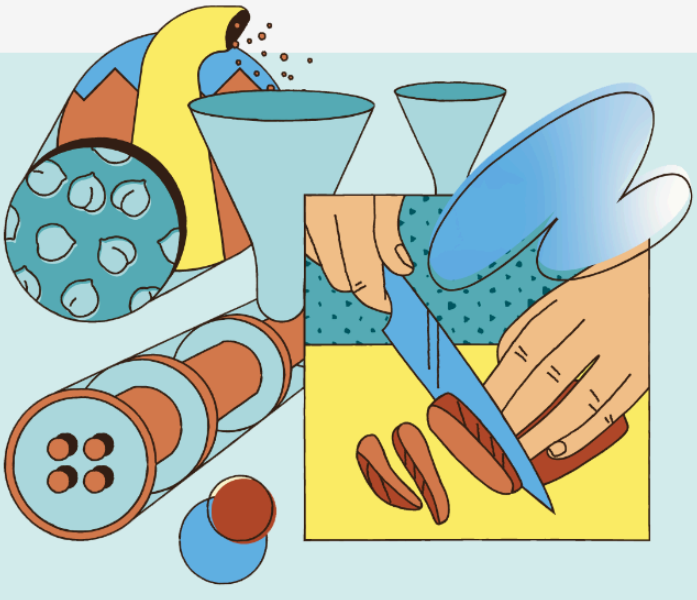
Technical report

Safety aspects in the production of plant-based meat

Plant-based beef - Incrível!



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Credits

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The Good Food Institute is a non-profit organization working globally to accelerate innovation in the alternative protein market. We believe that the transition to a more sustainable food system is fundamental to addressing the climate crisis, decreasing the risk of zoonotic diseases, and feeding more people with fewer resources. That is why we collaborate with scientists, investors, entrepreneurs and government agents to develop plant-based, cultured, or fermentation-enabled food products.

Our work is focused on three main areas:

In **Corporate Engagement**, we support companies of all sizes to develop, launch, and market alternative protein products, offering tools that assist startups and entrepreneurs in their business strategies. We also provide market intelligence to help companies make decisions, conducting research to identify and overcome challenges.

In **Science and Technology**, we fund cutting-edge research on alternative proteins, foster collaboration between scientists, companies, and governments, publish data and findings to drive scientific progress, and design educational programs to build the capacity of the next generation of leaders in alternative proteins.

In **Public Policies**, we advocate for public policies that support the development and marketing of alternative proteins, work with governments to create a favorable regulatory environment, educate the public about the benefits of alternative proteins, monitor the political landscape, and defend the interests of the sector.

With this work, we seek solutions to:

- **Safely, equitably and sustainably feed nearly ten billion people by 2050;**
- **Contain climate change caused by the current food production system;**
- **Create a food production chain that does not depend on animals;**
- **Reduce the contribution of the food sector to the development of new infectious diseases, some of which with pandemic potential.**

In just over six years of operation in Brazil, GFI has already helped the country to become one of the main players in the global plant-based protein market. The goal is to continue conducting this work to transform the future of food, promoting new protein sources and offering alternative proteins analogous to animal-based proteins.

Executive Summary

This document is a summary of the most relevant information found in the publication "Development and application of the HACCP plan in plant-based meat," developed by the Institute of Food Technology (ITAL), in collaboration with Liner Consultoria and with support from The Good Food Institute Brasil (GFI). This summary presents the flowchart and description of the processing steps of four types of plant-based meats (plant-based fish, chicken breast, beef burger and sausage), the possible safety hazards identified in the Hazard Analysis and Critical Control Point (HACCP) plans developed, the measures defined in the HACCP plans for hazard control, and the gaps in scientific information identified during the study.

It is worth noting that the study covered from the raw material selection to the final product in order to exhaustively analyze the potential hazards inherent in the production of plant-based meats. Accordingly, it is important to emphasize that identifying these possible hazards does not imply their presence in the products, but rather the need to implement strict control measures to ensure food safety. It is also important to emphasize that the food industry has the tools and knowledge to minimize these risks, and the presence of these hazards in final products is not a rule, but a possibility that requires attention. Another important point is that no hazard found is exclusive to the category of plant-based foods; all are known and can or cannot be found in other similar foods.

Gluten and soy protein were identified as possible allergenic hazards for the four products. *Salmonella* spp, *Escherichia coli* (sanitary and hygienic quality indicator), *Listeria monocytogenes* and *Bacillus cereus*, common to the four products, and spores of *Clostridium botulinum*, relevant in plant-based fish, were identified as possible biological hazards. Sand, ferrous and non-ferrous metal fragments, and flexible plastic or non-rigid polymer, in the four products, and insect fragments, relevant in plant-based chicken and sausage, were identified as possible physical hazards. Acrylamide (propenamide), aflatoxins B1, B2, G1 and G2, arsenic, cadmium, lead, copper, chemical contaminants in water, DON (Deoxynivalenol or vomitoxin), 3-MCPD esters (3-monochloropropane-1,2-diol or 3-chloropropane-1,2-diol), glycidol esters, fumonisins (B1+B2), migration of packaging material components, ochratoxin A, pesticide residue (general) and ZEA (zearalenone), for the four products, dioxins, furan and methylfurans, relevant in plant-based chicken, burger and sausage, and ethylene oxide, exclusive for sausage, were identified as possible chemical hazards.

The study also showed gaps in scientific information necessary for a better understanding of food safety issues, which may guide future research. Notably, these gaps include a lack of data on the incidence and prevalence of microorganisms, mycotoxins, heavy metals, pesticide residues and natural plant toxins in ingredients and final products, as well as data on processing-induced toxic compound formation. Considering the identified gaps and the growing importance of plant-based meat for a more sustainable future, it is essential that regulatory agencies, industry, researchers and universities join efforts to advance the evaluation, proposition and implementation of actions that seek to ensure the safety of these products, including updating legislation, investing in research and development, implementing food safety management systems, and training qualified professionals.

Keywords: plant-based meat; hazard analysis; plant-based proteins.

Highlights

- The study highlighted the need for further research to understand the incidence and prevalence of contaminants in ingredients and final products, as well as the formation of toxic compounds during processing.
- The study emphasized the importance of implementing strict control measures at all key stages of production and ensuring the proper implementation of prerequisite programs to ensure the safety of these foods.

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α-ZEL	alpha-Zearalenol
β-ZEL	beta-Zearalenol
3-MCPD	3-monochloropropane-1,2-diol or 3-chloropropane-1,2-diol
ABNT	Brazilian National Standards Organization
AME	Alternariol Methyl Ether
ANVISA	Brazilian Health Regulatory Agency
AOH	Alternariol
HACCP	Hazard Analysis and Critical Control Point
CDC	Centers for Disease Control and Prevention
CFA	Chilled Food Association
DON	Deoxynivalenol
EFSA	European Food Safety Authority
ENA	Eniatine
EU-FORA	European Food Risk Assessment Fellowship Programme
ANFs	Antinutritional Factors
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GFI	The Good Food Institute
HAA	Heterocyclic Aromatic Amine
IAFP	International Association for Food Protection
IARC	International Agency for Research on Cancer
IgE	Immunoglobulin E
ISO	International Organization for Standardization
ITAL	Institute of Food Technology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MAS	Monoacetoxyscirpenol
MPN	Most Probable Number
NSW	New South Wales
OTA	Ochratoxin A
CP	Control Point
CCP	Critical Control Point
PE	Polyethylene
PET	Polyethylene terephthalate (acronym also used for polyester, which is the generic term)
PRP	Prerequisite Program
OPRP	Operational Prerequisite Program
RASFF	Rapid Alert System for Food and Feed
RBA	Risk-Benefit Assessment
RDC	Collegiate Board Resolution (ANVISA)
SCIRP	Scirpentriol
STEC	Shiga toxigenic *Escherichia coli*
CFU	Colony Forming Unit
WHO	World Health Organization
ZEA	Zearalenone

Content

1. Introduction

The production of plant-based meat (plant-based products that mimic the color, flavor, texture and appearance characteristics of products made from meat) has grown significantly worldwide in recent years, with the rapid development of new products, new ingredients and new production processes, involving manufacturers ranging from *startups* to large and well-established multinational meat product companies.

This growth requires careful safety inspection for consumer health, since the formulation of these foods tends to present a greater diversity of ingredients compared to conventional meat products, which presupposes a wide variety of sources from which hazards can arise. The protein sources most commonly used for the production of plant-based meat include legumes, seeds, cereals, and tubers; oil and fat sources include canola, soybean, sunflower, coconut, palm, and cocoa butter; and starch sources include corn, wheat, potatoes, and cassava.

The hazards arising from plant ingredients depend on the original raw material (soil, agricultural inputs in plant cultivation, harvest, storage, and transport methods) and how they are processed to obtain the final ingredients. Plant raw materials in general carry microorganisms, including pathogenic species, and can also be contaminated with mycotoxins, heavy metals, pesticide residues, and other contaminants, which can reach the final product, in addition to toxic compounds that can be induced by processing.

A fact to be noted in this context of rapid evolution and constant innovation is the lack of a systematic assessment of safety aspects in the manufacture of plant-based meat, as is the case for animal-based equivalents. It is also noted that several products available on the market are manufactured by the meat derivatives industry, whose hazards and controls are already well established; therefore, there is a need for assessment and further understanding of the suitability of these controls to ensure the safety of plant-based products.

Thus, the objective of this study was to conduct an assessment of the safety aspects in the production of plant-based meat. The reference products for the study were chosen so as to include in the project the main plant ingredients commonly used in the formulation of plant-based meat (six types of proteins, four types of lipids, and three botanical sources of starch), two technological production processes (dry extrusion and wet extrusion), two types of product format (ready-to-eat and raw) and three preservation methods (sterilization, product stable at room temperature, freezing and refrigeration).

It is important to note that the product descriptions included in this document were not intended to present a formulation to be followed by manufacturers, even because there is a huge range of ingredients that can be used, in different combinations, to meet technological, nutritional, functional, and consumer preference needs. This study also had no objective of exhausting the assessment of hazards associated with such products, due to the diversity of sources from which such hazards may arise. The objective of the study was to trace and establish the main points that must be controlled in the production chain of plant-based meat, as well as to understand the points that require more in-depth scientific research to trace and control the hazards.

This study is the first conducted under this entire national and international theoretical and regulatory framework, and provides valuable technical and scientific inputs for legislators, researchers, teachers, rural producers and entrepreneurs in this segment, as well as to professionals working in the area of Quality Assurance Management. Its results may serve to support the implementation of effective controls in plant-based meat processing industries, as well as for the discussion of regulatory measures to be adopted by the agencies responsible for their regulation.

2. Methodology for identifying safety hazards

To identify biological, chemical, and physical hazards relevant to the safety of products covered by the study regarding public health, we examined the legislation of the Brazilian Health Regulatory Agency (Anvisa), subordinated to the Ministry of Health of Brazil, which establishes a standard for contaminants in food. Brazil's legislation is one of the most complete and comprehensive in the world, setting standards for a multiplicity of contaminants in an extensive range of products, in an organized and systematic manner. However, such standards apply to the end of the shelf life of food products intended for final consumers, offered for sale at retail. Although not applied to ingredients intended for the manufacture of food products, whose standard must be that of the newly produced product and not that at the end of the shelf life, such legislation can be used as a guide in the case of dehydrated ingredients used in the formulation of plant-based meat analogs, which, due to drying, do not undergo change in the contaminant load over the storage time.

The *Codex Alimentarius* recommendations regarding standards, guides and codes that aim to ensure food safety were also considered, as well as publications from several international organizations and committees such as the CDC (Centers for Disease Control and Prevention) in the United States, EFSA (European Food Safety Authority) in Europe, FDA (Food and Drug Administration) in the United States, IARC (International Agency for Research on Cancer), JECFA (Joint FAO/WHO Expert Committee on Food Additives), WHO (World Health Organization), and European Union regulations.

3. Flowchart, description of product processing steps, and considerations on product shelf life, distribution, and preparation instructions

The processing flowchart for the plant-based chicken breast is shown in Figure 1, and the description of the product processing steps and considerations on product shelf life, distribution, and preparation instructions are shown in Chart 1.

The processing flowchart for the plant-based fish is shown in Figure 2, and the description of the product processing steps and considerations on product shelf life, distribution, and preparation instructions are shown in Chart 2.

The processing flowchart for the plant-based beef burger is shown in Figure 3, and the description of the product processing steps and considerations on product shelf life, distribution, and preparation instructions are shown in Chart 3.

The processing flowchart for the plant-based sausage is shown in Figure 4 and the description of the product processing steps and considerations on product shelf life , distribution and preparation instructions are shown in Chart 4.

It is worth noting that the HACCP plans prepared in the complete study present a detailed description of each material used during product processing, including origin, geographical origin, composition, production method, packaging, transport, storage, shelf life, use method, acceptance criteria, hazards, and associated legislation.

FIGURE 1: PROCESSING FLOWCHART FOR THE PLANT-BASED CHICKEN BREAST.

