Chapter 5

Probiotic Plant-Based Beverages

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Abstract

Vegetable matrices are suitable substrates for obtaining probiotic plant-based beverages. The development of these products aims to serve a new segment of consumers who prefer plant-based foods or have restrictions on consuming dairy products. Here we will describe the process of obtaining soy, oat, and rice extracts. Next, we will discuss the fermentation process to obtain probiotic beverages.

Key words Fermentation, Soy, Oat, Rice, Functional food, Plant-based drinks, Milk alternatives

1 Introduction

Demand for probiotic plant-based beverages has grown worldwide among dairy and non-dairy consumers. Consuming probiotic beverages to improve gut health is not new [1]. However, the demand for these products also includes rising vegetarianism and emerging veganism, lactose intolerance, allergenicity for dairy products, dyslipidemia, and consumer demand for differentiated products [2, 3].

Non-dairy plant-based food matrices such as aqueous extracts of plant-based cereals and legumes have successfully been used to produce probiotic beverages. These substrates contain nutrients easily assimilated by probiotics, stimulating the growth of single and mixed cultures during fermentation [3–5] and providing better food product digestibility. The primary sources used to develop probiotic plant-based beverages are soybean, malt, wheat, barley, tree nuts, rice, and oat, which are suitable substrates for microbial growth [3].

Soy extract (commonly known as soymilk) is rich in compounds important for nutrition and beneficial for health [6]. Soy extract has a similar appearance and chemical composition to animal milk and can be used as a substrate for fermentation by lactic acid bacteria [7]. Soy extract has 3.0% protein, 2.0% lipids, and 2.0%

Adriano Gomes da Cruz et al. (eds.), *Probiotic Foods and Beverages: Technologies and Protocols*, Methods and Protocols in Food Science, https://doi.org/10.1007/978-1-0716-3187-4_5,

carbohydrates, mainly sucrose, raffinose, and stachyose [8]. This product is an alternative to milk due to its protein quality, absence of cholesterol and lactose, and functional properties. However, consumption of this product is limited due to the characteristic flavor and astringent potential of soybeans due to the presence of lipoxygenase enzymes and non-digestible oligosaccharides [9].

Oat (*Avena sativa* L.) is a gluten-free cereal with significant nutritional and functional values, mainly due to its high content of β-glucans. Intake of these soluble fibers has been associated with reduced serum cholesterol and risk of cardiovascular disease [10]. The utilization of this information is authorized by the U.S. Food and Drug Administration (FDA) and European Food and Safety Agency (EFSA) to appear on food labels that contain the minimum amount of β-glucan required for such a health claim. Other oat constituents, such as avenanthramides, tocols, sterols, phytic acid, and avenacosides, have also demonstrated diverse health benefits, including anticarcinogenic and antihyperglycemic activities and improvements in gastrointestinal disorders [11].

Rice is a basic and important food for many of the world's population, and the *Oryza sativa* L. is the most widely grown. The rice grain has a high concentration of carbohydrates, mainly starch, and lower concentrations of vitamins and minerals [12]. The rice grain has potential health benefits so that it can be used as a matrix for plant-based extracts and a vehicle for producing probiotic foods [2]. Plant-based rice extract is a non-dairy beverage extracted from ground rice grain in water followed by homogenization, obtaining emulsions in the water phase of the components present in the rice grain [13–15]. The emulsion formed is a colloidal system constituted by a continuous phase (water) and a dispersed phase (particles). Fraction protein, starch granules, solid parts of rice matrices, and lipid droplets are particles that may be present in the dispersed phase [16, 17]. Plant-based rice extract has been considered an excellent alternative to cow's milk due to health concerns [18–20], is cheaper, and is environmentally friendly [13, 17]. However, plant-based rice extract has different sensory characteristics, stability, and nutritional composition than cow's milk [15].

