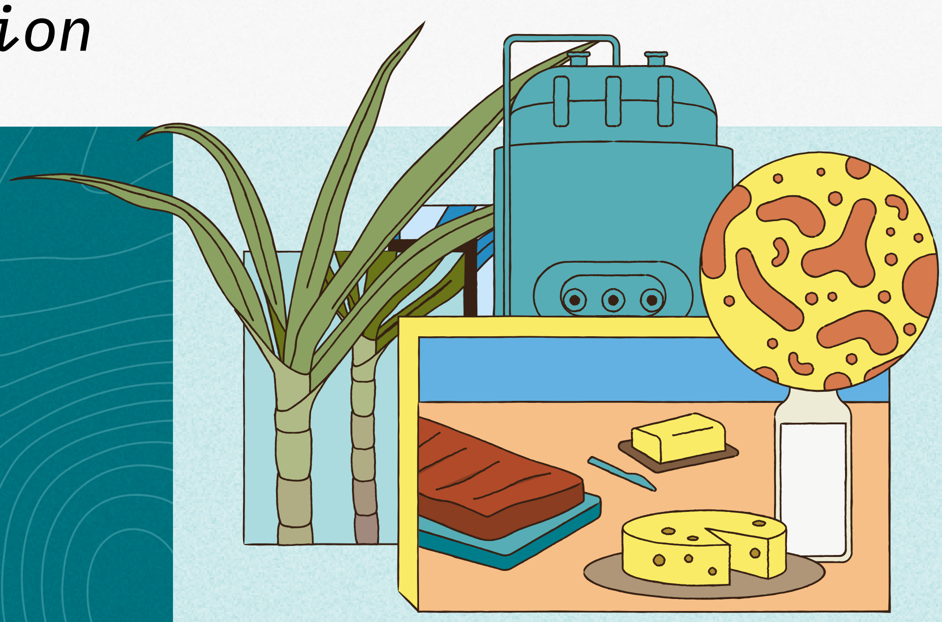
Fermentation in Brazil

Potential for alternative protein production

Image: Plant-based meat made from fermented soy flour - Chunk Foods.



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Executive summary

Whole cuts made from fermented soybean flour - Chunk Foods.

Globally, Brazil plays a key role in food production and can participate actively in building a more sustainable food system. The growing demand for foods and proteins and the impacts associated with the current production chain—such as aspects related to the environment, health risks, production efficiency, and resource use—have led to the pursuit of alternative sources of high-quality proteins that make the food production chain more efficient, safe and resilient.

In this context, fermentation technology—due to the microorganisms' rapid growth and high productivity—is an excellent opportunity for food industries seeking to meet consumers' new demands for healthier and more sustainable products. Ingredients produced through fermentation can contribute to developing healthy alternative protein products with high protein content and improved sensory, nutritional, and technological aspects. Moreover, this technology can be used to produce specific ingredients for cultivated meat, as is the case with culture media.

Fermentation processes comprising the growth of microorganisms to develop specific sensory and/or nutritional profiles in foods or ingredients are called traditional fermentation. The growth of microorganisms to use the cell biomass as a primary protein source is known as biomass fermentation. Finally, precision fermentation is the obtention of specific purified ingredients such as flavors, enzymes, proteins, and fats for incorporation into analogous plant products or cultivated meat.

Mycelium-based fish analogue - Aqua Cultured Foods.

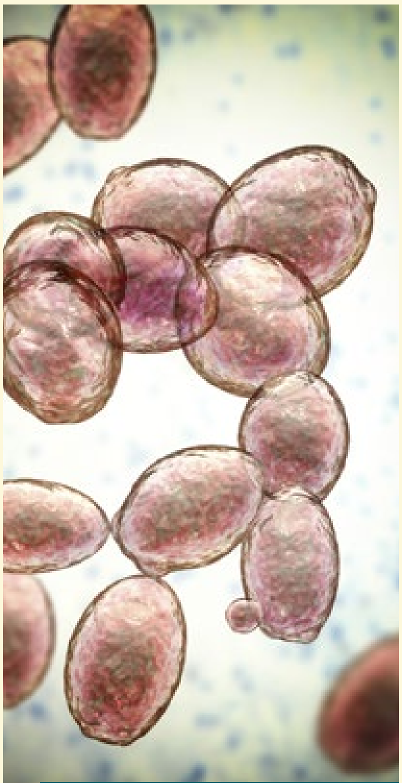
The global market for alternative products derived from fermentation has grown in recent years, reaching 4.1 billion dollars invested between 2013 and 2023 (Good Food Institute, 2023). Regarding established businesses, currently, 158 startups are developing options for alternative proteins produced through fermentation. Several development opportunities can be explored at all stages of the fermentation process chain, in particular, to optimize the processes and maximize the resulting products' competitiveness.

Brazil has the potential to lead the production of alternative proteins, aligning with the global growth of this industry. The country can actively build this new chain by coordinating stakeholders such as research institutions and industries and leveraging national strengths and established capacities. Such strengths include the wide availability of raw materials for fermentation, such as sugarcane, corn, agro-industrial wastes and by-products of the food and beverage industries, along with the significant technical and productive capacity, driven by the numerous companies using large-scale fermentation in the food, chemical, health and biofuel areas.

This document provides information on fermentation processes, their applications in the production of alternative proteins, investments, businesses and regulation of the sector regulation. In addition, it contains unprecedented findings from a survey of the development of fermentation technology applied to alternative proteins in Brazil. Such content is the base for the discussion on the potential, challenges, bottlenecks and opportunities for large-scale production.

BOX 1

Benefits and advantages of Brazil for the production of fermentation-derived proteins.


3D illustration of yeasts - Adobe Stock.

- Natural biodiversity: the country's biodiversity is vast, with microorganisms, plants, grains, seeds, etc., to be explored in fermentation;
- Availability and diversity of substrates for use in fermentation;
- Already established fermentation companies can be adapted to produce proteins;
- Potential on collaboration between agro-industries and fermented-protein producing companies, using residues as a culture medium input, resulting in a circular economy;
- Established regulation for fermentation-derived proteins.

Key-words: *traditional fermentation, biomass fermentation, precision fermentation, food, alternative proteins, bioeconomy, sustainability.*

Abbreviations and acronyms

LCA
Life Cycle Assessment

ANVISA
Brazilian Health Regulatory Agency

ABBI
Brazilian Association of Bioinnovation

LAB
Lactic Acid Bacteria

CAPEX
Capital Expenditure

COP
Conference of the Parties

CRISPR
Clustered Regularly Interspaced Short Palindromic Repeats

DNA
Deoxyribonucleic acid

EMBO
European Molecular Biology Organization

EMBRAPA
Brazilian Agricultural Research Corporation

USA
United States of America

GFI
Good Food Institute

SDGs
Sustainable Development Goals

GMO
Genetically Modified Organism

pH
Power of Hydrogen or Potential of Hydrogen

GDP
Gross Domestic Product

RDC
Collegiate Board Resolution

RNA
Ribonucleic acid

SCO
Single-Cell Oil

SCP
Single-Cell Protein

TEA
Techno-economic assessment, or techno-economic analysis

PBI
Precision Breeding Innovation

UN
United Nations

Content

1

Introduction


Mycelium-based chicken analogue - Typcal.

1.1. The food system and alternative proteins

Population growth, changing dietary patterns and concerns about global warming make reformulating the world's food system urgent. The world population is estimated to be approximately 9.7 billion people by 2050 (United Nations, 2022), requiring a proportionally up to 73% increased food production to meet the growing demand (Bonny et al., 2015). Additionally, the environmental impacts of food production and the food system, which currently represent almost a third of the global greenhouse gas emissions (Food and Agriculture Organization, 2021), also warrant attention. In Brazil, this system represented 73.7% of total emissions in 2021, with a majority contribution related to beef production, equivalent to 57.2% of total emissions (*Sistema de Estimativas de Emissões e Remoções de Gases de Efeito Estufa*, 2023). In face of these challenges, it is imperative to seek solutions that reconcile the need to feed the growing population with the mitigation of the environmental impacts associated with food production.

Reducing greenhouse gas emissions by 2030 has become a goal to address global warming. Recently, the food system's impact was noted in COP27 and detailed in the COP28 global assessment (United Nations, 2023a). Changing production methods would not only optimize land use, but also reduce greenhouse gas emissions, thereby enabling the maintenance of ecosystems. In this context, alternative, more sustainable food production methods have attracted consumers' attention (Fasolin et al., 2019), driving continuous innovations in the alternative protein sector.

Alternative proteins include those derived from microorganisms such as yeast, bacteria, microalgae, and filamentous fungi, in addition to plant proteins and cell culture proteins. Compared to conventional proteins, alternative proteins have the potential to reduce environmental impacts, lower the risk of zoonoses and antimicrobial resistance, and contribute to animal welfare (United Nations, 2023b). Moreover, these alternatives can provide amounts of proteins and nutrients that are similar to, and in some cases even higher than, those of conventional meat. For example, plant-based meat analogs are notable for their high fiber content compared to conventional meat (Karatay; Ambiel, 2024). Although soy and wheat are already widely used as alternative protein sources, other inputs have been explored to provide a higher diversity of products to replace meat, fish, eggs, milk and derivatives; these substitutes are produced with plant proteins and through bioprocesses such as fermentation and cell culture.

Specifically regarding fermentation, since antiquity humans have utilized this natural process to produce a variety of products, of greater or lesser degree of complexity. Initially, fermentation was used to preserve food for longer periods and improve its nutritional value (Mannaa et al., 2021). Today, fermentation plays an important role in the expansion of options for already-known products, such as yogurts and cheeses, and in the creation of new foods, such as mycelium-based meat analogs[1].

Meat analogue made from mycelium - Nature's Fynd.

1 See Box 3 "What is mycelium? And mycoprotein?"

Microbial protein production through fermentation processes emerges as an effective and sustainable solution to meet the growing demand for alternative proteins. Unlike in animal production, this method provides a shorter and continuous production cycle, without relying extensively on natural resources such as arable land and water, and exhibits greater resilience to environmental events, such as periods of drought (Durkin et al., 2022). Additionally, agro-industrial waste can be used as raw material for the development of microorganisms, increasing the environmental efficiency of these processes. With more than 50 microorganism strains already used for food, including bacteria, microalgae, filamentous fungi and yeasts (Banks et al., 2022), microbial production offers a wide range of products, such as proteins, amino acids, oils, enzymes and vitamins, with potential for short- to long-term industrial scale-up. Thus, from a market point of view, fermentation applied to alternative protein production can provide numerous new options for consumers—with tasty, nutritious and sustainable products—and create business opportunities.

1.2. National and international market contextualization

The alternative protein sector reached a total value of US$ 59.1 billion in 2023 (Alternative Protein Market, 2023). Current projections estimate that these proteins will have five times lower costs by 2030 and ten times lower costs by 2035. These projections rely on the advancement of technology and resolution of bottlenecks so, by 2030, alternative protein products have better quality and cost less than half the production cost of animal products (Tubb; Seba, 2019). Regarding fermentation, current technical-economic analysis models estimate that mycoprotein, for example, can be produced for US$ 3.55/kg and a mycoprotein-based meat analog product can be produced for US$ 4.03/kg, indicating that the alternative is economically competitive with the price of beef cuts, especially when examining costs concerning protein content. These analyses also highlight the impact of the chosen type of process, indicating that continuous fermentation (as opposed to a batch system) was necessary to achieve cost parity with bovine protein. The authors also note that potential cost reductions to reach price parity with cheaper products such as chicken can be achieved through advances in specific microorganism parameters, such as protein content, biomass concentration reached (g/L), and specific growth rate (Risner et al., 2023).