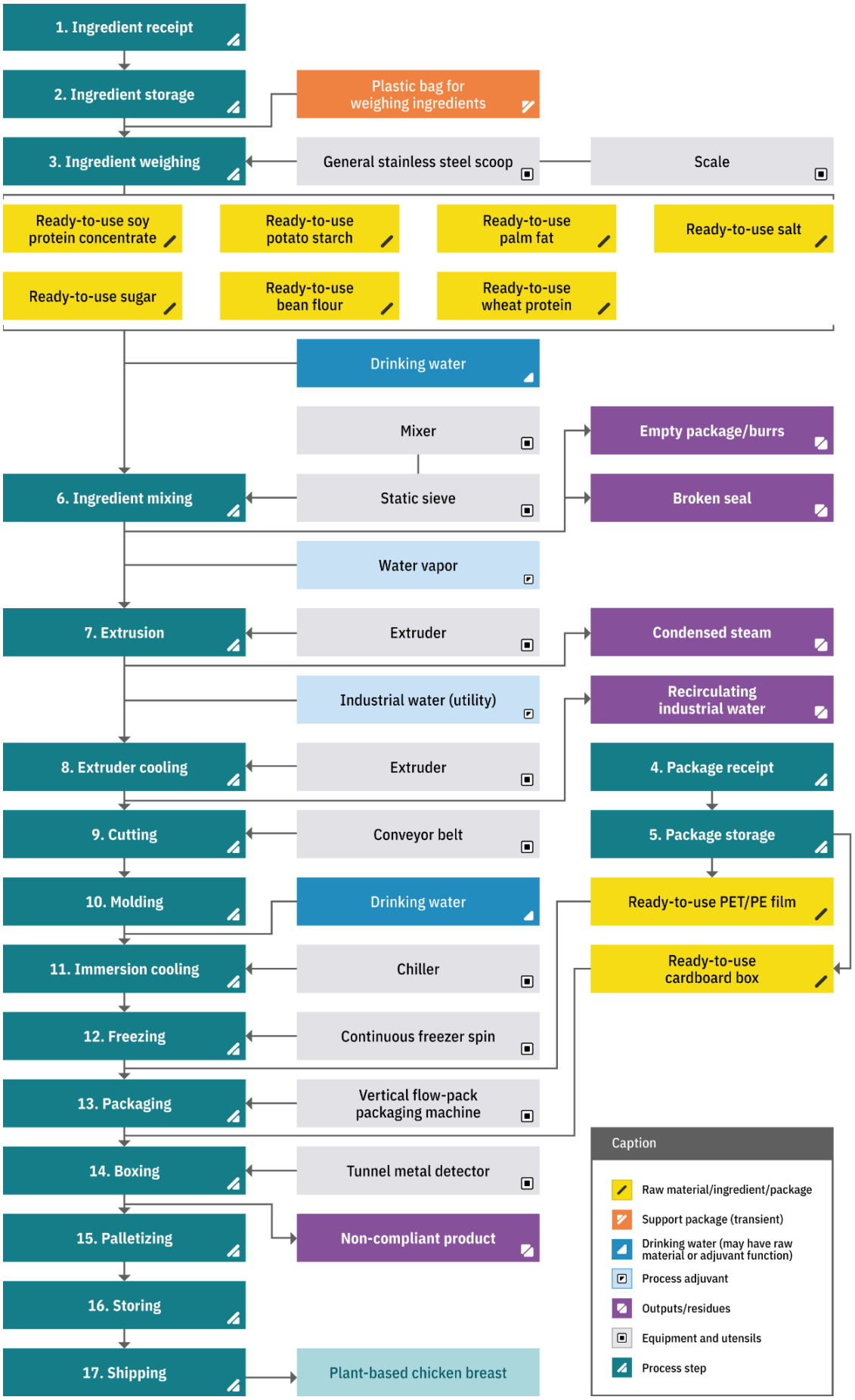

CHART 1: DESCRIPTION OF THE PRODUCT PROCESSING STEPS AND CONSIDERATIONS ON PRODUCT SHELF LIFE, DISTRIBUTION, AND PREPARATION INSTRUCTIONS FOR THE PLANT-BASED CHICKEN BREAST.

Step	Description
1. Ingredient receipt	Upon receipt, supplier documentation is checked, and an inspection is carried out to check the integrity of the material received and the hygiene conditions/presence of pests in the transport.
2. Ingredient storage	Ingredients are transported to the warehouse for storage on-site and under designated conditions.
3. Ingredient weighing	The ingredients are transported in their closed packages to the weighing room, where manual weighing is carried out with the aid of scoops, according to product formulation. The heavy material is packed in plastic boxes.
4. Package receipt	Upon receipt of the packages, an inspection is carried out to check the integrity of the material received, the hygiene conditions/presence of pests in the transport, and the specific characteristics for packaging frozen products.
5. Package storage	The packages are transported to the warehouse for storage at the designated location.
6. Ingredient mixing	Heavy ingredients are transported in closed plastic boxes to the mixer, which is fed manually.
7. Extrusion	Weighed and mixed powder ingredients are transported to the co-rotating twin screw extruder with high moisture die, manually or through helicoidal screw conveyor, being added to the extruder feed hopper by a gravimetrically controlled feeder, for mixing before hydration. The feed system is horizontal and gravimetric, regulated at a constant feed speed of 6 kg/h (dry basis). The mixed ingredients move through the equipment to the compartment, where they are hydrated with drinking water fed through an automatically controlled peristaltic pump. The moisture content during the extrusion process is 40% to 70%, and to reach these values, the indicated speed of water addition is 4.8 to 8.4 kg/h, calculated considering the initial moisture of the powder ingredients (proteins, flours and starches). In this step, oil is also added using an individual pneumatic pump system for each of the liquids. In the co-rotating twin-screw extruder's barrel, in a continuous process, the hydrated mixture enters the worm screw, where it is submitted to shear, high pressure, and heating. These operations result in cooking, denaturation and loss of solubility of proteins and gelatinization of starch. The extrusion parameters are rotation at 110-220 rpm and barrel temperature of 25-60 °C (1st zone), 35-90 °C (2nd zone), 105-155 °C (3rd zone), 136-155 °C (4th zone), and 110-165 °C (5th zone).
8. Extruder cooling	Cooling matrix temperature ranges from 30 to 80 °C, being controlled by the circulation of cooled water. It is the temperature gradient in the slow cooling and the shear stress when crossing the long cooling matrix (about 1.2 meters with reduced exit hole) that promote the direction and formation of the fibers of the plant-based product.
9. Cutting	Upon exiting the extruder die, the textured extrudate is deposited on a conveyor belt and cut by cutting blades according to the specified size.

10. Molding	On the conveyor belt, the textured and cut extrudate is submitted to molding, acquiring the final shape after passing through a heated grid-type stamping press.
11. Cooling	On the conveyor belt, the material is cooled using *chillers* or a mechanical cooling system until the internal temperature of the product reaches about 4° C.
12. Freezing	The cut and molded extrudate follows on the conveyor belt passing through the freezing tunnel with a temperature ranging from minus 25 °C to minus 35 °C, for an exposure time that is sufficient so that the product core reaches a temperature of minus 18 °C.
13. Packaging	Upon exiting the freezing tunnel, the frozen product is directed to the packaging system equipped with rolls of polyethylene terephthalate (PET) plastic film. The formed plastic bags are filled with the frozen product units according to the weight specified for the package. Then, each bag is automatically packed in a pre-formed carton cartridge or sleeve.
14. Boxing	The carton packages are packed in corrugated cardboard boxes in an automated process. The boxes are identified with coded labels. In this step, the packages also pass through a metal detector, with separation of those containing any metallic particles equal to or greater than 2 mm.
15. Palletizing	In this step, the boxes are manually placed on pallets. Once formed, the pallet is wrapped with stretch film and identified with coded labels, forming a loading unit.
16. Storing	The loading units are stored in a cold chamber at a temperature not higher than minus 25°C.
17. Shipping	This step involves loading and shipping the finished product, which must be transported in refrigerated trucks at a temperature of no higher than minus 12 °C.
Considerations on product shelf life, distribution, and preparation instructions	For the pilot study, this product was considered a frozen food with a shelf life of 12 months. The recommendation is that the product is transported in conditions such so as not to reach a temperature above minus 12°C, palletized and organized in vehicles in good condition, permanently equipped with a calibrated easy-to-read thermometer, clean, closed with protection against weather and contaminants, without evidence of pests and/or other animals, without sharing space with non-food products, free of toxic products, substances and objects foreign to the activity, thus ensuring the integrity and quality of the products. The recommendation is refrigerator defrosting (product surface temperature should not exceed 10°C) or, optionally, rapid defrosting as in a microwave oven, for example. In case of leftovers after consumption, disposal is recommended.

FIGURE 2: PROCESSING FLOWCHART FOR THE PLANT-BASED FISH.

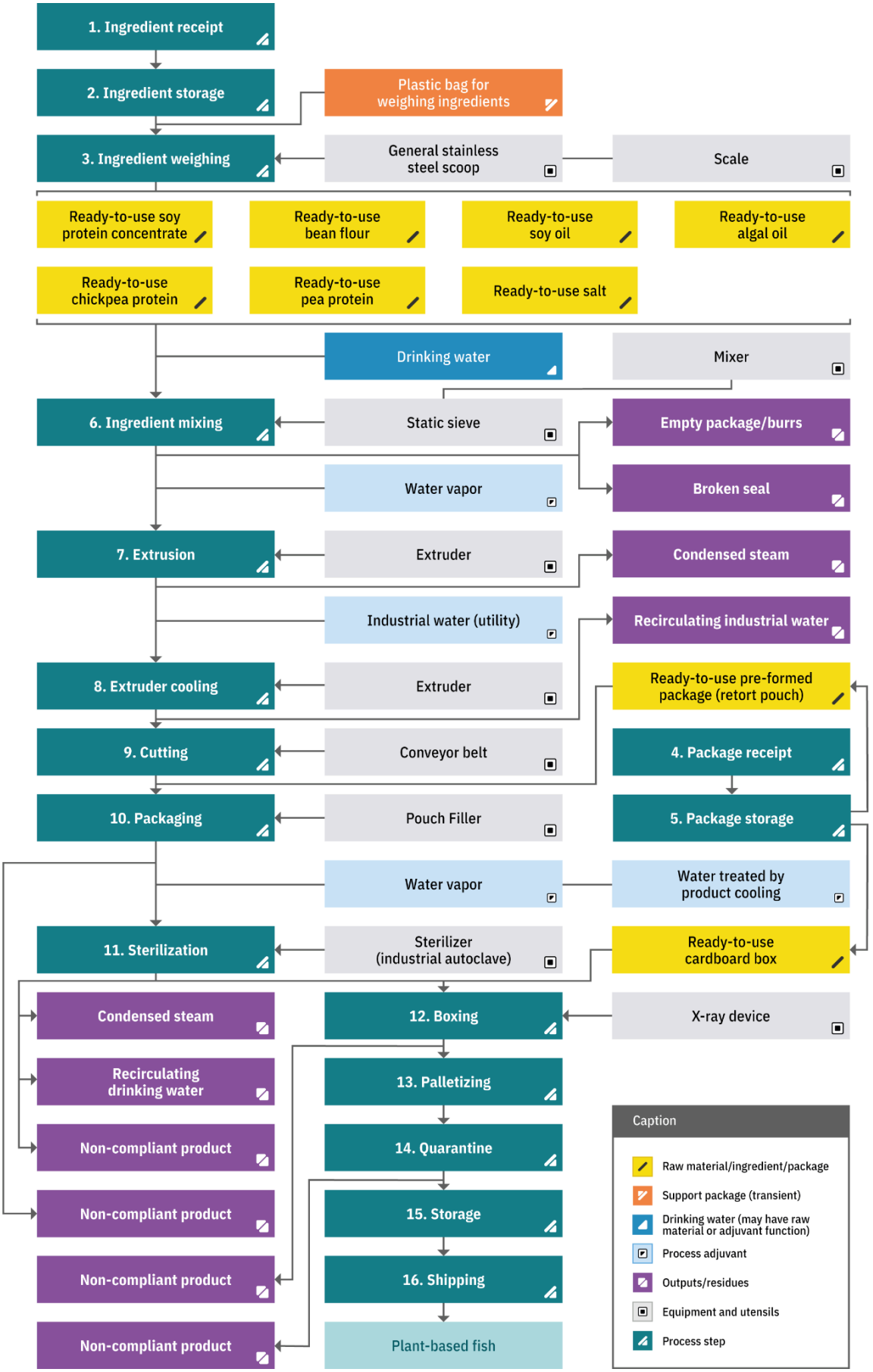

CHART 2: DESCRIPTION OF THE PRODUCT PROCESSING STEPS AND CONSIDERATIONS ON PRODUCT SHELF LIFE, DISTRIBUTION, AND PREPARATION INSTRUCTIONS FOR THE PLANT-BASED FISH.

Step	Description
1. Ingredient receipt	Upon receipt, supplier documentation is checked, and an inspection is carried out to check the integrity of the material received and the hygiene conditions/presence of pests in the transport.
2. Ingredient storage	Ingredients are transported to the warehouse for storage on-site and under designated conditions.
3. Ingredient weighing	The ingredients are transported in their closed packages to the weighing room, where manual weighing is carried out with the aid of scoops, according to product formulation. The heavy material is packed in plastic boxes.
4. Package receipt	Upon receipt of the packages, an inspection is carried out to check the integrity of the material received and the hygiene conditions/presence of pests in the transport.
5. Package storage	The packages are transported to the warehouse for storage at the designated location.
6. Ingredient mixing	Heavy ingredients are transported in closed plastic boxes to the mixer, which is fed manually.
7. Extrusion	Weighed and mixed powder ingredients are transported to the co-rotating twin screw extruder with high moisture die, manually or through helicoidal screw conveyor, being added to the extruder feed hopper by a gravimetrically controlled feeder, for mixing before hydration. The feed system is horizontal and gravimetric, regulated at a constant feed speed of 6 kg/h (dry basis). The mixed ingredients move through the equipment to the compartment, where they are hydrated with drinking water fed through an automatically controlled peristaltic pump. The moisture content during the extrusion process is 40% to 70%, and to achieve these values, the indicated speed of water addition is 4.8 to 8.4 kg/h, calculated considering the initial moisture of the powder ingredients (proteins, flours and starches). In this step, oil is also added using an individual pneumatic pump system for each of the liquids. In the co-rotating twin-screw extruder's barrel, in a continuous process, the hydrated mixture enters the worm screw, where it is submitted to shear, high pressure, and heating. These operations result in cooking, denaturation and loss of solubility of proteins and gelatinization of starch. The extrusion parameters are rotation at 110-220 rpm and barrel temperature of 25-60°C (1st zone), 35-90°C (2nd zone), 105-155°C (3rd zone), 136-155°C (4th zone), and 110-165°C (5th zone).
8. Extruder cooling	The cooling matrix temperature ranges from 30 to 80°C, and it is controlled by the circulation of cooled water. The temperature gradient in the slow cooling and the shear stress when crossing the long cooling matrix (about 1.2 meters with reduced exit hole) promote the direction and formation of the fibers of the plant-based product.
9. Cutting and cooling in the cooling tunnel	Upon exiting the extruder die, the textured extrudate is deposited on a conveyor belt and cut by cutting blades according to the specified size and submitted to another cooling step in a cooling tunnel before packaging.

