Chapter 6

Probiotic Plant-Based Cheese

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Abstract

Due to several factors, the demand for plant-based cheese has increased. However, formulating products with characteristics similar to cheese made with animal milk is still a challenge for researchers and industries. Here we will describe the process of obtaining probiotic pea cheese, probiotic tofu, probiotic soy-based cream cheese, and probiotic chickpea petit suisse cheese.

Key words Pea cheese probiotic, Probiotic tofu, Chickpea probiotic petit suisse cheese, Functional food

1 Introduction

Probiotic plant-based cheeses are products made from vegetable sources, usually legumes, including non-dairy fats or proteins, which result in a cheese analogous to that made with animal milk. It has benefits for the health of the consumer. Ethical reasons, sustainability, animal welfare, and health, such as lactose intolerance, milk allergy, and cholesterol issues, are the main consumer concerns that drive interest in plant-based dairy alternatives [1, 2]. The increased production and demand for plant-based cheese alternatives are also due to the increasing number of people following vegan diets [3].

The cheeses of vegetable origin differ sensorially from the cheeses of animal origin due to their composition. The plant-based cheese does not present the physical and sensory characteristics of dairy-based cheese, whereas the comparisons between flavor, taste, aroma, mouthfeel, and meltability limit consumer acceptability [2]. In cheese made with animal milk, casein-casein interactions promote stretch and flow functionality, which provides structure to the cheese matrix. In plant-based cheese, the formation of compact gel networks does not occur in the same way as with

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_6,

casein. As rennet does not induce coagulation in plant milk, other protein coagulation methods should be employed [4–6]. Therefore, applying enzymes, acids, lactic acid fermentation, or heat treatments are technologies that need to be considered [7]. This is because plant proteins have larger molecule sizes and more complex quaternary structures than milk proteins, in addition to having properties such as cross-linking and hydrophobicity [8]. Thus, these products are formed by lipids embedded in polysaccharides or protein matrices, forming a colloidal dispersion [6].

Many types of plant-based cheeses are formulated using combinations of oils (e.g., coconut or palm oil) and starches (e.g., potato or tapioca starch) [1]. The main ingredients for the formulation of a plant-based cheese are water, lipids, and vegetable proteins, which can be included as stabilizers, emulsifying salts, acidifying agents, preservatives, and flavors [9, 10]. In addition, a combination of ingredients has been used to provide a product with structure, viscosity, and melting similar to traditional cheese [5]. Although most of the time, a combination of ingredients is necessary, the primary raw materials used in producing plant-based cheeses are soybeans, peas, cashew nuts, coconut oil, oats, almonds, palm fruit oil, and corn zein [3, 11].

Peas represent a good alternative to produce plant-based cheeses as they have a low production cost and high protein content, which also stand out for their excellent gel formation. The gel is mainly formed by the presence of globulins, such as legumin and vicilins, representing 70–80% of the total protein content [11, 12]. In addition, pea cheese proved to be a suitable substrate for fermentation, and protein gels can be produced with 10% protein content and 10% olive oil levels without compromising gel hardness [11].

Soybean is a food cholesterol-free, low in sodium, a good source of nutrients, and a suitable medium for probiotic growth [13, 14]. Tofu is a plant-based cheese made from soy milk, one of the most important and popular foods in Asian countries, and is widely accepted worldwide. It is gaining popularity in Western countries due to its health benefits [15]. The fermentation increases the nutritional value and helps remove soy's taste, which many consumers do not accept [16]. Probiotic tofu can be a healthy alternative for vegans and vegetarians while positively affecting consumers' health and improving the taste of soy cheese.