Therefore, fermentation technology is a promising alternative for the food industry due to the wide range of inputs and variety of products obtained, encompassing both established ingredients such as yeast extracts—used to enhance the flavor of meat analog products and cheeses or to increase the nutritional value of products (Kale; Mishra; Annapure, 2022)—and novel products such as microbial protein-based dairy and meat analog products.

According to the GFI report (Good Food Institute, 2023), the alternative protein production-related fermentation sector received significant investments, totaling US$ 515 million in 2023, with a cumulative investment of US$ 4.1 billion since 2014. Currently, 158 companies use fermentation technology to produce alternative proteins: 80 companies use it for biomass fermentation; 73 companies, for precision fermentation; and 5 companies, for traditional fermentation.

To further the development of this growing market, companies specializing in processing meat, milk, and their derivatives, as well as food and beverage companies, are establishing strategic collaborations with fermentation protein companies. These companies already have substantial financial resources, robust infrastructure, and consolidated access to the supply chain. The partnerships contribute to advancing the technology, enhance manufacturing processes, and effectively introduce fermentation ingredients and products to the market. An example is the partnership between the multinational company **Nestlé and Perfect Day**, which teamed up to create a dairy beverage without animal ingredients, made with whey protein produced by the startup through precision fermentation. Other multinational giants in addition to Nestlé, such as Kraft Heinz, Cargill, Danone, ABInBev, General Mills and Tyson are involved in the fermentation sector either through investments, partnerships or R&D and production, which demonstrates the potential and importance of this rising market (Good Food Institute, 2023a).

In the Brazilian context, as of August 2024, at least nine national startups are developing alternative protein businesses and products using fermentation technology. Three of these startups are currently focused on the production of milk proteins by precision fermentation: Updary, **Ark Bio Solutions**, and **Future Cow**. Also, using precision fermentation, **Biolinker** operates in the production of growth factors for cultivated meat. Three other startups are using biomass fermentation to obtain protein ingredients and products: **Tekohá**, **Hyph** and **Typcal**, which **recently announced the development of a mycelium-based chicken breast analog**[2] with only two ingredients in addition to mycelium in the formulation and obtaining a product with satisfactory protein content, zero fat and containing fiber. The startup participates in the **Fungi Protein Association**, which aims to foster knowledge of fungi-based proteins and public policies on this new technology. **ProVerde** uses traditional fermentation to develop protein ingredients using food industry by-products, aiming at application in meat analogs. Finally, the **BioInFood** startup is focused on developing customized yeasts, ingredients, and processes, an example of different business opportunities, such as providing services and supplies for this fermentation-based alternative protein product production chain. All these initiatives illustrate Brazil's growing potential and diversification of the fermentation field, aligning with global innovation trends in the food industry.

1.3. Regulatory demands

The development of the alternative protein industry worldwide requires the establishment of appropriate regulatory framework and governance instruments. Alternative protein regulation is evolving worldwide, with several governments formulating and implementing new policies for product approval. Furthermore, implementing regulations reduces the risk associated with investments by established companies in the sector and new investors interested in startups, thereby creating business opportunities.

2 See Box 3 "What is mycelium? And mycoprotein?"

Mycelium-based chicken analogue - Typcal.

Currently, the food industry employs microbial fermentation to process and develop ingredients, making the most of this technology. This technology is also employed in the production of flavorings, sweeteners, and thickeners for foods and beverages. Most governments establish well-defined regulatory systems to ensure the safety of new products. Some countries evaluate new fermentation products by implementing specific regulations, requiring prior authorization for marketing. For example, the United States applies a more comprehensive regulation (Good Food Institute, 2022b).

Two prominent global initiatives for building fermentation regulation were the creation of the Fungi Protein Association in 2022 and the Precision Fermentation Alliance in 2023. The first includes over 30 startups, including Quorn, ENOUGH, and the Brazilian company Typcal. The second involves 13 precision fermentation startups, such as EVERY and Perfect Day. Both initiatives aim to pursue public policies, consumer engagement, and establishing regulation for products obtained through fermentation processes. In addition, the **ProVeg International** accelerator also supports the Fungi Protein Association. Such associations are key agents in knowledge exchange, institutional support engagement, organization around regulatory standards, and raising people's awareness of the advances and benefits of fermentation in food production.

Currently, the Ministry of Health is Brazil's main authority responsible for food safety regulation and inspection. It performs the function through the autonomous Brazilian Health Regulatory Agency (ANVISA), which evaluates the safety of additives and ingredients in foods and regulates labeling. Brazil is gradually replacing many of its food standards with official Mercosur standards, which, in turn, are influenced by European and North American standards.


Mycelium-based meat analogue - Enough.

ANVISA recently published RDC Resolution No. 839, of December 14, 2023, regulating the registration of new foods and ingredients without a history of safe consumption in the country, including those based on cell culture and fermentation. The resolution includes food obtained from plants, animals, minerals, microorganisms, fungi, algae or synthetically. This advancement positions Brazil prominently in the global application of alternative proteins, creating opportunities to attract investments in novel sustainable food production methods. The document contains the stages of exposure and risk assessment, characterization, studies of possible interactions, adverse health effects, composition specification, food/therapeutic purpose, nutritional quality, and toxicity, among others.

According to the document, new foods and new ingredients that are constituted, isolated or produced from microorganisms must be characterized by the scientific name and origin of the organism, proving that it is an internationally recognized culture. In the case of precision fermentation, if the ingredients and foods contain GMOs or GMO derivatives, they must comply with the requirements established by Law No. 11,105, of March 24, 2005, of the National Technical Commission on Biosafety (CTNBio).

ANVISA's publication was a prerequisite for the next steps of the regulatory process, which will define the product registration standards, including the labeling rules, the identity and quality standards to be complied with, and the rules for inspection of manufacturing units, all under the responsibility of the Ministry of Agriculture. Further details on the Brazilian and global context of public policies involving alternative proteins can be found in The Good Food Institute's State of Global Policy report.

2

Fermentation technology application in the production of alternative proteins

Mycelium-based meat analogue - Meati.

Fermentation has been used in food production for millennia, mainly as a food production and preservation technique, as previously mentioned. In this process, microorganisms consume raw materials and nutrients and produce macromolecules, such as proteins and fats, and other molecules produced through microbial metabolism, such as flavoring agents (alcohols, organic acids), peptides, and amino acids.

The production of alternative proteins and food ingredients through fermentation processes relies on the establishment of a bioprocess that invariably involves two main phases: upstream and downstream. In the upstream phase, the microorganism is selected and developed and then inoculated and cultured in bioreactors. The downstream phase consists of recovering and, when necessary, purifying the product of interest (Gupta, 2023).

The core of these processes is the bioreactor, which are tank in which microorganisms consume available nutrients and multiply under controlled conditions. They are thoroughly designed to provide the optimal setting for fermentation considering the optimal conditions of temperature, aeration, agitation, pH, etc., resulting in the desired product being obtained (Arora; Rani; Ghosh, 2018).


Bioreactors for biomass fermentation - Solar Foods.

Scale-up is a particularly challenging phase in the development of these bioprocesses, as maintaining yield relative to the laboratory scale is critical and necessitates specific strategies for each bioreactor design, product, and process (Mello et al., 2024). The effectiveness of this scale-up is significantly influenced by several variables impacting microbial performance. This includes factors that may be affected by increased culture volume, such as the accessibility of the substrate used to feed the microorganisms, as well as precise control of factors such as temperature, pH, humidity, pressure, aeration, and agitation in the culture medium.

The following topics will detail the different methods of using fermentation technology to produce alternative proteins. Each method involves specific processing challenges and requires some scientific and technological advances to gain scale and be integrated into the market.

2.1. Traditional fermentation

As mentioned above, fermentation is a process that has been used ancestrally in the production of foods such as bread, yogurt, and beer, and today, it acquires new aspects of innovation to obtain alternative protein products. Accordingly, traditional fermentation can be used to create new plant-based products and ingredients through the action of one or more live microorganisms, which can be bacteria, yeast or filamentous fungi (called “starter cultures”). In this process, plant-derived ingredients such as soybeans or other plant substrates are fermented, thereby providing products with distinct flavors, improved nutritional profiles, and modified textures (Figure 1).

Among the three technological approaches to fermentation, traditional fermentation is the simplest and requires less infrastructure investment. The processes are normally solid-state fermentation[3] and can occur in a simple, temperature-controlled incubator or, on a larger scale, in low-complexity and low-cost bioreactors, such as rotary drum or tray bioreactors.

3 Biotechnological process in which microorganisms are grown in a solid medium, where water is present in very low amounts, usually below 20% (Jin et al., 2019).

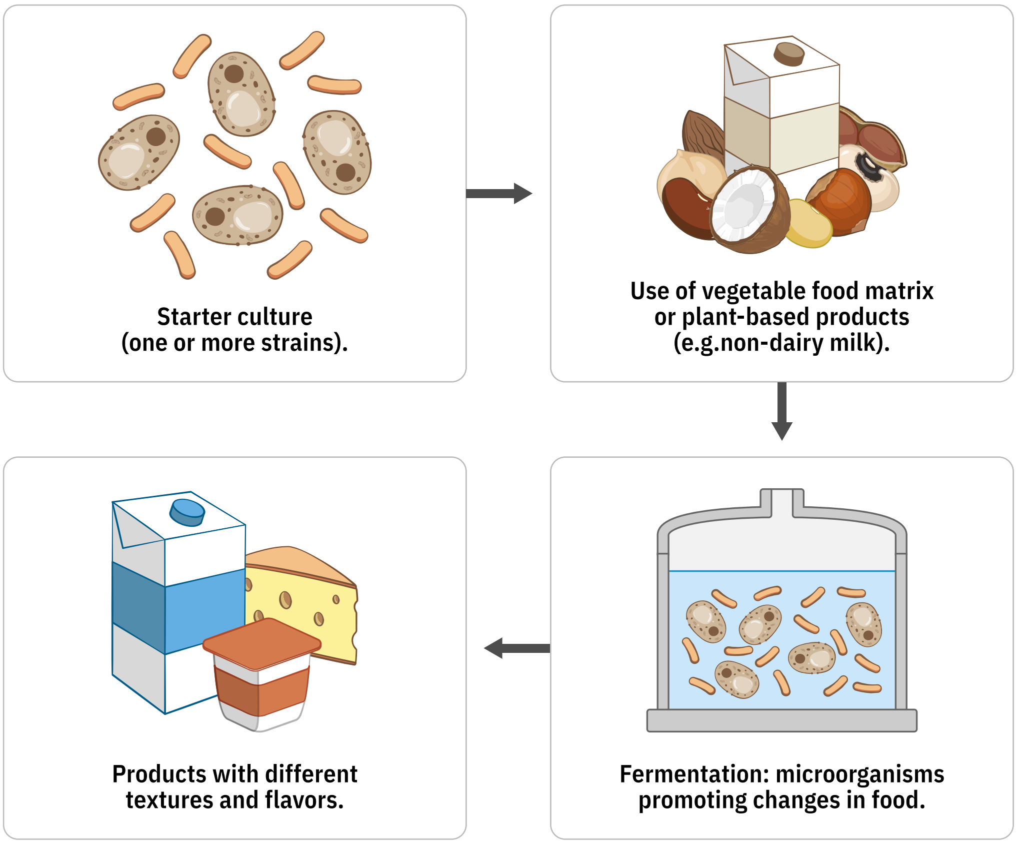


FIGURE 1
Traditional fermentation.