The aqueous extracts of plant materials, which form the base for probiotic plant-based beverages, are prepared by cleaning, dehulling, soaking, and milling the raw materials in water. Alternatively, it can also be dry milled and subsequently solubilized in water. Then, the slurry is filtered to separate the residues [1, 21]. As a result, these products have been noted for having adequate sensory characteristics, minimum recommended viable probiotic numbers, and a high level of beneficial substances in human nutrition, such as vitamins, minerals, antioxidants, dietary fibers, and natural prebiotic compounds [2, 4, 22]. However, the food matrix is significantly changed during the fermentation

process due to the production of acids, volatile compounds, and other transformations that alter the sensory characteristics of the beverage. Therefore, preparing probiotic plant-based beverages requires they possess desirable sensory, physical, and chemical characteristics and meet probiotic requirements.

Plant-based fermented beverages can be produced through microbial fermentation processes that can occur by the addition of starter cultures (culture-dependent ferments) or naturally (spontaneous ferments) [23]. Industrially produced probiotic products often use starter cultures to ensure product standardization. Industrial microbial cultures include liquid, frozen, freeze-dried, or atomized concentrated microorganisms for starter culture propagation and subsequent inoculation or the use of readily soluble cultures that allow direct vat set (DVS) inoculation [24]. The DVS inoculation of probiotics is convenient for storage and commercial applications. At the same time, freeze-dried concentrated microorganisms are widely used due to their intense fermentation activity and low storage and transportation costs [25, 26].

The probiotic strains used, besides having GRAS (Generally Recognized As Safe) and QPS (Qualified Presumption of Safety) status, recognized by the European Safety Authority—EFSA, must comply with the requirements to be considered probiotic and technological criteria to be employed in beverages, such as: grow in the food matrix, resist the technological processing steps, not produce undesirable sensory compounds, tolerate storage conditions, and resist marketing conditions [24]. Different species of *Lactobacilli* and *Bifidobacteria* have been reported in probiotic plant-based beverages, such as *Limosilactobacillus fermentum*, *Limosilactobacillus reuteri*, *Lactobacillus acidophilus*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus*, *Lacticaseibacillus casei*, and *Bifidobacterium* sp. In addition, other studies of mixed plant-based substrates and probiotic strains are being developed to produce beverages of particular flavor characteristics [3]. This chapter describes the processes for obtaining soy (*see* Subheading 2.1), oat (*see* Subheading 2.2), and rice extract (*see* Subheading 2.3). After obtaining one of the extracts, the fermentation procedure can be carried out according to Subheading 3.

2 Plant-Based Aqueous Extract

The processes for obtaining soy (*see* Subheading 2.1), oat (*see* Subheading 2.2), and rice extracts (*see* Subheading 2.3) will be described.

2.1 Soymilk

2.1.1 Materials

1. Food-grade whole soybeans, full-fat soy flakes, or full-fat soybean flour can be used (*see* **Note 1**).
2. Drinking water.
3. Container or tank for soaking soybean.
4. Stainless steel semi-industrial blender or grinder.
5. Filtration system.
6. System for heat treatment.

2.1.2 Methods

The soy extract can be obtained from the following steps:

1. Selection and removal of major and minor dirt from soybeans through sieving (*see* **Note 2**).
2. Soak soybeans in drinking water at a ratio of 1:3 soy: water, for 3 to 15 h, depending on conditions (*see* **Note 3**).
3. Grind the wet soybeans in a stone mill or hammer mill with additional fresh water. The ratio of water to soybeans is usually 6:1 to 10:1 (*see* **Note 4**).
4. Filter the slurry to separate the soybean extract from the insoluble fiber (okara) through a sieve, cloth, or pressing bag, with or without a wooden press. The slurry also can be filtered on one or more scraped filters in batch or semicontinuous mode or by continuous centrifugation filtration. The okara is usually washed once or twice with cold or hot water, stirred, and re-pressed to maximize soymilk yield. The total volume of the combined filtrate (raw soymilk) is about 6–10 times the original soybean volume.
5. Carry out the heat treatment followed by rapid cooling of the soymilk before fermentation. Heat treatment can be carried out by pasteurization or sterilization using ultra-high temperature (UHT). At the pasteurization, soybean extract is boiled for 10 min (95–98 °C) from the start of boiling, continuously stirring. The processing by UHT of soymilk is usually done by using UHT directly with steam injection, followed by a short holding time of about 5 s at about 145 °C, followed by flashing to remove unpleasant odors and excess steam.