Soy-based cream cheese is a cream cheese analog known for its creaminess and spreadability. Chemically, it can be defined as a microgel with a structure formed of protein-covered soy fat globules [4, 17], resulting from the homogenization of tofu, fat, and stabilizers. Therefore, its processing begins with the acidic coagulation of soymilk to obtain tofu. However, there are still few studies [18–20], on this cream cheese analog, particularly with the addition of probiotics. Nevertheless, advances in processing, such as membrane technology, enzymatic protein modification, and high hydrostatic pressure and ultrasound treatments, are promising to contribute to this product's sensory characteristics, texture, and cost efficiency [21].

The fermentation of these substrates to obtain a probiotic plant-based cheese is an alternative that can improve the products' nutritional, sensory, and shelf life since starter cultures can be selected that, in addition to promoting acidification, can produce extracellular polysaccharides that collaborate with the firmness of the product. This chapter describes the processes for obtaining probiotic pea cheese (*see* Subheading 2), probiotic tofu (*see* Subheading 3), probiotic soy-based cream cheese (*see* Subheading 4), and probiotic chickpea petit suisse cheese (*see* Subheading 5).

2 Pea Cheese Probiotic

Pea cheese is plant-based, produced by solubilizing pea protein in water and blending it with oil to create a plant-based emulsion. Coagulation of pea protein for gel formation can occur through the action of heat, high pressure, acidification, and fermentation. There is still a little exploration of fermentation-induced pea gel formation in this last one. Concerning the production of probiotic pea cheese, there are still few studies that explore this process. The primary materials and processing steps for the pea cheese probiotic are described below:

2.1 Preparation of Probiotic Pea Cheese

2.1.1 Materials

1. Pea protein isolate (PPI).
2. Extra-virgin olive oil.
3. Sucrose.
4. Glucose.
5. Salt.
6. Starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*).
7. Probiotic culture (e.g., *Lactobacillus acidophilus, Lacticaseibacillus paracasei,* and *Bifidobacterium*).

2.1.2 Methods

1. Suspend a pea protein isolate (PPI) in distilled water until a final protein concentration of 10% (w/w) (*see* **Note 1**).
2. Stir the mixture at 9500 rpm for 3 min with Ultra-Turrax, to ensure protein hydration.
3. Add 1% glucose (w/w) and 1% (w/w) sucrose during the stirring step to ensure that there is a substrate for the bacteria to grow and acidify the matrix, given the low sugar content of the PPI.

4. Emulsify the mixture using extra-virgin olive oil (around 10% olive oil, w/w) with Ultra-Turrax at 13,500 rpm for 3 min (*see* **Note 2**).
5. Homogenize the sample under continuous operation and high pressure at two stages (150 and 50 bars) in one pass to decrease oil droplet size and avoid phase separation (*see* **Note 3**).
6. Pasteurize (*see* **Note 4**) the matrix at 95 °C for 5 min in a water bath and cool down to 43 °C before microbial inoculation. For starter culture inoculation utilize 0.02% inoculum. Add probiotic culture and mixture. Containers used for preparation must be previously dry autoclaved at 121 °C for 20 min.
7. Ferment at 43 °C until reaching pH 4.5 (*see* **Note 5**).
9. Mold the product.
10. Store at refrigeration temperature.

2.1.3 Notes

1. It is known that 10% protein is an optimal protein concentration for a stable liquid matrix prepared with the PPI. This concentration keeps the matrix density low, which allows easy pre-processing and homogeneous inoculation in a liquid media.

   The use of PPI is a functional starting raw material for fermentation-induced pea protein gels.

   In the case of using another source of raw material for the production of probiotic pea cheese, such as pea protein concentrate (78% protein), it is recommended to adjust the pH of the suspension, as different pH values during pre-treatment of pea protein led to different ratios between soluble and insoluble protein aggregates in the protein slurry before fermentation. Therefore, these are determining factors during the formation of the gel, directly influencing the rheological properties. For example, the solubility of pea protein concentrate is highest at pH 8.0 and lowest at pH 6.0.
2. Olive oil or other oil types provide the matrix with the fat needed for the cheese and help to stabilize the pea proteins in suspension, which will likely sediment over time if only suspended in water. It is recommended that the initial pH of the PPI emulsions be 7.0. In pea protein emulsions, this protein acts as a surfactant due to its excellent emulsifying properties, which contribute to the stability of the emulsion. Thus, avoiding the need to use surfactants.
3. Hydration and homogenization of proteins with two pressure stages are important operations in producing a PPI matrix that guarantees stability and avoids phase separation during fermentation.