Tempeh is a classic example of a vegetable protein product made by traditional fermentation using whole soybeans cooked and fermented by the fungus *Rhizopus oligosporus*. This product has also been marketed in new versions, such as those produced by Mun Alimentos for example, using other grains such as cowpea and black rice in addition to soybeans, and delivering products with convenience (frozen and ready-to-eat) with suggestions for consumption in meals, sandwiches and also indulgence products such as tempeh stuffed sfihas.

Vegetable milk-based cheeses and yogurts are also examples of how to exploit traditional fermentation to obtain dairy product analogs. The products are obtained by employing lactic acid bacteria (LAB), which modify plant milk, affording a flavor similar to conventional milk-based products (Harper et al., 2022). That is the case for cashew nut milk-based yogurts and cheeses marketed by VidaVeg, for example.

Aiming at developing and improving vegetable ingredients, some studies with pea protein (Shi et al., 2021) and soy protein (Behrens; Roig; Silva, 2004) demonstrate that the use of fermentation with LAB disguised undesirable flavors and aromas and increased the acceptance of analogous products formulated with these ingredients. Commercially, companies such as Chunk Foods and Planetarians use traditional fermentation and by-products of these processes to create plant-based meat analogs and whole cuts.

The final product in traditional fermentation is commonly composed of the substrate used for growing microorganisms and the microorganism itself that was grown there. Fermentation agents in the final product contribute to an improved nutritional profile by increasing nutrients such as proteins and fibers derived from the microorganisms' cell biomass. Beyond the nutritional profile of these products, several studies have found evidence of the health benefits of consuming fermented foods. This growing interest is driven by the finding that foods submitted to fermentation may present superior nutritional quality, including the profile of proteins, amino acids, vitamins, fats, fatty acids, and higher bioavailability of nutrients and digestibility (Adebo et al., 2022). In addition, traditional fermentation products may contain microorganisms that are still alive, which have beneficial effects on the consumer's organism (Dahiya; Nigam, 2022). Thus, functional probiotic foods can be produced (find further information in Box 2 - Learn more about probiotics).

The development of foods that not only provide a pleasant sensory experience but also contribute to health promotion through the incorporation of functional microorganisms represents a significant area for innovation in this technology and offers a potential avenue to meet the growing demand for plant-based analog products with desirable health characteristics (**Lupetti; Casselli, 2024**).

Starter cultures used in traditional fermentation include microorganisms of the genera *Saccharomyces, Lactobacillus, Lactococcus, Streptococcus* and *Enterococcus*. The diversification and optimization of these microorganisms employed, aiming at the plant-based analog product market, also represents a research and development opportunity with major potential. British company Myconeos Limited, for example, specializes in developing new fungal strains for food applications and works on creating new strains designed to achieve similar levels of performance in plant-based dairy analog products. Recently, **the company published a scientific discovery** that opens possibilities for the use of species of *Penicillium cameberti* and *Penicillium roqueforti* optimized for the development of vegan Brie & Camembert.

Brazil is notable for its vast diversity of nuts, such as Brazil nuts and cashew nuts, oilseeds such as peanuts and soybeans, and grains such as corn, rice, beans and quinoa, which makes it a favorable country for exploiting these substrates in the production of fermented foods. In addition, the knowledge established in the research and development of conventional fermented products can be used to develop these plant-based products, enabling the study of the behavior of traditional cultures on these different substrates.

Plant-based cheese analogues - Miyoko's Creamery.

BOX 2

Probiotics and paraprobiotics: the microorganisms and their health benefits.

Probiotics are live microorganisms that, when consumed in adequate amounts, provide health benefits to consumers. Plant-based probiotic foods serve a public that seeks not only a vegan diet, but also functional products (Latif et al., 2023). To be considered a source of probiotics, the food needs to have microorganisms scientifically proven to provide benefits and it is necessary to guarantee the content of viable cells for the effect to be verified in the body. However, scientific research has reported that non-viable probiotic microorganisms, known as paraprobiotics, can also provide health benefits (Almada et al., 2016; Piqué et al., 2019; Taverniti; Guglielmetti, 2011).

Paraprobiotics are non-viable microbial cells or fractions that can still have a positive effect on the body and provide health benefits to consumers. This field is still under study, especially regarding the paraprobiotic action in food and not the consumption of these microorganisms in the form of capsules or powders (**Siciliano et al., 2021**).

2.2. Biomass fermentation

While traditional fermentation dates back to ancestral times, biomass fermentation technology arose in the postmodern era. It is based on the rapid growth of microorganisms, such as bacteria, yeasts, microalgae and fungi, with high protein content, which enables efficient protein production on a large scale (Aggelopoulos et al., 2014). This process is also performed in bioreactors, creating a favorable microbial growth setting. In this technology, the microorganism itself is the product of interest, called biomass (Figure 2). After fermentation, biomass is recovered and washed, if necessary, and a heat treatment can be applied to reduce RNA content or inactivate the microorganism. Finally, biomass is conditioned to the final form of the ingredient, either a powder or a wet biomass. Then, it is possible to create a variety of products that mimic traditional meat products (**Quorn**), such as restructured (**Nature's Fynd**) and whole cuts analogous to chicken (**Bosque Foods**), beef (**Meati**), bacon (**Hyph**), seafood (**Aqua Cultured Foods**), dairy analogs such as **cream cheese** and **yogurts**, or even protein ingredients (**Typcal**), suitable for use in different food products. Dried yeast powder and yeast extracts are also examples of ingredients that can be used similarly, increasing protein content and improving the flavor of plant-based analog products.

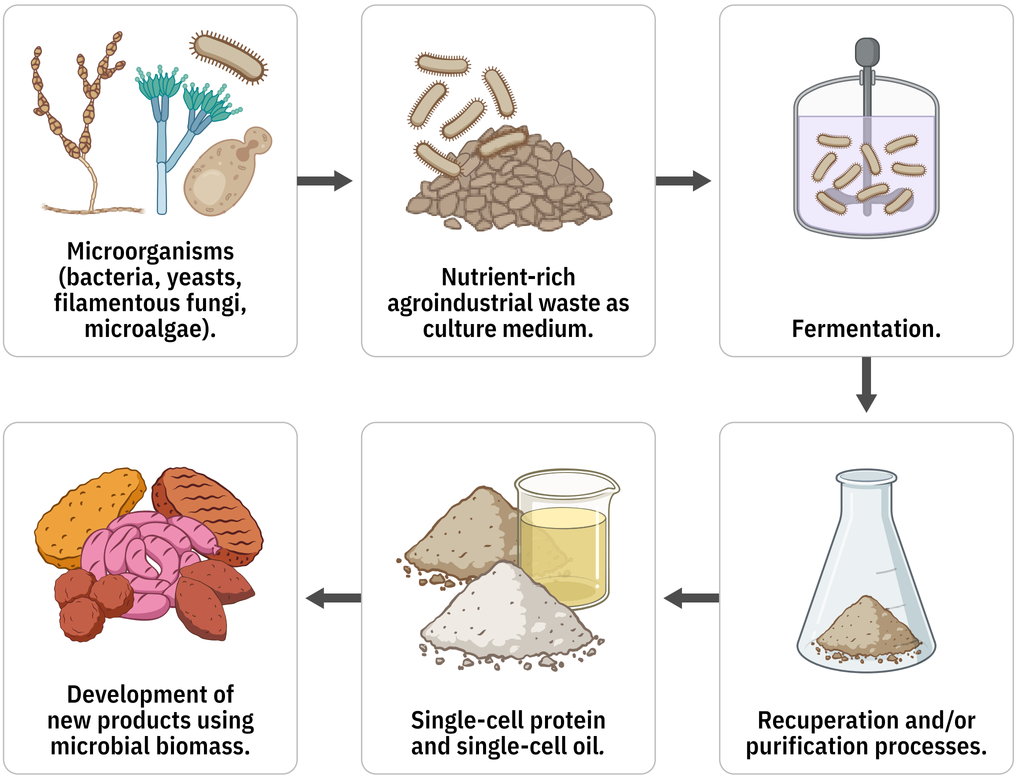


FIGURE 2
Biomass fermentation.

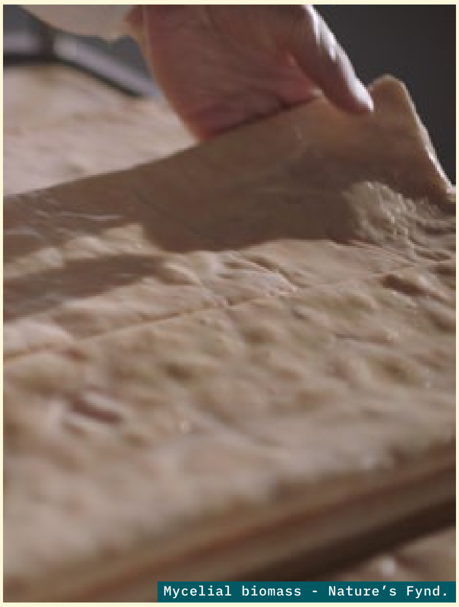
Depending on the microorganism chosen for the fermentation process, biomass fermentation products are single-cell proteins (SCP) or single-cell oils (SCO). Microorganisms classified as single-cell proteins and single-cell oils have in their composition very high levels of proteins and oils, respectively. Some examples of species used as a protein source include yeasts such as *Saccharomyces cerevisiae* — with a long history of consumption and use as nutritional yeast, backer's yeast and also a source of extracts used as flavoring agents in food formulations — and filamentous fungi such as *Fusarium venenatum*—used by Quorn—and *Fusarium flavolapis*—used by Nature's Fynd. Yeast *Yarrowia lipolytica* and fungi of the genus *Mortierella* are examples of microorganisms that accumulate significant amounts of lipids (Ochsenreither et al., 2016).

BOX 3
What is mycelium? And mycoprotein?

Some of the main agents used in biomass fermentation are filamentous fungi. Mycelium is the basic structure of these fungi. Just as animals are composed of organs and tissues (which are a collection of cells), fungi are composed of mycelium, made of a tangle of fibrillar structures (called hyphae) that are cell filaments of these microorganisms. Thus, from the structure responsible for support and nutrition, which grows within the substrate like a root (called vegetative mycelium), to the mushrooms and cotton-like structure we see in the growth of mold (also called aerial mycelia), everything is made of mycelium.

Mycoprotein is the name given to the protein that composes fungus biomass. The term originates from the Greek prefix "mico," meaning fungus.

Some companies use this name to designate the main ingredient in the formulation of products obtained from fungi. Thus, we can say that the mycelium contains mycoprotein.