10. Packaging	Upon exiting the cooling tunnel, the cut and cooled extrudate is directed to the automated packaging system. The packaging equipment is equipped with retort pouch packages, which are opened and filled according to the specified weight. As the packages are welded, they are collected on a side table, from where they proceed to autoclave sterilization.
11. Sterilization	The product, hermetically packed in the packages, must be sterilized in appropriate autoclaves, according to the processing temperatures and packaging types. Flexible packaging, such as pouches, requires autoclaves that operate with overpressure; that is, the autoclave pressure is the vapor pressure corresponding to the operating temperature. The most common autoclaves, in addition to immersion autoclaves, operate using water cascade, water spray, or air/steam mixture as heating media. The processing conditions (times, temperatures, and pressures) of the heating, level/processing, and cooling phases must be configured so that the product reaches the minimum required lethality. For low-acidity and high water activity products, such as the fish analog, the sterilization process targets *Clostridium botulinum* spores. The treatment must be sized to ensure at least 12 decimal reductions in the number of spores eventually present, which corresponds to F0=2.52 min. For higher safety from the point of view of public health, the minimum value applied is three minutes (Lewis, 2006; Lewis & Deeth, 2009). It is important to consider that, in order to achieve commercial sterility, the reduction of deteriorating mesophilic and thermophilic sporogenic bacteria, which are more resistant to heat than *Clostridium botulinum*, must also be ensured. Stumbo (1973) indicates F0 of at least five minutes and, in cases requiring the reduction of thermophiles, F0 between 14 and 16 minutes. In the case of the fish analog, because it is a product that involves the combination of ingredients from different sources, varied microbiota, and a lack of studies on the species present and on the interaction between them, it is suggested, a priori, a more intense heat treatment. Based on the thermal resistance of the spores of *Geobacillus stearothermophilus*, one of the most heat-resistant thermophiles (D value at 121.1°C = 4 to 5 minutes) (Stumbo, 1973) and the objective of achieving a reduction of five logarithmic cycles, lethality values of 20 minutes are recommended, that is, F0=20 minutes. Cooling occurs inside the autoclave, with water that underwent sterilization during processing and was later cooled indirectly, without contact with the external environment, which prevents any product recontamination issues. Each manufacturer must conduct heat penetration tests at the plant where the product will be processed, since the process times and temperatures to achieve the same lethality may vary depending on the type of autoclave, packaging, product composition, filling conditions, heating medium and other processing factors. Once the autoclave is opened, the packages are removed and sent directly to labeling and boxing in support carts.
12. Boxing	The cardboard packages are packed in corrugated cardboard boxes in an automated process. The boxes are identified with coded labels. In this step, the packages also pass through an X-ray equipment for detection and separation of those containing any metallic particles equal to or greater than 2 mm.
13. Palletizing	In this step, the boxes are manually placed on pallets. Once formed, the pallet is wrapped with stretch film and identified with coded labels, forming a loading unit.
14. Quarantine	A sample is taken from each batch and incubated at 35-37°C for 10 days for observation of possible package stuffing and altered pH of the product, indicating

	processing failure. Other sensory parameters can also be observed at the discretion of the manufacturer.
15. Storage	The loading units are stored at room temperature (maximum 30°C) until shipping.
16. Shipping	In this step, the finished product is loaded and shipped, being transported in trucks at room temperature (maximum 30°C).
Considerations on product shelf life, distribution, and preparation instructions	For the pilot study, this product was considered with a shelf life of 12 months. The recommendation is to transport the product at room temperature (maximum 30°C), palletized and organized in vehicles in good condition, clean, closed with protection against weather and contaminants, without evidence of pests and/or other animals, without sharing space with non-food products, free of toxic products, substances and objects foreign to the activity, thus ensuring product integrity and quality. The product is ready for consumption and can be consumed alone or in combination with other foods. After opening, keep it under refrigeration at up to 10°C and consume it within a maximum period of two days. In case of leftovers after consumption, disposal is recommended.
References cited	LEWIS, M. J. Thermal Processing. In: BRENNAN, J. G. (ed.). *Food Processing Handbook*. Weinheim: Willey-VCH, 2006. p. 33-69. LEWIS, M. J.; DEETH, H. C. Heat Treatment of Milk. In: TAMIME, A. Y. (ed.). *Milk Processing and Quality Management*. Hoboken: Wiley-Blackwell, 2008. p. 168-200. STUMBO, C. F. *Thermobacteriology in Food Processing*. 2. ed. New York: Academic Press, 1973.

FIGURE 3: PROCESSING FLOWCHART FOR THE PLANT-BASED BEEF BURGER.

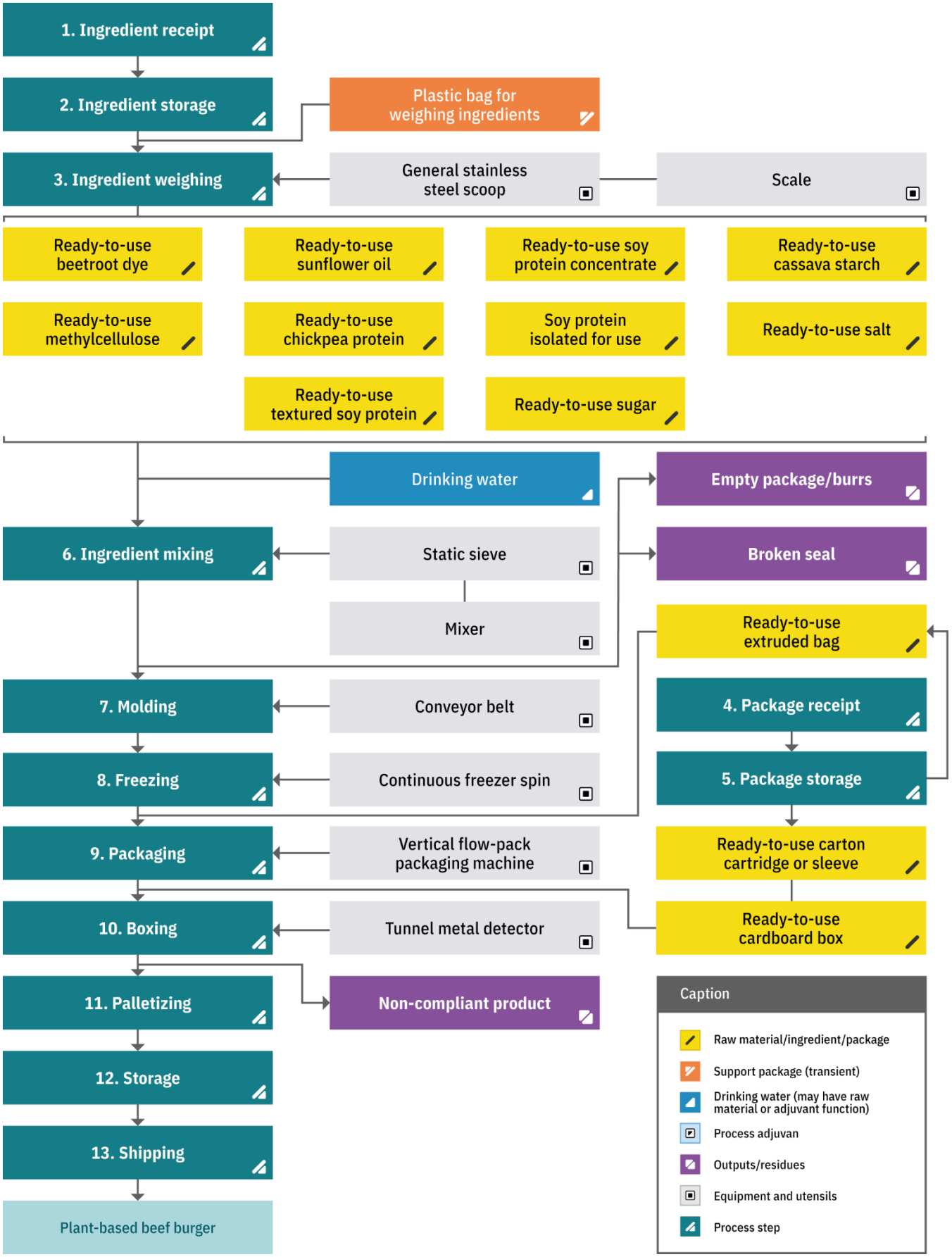

CHART 3: DESCRIPTION OF THE PRODUCT PROCESSING STEPS AND CONSIDERATIONS ON PRODUCT VALIDITY, DISTRIBUTION, AND PREPARATION INSTRUCTIONS FOR THE PLANT-BASED BEEF BURGER.

Step	Description
1. Ingredient receipt	Upon receipt, supplier documentation is checked, and an inspection is carried out to check the integrity of the material received and the hygiene conditions/presence of pests in the transport.
2. Ingredient storage	Ingredients are transported to the warehouse for storage on-site and under designated conditions.
3. Ingredient weighing	The ingredients are transported in their closed packages to the weighing room, where manual weighing is carried out with the aid of scoops, according to product formulation. The heavy material is packed in plastic boxes.
4. Package receipt	Upon receipt of the packages, an inspection is carried out to check the integrity of the material received, the hygiene conditions/presence of pests in the transport, and the specific characteristics for packaging frozen products.
5. Package storage	The packages are transported to the warehouse for storage at the designated location.
6. Ingredient mixing	Heavy ingredients are transported in closed plastic boxes to the mixer, which is fed manually. First, there is the addition of soy proteins and water, introduced by a closed system. Once the soy proteins are hydrated, the other ingredients are then added, except for methylcellulose, which is added after prehydration. It is recommended that the mixing operation be conducted in an air-conditioned environment at a temperature between 12 and 15°C after the ingredients have hydrated.
7. Molding	The mixture is placed in the former/molder feeding, where it is cut and molded into the shape of discs (burgers).
8. Freezing	The molded product is transported on conveyor belts to the freezing tunnel, where it reaches minus 18°C.
9. Packaging	Upon exiting the freezing tunnel, the frozen product is directed to the packaging system equipped with rolls of polyolefinic plastic film. Each unit is packaged in an automated horizontal form-fill-seal process. Then the units are automatically packaged in pre-formed carton paper packages, in number of units according to the weight specified for the secondary packaging.
10. Boxing	The carton packages are packed in corrugated cardboard boxes in an automated process. The boxes are identified with coded labels. In this step, the packages also pass through a metal detector, with separation of those containing any metallic particles equal to or greater than 2 mm.
11. Palletizing	In this step, the boxes are manually placed on pallets. Once formed, the pallet is wrapped with stretch film and identified with coded labels, forming a loading unit.
12. Storage	The loading units are stored in a cold chamber at a temperature not higher than minus 25°C.
Considerations on product shelf life, distribution, and preparation instructions	For the pilot study, this product was considered a frozen food with a shelf life of 12 months. The recommendation is that the product is transported in conditions such so as not to reach a temperature above minus 12°C, palletized and organized in vehicles in good condition, permanently equipped with a calibrated easy-to-read

	thermometer, clean, closed with protection against weather and contaminants, without evidence of pests and/or other animals, without sharing space with non-food products, free of toxic products, substances and objects foreign to the activity, thus ensuring product integrity and quality. The product should not be consumed raw, requiring application of heat (cooking, frying, grilling, baking) before consumption, to develop the desired texture characteristics. Heat treatment must ensure that all parts of the food reach a minimum temperature of 74°C. Lower temperatures may be used, provided that the temperature and time combinations are sufficient to ensure food hygienic-sanitary quality, such as 70°C for two minutes or 65°C for 15 minutes. The product should preferably be prepared without defrosting, but may be partially defrosted at the time of preparation. In case of prior defrosting, it must be done in a refrigerator, and the product's surface temperature must not exceed 10°C. In case of leftovers after consumption, disposal is recommended.

FIGURE 4: PROCESSING FLOWCHART FOR THE PLANT-BASED SAUSAGE.