4. Pasteurization of the matrix before fermentation is necessary to ensure the safety and growth exclusivity of the inoculated starter culture. Matrices with high starch contents will form a gel before fermentation, and it will be impossible to inoculate bacteria into a liquid medium. Therefore, less purified pea protein ingredients can present challenges during matrix processing. Thus, it is necessary to use purer protein fractions, such as protein isolates (80–95% protein).
5. The time required to reach pH 4.5 can vary between 5.5 and 7 h. The final pH affects rheology and meltability. Higher pH values (6.0–7.0) provide the cheese analog with more viscous properties and lower pH (4.5–5.5) with higher elasticity.

3 Probiotic Tofu

Tofu is a soy-based product that is precipitated with coagulants in the form of curds. The curds' size and the pressing time length determine the style of tofu, which can be soft, regular, firm, or extra firm. The probiotic culture can be added before or after the coagulant. However, there are still few studies that have investigated the incorporation of probiotics in tofu. The primary materials and processing steps are described below:

3.1 Preparation of Probiotic Tofu

3.1.1 Materials

1. Food-grade whole soybeans (*see* **Note 1**), full-fat soy flakes, or full-fat soybean flour.
2. Drinking water.
3. Coagulant (*see* **Note 2**).
4. Probiotic cultures.
5. Container or tank for soaking soybean.
6. Stainless steel semi-industrial blender or grinder.
7. Filtration system.
8. System for heat treatment.
9. Incubator for fermentation.

3.1.2 Methods

1. Wash dry whole soybeans and soak them in water overnight. The volume of water is usually 2 to 3 times the volume of the bean.
2. Drain the soaked beans and rinse with fresh water 2–3 times.
3. Ground the wet, clean soybeans in a mill with fresh water. The water:bean ratio is usually in the range of 6:1 to 10:1.
4. Filter the soy milk through a sieve, cloth, or press bag. Remove residue and wash once or twice with water (cold or hot), stirring and pressing again to maximize milk production.

5. Heat raw milk at a boiling temperature and keep it at this temperature for 5–10 min (*see* **Note 3**).
6. Once the milk is heated, transfer it to another container. At the same time, mix the powdered coagulant with a small amount of hot water to make a coagulant suspension. Then add the coagulant to the hot soy milk at 70–85 °C; depending on the type of coagulant used, let the mixture sit for about 10 min so that the proteins can coagulate (*see* **Note 4**).
7. After noting the beginning of coagulation, add the probiotic culture and mix.
8. Transfer the mixture to a sterile press cloth and place it into the mold. Discharge excess liquid by pressing (*see* **Note 5**).
9. Ferment at the temperature indicated for the microorganism (usually 37 °C) until the desired final pH, for about 20 h.
10. Store at refrigeration temperature.

3.1.3 Notes

1. Preferably beans with large seed sizes and light hilum to produce tofu of whiter color, higher yield, and better overall quality.
2. Coagulants can be enzymes, salts, or acids. The widely used ones for tofu making are calcium sulfate, magnesium chloride, or a mixture of both, nigari, and glucono-6-lactone (GDL, known as lactone).
3. To avoid burning the milk at the bottom of the cooking vessel, slow heating with frequent stirring is necessary. Alternatively, soy slurry may be heated before filtering into soymilk. Heating soy milk is important to denature the proteins so that they coagulate into curds in the presence of the coagulant. However, prolonged heat treatment should be avoided as it can destroy nutrients such as essential amino acids and vitamins, Maillard browning, and the development of cooked flavors, leading to lower yields and poor-quality tofu.
4. Factors affecting coagulation include the temperature at which a coagulant is added, the type and concentration of coagulants, the mode of adding coagulants, and the duration of coagulation [14]:
   - The suitable amount of coagulant: coagulates all soy milk and generates a clear whey with an amber or pale yellow color and sweet taste.
   - Excessive coagulant: the whey has a slightly bitter taste and a yellowish color, and the curd has a coarse, hard texture.
   - Insufficient coagulant: the resulting whey is cloudy, with some remains of uncoagulated soy milk.