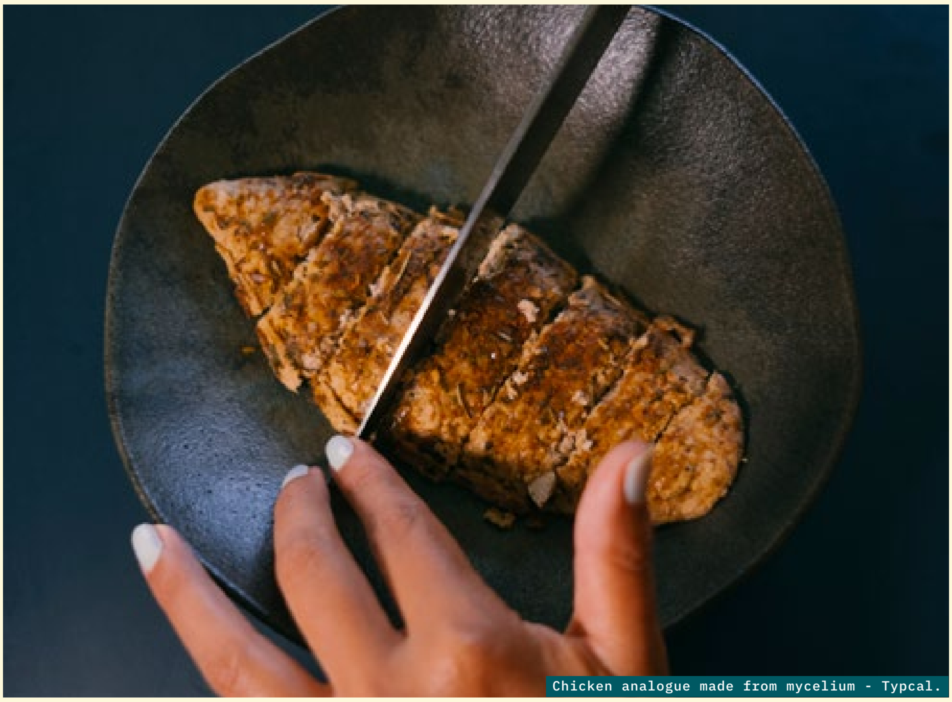

Mycelial biomass - Nature's Fynd.


Chicken analogue made from mycelium -Typcal.

BOX 4

Mycelium-based meat analogs.

As previously mentioned, mycelium is the part of the filamentous fungus that develops within the substrate, similar to a fungus 'root' (in the case of solid-state cultures). This fiber structure gives the products a texture very similar to meat's, satisfactorily mimicking meat products. In addition, mycelium is a rich source of proteins and fibers and contains no fat, which adds nutritional quality to the product.


Chicken analogue made from mycelium - Typcal.

In addition to producing protein-rich foods, biomass fermentation provides a unique opportunity to transform wastes from the food and beverage industries into high-value-added products (Gupta; Lee; Chen, 2018). This practice of using wastes contributes to advancing the circular economy and reducing processing costs since these inputs have a lower value than traditionally used substrates.

In addition to promoting a circular economy and having a vast potential to replicate desirable sensory characteristics of conventional products, biomass production provides an alternative that is not only sustainable but also nutritious.

In general, microorganisms are rich in proteins, fibers and micronutrients and have low fat content (Choi; Yu; Lee, 2022). Table 1 shows the protein, fiber and micronutrient contents of the four types of microorganisms used in biomass fermentation: bacteria, microalgae, filamentous fungi and yeast. The arrows indicate the relative quantity of macronutrients and micronutrients present in the microorganisms, and the number of arrows represents the quantity, ranging from one arrow (↑) for a lower quantity to three arrows (↑↑↑) for a higher amount.

TABLE 1
Relative average nutrient content of microorganisms used in biomass fermentation.

Microorganisms	Proteins	Fibers	Micronutrients
Bacteria	↑↑↑	↑	↑↑
Microalgae	↑↑↑	↑↑↑	↑↑↑
Filamentous fungi (mycelium)	↑↑	↑↑↑	↑↑
Yeast	↑↑	↑↑	↑↑

Source: Adapted from Graham and Ledesma-Amaro (2023).

As for proteins, bacteria may have the highest quantities. In addition to the high content, they can also be a source of complete proteins: many species of microorganisms contain essential amino acids, which are those that we need to obtain through the diet. As for fibers, there is a notably high amount of insoluble fibers in microalgae, in addition to beta-glucans and monoligosaccharides present in filamentous fungi, which provide intestinal health benefits. Finally, microalgae should be noted in terms of the availability of micronutrients, which generally include vitamins of the B, C and E complexes, zinc, selenium, iron and copper (Demirgul et al., 2022; Leblanc et al., 2011; Ślusarczyk; Adamska; Czerwik-Marcinkowska, 2021).

2.3. Precision fermentation

The last approach to fermentation technology to elucidate here is also the latest to be used for obtaining food ingredients. Initially used in the production of drugs, such as insulin, and food, such as chymosin (a fundamental enzyme in cheese production), citric acid and flavors, precision fermentation technology has undergone significant advances. Currently, this technique is also employed to obtain animal proteins, among other essential inputs for the alternative protein industry. These inputs — milk proteins, egg proteins, collagen, oils and enzymes — are produced by recombinant microorganisms and, after fermentation, are isolated and purified for later application in food (Knychala et al., 2024) (Figure 3).

To this end, the initial step involves identifying the gene responsible for the production of the molecule of interest, which may be of animal or plant origin (Box 5). This gene is then inserted into an expression vector or directly into the microorganism's genome, which starts to produce the molecule when replicating. Microbial cells then act as tiny "factories" during fermentation (Good Food Institute, 2023a).

These genetic modifications are carried out through molecular biology techniques such as recombinant DNA technology or the CRISPR approach[4]. Such methods enable the insertion of a gene from a donor organism, i.e., a DNA sequence encoding the synthesis of a specific molecule, into a faster-multiplying host microorganism. Thus, substantial quantities of this molecule can be produced in bioreactors within a short timeframe (Augustin et al., 2023). In summary, a microorganism, such as a bacterium, that receives a gene to encode an animal protein may start to produce large amounts of the animal protein without it having to be obtained through the conventional chain using animals.

Some examples of microorganisms used in precision fermentation are bacteria such as *Escherichia coli, Bacillus subtilis, Corynebacterium glutamicum,* and *Lactococcus lactis*, yeasts, mainly *Saccharomyces cerevisiae, Komagataella phaffii,* and *Kluyveromyces sp*, and filamentous fungi such as *Trichoderma reesei, Aspergilus oryzae* (Eastam; Leman, 2024) (learn more about each of these microorganisms and their use in Table 2).

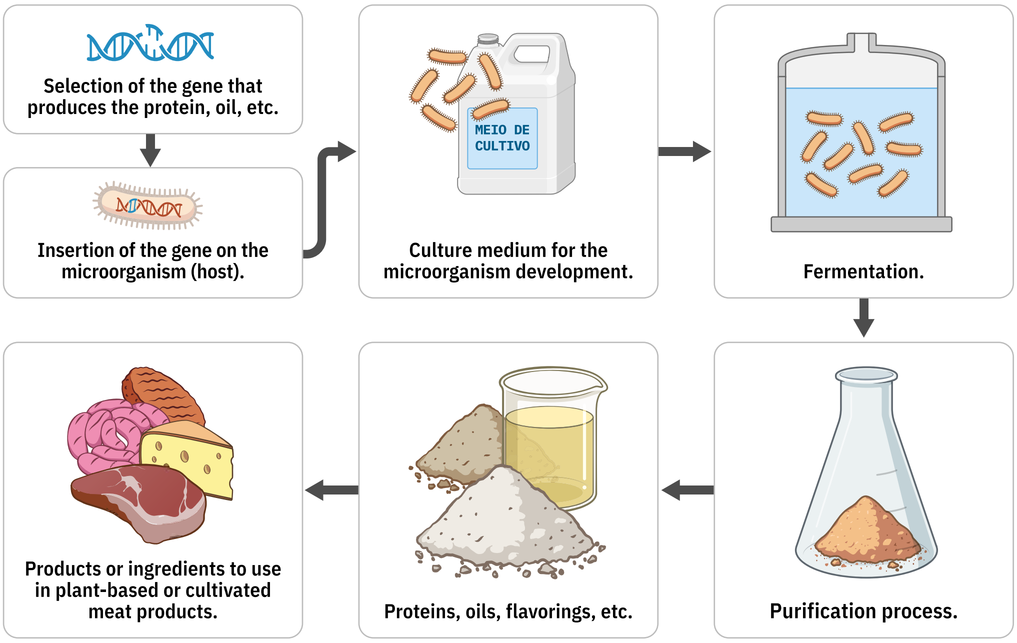


FIGURE 3
Precision fermentation.

4 CRISPR – Clustered Regularly Interspaced Short Palindromic Repeats.

BOX 5

The first step in a precision fermentation process development: the challenge of choosing the target molecule.

Target selection and design constitute the starting point for the precision fermentation process. The molecule or molecules of interest are called the target. The target may be a protein, a lipid, a flavor compound, an aroma, an enzyme, a growth factor, a pigment, or any other ingredient.

One of the most fundamental challenges in selecting these targets is determining which molecules contribute the most to the specific properties of animal products.

Some notable examples of ingredients already produced by precision fermentation in the context of alternative proteins are the use of heme proteins as ingredients to provide a set of sensory properties in the plant-based meat analog product, as conducted by the company **Impossible Foods**, which incorporates soy leghemoglobin produced by precision fermentation to improve taste, juiciness and appearance of their plant burger. Other recombinant proteins, such as casein and whey, are also important targets due to their unique functionality in dairy products. These proteins can be combined with plant-derived ingredients to create a final product. For example, sugar, coconut oil, and sunflower oil are combined with recombinant whey produced by fermentation to make an ice cream base by **Perfect Day**. We can also mention the choice of egg white proteins as a target, as in the case of **Every**, which delivers ingredients with specific functionalities important for food formulations such as foaming due to ovomucin, globulin and ovalbumin.

Although strain development, raw material optimization, and culture medium can substantially contribute to the process's viability, target selection is critical to achieving economic viability. Target choice is the base for determining demand, market share, and cost necessary to reach price parity, defined by similar ingredients already on the market.

Source: The Science Of… (2024)

Plant-based hamburger with fermentation-derived heme protein - Impossible.

Ice cream made with precision fermentation-derived dairy protein - Perfect Day.

TABLE 2
Microorganisms types in precision fermentation.

	Bacteria	Yeasts	Filamentous fungi
Main strains used	*Corynebacterium glutamicum, E. coli, Bacillus subtilis, Lactococcus lactis.*	*Komogaetella phaffii (Pichia pastoris), Saccharomyces cerevisiae, Kluyveromyces lactis.*	*Aspergillus niger, Aspergillus oryzae* e *Trichoderma reesei.*
Characteristics	Fast growth; Low titers, but high yield.	Protein folding capacity*; Absence of secondary metabolites (mycotoxins); Intermediate titers.	Protein folding capacity*; Higher titers; High protein secretion capacity; Several secondary metabolites require pathway repression/deletion.
Fermentation time	<48 hours	3-6 days	5-10 days
Titers	0.5-3 g/L	1-22 g/L	5-100 g/L

* The functional properties of proteins depend on the spatial structure resulting from this folding and the folding of the protein filament itself (Deckers et al., 2020).
Source: Adapted from Eastham and Leman (2024).

The recovery and purification steps of the target ingredients are fundamental and represent one of the primary cost drivers in the precision fermentation process, significantly impacting process yields. The complexity varies if the molecule is produced intracellularly or secreted into the culture medium. If it is an intracellular production, disruption of the microbial cells is also required to release the intracellular contents containing the target protein and separate cellular debris. Next, separation techniques, including centrifugation, filtration, precipitation, and chromatography, enable obtaining the target protein with a certain degree of purity. Finally, target molecule concentration is obtained, usually by additional chromatography or filtration (Augustin et al., 2023).