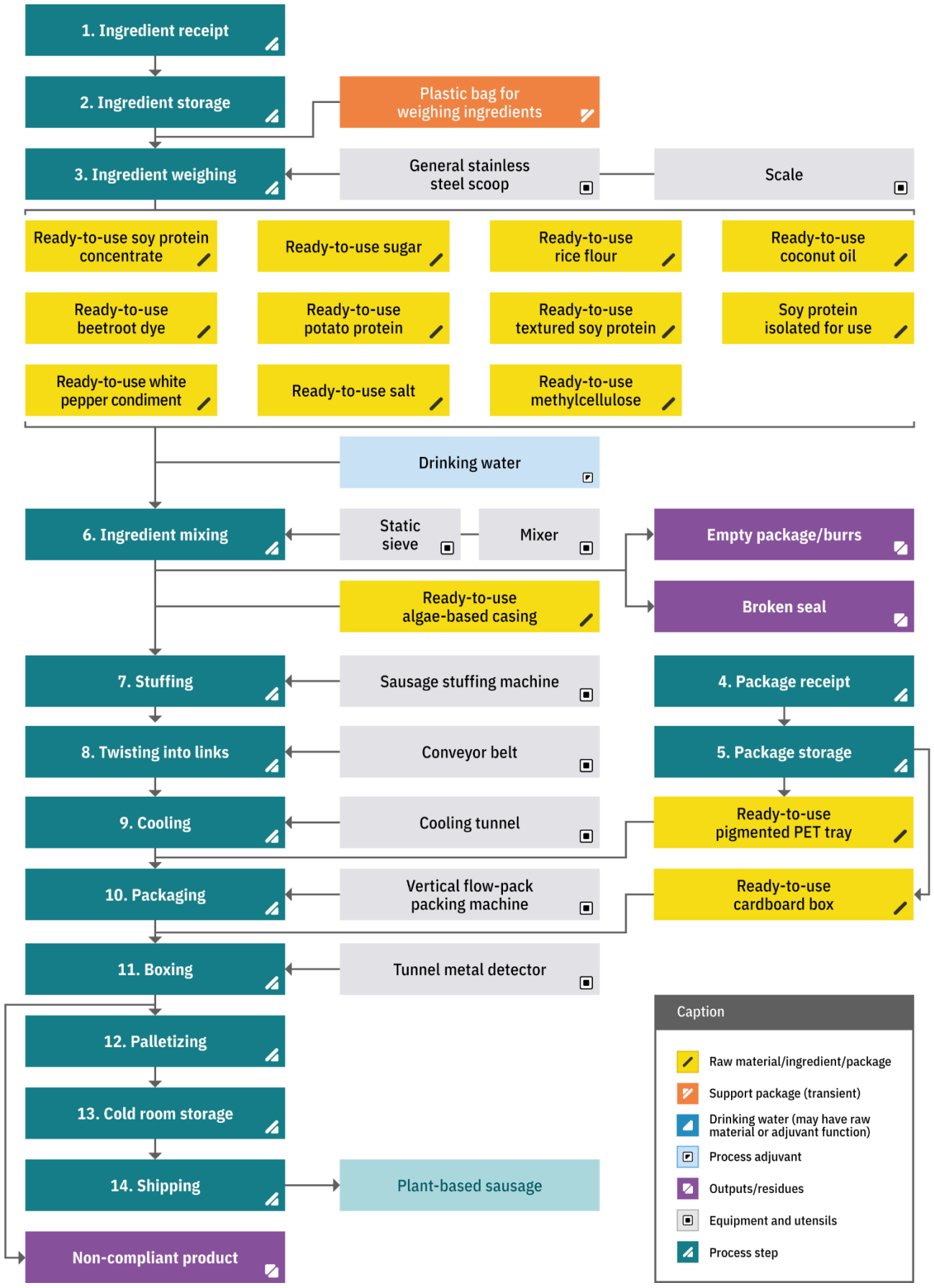

CHART 4: DESCRIPTION OF THE PRODUCT PROCESSING STEPS AND CONSIDERATIONS ON PRODUCT VALIDITY, DISTRIBUTION, AND PREPARATION INSTRUCTIONS FOR THE PLANT-BASED SAUSAGE.

Step	Description
1. Ingredient receipt	Upon receipt, supplier documentation is checked, and an inspection is carried out to check the integrity of the material received and the hygiene conditions/presence of pests in the transport.
2. Ingredient storage	Ingredients are transported to the warehouse for storage on-site and under designated conditions.
3. Ingredient weighing	The ingredients are transported in their closed packages to the weighing room, where manual weighing is carried out with the aid of scoops, according to product formulation. The heavy material is packed in plastic boxes.
4. Package receipt	Upon receipt of the packages, an inspection is carried out to check the integrity of the material received and the hygiene conditions/presence of pests in the transport.
5. Package storage	The packages are transported to the warehouse for storage at the designated location.
6. Ingredient mixing	Heavy ingredients are transported in closed plastic boxes to the mixer, which is fed manually. First, there is the addition of soy proteins and water, introduced by a closed system. Once the soy proteins are hydrated, the other ingredients are then added, except for methylcellulose, which is added after prehydration. It is recommended that the mixing operation be conducted in an air-conditioned environment at a temperature between 12 and 15°C after the ingredients have hydrated.
7. Stuffing	The mass is stuffed into edible plant-based casings, preferably using a vacuum stuffing machine.
8. Twisting into links	The stuffed mass is twisted to form links by using a link-forming system integrated into the stuffing machine.
9. Cooling	The stuffed product is transported on conveyor belts to the cooling tunnel, where it is cooled to below 5°C.
10. Packaging	Upon exiting the cooling tunnel, the cooled product is directed to the automated packaging system, where the links are placed in polyethylene terephthalate (PET) trays, in the quantity specified for the package. The PET packages are closed by sealing the film lid on the tray and are packaged in a carton, cartridge, or sleeve.
11. Boxing	The carton packages are packed in corrugated cardboard boxes in an automated process. The boxes are identified with coded labels. In this step, the packages also pass through a metal detector, with separation of those containing any metallic particles equal to or greater than 2 mm.
12. Palletizing	In this step, the boxes are manually placed on pallets. Once formed, the pallet is wrapped with stretch film and identified with coded labels, forming a loading unit.
13. Storage	The loading units are stored in a cold chamber at a temperature not exceeding 4°C.

14. Shipping	This step involves loading and shipping the finished product, which must be transported in refrigerated trucks at a temperature of no higher than 8°C.
Considerations on product shelf life, distribution, and preparation instructions	For the pilot study, this product was considered with a shelf life of 10 days at a temperature of 3 to 8°C. Refrigerated transport is recommended, under conditions such that the temperature of the product is maintained between 3 and 8°C, palletized and organized in vehicles in good condition, permanently equipped with an easy-to-read calibrated thermometer, clean, closed with protection against weather and contaminants, without evidence of pests and/or other animals, without sharing space with non-food products, kept organized and free of toxic products, substances and objects foreign to the activity, thus ensuring product integrity and quality. The product should not be consumed raw, requiring application of heat (cooking, frying, grilling, baking) before consumption, to develop the desired texture characteristics and reduce microbial load. Heat treatment must ensure that all parts of the food reach a minimum temperature of 74°C. Lower temperatures may be used, provided that the temperature and time combinations are sufficient to ensure food hygienic-sanitary quality, such as 70°C for two minutes or 65°C for 15 minutes. In case of leftovers after consumption, disposal is recommended.

4. Safety hazards identified in the HACCP plans

Five categories of hazards were identified, originating from ingredients, materials, and process steps: allergenic, physical, biological, chemical, and radiological hazards. Chart 5 shows each of them, their classification, and association with the manufacture of the plant-based products addressed in this study.

CHART 5: HAZARDS ASSOCIATED WITH THE MANUFACTURE OF THE PLANT-BASED MEAT.

Hazard	Classification	Plant-based product			
		Fish	Chicken	Burger	Sausage
Gluten*	Allergenic	X	X	X	X
Soy proteins*	Allergenic	X	X	X	X
Bacillus cereus	Biological	X	X	X	X
Clostridium botulinum spores	Biological	X	-	-	-
Escherichia coli (sanitary hygienic quality indicator)	Biological	X	X	X	X
Listeria monocytogenes	Biological	X	X	X	X
Salmonella spp.	Biological	X	X	X	X
Sand	Physical	X	X	X	X
Insect fragments	Physical	-	X	-	X

Ferrous and non-ferrous metal fragments	Physical	X	X	X	X
Flexible plastic or non-rigid polymer	Physical	X	X	X	X
Acrylamide (propenamide)	Chemical	X	X	X	X
Aflatoxins B1, B2, G1 and G2	Chemical	X	X	X	X
Arsenic	Chemical	X	X	X	X
Cadmium	Chemical	X	X	X	X
Lead	Chemical	X	X	X	X
Copper	Chemical	X	X	X	X
Chemical water contaminants	Chemical	X	X	X	X
Dioxins	Chemical	-	X	X	X
DON – Deoxynivalenol or vomitoxin	Chemical	X	X	X	X
Esters of 3-MCPD (3-monochloropropane-1,2-diol or 3-chloropropane-1,2-diol)	Chemical	X	X	X	X
Glycidol esters	Chemical	X	X	X	X
Fumonisins (B1+B2)	Chemical	X	X	X	X
Furan and Methylfurans	Chemical	-	X	X	X
Migration of packaging material components	Chemical	X	X	X	X
Ochratoxin A	Chemical	X	X	X	X
Ethylene oxide	Chemical	-	-	-	X
Residues of pesticides (general)	Chemical	X	X	X	X
ZEA – zearalenone	Chemical	X	X	X	X
Total Alpha radioactivity (drinking water)	Radiological	X	X	X	X
Total Beta radioactivity (drinking water)	Radiological	X	X	X	X

**Allergenic hazards were considered in all products, even those that do not contain the protein in question, due to the possibility of equipment of the same manufacturing unit being shared for different plant-based analogs.*

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The complete study contains a detailed description of each of these hazards and, where applicable, severity, action on the organism, foods involved in their transmission, symptoms, infectious dose, incubation period, toxicity, acceptable daily dose, and other relevant information. They also presented the origin of each hazard, the justification of the hazard, the degree of risk represented by the hazard (negligible, minor, medium, high) according to the severity, the probability of occurrence of the hazard, the acceptable level of the hazard in the final product, and the control measures adopted to prevent, eliminate or reduce the hazard to acceptable levels.

The complete study also describes critical control points (CCPs), prerequisite programs (PRPs), Control Points (CPs) and Synergistic Control Measures (SCMs) associated with each product. Critical limits for control measures classified as CCPs are established, as are monitoring and inspection procedures and guidelines for implementing the HACCP plan for the production of plant-based meat.

5. Control Measures

The control measures defined and established in the HACCP plans of the complete study were classified as CCP (critical control point), CP (control point), or PRP (prerequisite program), in addition to synergistic control measures (SCM), for which the following definitions apply:

Prerequisite Program (PRP). This designation is used when a significant hazard control measure is already clearly established in the good manufacturing practice standards established by Brazilian legislation.

Control Point (CP) Also called operational prerequisite program (OPRP), this designation is used when a hazard control measure is part of good manufacturing practices, but requires specific actions in addition to those proposed by Brazilian legislation, being considered essential for the control of identified hazards.

Critical control point (CCP) This designation is used for a process step in which one or more control measures that are essential to control and maintain a significant hazard at acceptable levels are applied in a HACCP plan, with significant hazards being defined as those identified by a hazard analysis as reasonably likely to occur at an unacceptable level in the absence of control and for which control is essential under the intended conditions of food use.

Synergistic Control Measure (SCM). In addition to direct hazard control, prerequisite programs can also fulfill a function so as to complement or enhance a critical control point (CCP). It is the combined action of two or more control measures whose result can be the sum of the effects of each individual measure or even a total effect that is greater than that sum.

Chart 6 shows the types of control measures established by the HACCP plans for each type of hazard identified in the study.

CHART 6: TYPES OF CONTROL MEASURES ESTABLISHED IN THE HACCP PLANS FOR PLANT-BASED MEAT.

Types of control measures associated with biological hazards*
Hazards: *Bacillus cereus, E. coli* (hygienic sanitary quality indicator), *Listeria monocytogenes, Salmonella*
• SCM-Inspection of raw material and ingredient suppliers
• SCM-Periodic Qualification Assessment of Raw Material and Ingredient Suppliers
• CP-Laboratory analysis of raw material or ingredient upon receipt for use upon release
• PRP-Guarantee of water potability control
• CP-Guarantee of pre-operational inspection of equipment/utensils through cleaning check by bioluminescence (ATP) (all products)
• PRP-Environmental monitoring plan (all products)
• PRP-Personal hygiene program for food handlers
• CCP-Control of extrusion time, temperature, and pressure variables (plant-based chicken and fish)
• CCP-Control of sterilization time, temperature, and pressure variables (plant-based fish)
• SCM-Temperature control in the handling and cold chain processes for product storage, transport, and sale
• SCM-Consumer label information and instructions on how to prepare and use the product
Types of control measures associated with physical hazards*
Hazard: Ferrous and non-ferrous metal fragments
• SCM-Inspection and qualification check of raw material and ingredient suppliers
• SCM-Screening of raw materials and ingredients before the mixing process
• SCM-Preventive maintenance of equipment
• SCM-Cross Contamination Control Program
• CCP-Guarantee of the perfect functioning of metal detectors
• CCP-Guarantee of the perfect functioning of X-ray devices (plant-based fish)
Hazard: Flexible plastic or non-rigid polymer
• PRP-Guarantee of the package opening procedure when adding ingredients
• SCM-Sieve use in the ingredient mixing process
• SCM-Food Handler Training and Capacity-Building Program
Hazard: Sand
• PRP-Guarantee of water potability control
• PRP-Exclusive receipt from approved supplier
• SCM-Periodic Qualification Assessment of Raw Material and Ingredient Suppliers
Hazard: Insect fragment
• PRP-Exclusive receipt from approved supplier
• SCM-Periodic Qualification Assessment of Raw Material and Ingredient Suppliers
Types of control measures associated with chemical hazards*
Hazards: Acrylamide, arsenic, cadmium, lead, copper, dioxins, furan and methylfurans, mycotoxins, migration of packaging material components, residues of pesticides (general)
• PRP-Exclusive receipt from approved supplier
• SCM-Periodic Qualification Assessment of Raw Material and Ingredient Suppliers
Hazards: 3-MCPD esters, Aflatoxins B1, B2, G1 and G2, deoxynivalenol, fumonisins (B1, B2), glycidol esters, ochratoxin A, ethylene oxide, zearalenone
• CP-Receipt of raw material/ingredient with batch analysis report.
• SCM-Periodic Qualification Assessment of Raw Material and Ingredient Suppliers
Hazard: Chemical water contaminants

- PRP-Guarantee of water potability control
- SCM-Certification check for water collection sources.
- SCM-Control of the use of water treatment chemicals registered and approved by official agencies

Types of control measures associated with allergenic and radiological hazards**

Allergenic hazards: Soy, gluten
- CP-Guarantee of pre-operational inspection of equipment by a specific allergen swab test
- PRP-Use of specific utensils for each type of allergen contained in raw materials and ingredients
- SCM-Food Handler Training and Capacity-Building Program
- SM-Allergen control program
- SM-Production start control program with labeling check
- PRP-Guarantee of label information and warning for allergens and legal aspects
- SM-Plan for label check and approval upon receipt

Radiological hazards: Total alpha radioactivity and total beta radioactivity (drinking water)
- PRP-Guarantee of water potability control
- SCM-Certification check for water collection sources.