- High temperature: rapid coagulation resulting in tofu with low water-holding capacity, hard and coarse texture, and thus a low bulk yield.
- Temperature too low: coagulation becomes incomplete, making tofu too soft to retain its shape.
- Time too short: coagulation is incomplete.
- Time too long: the system's temperature decreases to such an extent that the subsequent shaping step becomes complex. For silken tofu, the dwell time should be about 30 min; for regular tofu, 20–25 min; and for firm tofu, 10–15 min.

  The suggested additional temperature and concentration for the main coagulants are: calcium sulfate ($CaSO_4$) 0.5% (w:v) at 80–85 °C, magnesium chloride ($MgCl_2$) 5% (w:v) at 70–72 °C; Glucono-δ-lactone (GDL) 0.020 mM at 80 °C. The incubation time for coagulation is 10 min.

5. The curd is pressed to form tofu. The size of the curd and the length of the pressing time determine the style of tofu produced, which can be soft, regular, firm, or extra firm tofu—the softer the tofu, the lower the protein and fat levels and the higher the water content.

4 Soy-Based Cream Cheese Probiotic

This product is mainly obtained from tofu, which results from the acidic coagulation of soymilk. However, there are still few studies that have investigated the incorporation of probiotics in soy-based cream cheese. The main materials and processing steps are described below:

4.1 Preparation of Probiotic Soy Cream Cheese

4.1.1 Materials

1. Soft or firm tofu (*see* Subheading 3).
2. Palm oil.
3. Carrageenan gum.
4. Pectin.
5. Maltodextrin.
6. Salt.
7. Stainless steel semi-industrial blender or high-pressure homogenizer.
8. Probiotic cultures.

4.1.2 Methods

1. Homogenize (*see* **Note 1**) the tofu with palm oil (5–10%, w/w), carrageenan gum (4%, w/w), pectin (1%, w/w), maltodextrin (2–4%, w/w), and salt (1–3%, w/w). These ingredients

are responsible for the stability and texture of probiotic soy cream cheese (*see* **Note 2**).

2. Add the probiotic culture (*see* **Note 3**) to the soy cream cheese using sanitized or sterilized utensils.
3. Aseptically fill in appropriate packaging and store under refrigeration.

4.1.3 Notes

1. Homogenization time in stainless steel semi-industrial blenders varies from 2 to 5 min to produce a homogeneous emulsion. On an industrial scale, the mixture is homogenized in a high-pressure homogenizer with pressure ranging from 10 to 25 MPa. It is noteworthy that high shear rates make formulations more spreadable.
2. In addition to maltodextrin, soy protein concentrate or isolate can be added to the mixture to obtain a smooth soy cream cheese. Carrageenan gum and pectin increase the viscoelasticity of the cream and reduce product syneresis during cold storage. Furthermore, salt concentrations above 6 g/kg and high-fat content (>280 g/kg) can also reduce syneresis [17].
3. It is recommended that the probiotic concentration be at least 8 log CFU/g because there is a reduction in this value during storage and after ingestion of the product. Therefore, viability tests of probiotics are necessary to ensure a concentration above 6 log CFU/g of product at the time of consumption and in the distal part of the gastrointestinal tract.