This approach to fermentation exhibits greater complexity than those previously presented, stemming from its more intricate downstream processes and the initial stage of strain development. This technology necessitates a comprehensive understanding of the microorganisms employed in the fermentation process, including their genomes and metabolic functions. It requires the application of advanced technologies such as artificial intelligence, bioinformatics, and systems biology to identify, manipulate, and optimize both the microorganisms and the fermentation process (Teng et al., 2021).

All of the aspects above contribute to the main challenge of precision fermentation: improving the economic viability of production. To compete with animal proteins, researchers and companies must increase titer (quantity of a target molecule expressed relative to the total volume of upstream liquid produced containing the agent—the main upstream efficiency benchmark) and yield (the final purified protein mass ratio relative to its mass at the start of purification—the main downstream efficiency benchmark) of the target molecules and protein biomass.

TABLE 3
Understand the differences between each fermentation technology approach and alternative protein production.

	Fermentation technology approaches applied to alternative proteins		
	Traditional	**Biomass**	**Precision**
Is the microorganism present in the final product?	Yes, it can have beneficial biological activity when the product undergoes no thermal process.	Yes, always in the inactive form.	No.
What is the source of protein contained in the product/ingredient obtained?	The protein of the plant-based ingredient used and the protein of the microorganism used for fermentation.	The protein content of the microorganism.	A purified protein identical (or similar) to the animal protein.
Does it require purification processes?	No.	It is not necessary, but it is possible to develop an ingredient with a concentrated microbial protein.	Yes.
Main examples of products	Fermented plant-based drinks, tempeh, plant-based cheese and meat analog products	Mycelium-based meat (chicken, beef, bacon) and dairy analog products (cream cheese, yogurt)	Whey proteins, casein, egg proteins, collagen, hemeproteins, enzymes
Technological differential	Sensory and nutritional improvement of plant ingredients; and development of the category of fermented plant-based dairy and cheese analog products	Obtaining protein ingredients in bioprocesses with well-established technologies, with high productivity and efficiency.	Efficiently produce animal-free ingredients with specific functionalities that are essential in food formulations.
Recommended reading	Fact Sheet: Traditional fermentation	Fact Sheet: Biomass fermentation	Fact Sheet: Precision fermentation

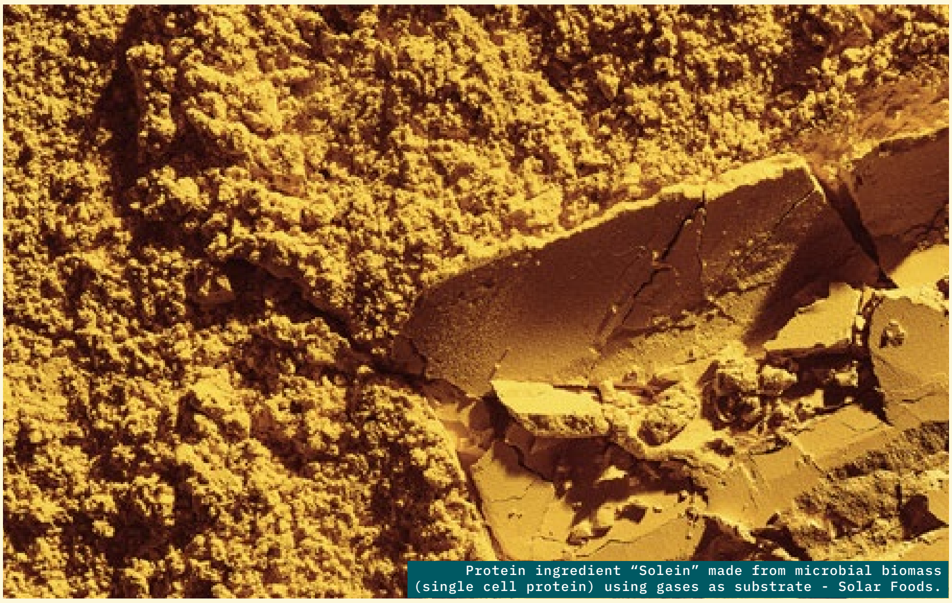
BOX 6

Proteins obtained from air.

Recently, an innovative approach to protein production—using air-based fermentation—was announced to be under development. In other words, these microorganisms use air components as a carbon source for their growth and formation of microbial biomass.

The company **Air Protein** produces proteins from carbon dioxide (CO_2), while **Solar Foods** uses both CO_2 and hydrogen (H_2) and oxygen (O_2) obtained from air. Both produce a protein ingredient that is incorporated into the production of various food products.

This is a new sustainable approach to food production, since it requires no agricultural substrates, which reduces land use and minimizes the use of other resources, in addition to mitigating polluting gases in the atmosphere, such as CO_2.



Protein ingredient "Solein" made from microbial biomass (single cell protein) using gases as substrate - Solar Foods.

3

Fermentation as a sustainable solution

Product analogous to tuna obtained by biomass fermentation - Aqua Cultured Foods.

Fermentation in the context of alternative protein food production represents a production model that follows a circular logic, in which the waste flows of one chain can be valuable inputs for others and in which there is a pursuit of the development of more efficient and less input-intensive and energy-intensive production processes, strongly supported by biotechnology. This aligns the technology with the concept of bioeconomy, defined as any economic activity utilizing bioprocesses and generating bioproducts that contribute to efficient solutions in the use of biological resources (Centro de Gestão e Estudos Estratégicos, 2021).

All technological approaches to fermentation have a potential for scalability and production efficiency, reducing environmental impacts, land use and water consumption. Life Cycle Assessment (LCA) studies demonstrate that biomass fermentation and precision fermentation can significantly decrease land demand and land occupation compared to conventional protein production, and can also reduce water consumption relative to other protein sources. The data indicate that the protein produced by biomass fermentation can have 53–100% less environmental impact, depending on the reference product and the energy source assumed for its production (Durkin et al., 2022; Järviö et al., 2021) and 83.9% reduction in drinking water consumption (Durkin et al., 2022). Other studies indicate that, when compared to ruminant meat production, mycoprotein has a significant potential to reduce water and land use, which may reach 90% reduction, in addition to a decrease of up to 80% in greenhouse gas (GHG) emissions (Hashempour-Baltork et al. 2020; Rubio; Xiang; Kaplan, 2020). According to a more recent study by Kobayashi et al. (2023), the environmental impacts of microbial protein production using oat by-products, compared to other conventional soy products, for example, result in a reduction of more than 60% in land use.

Perfect Day, one of the pioneers in the development of alternative proteins through precision fermentation, **conducted an LCA** study of their ingredients. The results demonstrate that, compared to total milk protein, its whey protein emits 91% to 97% less GHG, reduces energy demand by 29% to 60%, and reduces water consumption by 96% to 99%.

It is important to note that these studies consistently indicate that the reductions in greenhouse gas emissions are intrinsically linked to the energy source utilized in the production process, highlighting the use of renewable sources as a key solution. In this sense, **Brazil stands out as a benchmark in bioenergy**, with one of the cleanest energy matrices on the planet. While the world has, on average, 84% of primary energy generated by fossil sources, Brazil has 43% of its energy matrix generated by renewable sources (**Toledo, 2020**), and when we analyze the electric matrix, this number rises to 84.25% from renewable sources (**Matriz Elétrica Brasileira..., 2024**). Data from the **Technology and Innovation Report 2023** of the United Nations Conference on Trade and Development (UNCTAD) (2023) show that Brazil is in second position in bioenergy, ahead of the United States and only behind China.

After the energy source, the second major contributor highlighted in these LCAs is the carbon source used in the medium: glucose. In the Perfect Day whey protein LCA mentioned above, for example, the protein production (fermentation) phase contributed 25% to the total GHG emissions, due to the step of glucose production through starch hydrolysis, which alone contributes 83% of the emissions in this fermentation phase. In this regard, **Brazil's position as the world's largest producer of cane sugar, with record harvest results of 713.2 million tons of sugarcane in 2023/24**, represents a significant advantage, as the sugars from sugarcane juice (sucrose) are directly fermentable and do not require hydrolysis.

In addition, another solution to reduce the life cycle impacts of this chain is the use and valorization of by-products and waste as raw materials for fermentation processes, or the use of locally available resources and raw materials to reduce transport distances (Good Food Institute, 2022a; Koutinas et al. 2014; Smetana et al. 2018). Accordingly, Brazil also holds a prominent position due to the billions of tons of agro-industrial waste generated annually, originating from both minimally processed foods (fruits and vegetables) and those undergoing processing to separate fractions of interest, whether for food or bioenergy applications (Woiciechowski et al., 2013). Table 4 shows some of the main crops processed in Brazil and their main wastes.

TABLE 4
Examples of crops processed in Brazil and their main wastes.

Crop	Annual production (million tons)	Type of waste	Waste generation (million tons)
Sugarcane	685.85[1]	Bagasse	174[2]
Soy	147.68[1]	Bran	41.7[3]
Corn	111.64[1]	Bran	2.4[4]
Total	945.17	-	218.1

Source: Ajustes na Área… (2023) (1); Vilella e Hofsetz (2019) (2); Projeções para o… (2024) (3); Anec Eleva Previsões… (2024) (4).

These wastes, such as bran, bark, straw, bagasse, and fruit and seed residues, are excellent substrates for the growth of microorganisms, providing nutrients for their development. Depending on the type of agro-industrial waste used, different processes must be applied to pretreat the biomass and release the nutrients that will compose the fermentation culture medium, involving a field of studies in which **Brazil is undoubtedly a pioneer**.

As this is a developing technology, LCA data are still somewhat limited and may not represent the real potential to reduce the impacts of this chain, since the gain of scale and higher investments in research and development can provide higher efficiency gains than those expected in current LCAs. It is also noted the need to expand studies with representative data from the Brazilian production chain to address the potential for sustainable protein production by fermentation in the country.

FIGURE 4
Fermentation as a sustainable solution.

SUSTAINABLE DEVELOPMENT GOALS (SDG)
Fermentation to produce alternative proteins meets several SDGs, such as food safety and adequate management of waste.