**CCP (Critical Control Point) = a process step in which one or more control measures that are essential to control and maintain a significant hazard at acceptable levels are applied in a HACCP plan; PRP (Prerequisite Program) = his designation is used when the significant hazard control measure is already clearly established in the good manufacturing practice standards established by Brazilian legislation.; CP (Control Point) = this designation is used when a hazard control measure is part of good manufacturing practices, but requires specific actions in addition to those proposed by Brazilian legislation and are considered essential for control of the identified hazard; SCM (Synergistic Control Measure) = is an interaction that multiplies the results. It is the combined action of two or more control measures whose result can be the sum of the effects of each measure or a total effect that is greater than that sum.*

6. Research gaps identified to complement and/or validate the study of the safety aspects of plant-based meat

During this study, relevant information gaps — notably the almost unavailability of data on the quality of the plant proteins used and on the presence of biological and chemical contaminants — were identified in the safety assessment in the production of plant-based meat, as well as in the final products available on the market.

6.1. Data on biological contaminants in plant-based meat and in plant proteins used in their formulation

A survey of studies reported in the scientific literature on biological contaminants in plant-based meat and in the proteins used in their formulation found few publications, which are summarized in Chart 7.

One of these studies is a research project commissioned by the European Union (LikeMeat Project) (European Commission, 2021), which evaluated the presence of microorganisms in six types of proteins: lupins, peas, soybeans, wheat, rice and potatoes. The highest microbial load was found in lupin proteins (mean mesophilic aerobic count of $2.4x10^4$ CFU/g) and pea (mean of $3.4x10^3$ CFU/g). In the other proteins, the count of mesophilic aerobes was below 10^3 CFU/g on average. Contamination with enterobacteria and coliforms was observed, especially in isolated pea protein ($1.1x10^3$ CFU/g and $4.6x10^2$ CFU/g, respectively). As for contamination with *Bacillus* species, isolated pea protein ($2.6x10^2$ CFU/g) and isolated lupin protein ($9x10^2$ CFU/g) are noted. Wheat gluten also showed contamination

with *Bacillus* species, but with lower counts. *Salmonella*, *Listeria monocytogenes*, *Bacillus cereus* and *Clostridium* species were not found in any of the samples tested in this project.

CHART 7: SCIENTIFIC STUDIES FOUND IN THE LITERATURE ON BIOLOGICAL CONTAMINANTS IN PLANT-BASED MEAT AND IN THE PLANT PROTEINS USED IN THEIR FORMULATION.

Reference	Study summary	Findings
Martins *et al.* (2014)	Analysis of samples of several soy-based products in Brazil, including textured soy protein.	*Bacillus cereus* count: above 10^5 CFU/g in textured soy protein samples.
Canadian Food Inspection Agency (2019)	Survey of the microbial contamination of 245 samples of plant proteins collected at retail in 11 cities in Canada.	Quality was satisfactory in 95% of the samples: *Salmonella*, absent in 25 g, *E. coli* ≤1.8 NMP/g, *Bacillus cereus* (presumptive) ≤10^2 CFU/g, *Staphylococcus aureus* ≤25 CFU/g, and *Clostridium perfringens* ≤10^2 CFU/g. In the other samples, the *E. coli* count did not exceed 10^2 NMP/g. In 4.1% *Bacillus cereus* presented counts between 10^2 and 10^4 CFU/g. In 0.8% *Clostridium perfringens* presented counts between 10^2 and 10^3 CFU/g.
Pernu *et al.* (2020)	Analysis of 74 vacuum-packed plant-based sausage samples sold under refrigeration in Finland and Germany for the presence of *Clostridium botulinum spores.*	Counts of up to 1.2x10^3 spores/kg of type II psychrotrophic strains (also called non-proteolytic *Clostridium botulinum*), which are capable of growing and producing toxins under refrigeration, with overall prevalence of 32%.
Luchansky *et al.* (2020)	Study on the behavior of Shiga toxigenic *Escherichia coli* (STEC), *Salmonella,* and *Listeria monocytogenes* artificially inoculated in plant-based and bovine burgers during storage at 4°C and 10°C for 21 days.	Plant-based burger storage at 4°C for 21 days: *Listeria monocytogenes* increased by 1.3 log CFU/g, *Salmonella* and *E. coli* decreased by 0.4 log CFU/g. Bovine burger storage at 4°C for 21 days: *Listeria monocytogenes, E. coli,* and *Salmonella* decreased by 0.7, 0.3, and 0.6 log CFU/g, respectively. Plant-based burger storage at 10°C for 21 days: *Listeria monocytogenes, E. coli* and *Salmonella* increased by 2.6, 2.4, and 0.8 log CFU/g, respectively. Bovine burger storage at 10°C for 21 days: *Listeria monocytogenes, E. coli,* and *Salmonella* decreased by 0.9, 0.2, and 1.2 log CFU/g, respectively. These data showed greater susceptibility of plant-based burgers to the multiplication of these pathogens compared to beef burgers.
European Commission (2021)	Lupin, pea, soy, wheat, rice, and potato proteins from the European Union were analyzed. Evaluation of the effect of extrusion in reducing the microbial load of proteins.	Highest total count of mesophilic aerobes: mean of 2.4x10^4 CFU/g in lupin protein isolate. Highest count of enterobacteria: mean of 1.1x10^3 CFU/ g in isolated pea protein. Highest count of coliforms: mean of 4.6x10^2 CFU/ g in isolated pea protein. Highest count of *Bacillus* spp: mean of 9.0x10^2 CFU/ g in lupin protein isolate. *Salmonella, Listeria monocytogenes, Bacillus cereus* and *Clostridium* species not detected in any sample. After extrusion, the total count of mesophilic aerobes in the samples decreased to <100 CFU/g, and the presence of enterobacteria and coliforms was not detected.
NSW Food Authority (2021)	Survey of local or international reports of outbreaks of diseases transmitted by plant-based meat.	No reports of diseases were found.

NSW Food Authority (2021)	Analysis of 85 samples from plant-based meat sold in Australia and other countries.	The presence of *Salmonella* (absence in 25 g), coagulase-positive *Staphylococci* (<100 CFU/g), and *Clostridium perfringens* (<10 CFU/g) was not detected in any sample. *E. coli* was detected in one sample (10 CFU/g). *Listeria monocytogenes* was detected in two samples (<100 CFU/g). *Bacillus cereus* was detected in nine samples (six with 100 CFU/g, two with $2.0x10^2$ CFU/g, and one with $1.3x10^3$ CFU/g). Total count of mesophilic aerobes in cooked products: 74% of the samples were considered good (<10^6 CFU/g) or acceptable (<10^7 CFU/g). Total count of mesophilic aerobes in raw products: ranged from <10 to $2.2x10^6$ CFU/g (mean of $9.3x10^4$ CFU/g). The conclusion of the inspection was that the products analyzed did not present contamination that could represent a risk to health.
Tóth *et al*. (2021)	Analysis of 15 samples from five types of plant proteins: wheat gluten, soy protein, textured soy protein, and textured pea protein produced by two manufacturers (Norwegian Vestkorn and North American DuPont).	Wheat gluten samples had a mean total mesophilic aerobic count of 3.32 log CFU/g. In the other samples, the total mesophilic aerobic count was below 10 CFU/g. The presence of enterobacteria, molds and yeasts was not detected (<10 CFU/g) in any of the samples analyzed.
Wells-Bennik (2022)	Results of surveys of microbial contamination in pea, fava bean, mung bean, and chickpea protein isolates or concentrates with identification of isolates.	A significant fraction of the total bacterial count in these products consisted of spores (up to approximately 10^3/g), predominantly *Bacillus cereus, Bacillus licheniformis*, *Bacillus subtilis, and Bacillus amyloliquefaciens*, but also present were sulfite-reducing clostridia and *Geobacillus stearothermophilus.*
Kyrylenko *et al*. (2023)	Analysis of 88 samples of plant-based ingredients obtained from 12 different suppliers in Europe, America and Asia, including proteins (from peas, fava beans, mung beans and chickpeas) and flours (from peas, mung beans, rice, quinoa, amaranth, oats, coconut and almonds), with identification of isolates.	Total count of mesophilic aerobic: ranged between <1.0 and 5.3 log CFU/g. Count of mesophilic aerobe spores: ranged between <1.0 and 4.1 log CFU/g. Count of thermophilic bacteria spores, *Bacillus cereus* spores, and sulfite-reducing clostridia spores: close to or below 1.0 log CFU/g. Most commonly found *Bacillus* species: *Bacillus licheniformis and Bacillus cereus*, mainly in pea and oat samples. Most commonly found thermophile species: *Geobacillus stearothermophilus.* Most commonly found clostridium species: *Clostridium sporogenes* and *Clostridium tepidum,* mainly in pea and almond samples.
Liu *et al*. (2023)	Study of changes in the microbial population of soy-based and pea-based meat analogs stored at 4°C, examining both naturally present microbiota (total mesophilic aerobes, lactic acid bacteria, coliforms, molds and yeasts) and artificially inoculated bacteria (*Pseudomonas fluorescens, Brochothrix thermosphacta, Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes*).	Initial contamination in the soy-based analog: 3.10 log CFU/g for total mesophilic aerobes, 2.04 log CFU/g for lactic acid bacteria, 2.00 log CFU/g for coliforms, and 1.95 log CFU/g for molds and yeasts. Initial contamination in the pea-based analog: 3.82 log CFU/g for total mesophilic aerobes, 3.61 log CFU/g for lactic acid bacteria, 2.51 log CFU/g for coliforms, and 1.44 log CFU/g for molds and yeasts. Count after 10-day storage at 4°C: Total counts of mesophilic aerobes and lactic acid bacteria were close to 7.00 log CFU/g in both products. *Pseudomonas fluorescens* increased by 0.40 log CFU/g (soy) and 3.00 log CFU/g (pea). *Brochothrix thermosphacta* increased by 0.96 log CFU/g (soy) and 1.58 log CFU/g (pea). Count after 7-day storage at 4°C: *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* had no change in the soy-based analog. *Listeria monocytogenes* increased by 0.74 log CFU/g in the pea-based analog.

The LikeMeat Project (European Commission, 2021) also evaluated the effect of extrusion on the microbial load of the proteins under study, noting that after extrusion all presented mesophilic aerobe counts below 100 CFU/g and the presence of enterobacteria and coliforms was not detected. This leads to the assumption that the process of extruding protein sources is effective in destroying most of the microorganisms present in the ingredients. On the other hand, they noted the need to adopt care to ensure that recontamination does not occur.

In fact, there are reports, albeit rare, of relatively high counts of bacteria in already textured proteins. Martins *et al*. (2014), for example, analyzed samples of several mixed cereal flours and soy-based products in Brazil, including textured soy protein, in which they found a *Bacillus cereus* count above 10^5 CFU/g.

Recontamination in the manufacturing setting is an issue in industries that process dehydrated plant products, particularly soy derivatives. Rocha *et al*. (2022), in a study aimed at determining the occurrence of *Salmonella enterica* along the soybean meal production chain (raw material, in-process samples, final products and setting of five processing plants in Brazil), found that 12.9% of the 713 samples analyzed (n = 92) were positive for *Salmonella* spp. The fact to be noted is that the highest percentage of contamination was found in setting samples, representing 76.1% of all positive samples.

In Canada, the Canadian Food Inspection Agency (2019) conducted a survey of the microbial contamination of 245 samples of plant-based proteins collected at retail in 11 cities in the country. The results showed that 95% of the samples had satisfactory quality: *Salmonella*, absent in 25 g, *E. coli* ≤1.8 NMP/g, *Bacillus cereus* (presumptive) ≤10^2 CFU/g, *Staphylococcus aureus* ≤25 CFU/g, and *Clostridium perfringens* ≤10^2 CFU/g. *Salmonella* and *Staphylococcus aureus* were not found in any sample, and neither was *E. coli* count above 10^2 NMP/g. *Bacillus cereus* at counts between 10^2 and 10^4 CFU/g was found in 4.1% (10/245) of the samples. *Clostridium perfringens* at counts between 10^2 and 10^3 CFU/g was found in 0.8% (2/245) of the samples.

At the 2022 annual congress of the International Association for Food Protection (IAFP 2022), Nizo Company, a Dutch company that provides research and development services to the industry, presented data from its work to survey microbial contamination in pea, fava bean, mung bean and chickpea protein isolates or concentrates. The results demonstrated that a significant fraction of the total bacterial count in these products consisted of spores (up to approximately 10^3/g), predominantly *Bacillus cereus, Bacillus licheniformis, Bacillus subtilis,* and *Bacillus amyloliquefaciens,* but also present were sulfite-reducing clostridia and *Geobacillus stearothermophilus* (Wells-Bennik, 2022).

Kyrylenko *et al*. (2023) conducted a study of 88 samples of plant ingredients used in the production of plant-based analogs, obtained from 12 different suppliers in Europe, America and Asia, including 34 pea samples (26 protein isolates, six protein concentrates and two flours), 13 fava bean samples (seven protein isolates, three protein concentrates and three flours), two mung bean samples (one protein isolate and one flour), one chickpea protein isolate sample, 20 oat samples (two grains, three flakes, seven flours and eight syrups, pastes and hydrolysates), one rice flour sample, one quinoa flour sample, one amaranth flour sample, five coconut samples (flour, milk or cream), nine almond samples (flour, paste or syrup) and one cashew sample. The total count of mesophilic aerobes in the samples ranged between <1.0 and 5.3 log CFU/g, and that of mesophilic aerobe spores between <1.0 and 4.1 log CFU/g. Several of the ingredients studied showed a high proportion of mesophilic spores as part of the total mesophilic aerobe count; in 63% of the samples, the spore count was only one or less than one log unit lower than the total count. This was particularly the case for most pea protein

isolates and concentrates, fava bean and chickpea protein isolates, and rice, quinoa, amaranth, almond, and coconut flours. Counts of thermophilic bacteria spores, *Bacillus cereus* spores, and sulfite-reducing clostridia spores were close to or below 1.0 log CFU/g.

