBB b1 and b2; PBF b1, b2, b3 and b4; PBFC d1 and d2) (AOAC, 2023).

Table 1 Identification and main information from sample labels ($n = 27$)

Sample	Brand	Commercial name	Ingredients*	Total ingredients**
PBC-a1	a	Chicken	Water, *concentrated soy protein*, *pea protein*, *chickpea protein*, plant oil, salt, *plant fibers*, chicken aroma identical to natural, *spices*, ascorbic acid antioxidant and titanium dioxide dye.	11
PBC-a2			Water, *concentrated soy protein*, *pea protein*, sunflower plant oil, salt, *plant fibers*, yeast extract, chicken aroma identical to natural, ascorbic acid antioxidant and titanium dioxide dye.	10
PBC-e1 PBC-e2	e	Chicken Fillet	Water, *soy protein*, plant oil, *fava bean protein*, gluten, *bamboo fiber*, salt, minerals (Iron and Zinc), Vitamin A, Vitamin B9, Vitamin B12, *strawberry extract*, *tomato extract*, methylcellulose stabilizer and natural flavors.	15
PBGB-a1 PBGB-a2	a	Ground Meat	Water, protein preparation (*Textured soy protein*, *soy protein isolated and pea protein*), coconut fat, canola oil, natural flavor, methylcellulose stabilizer, salt and *beet powder*.	10
PBGB-e1 PBGB-e2	e	Ground Meat	Water, *pea protein*, plant oil, coconut oil, *bamboo fiber*, *fava bean protein*, *cocoa powder*, salt, *onion powder*, *beet powder concentrate*, *garlic powder*, *spinach powder*, *black pepper powder*, minerals (iron and zinc), Vitamin A, Vitamin B9, Vitamin B12, methylcellulose stabilizer and natural flavors.	19
PBK-b1 PBK-b2	b	Kibbeh	Water, *Soy Protein*, *Wheat*, Plant Oil, *Onion*, Gluten, Salt, *Garlic*, Malt Extract, *Parsley*, Iron, Vitamin B12, Flavoring, Methylcellulose Thickener.	14
PBP-b1 PBP-b2	b	Penne	Water, Penne (Iron and Folic Acid Enriched *Wheat Semolina*, Colors: *Urucum and Turmeric*), Plant-based Ground Beef (water, *Soy Protein*, Plant Oil, Salt, *Pea Flour*, *Chickpea Protein*, *Onion*, *Beet Flour*, Iron, Vitamin B12, Flavoring, Methylcellulose Thickener), *Tomato Pulp*, *Tomato*, Grated Plant-based Cheese (*cashew nut*, *Seasonings*, Salt), *Onion*, *Garlic*, Plant Oil, Salt, *Modified Starch*, Sugar, *Parsley*, *Basil*, *Black Pepper*, Iron, Vitamin B12, Flavoring.	25
PBB-a1	a	Burger with Beef Flavor	Water, protein preparation (*Textured soy protein*, *soy protein isolate*, *pea protein and chickpea flour*), coconut fat, canola oil, natural flavor, methylcellulose stabilizer, salt and *beet powder*.	11
PBB-a2 PBB-a3			Water, protein preparation (*textured soy protein*, *soy protein isolated and pea protein*), plant fat, salt, *beet powder*, natural flavor, methylcellulose stabilizer and charcoal dye.	10
PBB-b1 PBB-b2	b	Burger with Beef Flavor	Water, *soy protein*, plant oil, plant fat, wheat gluten, *pea protein*, salt, *onion*, *garlic*, iron, vitamin B12, methylcellulose thickener, natural flavorings, *beet red dye*.	14
PBB-c1 PBB-c2	c	Burger with Beef Flavor	Water, *textured pea protein*, coconut fat, *modified starch*, *onion*, yeast extract, *beetroot powder*, pink Himalayan salt, vitamin and mineral mix, *black pepper*, *garlic*, methylcellulose stabilizer, aroma and ascorbic acid antioxidant.	14
PBB-c3 PBB-c4	c	Burger with Chicken Flavor	Water, *textured pea protein*, coconut fat, *modified starch*, *onion*, yeast extract, pink Himalayan salt, mix of vitamins and minerals, *black pepper*, *garlic*, methylcellulose stabilizer, aroma and ascorbic acid antioxidant.	13
PBF-b1 PBF-b2	b	Breaded Fish Strips	Water, *breadcrumbs*, *soy protein*, plant oil, plant fat, *rice flour*, *cassava starch*, *corn flour* enriched with iron and folic acid, *onion*, gluten, *wheat flour* enriched with iron and folic acid, *wheat protein*, salt, *garlic*, *black pepper*, *dill*, *white pepper* ,iron, vitamin B12, thickeners: methylcellulose and carrageenan, flavorings.	21
PBF-b3 PBF-b4	b	Breaded Fish fillet	Water, plant oil, *breadcrumbs*, *soy protein*, plant fat, *onion*, wheat gluten, *wheat protein*, *rice flour*, salt, *cassava starch*, *corn flour* enriched with iron and folic acid, *wheat flour* enriched with iron and folic acid, *garlic*, *dill*, *black pepper*, iron, vitamin B12, *white pepper*, thickeners: methylcellulose and carrageenan, flavorings.	21
PBFC-d1 PBFC-d2	d	Codfish cake	Water, The New Protein® (*Textured Pea Protein*, *Isolated Pea Protein*, *Isolated Rice Protein*, *Concentrated Lentil Protein*), *Potato*, *Jackfruit*, *Rice Flour*, Olive Oil, Sunflower Oil, Cassava Starch, Corn Flour, *Garlic*, *Onion*, *Black Pepper*, *Parsley*, Himalayan Pink Salt, *Bamboo Fiber*, Rosemary Extract, [EPA (Eicosapentaenoic Acid), DHA (Decosahexaenoic Acid)], Methylcellulose Stabilizer, and Natural Flavorant.	20