2.1.3 Notes

1. For a better result, you should preferably use clear hilum soybeans to produce soy extract with whiter color, higher yield, and better overall quality.
2. At selection, discard the broken soybeans because the enzymatic reactions that cause the off flavor have already taken place. After the selection, optionally, the soybean can be dehulled to improve the flavor of the soy extract by removing bitter and astringent compounds from the husk.

3. Before soaking, the beans should be rinsed under running water, careful not to damage them. After soaking, the water can be removed and replaced with fresh process water. Soaking the soybeans in cold water causes little or no lipoxygenase activity. However, it requires more time than soaking in warm or hot water. Soaking in hot water has the advantage of removing all adverse enzyme reactions very quickly. Therefore, many processes include a hot blanching step for 15 to 20 min in 3–5 volumes of water at 85 to 90 °C. In addition, soaking in bicarbonate solution plays an essential role in softening the structure of soybeans, which will later create a finer slurry. As a result, soy extraction yield is higher because a large portion of the soybeans can seep through the filter cloth, resulting in higher protein content.
4. If heating has not yet taken place before grinding, hot water is used, or steam is injected to increase the temperature of the soybean slurry as quickly as possible. In addition to inactivating the enzyme, increasing the cooking temperature of soybeans will reduce the viscosity of the oil in soy extract and make it easier for the oil cells to break down. This phenomenon allows oil to be released and further increases the crude fat content of soy extract.

2.2 Oat Extract

2.2.1 Materials

1. Whole meal, hulled, or flakes oats.
2. Drinking water.
3. Container or tank for soaking oat.
4. Stainless steel semi-industrial blender or grinder.
5. Water bath.
6. Optional application of thermostable endo-acting enzymes: α-amylase (120 KNU-T/g) and β-glucanase (1 U/mg), both food grades.
7. Filtration system with mesh opening size up to 20 μm.

2.2.2 Methods

The preparation of oat extract, popularly known as oat "milk," can be obtained from the following steps:

6. Select and wash whole or hulled oats to remove dirt and unwanted material.
7. Soak whole meal, hulled, or flakes oats in drinking water at a ratio of 4 to 8% (w/v) at room temperature for 12 h (*see* **Note 1**).
8. Grind the oat with their soaking water in a stainless-steel blender or grinder for up to 5 min (*see* **Note 2**). Food industries generally do not perform the soaking step due to the long time required. In this case, the oat can be ground with hot

water at 80–90 °C for up to 5 min to adequately extract proteins, β-glucan, vitamins, minerals, etc.

9. If the objective is to reduce beverage viscosity and/or increase protein extraction, add 0.15% α-amylase (w/w; enzyme/oat) and 0.04% β-glucanase (w/w; enzyme/oat) to the slurry and keep it in a water bath at 80 °C for 2 h under moderate agitation (*see* **Note 3**).
10. Cool the slurry to 30 °C and then filter or centrifuge it to pass through a mesh opening up to 20 μm (*see* **Note 4**). The material retained in the mesh can be applied for the elaboration of bakery products.
11. This oat extract must be pasteurized in a water bath, discontinuous or continuous heat exchanger, before inoculating the probiotic culture (*see* Subheading 3).