In total, the work by Kyrylenko *et al*. (2023) isolated 845 bacterial colonies from the analyzed samples, belonging to 33 different genera. *Bacillus licheniformis* and *Bacillus cereus* were the most commonly found species among the isolated *Bacillus*, originating mainly from pea and oat samples. Among thermophile spores, *Geobacillus stearothermophilus* was the most frequent. Among the clostridia, the most commonly found were *Clostridium sporogenes* and *Clostridium tepidum,* mainly isolated from pea and almond samples.

The predominance of bacterial spores in proteins — the basic ingredients of plant-based meat — is very relevant due to their resistance to heat treatments applied in extrusion and sterilization.

Sulfite-reducing clostridia are of particular relevance from a public health point of view, because this group includes *Clostridium botulinum*, the most dangerous foodborne pathogenic bacteria.

In fact, a survey carried out in Finland and Germany to check the presence of *Clostridium botulinum* spores in 74 samples of plant-based sausage vacuum-packed and sold under refrigeration detected an overall prevalence of 32%, including counts of up to $1.2x10^3$ spores/kg of type II psychrotrophic strains (also called non-proteolytic *Clostridium botulinum*), which are capable of growing and producing toxins under refrigeration (Pernu *et al*., 2020). The authors concluded that the vacuum-packed plant-based sausages often contain *Clostridium botulinum* spores and may pose a high risk to consumers. It is therefore recommended that they be stored below 3°C and, even in the case of cooked products, that they should be heated before consumption. The Chilled Food Association (2018) established guidelines to define the shelf life of refrigerated foods packaged under vacuum or under modified atmosphere in relation to psychrotrophic strains of non-proteolytic *Clostridium botulinum*, defining storage temperature between 3 and 8°C and shelf life not exceeding 10 days, unless one of the following criteria is met to increase the shelf life: a) heat treatment at 90°C for 10 minutes (or equivalent) or b) pH reduction to ≤5.0 or c) addition of 3.5% NaCl or d) reduction of water activity to ≤0.97 or e) combination of heat and addition of preservatives under conditions that prevent multiplication and formation of toxins.

On the other hand, a data survey by the NSW Food Authority, a government agency in the state of New South Wales, Australia, indicated that there are no local or international reports of outbreaks of diseases transmitted by plant-based meat (NSW Food Authority, 2021). The same publication presented data from an inspection carried out on 85 products sold in supermarkets, "green" grocery stores and online stores in Australia and other countries, with microbiological analyses of the total count of mesophilic aerobes, molds and yeasts, *E. coli, Salmonella, Listeria monocytogenes*, coagulase-positive, Staphylococcus, *Bacillus cereus* and *Clostridium perfringens*. The presence of *Salmonella* (absence in 25 g), coagulase-positive *Staphylococcus* (<100 CFU/g), and *Clostridium perfringens* (<10 CFU/g) was not detected in any sample. *E. coli* was detected in one sample (10 CFU/g), *Listeria monocytogenes* in two samples (<100 CFU/g), and *Bacillus cereus* in nine samples (six samples with 100 CFU/g, two samples with $2.0x10^2$ CFU/g, and one sample with $1.3x10^3$ CFU/g). The conclusion of the inspection was that the products analyzed did not present contamination that could represent a risk to health.

Regarding the total count of mesophilic aerobes observed in the survey of NSW Food Authority (2021), among the cooked products requiring heating before consumption (27 samples of the 85 analyzed), 20 samples (74%) were considered good (total count $<10^6$ CFU/g) or acceptable (total

count <10^7 CFU/g) and the others were not categorized. Among the raw products requiring cooking before consumption (54 of the 85 samples), 10 presented a total count <10 CFU/g, 38 had a count between 10 and $2.2x10^6$ CFU/g (mean of $9.3x10^4$), and 10 had a count above $3x10^5$ CFU/g. Among the four products without instructions for preparation before consumption, two samples presented a total count <10 CFU/g, one had a count of $1.3x10^4$, and one had a count above $3x10^5$ CFU/g.

Tóth *et al*. (2021) also evaluated the total count of mesophilic aerobes, as well as enterobacteria, molds and yeasts in 15 samples of five types of plant proteins: wheat gluten, soy protein, textured soy protein, textured pea protein (with 70% protein) and textured pea protein (with 55% protein), with three samples of each type, produced by two manufacturers, the Norwegian Vestkorn (https://vestkorn.com/) and the North American DuPont (https://www.dupont.com/). Wheat gluten samples had a mean total mesophilic aerobic count of 3.32 log CFU/g. In the other samples, the total mesophilic aerobic count was below 10 CFU/g. The presence of enterobacteria, molds and yeasts was not detected (<10 CFU/g) in any of the samples analyzed.

Regarding studies of the multiplication potential of spoilage and pathogenic bacteria in plant-based meat during refrigerated storage, Liu *et al*. (2023) researched the changes in the microbial population of soy-based and pea-based meat analogs stored at 4°C, examining both the naturally present microbiota (total mesophilic aerobes, lactic acid bacteria, coliforms, molds and yeasts) and also artificially inoculated bacteria, including deteriorants (*Pseudomonas fluorescens* and *Brochothrix thermosphacta*) and pathogens (*Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes*).

In the soy-based analog food product, the results showed initial contamination of 3.1 log CFU/g for total mesophilic aerobes, 2.04 log CFU/g for lactic acid bacteria, 2.00 log CFU/g for coliforms, and 1.95 log CFU/g for molds and yeasts. In the pea-based analog, 3.82 log CFU/g for total mesophilic aerobes, 3.61 log CFU/g for lactic acid bacteria, 2.51 log CFU/g for coliforms, and 1.44 log CFU/g for molds and yeasts. At the end of 10-day storage at 4°C, both the count of total mesophilic aerobes and the count of lactic acid bacteria were close to 7.00 log CFU/g in both products. The count of *Pseudomonas fluorescens* in the soy-based and pea-based analogs increased by 0.4 and 3 log CFU/g, respectively. The count of *Brochothrix thermosphacta* increased by 0.96 and 1.58 log CFU/g in soy-based and pea-based analogs, respectively. In the soy-based analog, the count of *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* had no change after 7-day storage at 4°C, while the pea-based analog had *Listeria monocytogenes* increased by 0.74 log CFU/g.

Another study conducted by Luchansky *et al*. (2020) observed the behavior of shiga toxigenic *Escherichia coli* (STEC), *Salmonella,* and *Listeria monocytogenes* artificially inoculated into plant-based and beef burgers during storage at 4°C and 10°C for 21 days. In storage at 4°C, the count of *Listeria monocytogenes* in the plant-based burger increased by 1.3 log CFU/g, while the count of *Salmonella* and *E.coli* decreased by 0.4 log CFU/g. In the bovine burger, the count of *Listeria monocytogenes, E. coli,* and *Salmonella* decreased by 0.7, 0.3, and 0.6 log CFU/g, respectively. At 10°C, the count of *Listeria monocytogenes, E. coli,* and *Salmonella* in the plant-based burger increased by 2.6, 2.4, and 0.8 log CFU/g, respectively, while in the beef burger, the count decreased by 0.9, 0.2, and 1.2 log CFU/g, respectively. These data seem to show a higher susceptibility of the plant-based burger to the multiplication of these pathogens compared to the beef burger.

As a conclusion and for a better assessment of the research gaps regarding biological hazards, Chart 8 presents an overview of the scope of the information previously reported according to the type of material analyzed and microorganisms researched.

What is observed is that the number of studies available until the completion of this report (December 2023) was very small in view of the number of new products and new ingredients introduced into the market. When evaluating the availability of data according to the type of product, there are more studies on the quality of proteins than on the final products. However, only one or two studies were found for each particular protein type. When analyzing the data according to the types of contaminants researched, it is also observed that the studies are sparse by type of microorganism (one or two studies), with more data being found on the total count of mesophilic aerobes and *Bacillus cereus*.

In these few studies, the results were considered satisfactory from a safety point of view, but there are exceptions, such as the study of Martins *et al*. (2014), which found *B. cereus* count above 10^5 CFU/g in textured soy protein, and the study of Pernu *et al*. (2020), which found counts of up to $1.2x10^3$ spores/kg for non-proteolytic psychrotrophic strains of *Clostridium botulinum* in vacuum-packed plant-based sausage sold under refrigeration.

Thus, considering that the sampling is very small, the conclusion on the quality and safety of these foods still requires the involvement of other researchers and research groups in the microbiological evaluation of different products in different countries, at different sampling points, and at different stages of manufacture and marketing. This is particularly critical for finished products, given how quickly new alternatives are introduced into the market.

CHART 8: SCOPE OF INFORMATION FOUND IN THE LITERATURE ON BIOLOGICAL CONTAMINANTS IN PLANT-BASED MEAT AND IN THE PLANT PROTEINS USED IN THEIR FORMULATION, ACCORDING TO THE TYPE OF MATERIAL ANALYZED AND MICROORGANISMS RESEARCHED.

Material analyzed	Microorganisms researched							
Plant proteins	***Bacillus cereus***	***E. coli***	***Listeria mono***	***Salmonella***	**Total count**	***Bacillus* sp**	***Clostridium* sp**	**Spores**
Rice protein	*2, 3	-	2	2	2, 3	2	2, 3	3
Potato protein	2	-	2	2	2	2	2	-
Pea protein	2	-	2	2	2, 3, 8, 9	2	2, 3	3, 9
Fava bean protein	3	-	-	-	3, 9	-	3	3, 9
Bean protein	3	-	-	-	3, 9	-	3	3, 9
Sunflower protein	1	1	-	1		-	-	-
Chickpea protein	3	-	-	-	3, 9	-	3	3, 9
Lentil protein		-	-	-		-	-	-
Soy protein	2, 5	-	2	2	2, 8	2	2	-
Wheat protein	2	-	2	2	2, 8	2	2	-
Lupin protein	2	-	2	2	2	2	2	-

Identification of contaminants	-	-	-	-	3, 9	-	3	3, 9
Effect of texturing on contaminants	-	-	-	-	2	-	-	-
Finished product at retail	***Bacillus cereus***	***E. coli***	***Listeria mono***	***Salmonella***	**Total count**	***Bacillus* sp**	***C. botulinum* (spores)**	**Spores**
Miscellaneous	6	6	6	6	6	-	-	-
Plant-based sausage	-	-	-	-	-	-	7	7
Pea-based ground meat analogs	-	-	-	-	4	-	-	-
Soy-based ground meat analogs	-	-	-	-	4	-	-	-

*Referências: 1) *Canadian Food Inspection Agency* (2019), 2) *European Commission* (2021), 3) Kyrylenko *et al.* (2023), 4) Liu *et al.* (2023), 5) Martins *et al.* (2014), 6) *NSW Food Authority* (2021), 7) Pernu *et al.* (2020), 8) Tóth *et al.* (2021), 9) Wells-Bennik (2022).

Studies are also necessary to identify the contaminants found in non-specific microbiological analyses (total count, spores of mesophiles and thermophiles, bacilli and clostridia, for example), in order to obtain over time a comprehensive knowledge of the microbiota commonly present in this class of products.

Another gap that requires attention is the effect of extrusion on bacterial spores, since this process is considered a critical control point in reducing the contamination of plant proteins and meat analogs.

An example of this type of research initiative is a project underway in the Netherlands, coordinated by the Dutch company Nizo (https://www.nizo.com/) with the participation of the Dutch universities HAS Green Academy (https://www.has.nl/en) and Wageningen University & Research (https://www.wur.nl/en.htm) and several private companies, with the objective of collecting information on the levels and types of microbial contaminants in more than 80 plant ingredients, their potential to survive processing and the risk of multiplication and/or production of toxins in the foods in which they are used (Consortium Investigates, 2023).

6.2. Data on chemical contaminants in the plant-based meat and in the plant proteins used in their formulation

From the point of view of chemical contaminants, a project within the EU-FORA (European Food Risk Assessment Fellowship Programme) carried out a risk-benefit assessment (RBA) of the replacement of meat with plant-based analogs as to exposure to mycotoxins and natural toxins of plants. In the first stage of the project, there was a review on the occurrence of these contaminants in the products available on the market and in the plant sources most used in their production (soy, chickpea, pea and gluten), covering the period from 2001 to 2021. The result of this review was published by Mihalache *et al.* (2022).

6.2.1. Mycotoxins

Regarding mycotoxins, the results reported by Mihalache *et al*. (2022) showed that contamination is little studied or, in some cases, not studied. The vast majority of reports are focused on soy-based foods, while only a few of them consider foods containing other proteins, such as pea or chickpea proteins. Considering all the studies surveyed, a total of 25 mycotoxins were found, the most frequent being aflatoxin B1, ochratoxin A, and zearalenone. Overall, the available data were not considered sufficient to provide a reliable representation of the occurrence of mycotoxins in legume-based products, especially for peas and chickpeas.

According to the authors, there are more than 400 mycotoxins identified so far, but due to the unavailability of toxicological and occurrence data, only a few are regulated in plant sources (aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, zearalenone and patulin), but other mycotoxins, often referred to as "emerging," have also been found in legumes and grains (eniatins, beauvericin and moniliformin, for example).

The highest concentration of aflatoxin B1 (AFB1) was found in soy-based burgers in Italy (10.1 µg/kg).

The highest mean value for ochratoxin A (OTA) contamination was found in peas from Germany (49.4 µg/kg), with an incidence of 9.1%. In soybeans, the mean value was 2.26 µg/kg (pooled results from 30 European countries).

