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Review

Overview on Diversity and Microbiological Safety of Brazilian Artisanal Cheeses

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Abstract: Artisanal cheeses made with raw milk are highly appreciated products in Brazil. Most of these cheeses are produced in small properties across different production regions in the country, many of which have been granted a protected designation of origin. The most prominent state that manufactures these products is Minas Gerais, but production is also gaining strength in other Brazilian states. This text presents an overview of the many types of artisanal cheeses produced in the country, grouped by geographical regions, and reviews the current challenges faced by producers and government considering the safety of these cheeses.

Keywords: Brazilian artisanal cheeses; safety; challenges

1. Introduction

The history of artisanal cheese production in Brazil dates to the second half of the 18th century. After the discovery of Brazil in 1500, Portuguese settlers brought cattle from the Serra da Estrela, Portugal, to the region which now corresponds to the state of Bahia, in the North-Eastern part of the country. Herds of domesticated cattle expanded southwards along the São Francisco River, reaching the region of Serro, in Minas Gerais state, where gold explorers started the production of artisanal cheeses, using bovine raw milk and rennet from parts of the stomach of calves [1]. When gold mining and sugar cane exploration lost strength, the manufacture of artisanal cheeses gained economic importance, leveraged by the construction of a highway in 1929-1930 connecting the region of Serro with other municipalities in Minas Gerais state, and with Belo Horizonte, the state's capital. A great expansion of the market occurred in the following years, and artisanal cheese production became an autonomous element of the economy, not only for the region, but also for the entire state of Minas Gerais [2,3]. Although having started only recently in several parts of the country, artisanal cheese production is gaining increasing importance as economic income for thousands of families in rural areas, contributing to the local economies.

According to the Brazilian Institute of Geography and Statistics (IBGE – *Instituto Brasileiro de Geografia e Estatística*), Brazil encompasses twenty-six states and the Federal District, grouped into five geographical regions: Southeast (Sudeste), Northeast (Nordeste), South (Sul), North (Norte) and Central-West (Centro-Oeste) [4]. Besides Minas Gerais, in the Southeastern region, which is the largest cheese producer state, production is gaining strength in other Brazilian states, where artisanal cheese production is growing rapidly, with cheeses for all tastes and purchasing power.

2. Types of Artisanal Cheeses Produced in Brazil

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The best-known types of artisanal cheeses produced in Brazil, according to the producing region and state, are listed in **Table 1**. **Figure 1** shows the geographical location of the producing regions.

Table 1. Types of Brazilian Artisanal Cheeses.

Cheese type	Production region	Production state	Type of milk
Araxá	Southeast	MG	Cow
Cabacinha			Cow
Campo das Vertentes			Cow/Sheep
Canastra			Cow
Cerrado			Cow
Alagoa			Cow
Mantiqueira de Minas			Cow
Serra do Salitre			Cow
Serro			Cow
Triângulo Mineiro			Cow
Different Types of Cheese		SP	Goat/Cow/ Buffalo/Goat/ Sheep
Porungo			Cow
Coalho	Northeast	CE, PE, RN	Cow/Goat/ Buffalo/Sheep
Manteiga		CE, PE, RN BA, PB,	Cow/Goat
Galego		PE	Cow
Requeijão Pernambucano		PE	Cow
Flor de Mandacaru		PE	Cow
Cariri		PB	Goat
Dom Ariano		PB	Goat

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Dom Manelito			Cow
Colonial	South	PR, SC	Cow
Serrano		RS	Cow
Kochkäse		SC	Cow
Marajó	North	PA	Buffalo/Cow
Caipira	Central West	MS	Cow

MG = Minas Gerais, SP = Sao Paulo, CE = Ceará, RN = Rio Grande do Norte, PB = Paraíba, PE = Pernambuco, PR = Paraná, SC = Santa Catarina, RS = Rio = Grande do Sul, PA = Pará, MS = Mato Grosso do Sul.