*Faithful transcription of label information, **Estimated number of ingredients due to variation in product label description, *Italic*: possibility of identification by histological analysis

The selection of the method was based on the presence or absence of starch in the ingredient list.

Method No. 993.28 (samples without starches)

A 100 g aliquot of the homogenized sample was added to a 2000 mL beaker, along with 400 mL of hot filtered water, being stirred manually for 1 min. Then 200 mL of hot filtered water and 15 mL of Igepal® (Labware) were added, and the mixture was boiled on magnetic stirrer with heating (Model 752 A, Fisatom®) for 10 min. Still hot, the content was transferred to a No. 230 tamis sieve, whose retained material was washed with hot filtered

water (55–70 °C) until the effluent came out clear. The retained material was then transferred to a trap flask (2000 mL) with the aid of 600 mL of 40% isopropanol solution (Dynamic) and boiled for 10 min. After cooling to room temperature, we added 60 mL of P.A. mineral oil (Dynamic) and stirred for 10 min. The trap flask was completed with 40% isopropanol solution, allowed to rest for 20 min, and the oil phase was transferred to a beaker. In the same trap flask, a second extraction was performed by adding 35 mL of P.A. mineral oil, stirring for 30 s, complementing with 40% isopropanol and resting for 30 min, and the oil phase was recovered in a separate beaker.

Method No. 969.41 (samples with starches)

A 100 g aliquot of the previously homogenized sample was transferred to a 2000 mL beaker, adding 2000 mL of filtered water, 50 mL of P.A. hydrochloric acid (HCl, Dynamic) and 1 mL of ethyl ether. The mixture was homogenized with a glass rod, covered with watch glass and cartridge paper, and autoclaved at 121 °C for 15 min. After removing the beaker from the autoclave, the content was transferred, in small portions, to a No. 230 tamis sieve, using hot filtered water, washing successively until the washing liquid was clear and without foam. The retained material was then transferred again to the beaker, adding 30 mL of HCL and 50 mL of P.A. mineral oil, along with a magnetic bar, and the mixture was boiled under magnetic stirring for 5 min. Then the material was transferred to a percolator (2000 mL) containing approximately 250 mL of filtered water and the volume was completed with hot filtered water to about 1700 mL. After resting for 3 min, the liquid was drained until the oil phase lower meniscus reached the 250 mL mark, discarding the drained liquid. The washing and draining process with hot filtered water was repeated until the liquid was clear. At the end, the oil phase was collected in a beaker, and the percolator walls were washed alternately with hot water and ethyl alcohol until, visibly, no sample remained in the percolator.

The samples recovered in the beakers were filtered with creped qualitative filter papers (WHATMAN 40), through a vacuum pump (Model CA, Fanem LTDA). The material collected on the filter paper was observed under a stereoscope (Model SZ-III-BR-SIT, MICRONAL®) (30x magnification) and, when necessary, the impurities were removed, placed on a slide and observed under an optical microscope (model CBA-K, Olympus®) for identification (400x magnification).

All samples were analyzed in duplicate. According to the adopted criterion, analyses would be repeated if differences between replicates exceeded 10 units; however, this was not observed for any sample (Campolo et al. 2012). Results are expressed as number of foreign matter per 100 g of sample, and final values were calculated as the mean of duplicate analyses.

Analytical quality control for foreign matter assays

The foreign matter research methods were evaluated following the guidelines of INMETRO (2020) and the study of Fioravanti et al. (2024) for the figures of merit: limit of detection (LOD) and trend/recovery. The LOD was determined by performing 10 analytical blank assays contaminated with 1 IF standard. The recovery of the 10 readings was 100%, demonstrating that the LODs of the methods are 1 IF. For trend/recovery, all assays were performed independently and the samples were contaminated with IF standard at three different levels: 5, 10 and 15 for *Method No. 993.28;* and 5, 15 and 30 for *Method No. 969.41*. For *Method No. 993.28*, the results were 100% recovery of the standards at levels 5 and 15, and 87% for level 10. For *Method No. 969.41*, recovery was 93%, 100% and 100%, respectively, for the three levels. The recovery of the methods was considered satisfactory, as it presented results above 80% at the different levels.

Analysis and identification of histological elements

The isolation method for identification of histological elements was based on that described by Rodrigues et al. (1999). Briefly, the sample was dissolved in filtered water and the solution was vacuum filtered through 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 for staining the slide. A hydrolysis step (Rodrigues et al. 1999), with a sodium hydroxide solution (Dynamic) at 5%, was necessary in samples with a high number of ingredients, for better visualization and identification of histological elements.