Considering *Fusarium* mycotoxins, a 5% incidence of T-2 was reported in soy-based burgers in Italy, as well as deoxynivalenol (DON) and its acetylated forms (DON = 367.5 µg/kg, 3-AcDON = 154.7 µg/kg, 15-AcDON = 757.4 µg/kg). The incidence of DON and 3-AcDON was 5%. DON and 15-AcDON were also reported in soy sauce and roasted soybeans from Germany, with incidences of 25% and 20%, respectively.

Regarding contamination with zearalenone (ZEA), the highest mean level was found in German soy flour (214 µg/kg). The incidence in soy or soy-based products was 10% in soy flour and 100% in soy seeds. Alpha-zearalenol (α-ZEL) and beta-zearalenol (β-ZEL) ZEA metabolites have been reported in soy seeds, soy flour, and soy protein concentrate, with values ranging from 2 µg/kg to 100 µg/kg. The incidence was 10% in samples of soy flour and 20% in textured soy protein from Germany.

Fumonisins have also been found in legumes. The highest mean value reported was for fumonisin B1 (FB1), in Italian soy-based burgers (260.5 µg/kg); and lower concentrations were found in peas from Poland (0.02 to 0.9 µg/kg for FB1, FB2 and FB3).

Regarding emerging mycotoxins, one of the reported studies on the contamination of soy-based burgers of different brands marketed in Italy found enniatins (ENA, ENA1, ENB, ENB1) with an incidence ranging from 31% to 84% and Alternaria mycotoxins such as alternariol (AOH) and alternariol methyl ether (AME) with an average content of 184.4 µg/kg, and 207.5 µg/kg, respectively, in a frequency range of 5% to 9%.

A fact noted by Mihalache *et al*. (2022) in the project review was the occurrence, in several soy-based meat analogs, of mixtures of mycotoxins that can have proven additive or synergistic toxic effects. Examples: combination of [alternariol methyl ether (AME) + enniatins (ENAs)] with incidence ranging from 5% to 16% in soy-based burgers. Combination of [aflatoxin B1 (AFB1) + fumonisin B1 (FB1) + T-2 trichothecene] with an incidence of 20% in soy seeds. Combination of [trichothecenes types A and B + estrogen group toxins zearalenone (ZEA), alpha-zearalenol (α-ZEL), and beta-zearalenol (β-ZEL)] in soy-based foods. Combination of [scirpentriol (SCIRP) +

monoacetoxyscirpenol (MAS) + trichothecene HT-2 + Deoxynivalenol (DON) + Zearalenone (ZEA) + alpha-zearalenol (α-ZEL) + beta-zearalenol (β-ZEL)] with the incidence of 10% in soy flour. Combination of [SCIRP + MAS + T-2 Tetraol + DON + ZEA + α-ZEL] and [MAS + HT-2 + DON + ZEA + α-ZEL], with an incidence of 40% in partially defatted soy products. Combination of [MAS + HT-2 + DON + ZEA + α-ZOL] with 20% incidence in textured soy protein.

Rodríguez-Carrasco *et al*. (2019), who developed a quick and simple procedure for the simultaneous determination of isoflavones and mycotoxins in soy-based burgers, also found products contaminated with combinations of up to six mycotoxins per sample.

In conclusion, the results reported by Mihalache *et al*. (2022) showed that the contamination of plant-based meat by mycotoxins is still poorly studied, requiring further research to determine the situation of meat analog products on the market.

6.2.2. Natural plant toxins

Regarding the contamination of meat analogs or ingredients and their raw materials with natural toxins from plants, the review published by Mihalache *et al.* (2022) observed the almost unavailability of data on contamination by toxic alkaloids such as tropane and β-carboline, for example.

There were reports found on cases of contamination of soy-based products with tropane alkaloids (TAs) such as atropine and scopolamine. The highest contamination values originated from a notification made to the RASFF (Rapid Alert System for Food and Feed) by Germany, reporting levels of 19 µg/kg of atropine and 6.4 µg/kg of scopolamine in organic soy flakes imported from Austria. Pooled results from 16 European countries found the same alkaloids in soy flour, with an average limit of 1.54 µg/kg, for atropine, to 0.77 µg/kg for scopolamine.

Another type of alkaloid was found in soy sauce from Spain in 2004, β-carboline norharman (0.044 µg/kg) and β-carboline harman (0.18 µg/kg). A more recent study found higher levels of β-carbolines in soy sauce from Spain with mean values between 0.22 and 1.050 µg/kg. In Germany, soy sauce contaminated with carbohydrates derived from β-carbolines was also found, with mean values from 643.5 µg/kg to 1,819 µg/kg.

6.2.3. Agricultural pesticide residues

In addition to the chemical contaminants highlighted above, there are other potential hazards whose presence in plant-based meat and their main ingredients is practically unknown. Pesticide residues, for example, were detected in a study by Kolakowski *et al*. (2020) on the prevalence of glyphosate in soy-based products from Canadian retail markets (2015–2017). A soy-based beverage showed 0.0051 ppm, and meat alternatives showed concentrations of 0.015-0.016 ppm. In both cases, the author reports that the maximum allowed residue limit was not exceeded, but it should be noted that, like mycotoxins, residues of other pesticides may be present in the same product. In fact, Gionfriddo *et al*. (2020) evaluated the contamination levels for some pesticide residues in soy milk (two brands), finding dimethoate (118.9 and 6.5 µg/kg), malathion (27.4 and 28.2 µg/kg), chlorpyrifos (7.4 and 7.7 µg/kg), phosalone (40.1 and 33.6 µg/kg) and cyfluthrin (20.5 µg/kg).

6.2.4. Heavy metals

Similarly, there is an almost total lack of data on heavy metal contamination. In one of the rare studies found in the literature, Astolfi *et al*. (2020) analyzed 26 samples of plant-based milk analogs (four soybean, four rice, two oat, one spelt wheat, four almond, four coconut, two hazelnut, two nut, one cashew, one hemp and one quinoa) from the Italian market, with results showing low contamination with toxic trace elements, including arsenic, cadmium, lead and mercury.

Milani *et al*. (2023) researched trace element contents in 18 samples of soy-based beverages sold in the state of São Paulo (Brazil), noting that toxic metallic elements such as arsenic, cadmium, lead and tin were within the limits established by Brazilian and Mercosur legislation.

6.2.5. Process-induced toxic compounds

It is also necessary to consider the toxic compounds that can be formed during food processing, such as, for example, heterocyclic aromatic amines (HAAs), polycyclic aromatic hydrocarbons (PAHs), and N-nitroso compounds (nitrosamines, N-nitrosodimethylamine and N-nitrosodiethylamine) in meat products. According to a publication released by the United Nations Food and Agriculture Organization (FAO, 2022), meat analogs also have a risk of forming such compounds, as well as others resulting from processing at high temperatures, which still need to be researched. Notably, for example, the potential for the occurrence of glycidyl fatty acid esters, 2-monochloropropanediol (2-MCPD,) and 3-monochloropropanediol (3-MCPD), which are heat-induced contaminants in food, as well as the possible occurrence of trans fatty acids formed during partial hydrogenation of plant oils in certain plant-based meat analogs.

Heterocyclic aromatic amines (HAAs), considered carcinogenic and mutagenic, can be produced through Maillard reactions at cooking temperatures between 150 and 300°C (Lin *et al*., 2023), making it possible for their formation in the extrusion process. The application of heat by the consumer in the domestic preparation of products also offers opportunities for the formation of HAAs (Chen & Xi, 2022).

Xi and Chen (2021) submitted three plant-based meats to heat treatment at different temperatures (170, 190, and 210°C), observing much lower levels of HAAs than those found in meat-based products. Deng *et al*. (2022) studied the accumulation of HAAs at different stages of the processing of plant-based meat analogs, finding maximum levels of 160.3 ng/g in plant-based burger, lower than those of traditional beef burger (381.3 ng/g).

Polycyclic aromatic hydrocarbons (PAHs), considered carcinogenic, can be produced in the improper combustion of organic products (Wang *et al*., 2022). In the preparation of barbecue with charcoal or in smoking, meat-based products and their plant-based analogs may have contact with the flame, allowing the formation of PAHs (Rose *et al*., 2015; Urban & Lesueur, 2017).

Zastrow *et al*. (2021) studied the formation of PAHs in plant-based beef burgers grilled in charcoal, finding between 1.4 and 6.4 µg/kg, lower values than those found in beef burgers.

He *et al*. (2020) reported a screening by their research group for the detection of PAHs and N-nitroso compounds in commercially available plant-based meat analogs. In most products, these compounds were not detected in the samples after cooking under the conditions recommended by the manufacturers, except in one, which presented N-nitrosodiethylamine at a concentration of 15.19 ± 1.21 µg/kg (unpublished data).

All data presented in the previous items, sometimes discrepant between the reports found, make clear the need for more comprehensive research on the situation of meat analog products and their main ingredients available on the market regarding the presence of chemical contaminants. This knowledge is relevant not only from the point of view of consumer safety, but also from the point of view of protecting manufacturers and their brands.

6.3. Anti-nutritional factors in plant proteins

An issue that can be raised regarding plant-based meat is the presence of antinutritional factors (ANFs) in the proteins used in the formulation, since they are the ingredients present in greater quantity. Anti-nutritional factors are natural compounds present in certain plants that exhibit the ability to impair the digestibility and metabolic utilization of proteins and other nutrients. The main ones found in grains and legumes are oligosaccharides, trypsin inhibitors, phytic acid, tannin and hemagglutinins, also called lectins.

Oligosaccharides (raffinose, stachyose and verbascose, for example) are not digested in the intestine and can cause flatulence and abdominal swelling. Raffinose can cause nausea, diarrhea, indigestion and gas (Elango *et al*., 2022).

Trypsin inhibitors inhibit the activity of trypsin by reducing the digestion and absorption of dietary protein. They may cause pancreatic hypertrophy/hyperplasia (growth inhibition) (Liener, 2009).

Phytic acid can bind to minerals, proteins and starch. It decreases the availability of several minerals, such as calcium, iron, zinc, copper, cobalt, and manganese (Kies *et al*., 2006).

Tannin inhibits digestive enzymes, decreasing the digestibility of proteins and carbohydrates (Joye, 2019).

Absorbed hemagglutinins (lectins) can affect immunity, the endocrine system, and overall metabolism. They can bind to glycoprotein receptors on epithelial cells lining the intestinal mucosa, inhibiting growth by interfering with nutrient absorption (Bardocz *et al*., 1995).

The adopted plant protein production process (wet extraction or dry fractionation) has a different effect on the ANF content in the final product. Dry fractionation using air classification is a method applied to the flours of starch-rich crops, such as peas or beans, in which the flour is submitted to a spiral flow of air that separates the protein granules from the starch granules by difference in size and density. Air classification leads to enrichment in antinutritional factors such as tannins, phytic acid, and trypsin inhibitors (Banach *et al*., 2022).

Wet extraction is the standard method for producing protein isolates, mainly used in the processing of oilseeds and legumes. In the case of oilseeds (e.g., soybean or canola), it requires a defatting step (by pressing or extraction with hexane). In summary, wet extraction includes a hydration step, a settling step to remove starch and insoluble fiber, isoelectric precipitation to extract the globulin fractions from the proteins, and a drying step. The high purity of the protein obtained provides greater flexibility in food formulation and in the removal of antinutritional factors (especially during the isoelectric precipitation step) (Banach *et al*., 2022).

ANFs can be reduced or inactivated through the following processes:

- Oligosaccharides: maceration (soaking/resoaking), fermentation, and germination.
- Trypsin inhibitors: heat treatment.
- Phytic acid: maceration, germination, fermentation.
- Tannin: peeling, maceration, cooking, fermentation, germination.

- Hemagglutinins (lectins): heat treatment.

Heat treatments applicable for elimination or reduction include boiling, drying and roasting, pressure cooking (domestic, 30 minutes in a pressure cooker, autoclaving), and thermoplastic extrusion.

According to Nikmaram *et al*. (2017), extrusion is considered effective in reducing the majority of ANFs associated with plant proteins. Rathod & Annapure (2016) evaluated its effect on the inactivation of anti-nutritional factors of lentil protein, noting that, in the operation with moisture content of 22%, temperature of 180°C, screw speed of 150 to 250 rpm and constant feed rate of 16 rpm (340 g/min), it was possible to reduce trypsin inhibitors by 99.54%, phytic acid by 99.3% and tannin by 98.83%, without changing the protein content.

A study conducted by Kaur *et al*. (2015) evaluated the effect of extrusion process variables on phytic acid and trypsin inhibitor contents in wheat, rice, barley and oat bran fractions. For phytic acid, extrusion performed with a humidity of 20% and a temperature of 115°C showed a greater reduction in wheat bran (64.4%), barley bran (63.55%), and oat bran (26.47%). For rice bran, the highest phytic acid reduction (55.83%) was achieved at 140°C and 20% moisture. Similarly, the highest reductions in trypsin inhibitor content were obtained in extrusion performed at 140°C and 20% moisture, with a 71.2% reduction in wheat bran, 72.2% in oat bran, and 73.1% in rice and barley bran. These results may indicate that temperatures of 115 to 140°C, along with higher moisture contents (20%), could help reduce phytic acid and trypsin inhibitor contents in fiber-rich by-products.

On the other hand, Ainsworth *et al*. (2007) studied the effect of extrusion on a material composed of the brewing industry's by-product (barley grain hulls in combination with parts of the pericarp and layers of the barley seed coating) and starchy flours. They observed no reduction in phytic acid content. The authors concluded that the mechanical energy applied in the extrusion was not sufficient to cause the reduction, probably due to the presence of other components that have a protective effect against shear forces.