5 Chickpea Probiotic Petit Suisse Cheese

Chickpea probiotic petit suisse cheese is the food produced from the fermentation of chickpea extract, followed by obtaining quark cheese. This product can be flavored and added with emulsifiers and stabilizers. There are still few studies that investigate the use of chickpeas for the preparation of petit suisse. The main materials and processing steps are described below:

5.1 Preparation of Chickpea Probiotic Petit Suisse Cheese

5.1.1 Materials

1. Food-grade whole chickpea.
2. Drinking water.
3. Container or tank for soaking chickpeas.
4. Stainless steel semi-industrial blender or grinder.
5. Water bath.
6. Probiotic cultures (*see* **Notes 1** and **2**).
7. Stainless steel semi-industrial blender or grinder.
8. Filtration system.

9. System for heat treatment.
10. Incubator for fermentation.

5.1.2 Methods

The preparation of chickpea probiotic petit suisse cheese starts with the preparation of the chickpea extract, and can be obtained by the following steps:

1. Select and wash whole or hulled chickpeas to remove dirt and unwanted material.
2. Soak the chickpea in drinking water at a ratio of 1:3 chickpea: water (w/v) at room temperature for 12 h.
3. Drain the soaked chickpea and rinse with fresh water 2–3 times.
4. Grind the chickpea with water for up to 10 min. The water: chickpea ratio is usually in the range of 6:1 (w/v). Food industries generally do not perform the soaking step due to the long time required. In this case, chickpeas can be ground with hot water at 80–90 °C for up to 5–10 min for adequate extraction of compounds.
5. Cool the slurry to 30 °C and filter through a sieve, cloth, or pressing bag. The slurry also can be filtered on one or more scraped filters in batch or semicontinuous mode or by continuous centrifugation filtration.
6. Add sugar (5–15%, w/w) to the chickpea extract and give heat treatment. Before inoculating the probiotic culture, this chickpea extract must be pasteurized in a water bath, discontinuous or continuous heat exchanger (*see* **Note 3**).
7. Cool the aqueous extract rapidly to fermentation temperature (usually between 37 °C and 43 °C), according to the manufacturer's recommendations.
8. Add the inoculum, homogenizing with the help of sanitized or sterile utensils.
9. Ferment at the temperature indicated for the microorganism (usually between 37 °C and 43 °C) until the desired final pH (*see* **Note 4**).
10. Desorb for up to 12 h at 4–8 °C in a synthetic filter or sieve to get quark cheese (*see* **Note 5**).
11. Add guar gum (0.3–0.7%, w/w) and xanthan gum (0.3–0.7%, w/w), and mix with quark mass (*see* **Note 6**).
12. Add, if you prefer, fruit pulp (10 to 30%, w/w) previously heat-treated and other additives such as flavorings (*see* **Note** 7).
13. Aseptically fill in appropriate packaging and, preferably, store under refrigeration.

6 Notes

1. Check the manufacturer's instructions for use in less than the recommended volume in the microbial culture envelope. More instructions are given in Chap. 5, in the **Notes** to Subheading 3.
2. Exopolysaccharide-producing probiotic culture is recommended to obtain better texture properties.
3. The chickpea extract must have a typical taste and odor, an absence of spoiling microorganisms, pathogens, and an absence of fermentation-inhibiting substances. The heat treatment must be carried out in such a way as to guarantee the safety of the product. 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.
4. 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.
5. Additional hygienic care must be taken in the draining step to avoid microbiological risk. It is recommended that this step be carried out under refrigeration. The whey released to obtain quark cheese has a high concentration of water-soluble components, such as carbohydrates, salts, and proteins, so it can be used to manufacture other food products.
6. Other thickening agents can be used to ensure the texture properties of the product, such as carrageenan gum (2–5%, w/w) and pectin (0.5–2%, w/w).
7. The acidity of fruit pulp can affect the viability of probiotic microorganisms during product storage. Therefore, perform feasibility tests to verify that the probiotic count is as desired.