The histological elements characteristic of each sample and possible frauds were identified by comparing the standards available in the Adolfo Lutz Institute microscopy laboratory and the structural characteristics described by Winton and Winton (1906), Menezes Junior (1949; 1958) and Wallis (1968), such as: Palisaded cells and column cells (reels, hourglasses) of spermoderma – soy, pea and chickpea; starch grains – cassava, corn, wheat, pea and black pepper; dotted and crosslinked ducts – beet; thin-walled cells and vessels – onion and garlic; stone cells – black pepper; thin and oval mesocarp cells – tomato; pigmented cells (chloroplasts) - parsley.

All histological analyses were conducted independently by two trained professionals with experience in food microscopy. Identification was based on comparison with laboratory reference standards and well-established literature (Winton and Winton 1906; Menezes Junior 1949, 1958; Wallis 1968). While microscopy is a qualitative

technique and minor misclassification cannot be fully excluded, these procedures ensured robust and reproducible identification of the main ingredients present in the samples.

Results and discussion

Analysis and identification of foreign matter

Macroscopic foreign matter such as whole insects, rigid objects, plastic and metal were not found in the samples. Although foreign matter contamination can occur at different stages of the food production chain—from cultivation, through processing, to transportation and marketing —, the result was expected, since the food industry currently has several technologies capable of significantly reducing the presence of these contaminants in the final product. (Payne et al. 2023; Shi et al. 2023).

Table 2; Fig. 1 show the mean value of duplicate analyses of microscopic foreign matter (FM) identified in the samples, including insect fragments (IF), unidentified mammalian hair (MH), and mites. These can be broadly categorized into components associated with raw materials (e.g., IF and mites) and those potentially related to handling or processing conditions (e.g., MH). This distinction is important because some types of foreign matter are unavoidable, while others may indicate hygienic deficiencies.

A total of 99 microscopic FM were found in the 27 samples. Microscopic FMs were detected in 25 of the 27 samples analyzed (93%). Although two samples (PBC-e2 and PBGB-e1) showed no detectable FM, other batches of the same products (PBC-e1 and PBGB-e2) contained foreign matter (2 and 3 FMs, respectively). Therefore, considering batch variability, all evaluated PB foods were associated with the presence of microscopic foreign matter.

Insect fragments (IF) were the most frequent type of FM, accounting for 63% of the total and were found in 22 of the 27 samples (81%), being identified by their shape, presence of pores, bristles and color (Mattos et al. 2020). Their occurrence is likely associated with raw materials, particularly grains such as corn and soybeans, which are vulnerable to both external and internal insect infestation. Internal infestations are especially difficult to detect, and insects present in bulk grains are often fragmented during milling, resulting in their presence in processed products (Rodrigues et al. 2005; Campolo et al. 2012).

Unidentified mammalian hairs (MH) accounted for 32% of the total FM and were present in 20 samples (74%), with the highest number observed in samples

Table 2 Type and quantity of FM ($\bar{x}$) found in the 27 samples of PB meat analogs and derivatives

Commercial name	Sample	Total FM	$\bar{x}$ IF	$\bar{x}$ MH	$\bar{x}$ Mites	$\bar{x}$ Total for sample
PB Chicken	PBC-a1	23	3	3	1	7
	PBC-a2		10	2	4	16
PB Chicken Fillet	PBC-e1	2	-	2	-	2
	PBC-e2			-		0
PB Ground Meat	PBGB-a1	3	1	-	-	1
	PBGB-a2		2			2
PB Ground Meat	PBGB-e1	3	-	-	-	0
	PBGB-e2		1	2		3
PB Kibbeh	PBK-b1	11	8	1	-	9
	PBK-b2		2	-		2
PB Penne	PBP-b1	11	5	2	-	7
	PBP-b2		3	1		4
PB Burger with Beef Flavor	PBB-a1	12	3	3	-	6
	PBB-a2		4	-		4
	PBB-a3		1	1		2
PB Burger with Beef Flavor	PBB-b1	4	1	1	-	2
	PBB-b2		-	2		2
PB Burger with Beef Flavor	PBB-c1	7	2	2	-	4
	PBB-c2		2	1		3
PB Burger with Chicken Flavor	PBB-c3	9	2	1	-	3
	PBB-c4		5	1		6
PB Breaded Fish Strips	PBF-b1	4	1	2	-	3
	PBF-b2		-	1		1
PB Breaded Fish fillet	PBF-b3	3	1	-	-	1
	PBF-b4		1	1		2
PB Codfish cake	PBFC-d1	7	3	2	-	5
	PBFC-d2		1	1		2
TOTAL (FM)		99	62	32	5	
TOTAL (%)			63%	32%	5%	

FM foreign matter, *IF* insect fragments, *MH* unidentified mammalian hair, $\bar{x}$ mean value of duplicate analyses