Kelkar *et al*. (2012) evaluated the effect of low-temperature extrusion (85, 100 and 120°C) on the reduction in raffinose and stachyose in two varieties of beans (pinto bean and navy bean), observing a reduction in stachyose only in the pinto variety and in raffinose in both varieties, but at a lower degree than that obtained in steam cooking. Berrios *et al*. (2010) also observed a reduction in raffinose after extrusion of lentil and pea flour, while in chickpea flour, there was no significant difference. Pedrosa *et al*. (2021) reviewed studies on the effect of extrusion on legume oligosaccharides, including raffinose and stachyose, finding controversial results; several studies reported an increase, while others reported a decrease. In general, increments were found in association with the release of oligosaccharides from the food matrix due to cell damage or partial hydrolysis of longer-chain oligosaccharides, caused by extrusion temperature and pressure. The reduction was shown to be related to the formation of complexes between sugars and proteins during extrusion. The authors concluded that the different oligosaccharides can be affected in various ways depending on the extrusion parameters. In addition, the extent of these modifications may depend not only on the extrusion conditions but also on the characteristics of the raw materials being processed (Pedrosa *et al*., 2021).

However, it is important to consider that not all proteins added to the products addressed in this study are necessarily extruded, with each manufacturer being responsible for evaluating the situation of their product regarding this factor.

6.4. Allergens in alternative proteins

Another point that requires further studies in view of the trend of replacing meat-based products with plant-based analogs is the issue of allergens.

Food allergy is defined as an adverse effect on human health arising from an abnormal immune response to exposure to certain food proteins. Most reported and confirmed food allergies are categorized as IgE-mediated, as they trigger the immune system to produce immunoglobulin E (IgE) antibodies. Symptoms of IgE-mediated allergies can range from mild and transient to severe, including death without appropriate and urgent treatment. They typically develop within minutes to 1-2 hours after ingestion of minimal amounts of the implicated food. In non-IgE-mediated reactions, including celiac disease, symptoms usually occur several hours after exposure and are rarely acute or life-threatening (Kopko *et al*., 2022).

The *Codex Alimentarius* lists eight products as responsible for the majority of food allergies worldwide: crustaceans; eggs; fish; milk; peanuts; soybeans; nuts; and gluten-containing cereals (wheat, rye, barley, spelt or its hybridized strains, and oats, the latter included because, while it does not contain gluten, it is commonly produced in the same location as gluten-containing cereals, such as wheat, resulting in cross-contact with allergens) (*Codex Alimentarius* CXC 80-2020– Code of Practice on Food Allergen Management for Food Business Operators).

However, there are several other products that can cause allergic reactions in susceptible individuals, and the increasing use of new plant protein sources also implies greater exposure to new allergens, with studies demonstrating the influence of consumption habits on the development of food allergies (Kopko *et al*., 2022). The authors note, for example, an increased number of cases of allergy to buckwheat (*Fagopyrum esculentum* Moench) in Asia, as it is a pseudocereal that has been widely used as a replacement for wheat in foods because it does not contain gluten. Another example is that, in settings with regular exposure to a particular allergenic food, the prevalence of allergy to that food is lower among first-generation immigrants from settings with lower exposure than among the native population, but the prevalence among second-generation immigrants, already subjected to greater exposure, increases compared to the first generation.

An important fact to consider when observing an increase in the prevalence of food allergies is to assess if there was a real increase in the absolute number of occurrences due to the influence of environmental factors or if the increase is not also related to improved identification and diagnosis and enhanced notification systems (Savage *et al*., 2016).

Therefore, Kopko *et al*. (2022) note the need to study the new factors and trends that influence protein consumption patterns while evaluating the ability of existing monitoring and regulatory systems to manage the increased incidence of reactions to new allergens, as well as existing ones.

To this end, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have held a series of meetings in recent years to discuss the issue of food allergies, reported in three publications. The first deals with the review and validation of the list of priority allergens in the Codex Alimentarius (Food and Agriculture Organization of the United Nations/World Health Organization, 2022a). The second deals with the review and establishment of limits for priority allergens in foods (Food and Agriculture Organization of the United Nations/World Health Organization, 2022b) and the third deals with the review and establishment of preventive labeling for allergenic foods (Food and Agriculture Organization of the United Nations/World Health Organization, 2023).

7. Conclusion of the study on the safety aspects of the production of plant-based meat

From the point of view of their intrinsic characteristics, the four products addressed in this study are extremely susceptible to microbial deterioration and pathogen multiplication, since they do not contain preservatives and their pH and water activity are not restrictive to the growth of microorganisms. With the exception of the fish analog, which undergoes commercial sterilization heat treatment already in the hermetically sealed packaging, the safety of the others during the shelf life is totally dependent on the cold chain in transport and sale. In this aspect, plant-based sausage is the most vulnerable, since the fact that it is not frozen may allow the multiplication of psychrotrophic pathogens such as *Listeria monocytogenes*, non-proteolytic strains of *Clostridium botulinum,* and strains of *Bacillus cereus*, having its shelf life restricted to 10 days at temperatures not exceeding 8°C.

Hazard identification showed that there are hazards common to almost all plant ingredients used in the formulation of plant-based analogs, such as *Salmonella*, arsenic, cadmium, lead and pesticide residues, while others are characteristic of certain ingredients, such as aflatoxins B1, B2, G1 and G2 in rice flour and bean, soy and wheat proteins; ochratoxin A (OTA) in bean, soy and wheat proteins; deoxyvalenol (DON) and zearalenone (ZEA) in soy and wheat proteins; glycicidol and 3-MCPD (3-monochloropropane-1,2-diol or 3-chloropropane-1,2-diol) esters in oils and fats; dioxins, furan and methyl furans in sugar; and ethylene oxide in konjac and guar gums, which are part of the composition of edible plant-based casing.

What was concluded throughout the study is that most of the control measures necessary for the safety of these products are not categorized as CCPs (Critical Control Points), but as PRPs (Prerequisit Program) or CPs (Control Points). Such measures include those that aim to ensure the quality of purchased ingredients regarding the absence or reduction of hazards to the minimum feasible, such as exclusive receipt from approved suppliers, receipt with a batch analysis report, and laboratory analysis of material received before release for use, and those that aim to ensure adequate conditions during processing (such as environmental hygiene monitoring plan, personal hygiene program for handlers, and allergen cross-contamination control program in factories that process more than one type of product.

The study showed large gaps in information on the incidence and prevalence of biological and chemical hazards in final products and plant ingredients used in their formulation, notably data on quantification and identification of microorganisms present; data on contamination with mycotoxins, heavy metals, pesticide residues and natural plant toxins such as tropane alkaloids and β-carbolines; data on the formation of process-induced toxic compounds, such as heterocyclic aromatic amines (HAAs), polycyclic aromatic hydrocarbons (PAHs), nitrosamines, glycidyl fatty acid esters, 2-monochloropropanediol (2-MCPD) and 3-monochloropropanediol (3-MCPD) esters; data on the persistence of antinutritional factors (trypsin inhibitors, phytic acid, tannin, hemagglutinins and oligosaccharides) in plant proteins and final products; and studies on what effect the trend of higher consumption of new plant protein sources will have on the development of food allergies.

8. Path to follow

Considering the gaps identified in this study and the growing interest in plant-based, it is suggested that regulatory agencies, industries, researchers, universities, and accrediting institutions intensify their efforts toward the following goals:

Regulatory Agencies: Monitor advances in safety studies, especially regarding greater scientific evidence on the potential chemical and biological hazards identified in this study to conduct possible updates of legislation, implementing new criteria and limits or adapting existing ones to more specific values for the production and sale of these foods, ensuring their quality and safety. In addition, promote research and development programs to fill the identified knowledge gaps, fostering collaboration between research institutions and industry. Expand the development of mechanisms for assessing HACCP studies of industries through the use of online platforms and information systems, in order to anticipate and mitigate risks associated with plant-based meat.

Industry: Implement robust food safety management systems, such as HACCP, and invest in production and analysis technologies that enable more effective control of identified hazards. Promote staff training on food safety aspects and the importance of product traceability, and foster collaboration with researchers to fill scientific knowledge gaps.

Researchers: Increase studies on the incidence and prevalence of contaminants in ingredients and final products, as well as on the formation of process-induced toxic compounds. Develop new analysis methodologies for the detection of emerging contaminants and assess the effectiveness of different treatments for risk reduction.

Universities: Include issues related to plant-based meat in curricula of nutrition, food technology and food engineering programs and related fields, promoting the training of qualified professionals to work in this sector.

Accrediting institutions and/or private initiatives for food safety: Include issues related to plant-based meat as a specific group of foods to be considered for audits of standards and schemes of food safety management systems, establishing, within the applicable needs, additional requirements and additional notes that can better elucidate safe food production practices of this segment of the food industry.

Collaboration between these actors is essential to ensuring the safety and quality of plant-based meat and promoting public health.

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10. Glossary

Plant-based meat analog food products. Plant-based products that mimic the color, flavor, texture, and appearance characteristics of meat-based products.

Hazard Analysis and Critical Control Points (HACCP). Food safety control system by means of identification and control of biological, chemical, physical, allergenic, and radiological hazards at all steps of the production chain, from raw material production to manufacturing, distribution and consumption.

Critical limits. Measurable value that separates acceptance from rejection.

Control measure Action or activity that is essential to prevent a significant food safety hazard or reduce it to an acceptable level.

Synergistic measure. An interaction that multiplies the results. It is the combined action of two or more control measures whose result can be the sum of the effects of each measure or a total effect that is greater than that sum.

Monitoring of CCPs and CPs. It is the determination of the situation of a system, a process or an activity through measurements of the control variables established for CCP or CP.

Hazard. Contamination, at an unacceptable level, with the potential to cause an adverse health effect.

Allergenic hazard. Allergenic agent in food, with the potential to cause an adverse health effect (food allergies arising from an abnormal immune response to exposure to certain food proteins). The Codex Alimentarius (CXC 80-2020 – Code of Practice on Food Allergen Management for Food Business Operators) lists eight products as responsible for the majority of food allergies worldwide: crustaceans; eggs; fish; milk; peanuts; soybeans; nuts and gluten-containing cereals (wheat, rye, barley, spelt or its hybridized strains, and oats, the latter included because, although it does not contain gluten, it is commonly produced in the same setting as gluten-containing cereals, such as wheat, resulting in cross-contact with allergens).

Biological hazard. Biological agent, in food, with the potential to cause an adverse health effect. Including pathogenic bacteria (such as *Salmonella*, *Campylobacter*, pathogenic *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Clostridium perfringens*, and *Clostridium botulinum*), viruses (such as norovirus and hepatitis A virus), and parasites (such as *Taenia solium*, T*oxoplasma gondii*, *Cryptosporidium* spp., *Entamoeba histolytica* and *Trichinella spiralis*).

Physical hazard. Physical agent, in food, with the potential to cause an adverse health effect. Including, for example, sand, insect fragments, ferrous and non-ferrous metal fragments, flexible plastic, or non-rigid polymer.

Chemical hazard. Chemical agent, in food, with the potential to cause an adverse health effect. Including, for example, mycotoxins (such as aflatoxins, ochratoxin A, fumonisins, zearalenone, deoxynivalenol), heavy metals (such as arsenic, cadmium, lead, and copper), and pesticide residues.

Radiological hazard. Radiological agent, in food, with the potential to cause an adverse health effect.

Significant hazard. Hazard identified by a hazard analysis as reasonably likely to occur at an unacceptable level in the absence of control and for which control is essential under the intended conditions of use of the food.

Control Point (CP). Also called operational prerequisite program (OPRP), this designation is used when a hazard control measure is part of good manufacturing practices, but requires specific actions

in addition to those proposed by Brazilian legislation, being considered essential for the control of identified hazards.

Critical control point (CCP). A process step in which one or more control measures that are essential to control and maintain a significant hazard at acceptable levels are applied in a HACCP plan.

Hazard probability. It is related to the frequency of occurrence of the hazard in its source and the possibility of the hazard remaining in the food, with damage to the consumer. In this study, it was classified as high (there is knowledge of the existence of the hazard in the product(s)/inputs evaluated or in similar products based on epidemiological data from the country and from abroad, with occurrence of recall, and is described in specific national and international legislation); medium (there is knowledge of the occurrence of the hazard in the product(s)/inputs evaluated based on epidemiological data from abroad, and is described in specific national legislation); low (the occurrence of the hazard in the product(s)/inputs is known only by reports in the technical and scientific literature, without identification of epidemiological data, and is not described in specific national legislation, with indications of control only in some other countries); negligible (the occurrence of the hazard is known only by reports in the technical and scientific literature, it is not specific to the product(s)/inputs evaluated, and there are no legal recommendations in the country or abroad for its evaluation).

Prerequisite Program (PRP). This designation is used when a significant hazard control measure is already clearly established in the good manufacturing practice standards established by Brazilian legislation.

Operational prerequisite program (OPRP). This designation is used when a hazard control measure is part of good manufacturing practices, but requires specific actions in addition to those proposed by Brazilian legislation, being considered essential for the control of identified hazards.

Hazard severity. Severity of the effect of a hazard present in food on consumer health. It can be classified as high (it can be lethal or cause sequelae or serious injuries, requiring immediate hospital intervention, or cause disabling or long-term illness or have a carcinogenic or teratogenic effect); medium (it can cause severe discomfort, but without sequelae, usually having short duration and self-limiting symptoms, and medical intervention may be needed); low (it causes only discomfort, mild ill-being or minor injuries, with no need for hospital intervention).

Risk significance. It is a function of the probability of occurrence of an adverse health effect and the severity of that effect, as a consequence of hazards present in food or its sources.

CCP, CP and PRP check. It is the confirmation, by provision of objective evidence, that specified requirements have been complied with.

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Todo o trabalho desenvolvido pelo GFI é oferecido gratuitamente à sociedade e só conseguimos realizá-lo pois contamos com o suporte de nossa família de doadores. Atuamos de maneira a maximizar as doações de nossa comunidade de apoiadores, buscando sempre a maior eficiência na utilização dos recursos.

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