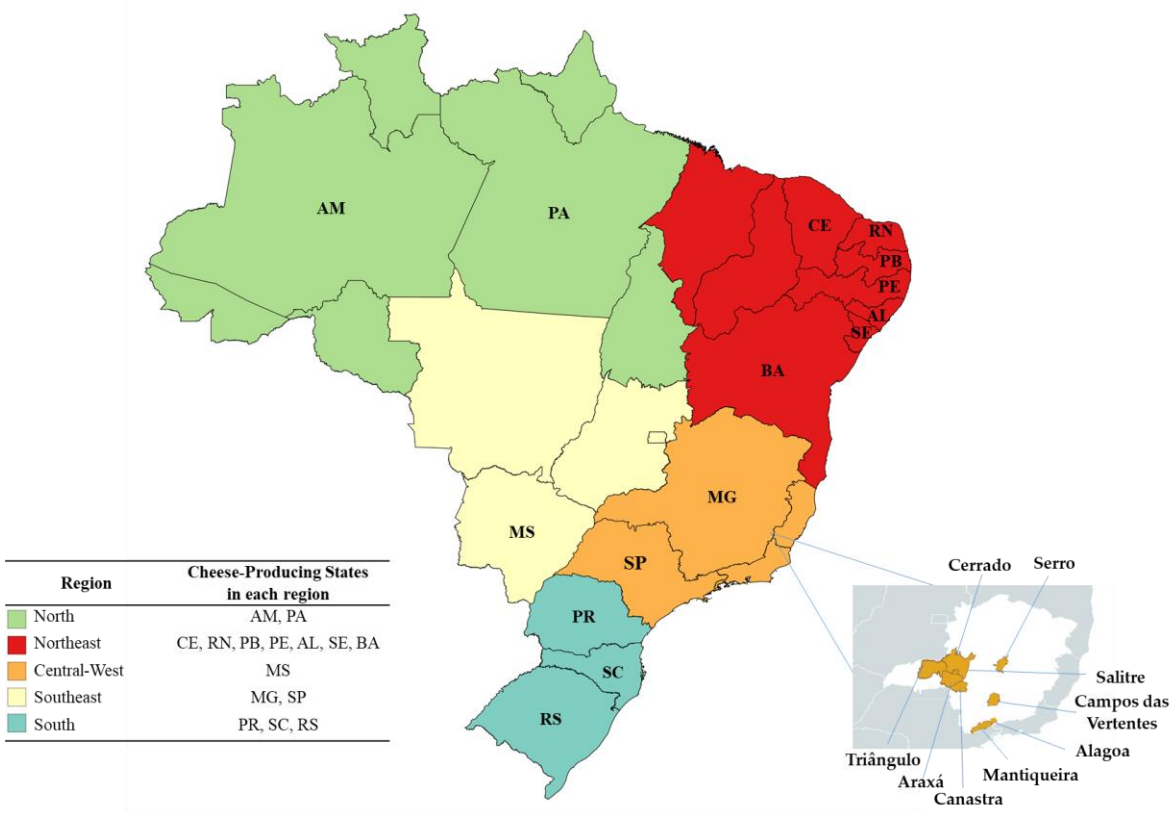

Figure 1. Artisanal cheese producing regions and states where they are located. North: AM – Amazonas, PA – Pará; Northeast: CE – Ceará, RN – Rio Grande do Norte, PB – Paraíba, PE – Pernambuco, AL – Alagoas, SE – Sergipe, BA – Bahia; Southeast: MG – Minas Gerais, SP – São Paulo; Central-West: MS – Mato Grosso do Sul; South: PR – Paraná, SC – Santa Catarina, RS – Rio Grande do Sul.

2.1 Artisanal Cheeses from the Southeast

2.1.1. Cheeses from the State of Minas Gerais

The state of Minas Gerais in Brazil is historically recognized for its secular tradition in the art of cheese making. This state is the largest cheese producer in Brazil and stands out because of the production of a large variety of artisanal cheeses, collectively named as *Minas artisanal cheese*.

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The manufacture of *Minas artisanal cheese* started with the Portuguese colonizers in the 18th century, becoming the most popular and consumed artisanal cheese in the country. *Minas artisanal cheeses* have a great socio-economic importance in the state, as thousands of rural families depend on them for their survival [5,6]. These cheeses are produced in nine spatially limited regions in the state harboring peculiar geomorphological and cultural characteristics: Araxá, Campo das Vertentes, Canastra, Cerrado, Serra do Salitre, Serro, Triângulo Mineiro [7], Alagoa and Mantiqueira [8,9] (**Figure 1**). The manufacturing procedure in these regions is similar and follows the Portuguese tradition, differing mainly in the curd pressing stage: in Serro, the curd is pressed with bare hands while in Canastra, Serra do Salitre and Cerrado a piece of cheese cloth is used. Thus, depending on the pressing method, more whey can be retained in the mass and, consequently, the product will present significant differences in flavor and texture [10].

Figure 2 shows a flowchart of the production of *Minas artisanal cheese,* the most studied Brazilian artisanal cheese. This cheese is manufactured with unpasteurized cow milk, added of liquid or powdered rennet, salt and endogenous cultures usually referred to as "*pingo*", known as the back-slopping method. *Pingo* is an endogenous starter culture composed of fermentative microorganisms, obtained from the whey that drains from freshly manufactured cheeses during the molding stage, and it is used to make the following day's batch [11]. The microbial diversity of *pingo* is characteristic of each production region, explaining the unique sensorial characteristics (taste, texture, color and aroma) that develop during ripening of cheeses produced in the different regions [6].