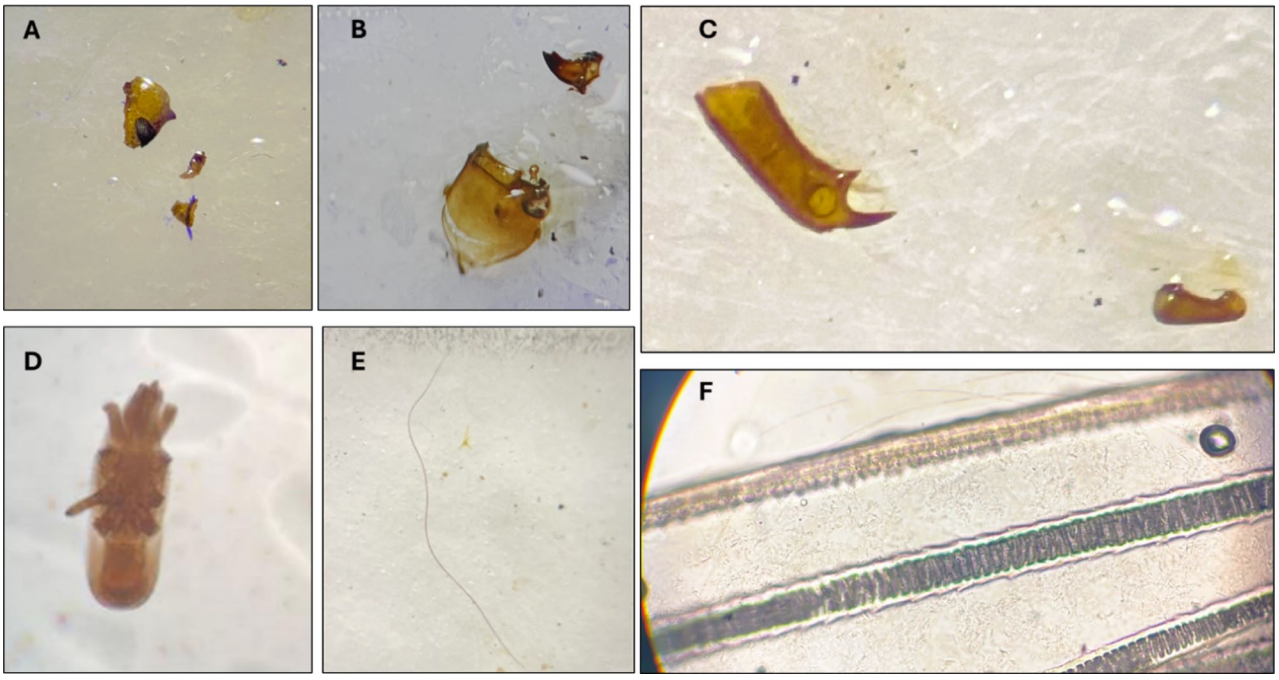


Fig. 1 Examples of FM found in the samples. **A**, **B**, **C** insect fragments; **D** Mite; **E** unidentified mammalian hair (30x magnification); **F** 3 unidentified mammalian hairs (400x magnification). All photos are cropped to highlight the FM (400x magnification)

PBC-a1 and PBB-a1 (three MHs in each). The MHs found were classified as undercoats, based on the characteristics of the marrow and scales (Aquino and Quadros 2022). However, it was not possible to determine the species of origin, since, as described by Aquino and Quadros (2022), only the medullary pattern present in the shield region of the guard hairs has diagnostic value for this purpose. Due to the lack of specific legislation and scarcity of studies available in the literature involving PB products, this finding may be associated with handling, processing environments, or raw materials, highlighting potential points of contamination along the production chain.

Mites represented 5% of the total FM and were found in two samples (7%), both corresponding to plant-based chicken from brand a (PBC-a). The identification was due to their color and shape: body fused in cephalothorax and abdomen, four pairs of legs as an adult and lack of antennae (Hagstrum et al. 2017). Sample PBC-a2 was the sample with the highest number of IFs and mites.

Unavoidable foreign matter (FM) may be present in foods even when good manufacturing practices are applied, and tolerance limits are established by regulatory agencies for specific food categories and ingredients. However, no specific tolerance limits are currently defined for plant-based products marketed as meat alternatives. Based on existing guidelines and the ingredient lists of the sample labels, the presence of FM may be considered acceptable in products containing ingredients such as tomatoes, wheat and corn flour, cocoa, spices, ground black pepper, and peas. The permitted levels of FM vary depending on the type and proportion of raw materials used in the final product (FDA, 2018; ANVISA, 2022a). This highlights a regulatory gap for plant-based meat analogues in Brazil, reinforcing the importance of monitoring and research to inform future standards.

Considering that all analyzed samples contained at least one of these ingredients (Table 1), the presence of FM can be regarded, at least in part, as inherent to this product category. Nevertheless, the levels and types of microscopic FM observed, particularly the presence of MH, suggest the need for improvements in hygienic and sanitary conditions throughout the production chain. It is important to note that these findings do not necessarily indicate a direct risk to consumer health but rather highlight aspects related to product quality and manufacturing practices. Similar considerations have been reported for alternative protein foods, in which the presence of contaminants is often related to raw materials and processing conditions, highlighting the importance of quality control measures rather than indicating an immediate health risk (Tan et al. 2024).