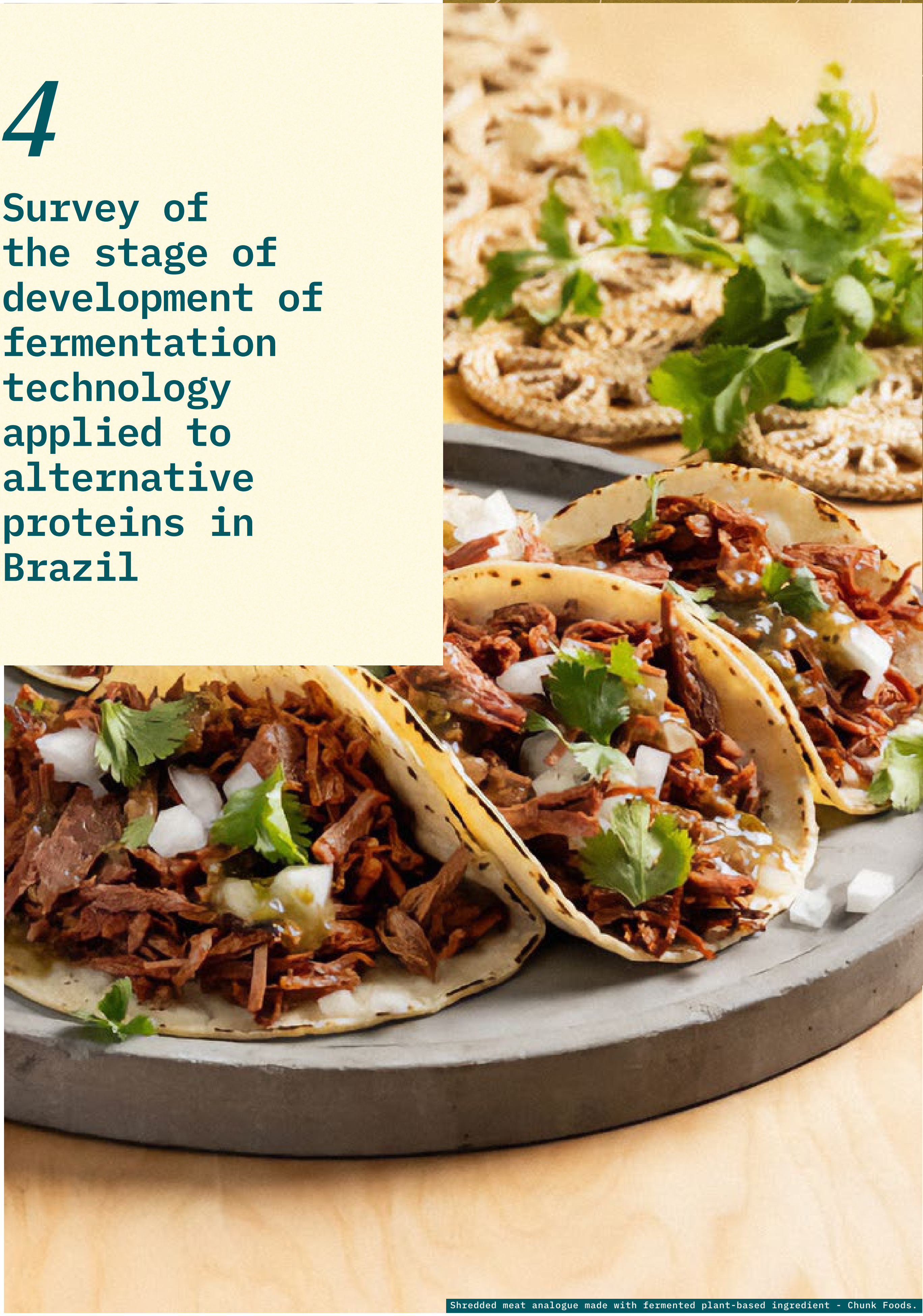
SUSTAINABLE PRODUCTION
Less greenhouse gases emitted and lower water consumption compared to conventional protein production.

EFFICIENT PRODUCTION
Regardless of the type of fermentation, protein production is efficient while reducing environmental impact, such as land use and pollution.

CIRCULAR ECONOMY
Potential to upcycling agro-industrial waste as culture media input to cultivate microorganisms, feeding back and integrating the chain.

4

Survey of the stage of development of fermentation technology applied to alternative proteins in Brazil



Shredded meat analogue made with fermented plant-based ingredient - Chunk Foods.

4.1. Survey overview and approach

In a partnership between The Good Food Institute Brasil and researchers from the Federal University of Paraná and the Getúlio Vargas Foundation, a survey was conducted throughout 2024 to trace the potential, opportunities and challenges for the development of the fermentation-derived protein ecosystem and production chain in the country. Data from this survey were collected using quantitative and qualitative approaches as described below:

1 Using search tools (Google), public databases (**Sucupira Platform**) and GFI's own database (**Alternative Protein Company Database**) 240 institutions, including universities, startups, research centers and companies relevant to the area of alternative proteins were identified.

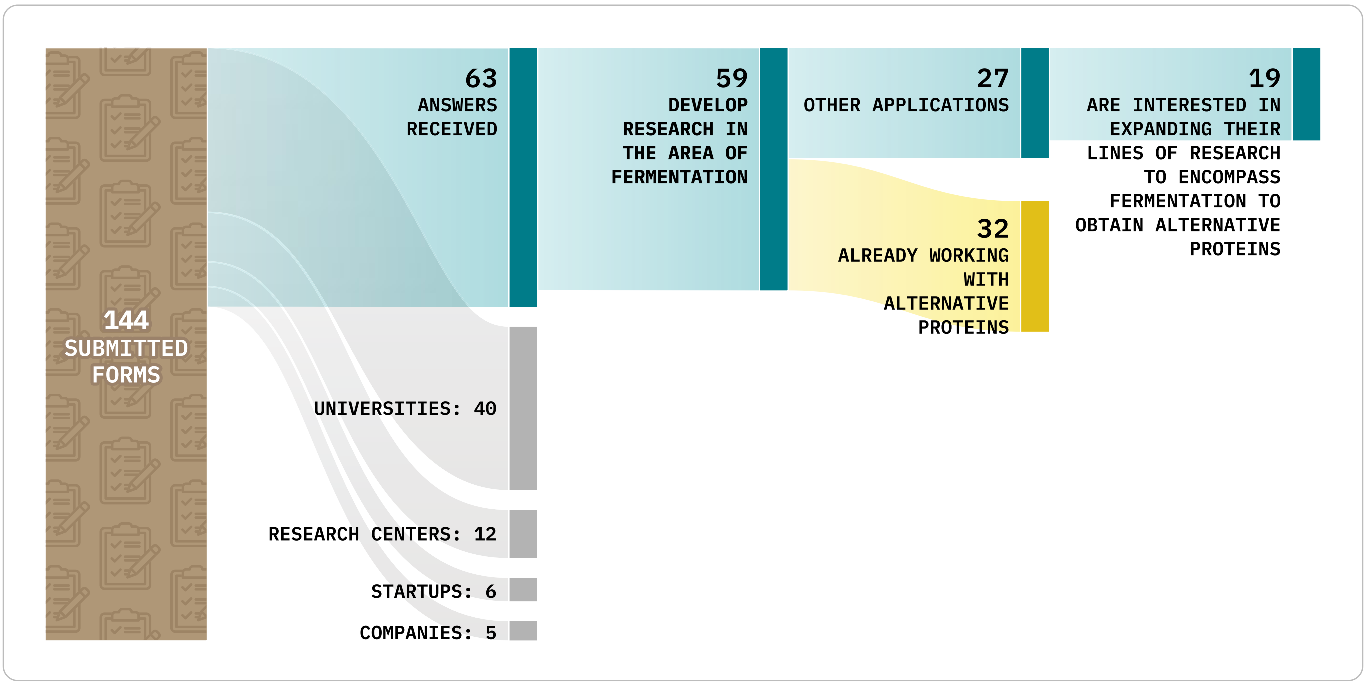
2 Of these 240 institutions, it was possible to obtain the specific contact (email) of 144 people linked to them, such as program coordinators, staff members, and researchers. These professionals received an **online questionnaire** that addressed the most varied questions, including products developed, lines of research, the institution's activities in alternative proteins, infrastructure installed for bench tests and scale-up, fundraising, contact with suppliers, and difficulties faced, among others.

3 From the specific contacts obtained, about 18 were selected for further information collection through consultations and interviews.

The data obtained through the survey provided an extensive analysis of fermentation technology applied to alternative proteins in Brazil that will support the conduct of GFI Brasil's activities in this area. Due to the relevance of the information to other actors in the alternative protein ecosystem, the main results obtained in this study will be shared in the following topics, in addition to the opportunities and challenges traced through the analysis of the surveyed situation.

4.2. Main findings

Of the 144 questionnaires submitted, 63 responses were obtained, including 40 universities, 12 research centers, 5 companies and 6 startups. Of these, 59 answered that they are active in the development of research in the fermentation area, with the majority (54%) already working with alternative proteins (32 respondents) and 46% working with fermentation for other applications (27 respondents). Of these 46%, a large part (71%) claims to be interested in expanding their lines of research to cover fermentation to obtain alternative proteins.



It was observed that, among the institutions working with alternative proteins, 43.8% carry out only one type of fermentation and have a more specific infrastructure dedicated to this single approach. Of the institutions with this profile, 18.8% perform only biomass fermentation, 12.5% perform only traditional fermentation, and 12.5% perform only precision fermentation. Institutions with a more diverse research profile, active in conducting two or more types of fermentation, represent 56.62% of the total. Of these, about one-third (37.5%) perform biomass or traditional fermentation and precision fermentation and have a more complete and versatile infrastructure for conducting broader research lines. The remaining 18.8% of institutions carry out research with traditional and biomass fermentation. These results demonstrate the wide scope of operation of the Brazilian institutions traced, as for technical and infrastructure aspects, within the field of fermentation, mainly being able to conduct research on the three different technological approaches.

The installed capacities of bioreactors were also surveyed (Figure 5), and the results showed a predominance of bench-scale research infrastructure (1-10L bioreactors) present in 70% of all institutions evaluated. Infrastructures of this type allow the evaluation of most process variables, such as agitation rate, temperature control, and pH control, but are insufficient to determine the ideal scale-up criterion and consequently enable the transition to industrial production scales.

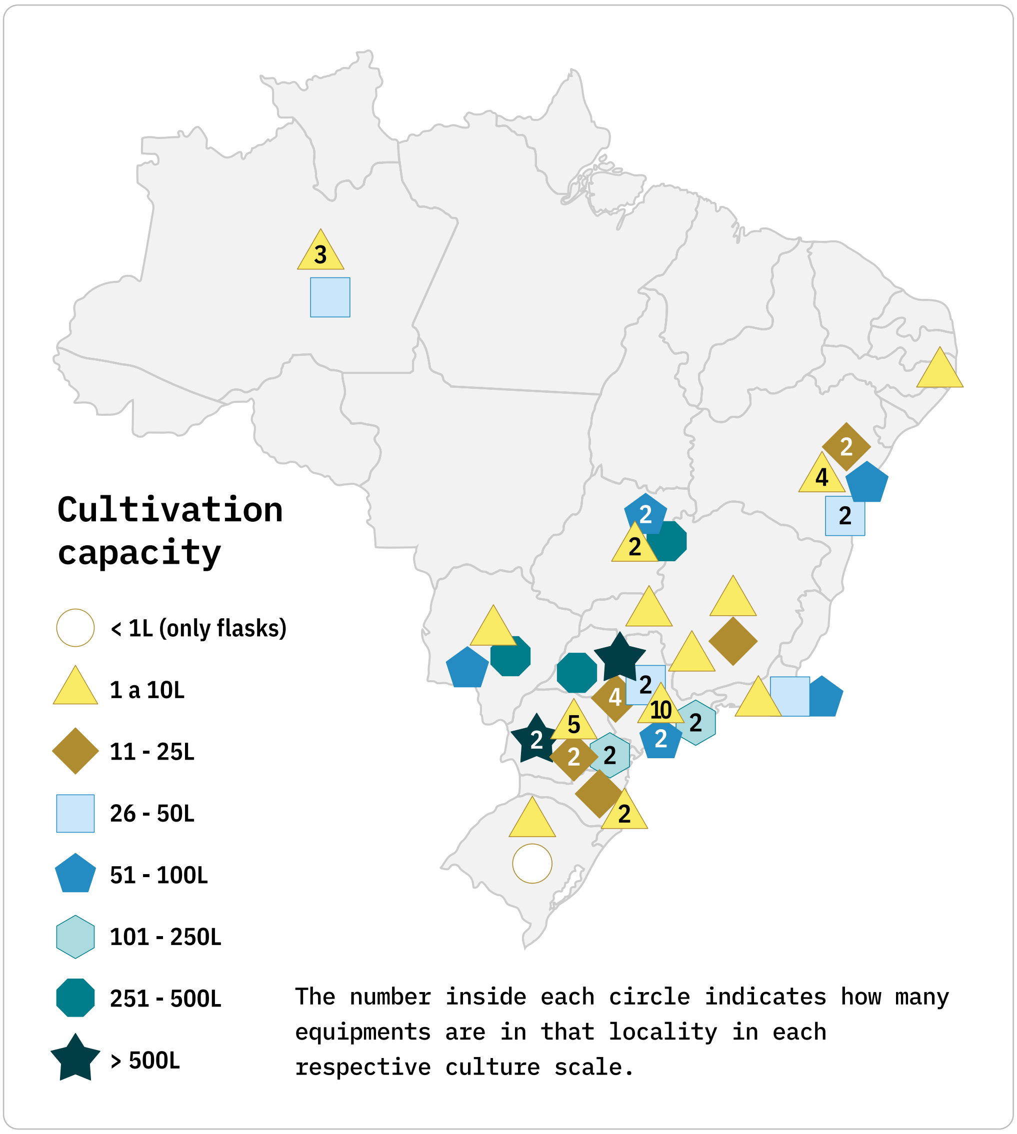
Pilot infrastructures for scale-up (above 11L) surveyed in the study are concentrated in research centers, technological institutes and private institutions. Only six institutions reported having bioreactors with volumes higher than 100 liters, which can represent a challenge for those seeking to scale up their processes. The establishment of the pilot scale contributes to a realistic view of process yields, analysis of energy expenditure and costs—thus called “demonstrative” or demo scale—and productions on this scale are essential to study the viability of the process, which is necessary for companies seeking financing and to enable technologies to move to the production phase on an industrial scale.