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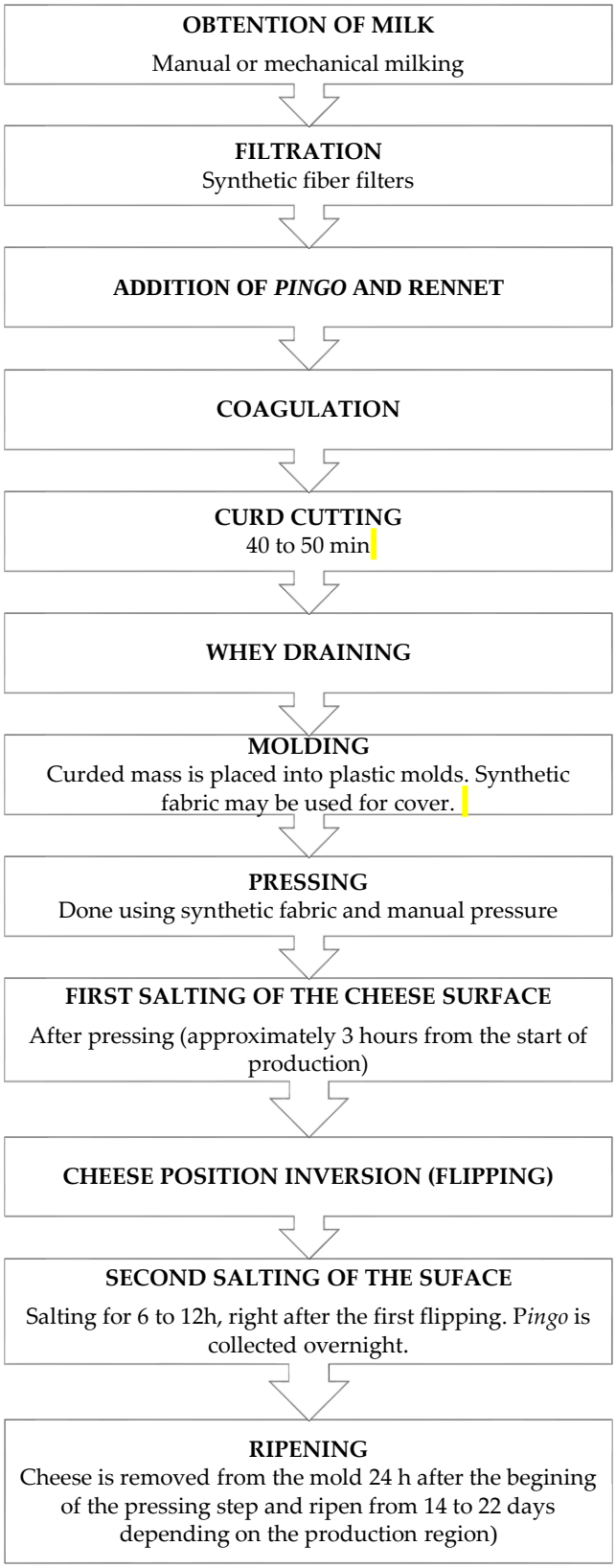

Figure 2. Flowchart of *Minas artisanal cheese* Production.

According to Minas Gerais State Law 23157 [12], *Minas artisanal cheeses* are prepared with fresh and raw whole cow milk and with specific identity and quality characteristics. Due to the traditional, cultural and economic importance, in 2008 the National Historic and Artistic Heritage Institute

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(*Instituto do Patrimônio Histórico e Artístico Nacional* - IPHAN) granted Cultural Property of Immaterial Nature status to Minas artisanal cheeses [13].

The most relevant types of *Minas artisanal cheeses* are described below:

a. Canastra cheese

The *Canastra cheese* is a typical product from the Serra da Canastra region that covers the municipalities of Delfinópolis, São Roque de Minas, Vargem Bonita, Tapiraí, Bambuí, Medeiros, Piumhi, São João Batista do Glória and Córrego D'Anta [14]. In this region, there are 4,813 dairy farms and 264,000 animals, resulting in an average of 55 animals per property [10]. Among these farms, approximately 800 are dedicated to production of *Canastra cheese*. Average milk production of matrices is about 1,400 liters/lactation, and the fat content is close to 3%, i.e, excellent for cheese production [10,6]. In 2002, around 60 producers joined efforts and created the Canastra Cheese Producers Association (*Associação dos Produtores de Queijo da Canastra* - APROCAN), aiming at increasing their reach and protecting their products. The total turnover of this group of producers is around R$ 60 million (11 million USD) per year, with an average of 25 cheeses produced every day in each farm [15]. After the official recognition in 2005, several new producers have joined APROCAN and many others are in the process of joining [15].