The study of Fioravanti et al. (2024) showed that, of 30 samples of plant-based beverages studied, only six samples contained some type of FM. The FM difference found in the study with plant-based beverages (23%) and in the PB samples of the present study (100%) is directly related to the manufacturing processes and raw materials used for the preparation of the final products. The review by Tan et al. (2024) on the safety of novel food sources

and production systems demonstrates that, as new ingredients, inputs, and production systems are explored, the safety profile of food products may be affected by the unintentional introduction of microbiological, chemical, or physical contaminants. This cautious interpretation frames the presence of mammalian hair as a potential indicator of hygienic weaknesses rather than proof of non-compliance or unsafe products.

Analysis and identification of histological elements

A characteristic observed in the 27 samples analyzed was the major variation in product composition. Even when they are proposed as substitutes to the same type of food, the formulas differ noticeably, including between different batches of the same product (PBC-a and PBB-a). This lack of standardization between PB products has been reported in other studies (Harnack et al. 2022; Bogueva and Marinova 2024; Miller et al. 2024). According to McClements and McClements (2023), much of the criticism of plant-based meat analogs is due to the complexity and long list of ingredients used in these products. This variability in formulation has also been associated with challenges in labeling accuracy and transparency, as the studies cited above have reported inconsistencies between declared ingredients and actual composition in plant-based meat products. In the context of food analysis, histological elements refer to microscopic structures derived from plant or animal tissues that compose the food matrix, such as cells, cell walls, fibers, and conducting vessels. Their identification by microscopy contributes to the determination of product composition, authenticity, and quality.

Based on the lists of ingredients described on the labels (Table 1), 29 histologically recognizable plant constituents were identified in the samples. Of these, five were not identified due to lack of reference standards or descriptions in the literature: bamboo fiber (PBC-e; PBGB-e; PBFC-d), plant fiber (PBC-a), jackfruit (PBFC-d), concentrated lentil protein (PBFC-d) and fava bean protein (PBC-e; PBGB-e). Microscopy has long been applied as a reliable tool for the identification of food components and detection of contaminants, particularly in complex matrices, supporting its use in authenticity and quality assessment studies (Winton and Winton 1906; Fontes and Fontes 2005).

Plant or bamboo fibers are described in 8 samples, and structures resembling plant fibers were observed in all of them, but no standards were found to confirm their histological characteristics. Fibers (from different sources) can act as binding agents to improve the stability, thickness and consistency of PB products and reduce volume loss by cooking (Vila-Clarà et al. 2024).

On the other hand, 10 vegetable ingredients mentioned on the labels were not found in the samples, even with descriptions of their histologies in specific literature of the field (Winton and Winton 1906; Fontes and Fontes 2005; Menezes Junior 1949): tomato and strawberry (PBC-e), cocoa and spinach (PBGB-e), cashew nuts, turmeric, basil and annatto (PBP-b), dill (PBF-b) and potato (PBFC-d). The lack of observation may be related to the manufacturing process, which can lead to degradation of these components, to the form of addition to the food (such as powder, extract or protein), and to the low concentration of these ingredients in the samples, which have complex formulations, containing between 15 and 25 ingredients (Table 1).

Considering the bibliographic references (Winton and Winton 1906; Fontes and Fontes 2005; Menezes Junior 1949) and reference standards available in the laboratory, it was possible to identify 14 ingredients based on the histological elements present in the samples: modified starch ($n = 6$), rice ($n = 4$), beet ($n = 6$), onion and garlic ($n = 14$), pea ($n = 11$), chickpea ($n = 2$), cassava ($n = 6$), corn ($n = 6$), pepper ($n = 15$), parsley ($n = 6$), soy ($n = 25$), tomato ($n = 2$) and wheat ($n = 8$). Table 3 shows the histological elements identified by sample.

Pepper, garlic and onion are added to PB products for flavor and aroma, acting as agents that mask any unpleasant aftertaste from the use of specific raw materials such as soybeans and peas (Vila-Clarà et al. 2024). The histological elements characteristic of these three ingredients are well described in the literature (Winton and Winton 1906) and easy to identify. Both black and white pepper are mentioned on the labels; however, the histological analysis does not enable distinguishing between them, since both originate from the same fruit, differing only by the stage of maturation and the removal or not of the peel during processing (Brasil 2021). In the PBC-a1 sample, histological elements of pepper were identified, and this fact is explained by its ingredient list including "spices." PBC-a1 and PBC-a2 are the same products, however, with different batches and list of ingredients as shown in Table 1. The spices ingredient mentioned in the PBC-a1 label was not mentioned in the PBC-a2 sample.

In the PBGB-e and PBFC-d samples, histological elements of onion and garlic were not found, probably due to the powder form and the high number of ingredients, 19 and 20 items, respectively (Table 1). The lack of detection of these histological elements does not affect the quality of the product, considering that the list of ingredients is described in descending order of proportion of the items (ANVISA, 2022b). It is important to note that the samples are composed of mixtures of raw materials and these may have their cellular structures modified due to the manufacturing processes that involve heat (Silva et al. 2020).