2.2.3 Notes

1. The ratio of 4 to 8% (w/v; oat/water) is suitable for extracting a significant amount of β-glucan and making the beverage prebiotic. However, oat extract with high amounts of this fiber can show undesirable sensory properties to consumers due to its high viscosity. Therefore, carrying out previous tests with consumers is important to assess the drink's acceptability.
2. High grinding time contributes to greater β-glucan extraction, but its impact on beverage viscosity must be investigated.
3. Termamyl® (Novozymes) is an endo-acting α-amylase used to liquefy oat starch and produces oat-based drinks with moderate viscosity. Beerzym BGHK4® (Erbslöh Geisenheim GmbH) is a β-glucanase that also reduces the viscosity of the beverage through the hydrolysis of β-glucan.
4. High pressure during filtration can make it easier for the slurry compounds to pass through the mesh, especially β-glucan and proteins.

2.3 Rice Extract

2.3.1 Materials

1. Rice grain (fully milled or partially milled), milled broken rice, or rice flour.
2. Drinking water.
3. Container or tank for soaking rice.
4. Stainless steel semi-industrial blender or grinder.
5. Enzymes: α-amylase and glucoamylase (Glucozyme).
6. Filtration system.

2.3.2 Methods

The process of extracting the rice extract ("rice milk") follows the following steps:

1. Prepare the grains, passing them through cleaning, selection, classification, and washing steps. The rice grains can be fully or partially milled (*see* **Note 1**).
2. Hydrate the grains in water in the proportion of 1:2 (w/v) at 2 °C for 5 h.
3. Grind the rice with the hydration water using the stainless-steel blender or grinder for about 3 min until a homogeneous mixture is obtained. Then, for liquefaction, add 0.1% enzyme α-amylase at 90 °C for 30 min in the homogeneous mixture and use 0.1% glucoamylase (Glucozyme) at 55 °C for 12 h to reduce the beverage's viscosity and convert rice extract's starch to simple sugars for consumption in the fermentation process of plant-based rice extract. Saccharification can also be carried out simultaneously with the fermentation step, when applicable, by adding 0.1% glucoamylase (Glucozyme), a fermenting microorganism, to 200 g of rice extract (*see* **Note 2**).
4. Remove coarse particles by filtration, decanting, or centrifugation. These larger or coarse particles can be used in other food products. Next, extract the soluble phase ("rice milk" or rice extract).
5. Add other necessary ingredients to improve the chemical and sensory characteristics of the beverage (*see* **Note 3**).
6. Pasteurize the rice extract before inoculation of the probiotic culture. Pasteurization can occur in a water bath, discontinuous, or continuous heat exchanger.

2.3.3 Notes

1. The process of rice extract involves grain preparation, hydration, and breakdown (size reduction) of grain extracted in water, treated with enzymes to partially break down the starch and facilitate a suspension mixture, filtered, and thermic treatment. Fully milled indicates that the husk, germ, and bran have been removed, and only the endosperm (white rice) remains, while the partially milled only husk has been removed. Rice flour can also be used as a raw material. Fully milled grain may result in better texture in the slurry (paste), but it has high starch content and low content of nutrients, fiber, vitamins, and bioactive components. Several rice grains can be used in plant-based rice processing, but polished rice is the most used raw material. Brown rice is also sometimes used. When rice flour is used to extract the beverage, there will be no need for a milling step, but an effective mixing solution is recommended to create a uniform slurry.
2. Cereals and pseudocereals have a high proportion of starch and form a thick paste when heated above the gelatinization temperature (55–65 °C). Therefore, to prevent and avoid problems

in the later stages of processing, the use of the α-amylase enzyme is required.

3. After obtaining the desired viscosity (thickness), other ingredients can be added [13, 14]. Protein, calcium, and vitamins are examples of essential nutrients required in the rice extract once they are limiting nutrients [13, 27].