Based on the study results, Brazil’s fermentation-derived alternative protein industry is in formation and characterized by high fragmentation of players and a high potential for product differentiation. The surveyed companies are predominantly startups in the early stages of development. Interviews revealed that these startups are primarily in the proof-of-concept, bench-testing, and product development phases, with few progressing to the pilot or initial industrial production stage. Companies with revenue lines or even in break-even face scalability challenges due to a lack of investments and more stabilized projections in terms of demand. In addition, startups reported difficulty finding structures for scaling up their products. It is important to note that among the institutions with available pilot infrastructure, only three possess food-grade certification. This likely reflects the objectives of institutions with this profile, primarily not focused on food research or production and/or do not intend to commercialize products. Properly certifying these infrastructures and facilitating access for startups to existing facilities are crucial for the evolution of these businesses and the development of the country’s fermentation-derived alternative protein production ecosystem. Such adaptations can facilitate, for example, the transition of startups through the challenging “valley of death[5].” During this stage, several startups fail due to a lack of resources or the inability to demonstrate the commercial viability of their product or service

5 Term referring to the critical period in the life of a startup or project in which the company faces major financial and operational difficulties to transform an innovative idea into a viable commercial product.

FIGURE 5

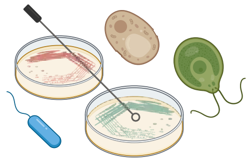
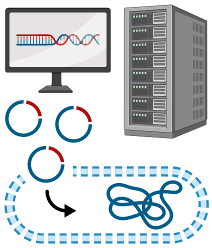
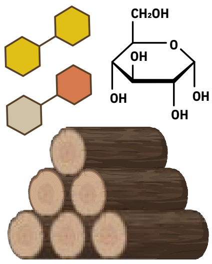
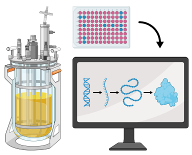
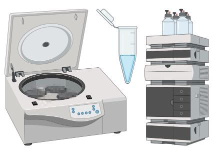
Mapping of the installed capacity of Brazilian institutions for research with microbial cultivation.



The survey also highlighted the essential role of universities within this ecosystem. Representing nearly two-thirds of the surveyed institutions, they play a crucial role in developing research in the country, thereby boosting scientific and technological advancements in the sector. As for the scientific potential and research lines developed (Table 5), the results showed a concentration of research involving the use of by-products for developing culture medium in about 90% of the surveyed institutions. However, only 29% of the researchers working with biomass and traditional fermentation develop downstream processes research, and the Brazilian researcher's topic less addressed. At the same time, it is the research focus for 64% of those active in precision fermentation. It is essential to develop new protein purification and drying approaches that reduce the cost and increase the yield and sustainability of downstream processes to enable alternative protein production by precision fermentation. Another highlight is that only 36% of the surveyed researchers are working on prospecting new microorganisms and exploring the microbial biodiversity of Brazilian biomes. This is a major opportunity to explore and value the country's significant biodiversity (Box 7).

TABLE 5

Percentage of Surveyed Brazilian Institutions Engaged in Research on Each Topic Within the Fermentation Process Chain.

Research topic		Precision fermentation	Biomass and traditional fermentation
	Prospecting for new microorganisms	na	36%
	Use of omics analyses to search for target molecules	57%	NA
	Development of microbial chassis for precision fermentation	86%	NA
	Optimization of cloning/ heterologous expression	86%	NA
	Use of industrial / agro-industrial wastes as culture medium components	93%	86%
	Optimization of components and/or quantities of synthetic medium components	50%	43%
	Optimization of fermentation conditions	100%	93%
	Metabolic engineering studies	79%	NA
	Optimization of downstream operations (processes of separation, purification, etc.)	64%	29%

NA: Not applicable. The topic was not included in the form of this technological approach.

BOX 7

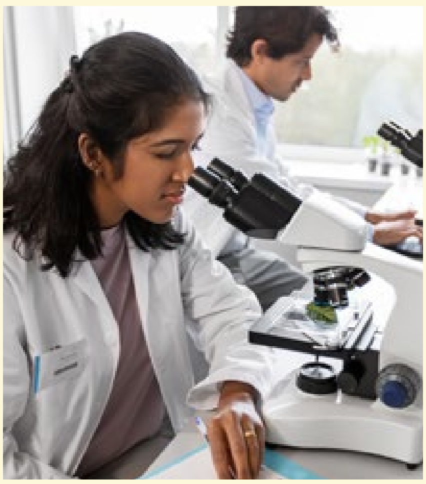
Research opportunities in Brazil.

In biodiversity

Bioprospection of new microorganisms in brazilian biodiversity.

In agribusiness

Large amount of low-cost agro-industrial waste to investigate, aiming to become culture medium inputs for fermentation of microorganisms.



4.3. Opportunities and challenges identified

Brazil has enormous potential to stand out and influence the advancement of fermentation technology for alternative proteins worldwide, especially for being notable in biodiversity and agriculture and for its human capital. Strengths identified include the abundant biodiversity of microorganisms and the availability of raw materials for fermentation. Some examples of raw materials are sugarcane, corn, agro-industrial wastes and by-products from the food and beverage industries, such as those from the brewing industry (yeast or malt bagasse). In addition, the country has a significant technical and productive capacity, driven by companies using large-scale fermentation for different commercial purposes, such as in the food, chemical, health and biofuel sectors.

Despite the similarity of fermentation processes adopted across various industries in the country, the production of alternative proteins using this technology possesses unique characteristics, rendering training specialized professionals in this area a crucial initiative for the industry's development. Expertise in industrial microbiology, bioprocess scale-up, bioreactor construction and operation, bioproduct separation and purification processes, aseptic techniques, expertise in molecular biology and biochemistry, and development of formulations and final products, for example, are essential for the execution of fermentation bioprocesses. The survey showed that the professionals involved in the fermentation area are mainly biologists, bioprocess engineers, chemical engineers, and biotechnologists, with the growing presence of food engineers in the case of institutions that research alternative proteins. The diversity of professionals underscores the multidisciplinary nature of the fermentation chain, the advancement of which also relies on professionals such as mechanical, mechatronic, and electrical engineers involved in the production of equipment and sensors for bioreactors—the latter representing a research area that remains underexplored in Brazil. New models of high throughput bioreactors have emerged as powerful tools for validating fermentation processes, in addition to using Artificial Intelligence and Machine Learning tools for process optimization.

Brazil also has the opportunity to foster strong partnerships with industries to overcome technological challenges and optimize production. Examples such as the cooperation of alcohol industries to make industrial fermentation equipment viable for alternative protein production demonstrate how the expertise and infrastructure already installed in the country can also be leveraged to achieve prominence in producing fermentation-derived alternative proteins (Box 8). The circular economy concept can also be applied in this chain, connecting agribusinesses and food industries with fermentation companies to supply their by-products as substrates for microorganisms.

The national regulation of new foods recently facilitated Brazil's entry into the fermentation-enabled protein sector. This regulation enables national production and commercialization, creating opportunities for established companies and emerging entities in this segment. This regulation also minimizes risks for investors, as alternative products become reliable and safe, fostering business opportunities for both established companies and new investors in startups.

Based on the survey and internal studies conducted at GFI Brasil, the development of this industry faces two primary challenges: attracting financial resources for its growth and producing products that are competitive in terms of price and quality. This requires investments in research, input development, process optimization, scale-up studies, and economic feasibility studies.

Resources can be mobilized through donations, private sponsorships, private investments, and public funding. Visibility, competitiveness, and regulatory anticipation strategies are examples of private investment drivers, mostly driven by the economic and financial viability of the business models presented. Especially for this sector in Brazil, this directly implies the need to create shared infrastructures for producing alternative proteins through fermentation. That is because one of the major challenges in expanding Brazil's fermentation capacity is posed by the lack of pilot-scale infrastructure to serve as a basis for industrial scale-up, as noted in the previous topic of this document, limiting the opportunities for scale-up, economic feasibility study with data on a representative scale, in addition to the feasibility of products and ingredients bring tested by potential partners and investors.

Finally, the development of competitive products implies overcoming the challenge of consumer acceptance. In this sense, the crucial characteristics influencing consumer behavior include price (Newton et al., 2024), sensory aspects, and health aspects (Good Food Institute, 2021). The price challenge tends to be mitigated as lower-cost processes and inputs are adopted and by scaling up production through consumption growth and economies of scale. In turn, achieving sensory and nutritional quality for the product requires investing in research and developing inputs and processes. It is worth noting, and widely discussed in this document, the potential for producing ingredients that promote sensory and nutritional improvements in plant-based analog products through fermentation.

BOX 8

Opportunities for retrofit and Contract Manufacturing Organizations (CMOs)

Fermentation-enabled protein production may involve high costs, including equipment, infrastructure, and operations. Additionally, such cost may vary for each type of fermentation, considering the upstream and downstream stages. In precision fermentation, purification processes increase equipment costs; in traditional fermentation, upstream costs tend to be lower due to the lower complexity of the bioreactors used. In addition to building a new plant and acquiring new equipment, the options include partnerships with Contract Manufacturing Organizations (CMOs), with production being outsourced, or retrofitting of facilities and equipment of an existing industry.

Investment in CMOs in the alternative protein sector in Brazil is also a major opportunity for the country, not only to meet the domestic demand of Brazilian startups but also a global demand for this type of infrastructure, considering the country's already established competitive advantages, such as the wide availability of low-cost substrates and well-established chain.

In Brazil, where the fermentation industry in some sectors is already well established, part of the existing infrastructure can be adapted to fermentation processes to produce alternative proteins. This could reduce the initial CAPEX by up to 70% and reduce the construction period by up to 6 months, depending on the adjustments required in equipment and facilities (Good Food Institute, 2023b).

Several parallel industries with facilities could be suitable candidates for adaptation to biomass fermentation, but three present higher potential for conversion or adaptability:

Breweries: Significant process similarities and advantageous market conditions indicate the availability of idle or decommissioned facilities and equipment.