Beet is a coloring agent added to PB products proposed as substitutes to beef, being used to simulate its reddish color. Maintaining color during the production

Table 3 Results and scientific name of the histological elements identified in the samples of PB meat analogs and derivatives

Scientific name	Popular name	Number of samples with the characteristic HE* identified	
-	Modified starch	6	PBP-b1; PBP-b2; PBB-c1; PBB-c2; PBB-c3; PBB-c4
Oryza sativa	Rice	4	PBF-b1; PBF-b2; PBF-b3; PBF-b4
Beta vulgaris	Beet	6	PBGB-a1; PBGB-a2; PBB-a1; PBB-a2; PBB-a3; PBB-c2
Allium sp	Onion/garlic	14	PBK-b1; PBK-b2; PBP-b1; PBP-b2; PBB-b1; PBB-b2; PBB-c1; PBB-c2; PBB-c3; PBB-c4; PBF-b1; PBF-b2; PBF-b3; PBF-b4
Pisum sativum	Pea	11	PBC-a1; PBC-a2; PBGB-a1; PBGB-a2; PBB-a1; PBB-a2; PBB-a3; PBB-b1; PBB-b2; PBFC-d1; PBFC-d2
Cicer arietinum	Chickpeas	2	PBC-a1; PBB-a1
Manihot esculenta	Cassava	6	PBF-b1; PBF-b2; PBF-b3; PBF-b4; PBFC-d1; PBFC-d2
Zea mays	Corn	6	PBF-b1; PBF-b2; PBF-b3; PBF-b4; PBFC-d1; PBFC-d2
Piper nigrum	Black/white pepper	15	PBC-a1; PBGB-e1; PBGB-e2; PBP-b1; PBP-b2; PBB-c1; PBB-c2; PBB-c3; PBB-c4; PBF-b1; PBF-b2; PBF-b3; PBF-b4; PBFC-d1; PBFC-d2
Petroselinum crispum	Parsley	6	PBK-b1; PBK-b2; PBP-b1; PBP-b2; PBFC-d1; PBFC-d2
Glycine max	Soy	25	PBC-a1; PBC-a2; PBC-e1; PBC-e2; PBGB-a1; PBGB-a2; *PBGB-e1; PBGB-e2*; PBK-b1; PBK-b2; PBP-b1; PBP-b2; PBB-a1; PBB-a2; PBB-a3; PBB-b1; PBB-b2; *PBB-c1; PBB-c2; PBB-c3; PBB-c4*; PBF-b1; PBF-b2; PBF-b3; PBF-b4
Solanum lycopersicon	Tomato	2	PBP-b1; PBP-b2
Triticum sp	Wheat	8	PBK-b1; PBK-b2; PBP-b1; PBP-b2; PBF-b1; PBF-b2; PBF-b3; PBF-b4

**HE* Histological element

process is still a challenge, and ensuring that the color changes similarly to meat when cooked is also another aspect to be considered (Sha and Xiong 2020; Vila-Clarà et al. 2024). Despite the difficulty in identifying the histological elements characteristic of beet, a hydrolysis step enabled identifying fragments of layer of tabular, slightly rounded cells and their characteristic large scalariform and crosslinked spiraloid vessels (Winton and Winton 1906). Another evidence of the presence of this vegetable was its characteristic color after dissolution in water.

In the PBGB-e and PBP-b samples, no histological elements of beet were found, which may be related to the number of ingredients used for their preparation (Table 1). The histological elements characteristic of beet were also not identified in the PBB-c1 sample, and this sample has a different color from the PBB-c2 sample, which differs only as to the batch (Fig. 2), being another indication that the beet ingredient was not added to the product.

Wheat, cassava and corn ingredients were easily identified in breaded samples (PBF-b and PBFC-d) and in PBK-b and PBP-b, due to the presence of their characteristic starches and distinct polarizations (Winton and Winton 1906; Menezes Junior 1949). Four Burger samples of brand c (PBB-c) also presented cassava starch, referring to the modified starch ingredient present in the labels. Characteristic rice starches were found in the 4 PBF-b samples, while in the PBFC-d samples they were not found, probably due to the high number of ingredients (Table 1). Histological elements of parsley (PBK-b, PBP-b and PBFC-d) and tomato (PBP-b) were found in all samples in which they are cited (Tables 1 and 2). Descriptions of these histological elements are well presented in the literature and identification is easily performed by a well-trained analyst.

As for protein ingredients, a total of 12 different types are mentioned in the 27 samples studied and most samples ($n = 19$) have more than one protein source on their label. The combination of different protein sources has been studied for several reasons, including the improvement of the sensory properties of the product, the reduction of undesirable characteristics, the enhancement of nutritional value, the availability and cost of protein sources, the influence of the color of each of them and the potential for improving texture (Miller et al. 2024). Figure 3 shows, visually, the list of the proteins most used in the preparation of the samples.

Soy protein ($n = 12$) and pea protein ($n = 11$) were the raw materials most used in the formulation of the analyzed samples, followed by their variations: textured pea protein ($n = 6$), isolated soy protein ($n = 5$) and textured soy protein ($n = 5$). These five are the main protein sources of the samples, since they appear among the first ingredients listed on the labels, not considering water (ANVISA, 2022b). Along with water, proteins represent the predominant components of several plant-based foods, being responsible for providing texture, flavor and appearance (Vila Clarà et al. 2024).

Proteins – such as fava bean, wheat, chickpea, lentil and rice proteins – are commonly used in PB products as fillers to improve texture and consistency, in addition to supplementing and enriching the protein profile, making these products more similar to animal proteins (Miller et al. 2024; Vila-Clarà et al. 2024). For fava bean, wheat, lentil and rice proteins, no histological standards or descriptions were found in the literature.