3 Fermentation

The general process of obtaining probiotic plant-based beverages using DVS culture will be described. However, depending on the culture used and the substrate, it will probably be necessary to adapt the inoculation and fermentation conditions. In some cases, it is necessary to reactivate the culture, which can be purchased in liquid, freeze-dried, or concentrate-frozen form, before inoculation, so follow the manufacturer's instructions.

3.1 Materials

1. The aqueous extract was obtained according to Subheading 2.
2. Microbial culture: add 2% to 3% of the microbial culture or use the manufacturer's recommendations (*see* **Note 1**).
3. Optional ingredients: Sucrose (6 to12%), thickeners (0.5%), flavorings (0.1%), and other optional ingredients such as pulps, fruits, and prebiotics are recommended.
4. Flasks and incubator oven or industrial fermentation tank.
5. Cooling heating system for beverages.

3.2 Methods

The preparation of probiotic plant-based beverages can be obtained from the following steps:

1. Add the non-volatile or thermolabile ingredients and homogenize for solubilization (aqueous extract, sucrose, thickeners, and others) (*see* **Note 2**).
2. Heat treat the mixture of aqueous extract and other ingredients before receiving the inoculum for fermentation (*see* **Note 3**). It is suggested to use 95 °C for at least 5 min (*see* **Note 4**).
3. Cool the aqueous extract rapidly to fermentation temperature (usually between 25 °C and 43 °C), according to the manufacturer's recommendations (*see* **Note 5**).
4. Add the inoculum, homogenizing with the help of sanitized or sterile utensils.
5. Ferment at the temperature indicated for the microorganism (usually between 25 °C and 43 °C) until the desired final pH (*see* **Note 6**).

6. Cool to 5 °C for 12 h for stabilization and then homogenize, preferably without incorporating air (*see* **Note 7**).
7. Add the flavorings and other thermolabile ingredients.
8. Aseptically fill in appropriate packaging and, preferably, store under refrigeration.

3.3 Notes

1. Check the manufacturer's instructions for use in volumes lower than the recommended in the microbial culture envelope. Generally, for mixed culture envelopes, you should sterilize 1 L of the fermentation substrate at 121 °C for 15 min. After cooling down (~10 °C), add the mixed culture (1 envelope of 5 UC for 1 L of the substrate), and homogenize with sterile utensils. Then distribute into sterile 10 mL containers and freeze quickly at −18 °C until use. In this example, each container will contain enough microbial culture to be used in 5 L of beverage. However, other proportions can be used according to the production scale.
2. Flavoring ingredients or ingredients with thermal instability can be added aseptically after heat treatment. The sanitary quality of these ingredients must be checked beforehand or ensured by the manufacturer through technical reports. Sucrose is usually added to improve flavor and consistency and is used as a fermentation substrate by some starter cultures. In addition, thickeners, fruit pulp or juice, and other compounds can improve the product's stability and acceptability.
3. The aqueous extract must have a typical taste and aroma, an absence of spoiling microorganisms, pathogens, and an absence of fermentation inhibiting substances. In addition, the heat treatment must be carried out in such a way as to guarantee the safety of the product.
4. If the heating is done in an open tank, at a lower temperature and longer time, an increase in the solids content of the aqueous extract will occur. The same effect can be observed with heating at 95 °C for 5 min in a plate heat exchanger under a partial vacuum, where part of the water is evaporated.
5. Heating and cooling operations can be performed on a plate heat exchanger.
6. Generally, aqueous extracts are fermented to a pH of about 4.3. The fermentation time depends on the characteristics of the culture and temperature employed and typically ranges from 4 to 30 h. Some probiotic microorganisms, such as *Bifidobacteria*, can accumulate compounds undesirable for the product during fermentation, such as acetic acid. Therefore, in some cases, the probiotic may not participate in the fermentation

process. In this case, the highly concentrated culture is added at the end of the process, followed by cooling and packaging.

7. Most probiotic microorganisms are anaerobic, and mixing can lead to the incorporation of oxygen into the beverage, reducing the probiotics' viability.

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