References

1. Harper AR, Dobson RC, Morris VK, Moggré GJ (2022) Fermentation of plant-based dairy alternatives by lactic acid bacteria. Microbiol Biotechnol 15(5):1404–1421
2. Short EC, Kinchla AJ, Nolden AA (2021) Plant-based cheeses: a systematic review of sensory evaluation studies and strategies to increase consumer acceptance. Foods 10(4):72
3. Craig WJ, Mangels AR, Brothers CJ (2022) Nutritional profiles of non-dairy plant-based cheese alternatives. Nutrients 14(6):1247
4. Jeewanthi RKC, Paik HD (2018) Modifications of nutritional, structural, and sensory characteristics of non-dairy soy cheese analogs to improve their quality attributes. J Food Sci Technol 55(11):4384–4394
5. Mattice KD, Marangoni AG (2020) Physical properties of plant-based cheese products produced with zein. Food Hydrocoll 105:105746
6. Grossmann L, McClements DJ (2021) The science of plant-based foods: approaches to create nutritious and sustainable plant-based cheese analogs. Trends Food Sci Technol 118: 207–229
7. Fu W, Yano H (2020) Development of "new" bread and cheese. PRO 8(12):154

8. Bachmann HP (2001) Cheese analogues: a review. Int Dairy J 11(4–7):505–515
9. Guinee TP, Mulholland EO, Kelly J, Callaghan DJO (2007) Effect of protein-to-fat ratio of milk on the composition, manufacturing efficiency, and yield of cheddar cheese. J Dairy Sci 90:110–123
10. Kamath R, Basak S, Gokhale J (2022) Recent trends in the development of healthy and functional cheese analogues-a review. LWT 155: 112991
11. Masiá C, Jensen PE, Petersen IL, Buldo P (2022) Design of a Functional pea Protein Matrix for fermented plant-based cheese. Foods 11(2):178
12. Lam ACY, Can Karaca A, Tyler RT, Nickerson MT (2018) Pea protein isolates: structure, extraction, and functionality. Food Rev Int 34(2):126–147
13. Matias NS, Bedani R, Castro IA, Saad SMI (2014) A probiotic soy-based innovative product as an alternative to petit-suisse cheese. LWT 59(1):411–417
14. Liu K (2008) Food use of whole soybeans. In: Johnson LA, White PJ, Galloway R (eds) Soybeans. AOCS Press, Urbana
15. Faisal M, Mulana F, Alam PN, Daimon H (2014) Wastewater characteristics from tofu processing facilities in Banda Aceh. Proc Ann Int Conf Syiah Kuala University-Life Sciences & Engineering Chapter 4(1):18–21
16. Zeybekoglu N, Ozhan AH, Boyacioglu O (2021) Probiotic tofu with Bacillus clausii spores to support gastrointestinal microflora. Adnan Menderes Üniversitesi Sağlık Bilimleri Fakültesi Dergisi 5(3):534–545
17. Wolfschoon-Pombo AF, Dang BP, Chiriboga BC (2018) Forced syneresis determination results from commercial cream cheese samples. Int Dairy J 85:129–136
18. Liong MT, Easa AM, Lim PT et al (2009) Survival, growth characteristics and bioactive potential of Lactobacillus acidophilus in a soy-based cream cheese. J Sci Food Agric 89(8):1382–1391
19. Lim TJ, Easa AM, Karim AA et al (2011) Development of soy-based cream cheese via the addition of microbial transglutaminase, soy protein isolate and maltodextrin. Br Food J 113(9):1147–1172
20. Rinaldoni AN, Palatnik DR, Zaritzky N, Campderros ME (2014) Soft cheese-like product development enriched with soy protein concentrates. LWT Food Sci Technol 55(1): 139–147
21. Wolfschoon Pombo AF (2021) Cream cheese: historical, manufacturing, and physico-chemical aspects. Int Dairy J 117:104948