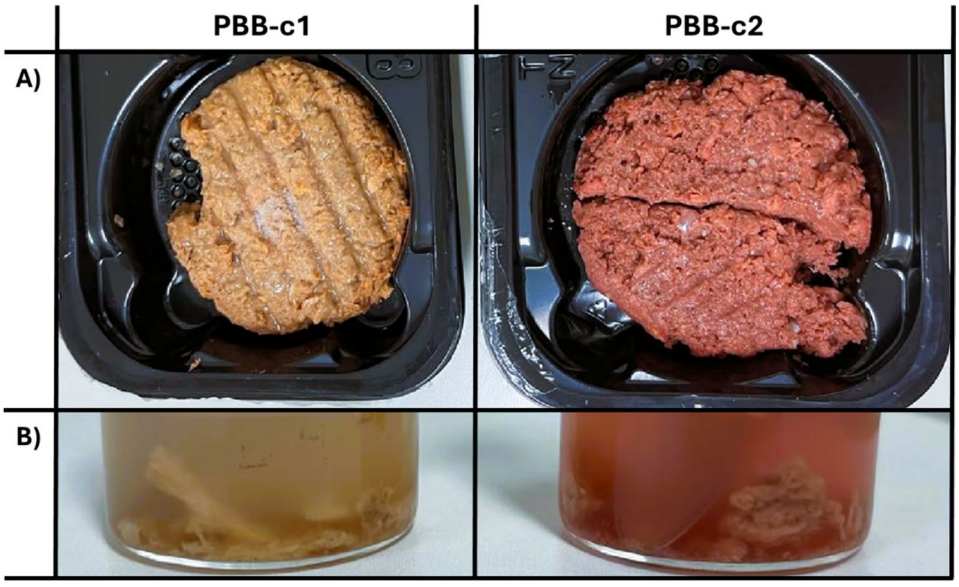


Fig. 2 color comparison for the PBB-c1 and PBB-c2 samples; **A** samples in the original packages; **B** samples diluted in filtered water



Fig. 3 Proteins listed on the labels of the 27 samples

Figure 4 shows the differences between the characteristic histological elements for the identification of products containing: peas, soybeans and chickpeas.

Chickpea was identified by hourglass-shaped column cells, with more rounded ends and their palisades in varying sizes characteristic of chickpea (Fig. 4 - D_1, D_2 and D_3). Another relevant characteristic is their little polarization when compared to the same cells of soybean and pea (Winton and Winton 1906). The detection of histological elements of chickpea in the PBC-a1 sample, similar to those observed in PBB-a1 (which declares chickpea flour in its composition, Table 1), suggests the presence of this ingredient in the sample. However, it is not possible to confirm whether it is specifically chickpea protein, due to the lack of reference standards. In the Penne samples (PBP-b), no chickpea elements were identified.

The identification of soy protein, concentrated protein and textured protein was possible through the presence of column cells that present intense polarization and long palisaded cells (Fig. 4 C_1), which, when compared to the other two legumes, peas and chickpeas, are larger (Fig. 4A) (Winton and Winton 1906). Isolated soy protein was identified through the presence of a spherical/oval structures, which had a characteristic reel shape inside and, when adding lugol, acquires an amber color (Fig. 4 - C_2 and C_3). This observation contradicts the study of Mattos et al. (2015), which concluded that soy protein isolates have round shapes without distinct microscopic structures. This difference may be due to changes in the

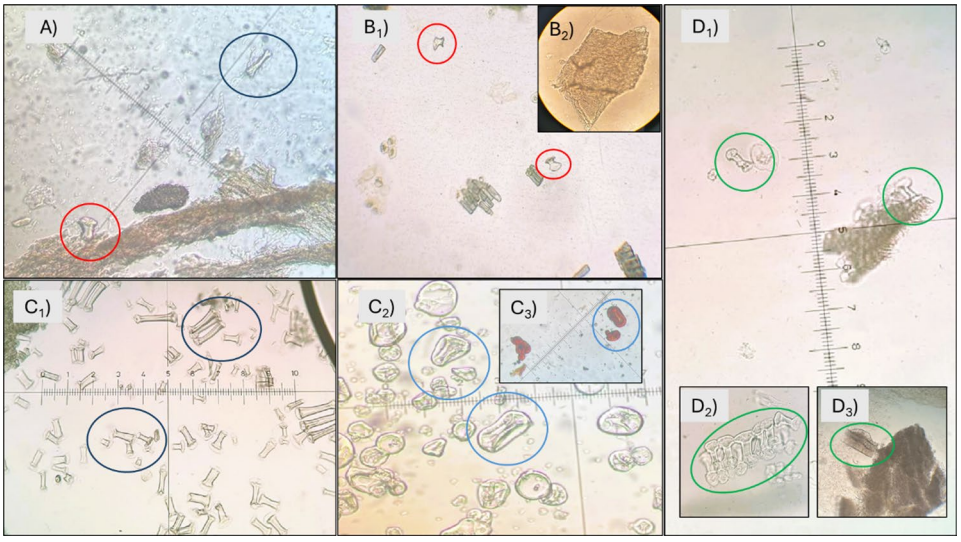


Fig. 4 **A** difference of reel cells of soybean (blue circle) and pea (red circle), sample PBC-a2; **B** Standards of characteristic elements of pea - **B1**) short and wide column cells (red circle), and short and linear palisaded cells, **B2**) standard of textured pea protein (amorphous structure); **C** Standards of characteristic elements of soybean - **C1**) long column and palisaded cells (blue circle), **C2**) spherical/oval structure, which had a reel shape inside (light blue circle), **C3**) highlight of isolated soy protein in the presence of lugol with amber coloration; **D** Standards of characteristic elements of chickpea - **D1**) reel cells, with more rounded ends and their palisades in varying sizes (green circle), **D2**) highlight of reel cells, **D3**) highlight of palisaded cells. 1 mm ruler. All photos are cropped to highlight (400x magnification)