Ethanol plants: The annex construction provides direct access to the cheapest raw material source needed for fermentation, thus reducing supply chain risk and operating costs (**Amyris** and **Lesaffre** are examples). Additionally, in Brazil, it is still possible to reduce the environmental impacts of the process by using renewable energy sources already produced in these facilities..

Wineries: Case studies in the United States demonstrate a potential to leverage 75% of equipment downtime throughout the year due to the seasonality of wine production, especially in wineries that use stainless steel (sterilizable) fermenters.

Learn more at: Manufacturing capacity landscape and scaling strategies for fermentation-derived protein.

5

Final considerations

Scallop analogue obtained through fermentation - Aqua Cultured Foods.

To ensure the viability of fermentation-derived protein industries, a solid supply chain and a robust entrepreneurial ecosystem are essential, which involves collaboration between agribusinesses, food industries, fermentation companies, and other stakeholders. Universities, startups, and companies drive innovation, while investors and government agencies provide financial and regulatory support; consequently, fostering a collaborative network and cooperation among these diverse actors can significantly boost the fermentation-derived protein industry.

Despite a significant increase in interest in alternative proteins in Brazil, investment data still reveal gaps compared to other countries. While countries such as the United States and some European countries have substantially increased investments in alternative proteins, Brazil still faces challenges. This investment disparity may hinder the full development of the alternative protein industry in the country, underscoring the need for a more proactive approach to attracting investment and fostering innovation within the sector. Obtaining investment and funding for researching and scaling up fermentation technologies leads to major opportunities.

According to the study by Moraes, Claro and Rodrigues (2023), which was conducted in consultation with alternative protein specialists, important factors driving innovation in this sector are tax incentives, access to financing and opportunity costs. Several hindering and facilitating factors identified in this study are matters of public interest, necessitating that organizations and stakeholders develop a collective understanding of the most sensitive and urgent issues requiring action.

Investing in research and development to obtain new technologies for fermentation processes can provide significant returns in the next decade (Visão de Futuro..., 2018). Brazil has major potential for innovation in this sector. With a focused and collaborative strategy, Brazil will be well-positioned to become a global leader in this emerging market, reproducing successful cases of the country's development and leadership in similar sectors, such as biofuels, and reaffirming its leadership in the development of the bioeconomy.

For the advancement and development of this novel approach to fermentation technology, it is essential to disseminate substantiated information on fermentation technologies for the production of alternative proteins to educate the population and increase consumer acceptance. Continuous investment in research and development and interdisciplinary collaborations are also necessary to engage and advance this emerging global value chain. Furthermore, implementing partnerships between research institutions, such as universities, startups and companies, enables technologies to go beyond the benches and reach the market effectively and safely. The scalability of the technology will be key to obtaining new products that are accessible and satisfactory to consumers.


Mycelium-based meat analogue - Meati.

BOX 9

Call to action: What can be done to boost this ecosystem?

The recommendations are key points identified to develop the value chain of fermentation for protein production in Brazil.

Disseminate technical content and promote forums for discussions and workshops with various actors in the ecosystem, addressing fermentation technologies for the production of alternative proteins with scientific foundations, clarifying their benefits and identifying the country's potential and competitive advantages in this sector;

Encourage and finance research and development in universities and research centers in Brazil on fermentation for protein production. Highlighting the topics previously identified to address specific technological bottlenecks:

- Prospecting new national low-cost substrates, such as industrial/agro-industrial by-products, that diversify and reduce competition for carbon and nitrogen sources already used;
- Development of technological routes for efficient and safe generation of cellulosic/hemicellulosic hydrolysates for use in food production;
- Prospecting for novel microorganisms and the development of microbial chassis with the potential for scaled-up production, also capable of utilizing various carbon sources beyond sugars (e.g., alcohols, organic acids, methane, CO_2), thereby enabling substrate diversification and minimizing competition with food production and other value chains;
- Optimization of bioprocesses and reduction of the use of resources such as energy and water;
- Recycling of waste media and the utilization of process by-products, developing processes aligned with the concept of a circular economy;
- Development of more efficient downstream processes, with lower cost and less use of resources (energy, water) for precision fermentation;
- Conducting LCA and TEA (Techno-economic analysis) studies using data from the Brazilian supply chain to understand its potential and identify areas for improvement.

Encourage the establishment of partnerships between institutions possessing infrastructure for pilot testing and those lacking such infrastructure to foster collaborations that enable proof-of-concept validation and analysis of process scalability—particularly for startups navigating the "valley of death";

Providing financing, through public and private subsidies, for the development of the fermentation value chain for alternative protein production in Brazil, engaging professionals from multiple disciplines;

Engage established fermentation industries and CMOs in Brazil to expand the installed capacity available for the production of proteins via fermentation;

Engage agro-industries and food and beverage industries in the fermentation chain for protein production and establish cooperation models for the provision of their wastes and by-products as inputs for fermentation culture media, thereby integrating food production into the concepts of a circular economy and biorefineries.

Glossary

Animal-based food
Products from animals. In this report, the term is used to define animal-derived foods such as beef, pork, mutton, poultry, seafood, eggs, and dairy.

Life Cycle Assessment (LCA)
Analysis of the environmental impacts of a product throughout all phases of its production, from the extraction of raw materials to disposal, to inform the environmental impacts of production as a whole.

Biomass
Organic matter produced through fermentation, consisting of the microorganism itself.

Lignocellulosic biomass
Plant material composed mainly of three components: cellulose, hemicellulose and lignin.

Bioreactor
Equipment used in fermentation processes to control the ideal conditions for the growth and production of microorganisms, such as temperature, humidity, pH, etc.

Breakeven
Also known within companies as the "equilibrium point," is the point at which revenues equal the costs and expenses of a business in a period. It is nothing more than the exact moment when the business's balance sheet is completely balanced, that is, there is no loss or profit.

CAPEX
The term CAPEX (Capital Expenditure) refers to a company's investments in fixed assets. It represents the capital used by companies to purchase, implement, expand or replace physical assets such as buildings, equipment, machinery, facilities and others.

Cultivated meat
Meat produced directly from animal cells. This is done by extracting cells from a living animal and culturing them in bioreactors. The cells can be differentiated into muscle, fat, or other cell types to create products that have three-dimensional structures, nutritional profiles, and organoleptic properties that are equal to or similar to conventional meat.

Strain
Refers to a specific strain of microorganisms that are used to perform fermentation.

CRISPR
Gene editing technology that enables precise modification of DNA sequences in living organisms.

Starter cultures
Refer to the specific microorganisms intentionally added to a fermentation medium to start and control the process.

Cellular debris
Refers to cellular fragments or residues resulting from the process of disruption of microorganisms after fermentation.

Downstream
Refers to the steps after the fermentation process, encompassing the recovery, purification and formulation of the desired products, such as proteins, after fermentation.

Circular economy
Is a sustainable model that aims to minimize waste and optimize the use of resources, promoting the reuse, recycling and recovery of materials or waste.

Expertise
Ability, knowledge and experience in a specific area.

Fermentation

Biological process that converts the nutrient-rich culture medium, such as sugars, into alcohol, acids, gases, proteins, etc., usually carried out by microorganisms such as bacteria, yeast or fungi.

Biomass fermentation

Fermentation process aimed at multiplying microorganisms and subsequently using the microorganism itself (i.e., cell biomass) as a source of proteins and other constituents of dietary interest.

Traditional fermentation

Fermentation process in which microorganisms are added to a plant matrix, aiming to improve sensory characteristics, increase nutritional value and improve the bioavailability of proteins through their multiplication and generation of metabolic compounds.

Precision fermentation

Fermentation process that uses genetically modified microorganisms to produce specific functional ingredients, including proteins, vitamins, and flavor molecules. These can be used in novel plant-based foods to improve taste or texture, and in cultivated meat to provide more efficient growth, for example.

High throughput

High-throughput screening (HTS) is a method for scientific discovery using robotics, data processing/ control software, liquid handling devices and sensitive detectors, which enables a researcher to quickly conduct more tests and speeds up the achievement of optimized results and processes.

Input

Elements used as raw materials to produce food.

Leghemoglobin

Heme-like protein found in the root of nodules of leguminous plants such as soybeans. It is used in plant-based hamburgers to mimic the taste and juiciness of conventional meat.

Machine learning

Is a branch of Artificial Intelligence that focuses on creating systems that can learn based on data. Rather than being programmed with specific rules to perform a task directly, these systems are trained using data and algorithms that allow them to improve their performance to accomplish their goal. In other words, Machine Learning allows computers to learn based on data without having to explicitly program that task.

Mycelium

Filamentous fungi structure formed by long cell filaments (hyphae) that branch and intertwine.

Mycoprotein

Is a term that defines the protein derived from fungi. The word comes from the Greek term “myco,” meaning fungus.

Neophobia

Fear or aversion to new experiences, such as refusal to try new foods.

Genetically modified organisms (GMOs)

Are organisms whose genetic material has been modified in a way that does not occur naturally through cross-breeding or natural recombination.

Conventional production

Refers to traditional animal production, which involves everything from animal breeding, feeding, and development, to slaughter.

Conventional product

Refers to animal-based products, such as meat, milk and eggs.

Fermentation-derived products

Products obtained by microbial processes, that is, by the culture of microorganisms such as bacteria, yeasts, microalgae and filamentous fungi.

Organoleptic properties

Are sensory properties such as taste, aroma, texture, moisture, mouth feel, appearance and color.

Alternative proteins

Unconventional protein sources (such as animals), often based on plant, cell culture, or fermentation.

Purification

Process of separating and removing impurities, such as other cellular components and by-products, to obtain a high-quality, purified protein.

RDC Resolution No. 839 (December 14, 2023, ANVISA)

Resolution that provides proof of safety and authorization to use new foods and new ingredients.

Food security

Refers to the guarantee that all people have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and preferences for an active and healthy life.

Single-Cell Oil (SCO)

Oils produced through single-cell microorganisms such as bacteria, yeast or algae.

Single-Cell Protein (SCP)

Proteins produced through single-cell microorganisms such as bacteria, yeast or algae.

Stakeholders

For alternative protein fermentation include producers, consumers, specific interest groups (environmental protection, reduction of animal suffering, comprehensive health care promotion, etc.), lawmakers, and the food industry.

Startup

Is a technology-based company with a repeatable, scalable and sustainable business model that operates in a context of risks and uncertainties. They can operate in different areas and markets, and use technology as the basis for their operations. They are created to solve previously unsolved pains or problems or improve old solutions that are no longer as efficient. This makes the challenge for those who are creating the business much greater. Another major difference lies in the goals: while a traditional company seeks long-term profitability and stable value, the focus of startups is to capture investments that enable them to consolidate their business model, grow and increase profits exponentially.

Upstream

Refers to the initial phase of the production process, involving steps such as selecting microorganisms, optimizing culture conditions and preparing biomass (living organisms used in the fermentation process). In protein production, this phase may also include genetic engineering to improve the expression and production of the desired protein. The term "upstream" highlights the part of the process that occurs prior to the fermentation itself.

Valley of Death

Term refers to the critical period in the life of a startup or project in which the company faces major financial and operational difficulties to transform an innovative idea into a viable commercial product.

Expression vectors

Or plasmids are circular DNA sequences that are used as a vehicle to carry foreign DNA sequences into a host cell. They enable the production of proteins that are different from those normally generated by the cell, through an external DNA. They are cloning vectors with all genetic elements that allow the expression of recombinant proteins.

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