industrial process for obtaining soy protein isolate in recent years (Banach et al. 2022; Alasi et al. 2024; Ihsan et al. 2024).

Characteristic histological elements were found in all 19 samples (Table 1) that declare soy protein and its derivations. In addition, six samples that do not have soy among their ingredients, four PBB-c and two PBGB-e samples, also presented histological elements of soy. This fact may be due to cross-contamination, label inconsistency or fraud. The "e" brand samples had on their labels the information "*Allergens: may contain oats, rye, barley, soy and wheat*," which is a mandatory requirement for labels of the main foods that cause food allergies marketed in Brazil (ANVISA 2015).

Pea protein and protein isolate were identified by verifying the presence of short and wide column cells, with many being together with short and linear palisaded cells (Fig. 4 - B_1 and B_2), inside which a thin line can be observed (Wallis 1968). These characteristics were also observed in PBP-b samples containing pea flour in their composition. Of the 17 samples that list pea protein and pea protein isolate, in 11 their identification was easily performed (Table 2). The textured pea protein standard obtained for this study presents amorphous structures, whose identification is not possible (Fig. 4 B_2). Despite the aforementioned difficulties, the histological structures observed in the PBGB-e and PBB-c samples were conclusive as to the identification of soy, ruling out the presence of pea.

Finally, the study of histological elements in the samples showed that 78% of the samples are consistent with the main protein sources described on their labels. However, the fact that 22% of the samples were inconsistent due to the presence of soy—a raw material not described on the label—indicates a concern about the quality of this category of products, considering that soy is an allergenic food. Food allergies, triggered by allergenic compounds present in foods and beverages, may pose potentially serious consequences. The growing popularity of plant-based and lactose-free diets has raised concerns about cross-contamination with allergenic proteins during manufacture (Karimi et al. 2025).

The distribution of histologically identified ingredients varied across product types and brands, with soy and pea being the most consistently detected protein sources. Inconsistencies between label information and histological findings were observed in 22% of the samples, particularly due to the detection of undeclared soy, indicating variability in formulation control.

These findings highlight the complexity of plant-based formulations and the challenges associated with ingredient verification, reinforcing the relevance of microscopy for food authenticity assessment. Although microscopy has limitations in highly processed and complex matrices, where components may be present in low concentrations or structurally altered, it remains a valuable analytical tool. While complementary techniques such as PCR-based methods, ELISA, and spectroscopic approaches may offer higher sensitivity, they are often costly and not always readily applicable, particularly for newly developed products such as plant-based meat analogues.

In this context, the detection of label inconsistencies or undeclared allergens remains analytically challenging, and microscopy continues to be a viable and effective approach, especially when performed by well-trained analysts. Despite the limited literature on physical contaminants in plant-based meat analogues, this study contributes to advancing knowledge in this emerging field.

Conclusion

Microscopic analysis is an effective tool for monitoring plant-based food products proposed as substitutes for meats and derivatives. The detection of foreign matter in all samples highlights the need for additional studies, especially due to the high number of mammalian hairs identified in a same sample. Such occurrence should be further researched in order to understand whether this contamination can be minimized through the improvement of good manufacturing practices. The research and identification of PB histological elements, despite limitations and complexity, proved effective in identifying the main components present in the products and revealed possible label inconsistency or undeclared allergen presence. The histological identification of protein sources was possible for soy and pea through acquired standards and literature descriptions.

The presence of soy, a known allergen, in six products (two different brands) that have no declaration of this ingredient on their labels represents an alert for better monitoring and control of this category of foods. In addition, the absence of the raw material (beet) declared in a sample proposed as substitute to beef burger shows a lack of standardization and control in the manufacture of this product.

This study is disruptive for plant-based meat analog food products and demonstrates the need for further studies and inspection in order to ensure consumer safety.

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Authors' contributions

Maria Isabel Andrekowisk Fioravanti : study concept, methodology, validation, analysis, and formal investigation, data curation, writing (original draft, review, and editing).Elaine Cristina de Mattos : methodology, validation, analysis, formal investigation, data curation, writing (review and editing). Isadora de Oliveira Silva : methodology, validation, analysis.Flávia de Carvalho : methodology, validation, analysis.Marcelo Antônio Morgano: project administration, study concept, writing (review and editing), resources. Adriana Pavesi Arisseto Bragotto : study concept, writing (review and editing), resources.

Data availability

The Data that support the findings of this study are available from Instituto Adolfo Lutz (IAL). The data are available upon request and with the permission.

Declarations

Competing interests

The authors declare no competing interests.

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