The classical *Canastra cheese* has the following characteristics: cylindrical shape, flat or slightly curved at the sides and a slightly acidic and non-spicy flavor. It has a yellowish-white color and a thin yellowish crust that may darken with ripening. The required ripening time is a minimum of 22 days, resulting in a semi-hard or slightly soft, buttery and compact cheese [16]. There are some variants of *Canastra cheese* (**Figure 3**):

- *Traditional*: cheeses produced in bottomless cylindrical containers, presenting 6-9 cm height, 17 cm diameter, 900-1,300 g weight, and 22 days of ripening [16,17].
- *Merendeiro*: smaller cheeses, presenting 10 cm diameter, 6 cm height, 300 to 400g weight, and 22 days of ripening [16].
- *Real* (also called *Canastrão)*: larger in size (28 to 35 cm diameter and 10 -18 cm height), these cheeses have 5,000 to 7,000 g weight and are ripened for at least 60 days [16, 18]. Their flavor is sweet and light, with a bitter taste at the end, reminiscent of Dutch cheeses. According to the local memory, this type of Canastra cheese was formerly produced for special occasions, such as visits of authorities from the church, the government or the military. The main characteristic is the presence of propionic bacteria, responsible for the curing process, and production of gas, which contributes to the puffiness and formation of round holes in the cheese, as occurs in Emmental and Gruyère cheeses.

Figure 3. A: *Traditional Canastra* (upper and lower shelves) and *Merendeiro* cheeses (middle shelves) ripening on wooden shelves; B: Slices of *Canastra Real* (or *Canastrão*) with 60 days of ripening.

In May 2008, the *Canastra cheese* was recognized as a Brazilian Intangible Cultural Heritage [13]. In 2012, the National Institute of Industrial Property (INPI - *Instituto Nacional de Propriedade Industrial*)

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granted these cheeses with the "Geographical Indication" seal [19, 20], and the Serra da Canastra region was recognized as a reference in production of cheeses [21]. *Canastra cheeses* are gaining international recognition, winning many international awards, such as the 24 super gold, gold, silver and bronze medals in the "*Mondial du Fromage et des Produits Laitiers*" competition, in France, in June 2019 [22,23].

The unique features of *Canastra cheese* can be attributed to the milk that comes from mixed-breed of *Bos taurus* and *Bos indicus*, and other variants, and to the characteristics of the natural pastures for cattle feeding, like *Capim meloso* (*Melinis minutiflora*) and native grasses, gradually replaced by more productive ones, such as *Brachiaria* spp. and *Panicum* [6,24].

b. Serro artisanal cheese

This type of cheese corresponds to a group of cheeses produced by approximately 760 cheese producers, located in the municipalities of Alvoradas de Minas, Conceição do Mato Dentro, Dom Joaquim, Materlândia, Paulistas, Rio Vermelho, Sabinópolis, Santo Antônio do Itambé, Serra Azul de Minas, Coluna and Serro, known for dairy farming. Serro and the neighboring municipalities produce around 10,000 cheeses daily [25].

According to Minas Gerais State Ordinance 1305/2013 [26], *Serro cheese* must be ripen for 17 days, resulting in products with firm consistency and mild and slightly acid flavor. They present a thin crust and a yellowish white color and a natural sheen. They present cylindrical shape, with 13 to 15 cm diameter, 4 to 6 cm height and 700 to 1,000 g weight [27]. *Serro cheeses* have also been recognized as a Brazilian Intangible Cultural Heritage in 2008 [12]. In 2011, INPI granted the "Geographical Indication" seal in the item of "Origin Indication" [19].

The peculiar sensorial properties of *Serro artisanal cheeses* are devoted to milk coming from European cow breeds such as Dutch, Jersey and Swiss Pardo. The Serro region has about 124,000 animals, distributed in 2,581 properties, usually small cattle rancher families, with an average of 50 animals per property [27]. Average production is 110 liters of milk per day, per property. It is estimated that *Serro cheese* is produced in 6000 properties, but only 756 are registered in this activity, where 10,000 pieces are produced per day [25]. The vegetation to feed the cattle in this region comprises *Brachiaria* spp., *Panicuns*, Jaraguá grass (*Hyparrhenia rufa*), Meloso grass (*Melinis multiflora*), and leguminous plants such as Carrapicho (*Cenchrus echinatus* L.), Beiço de boi (*Desmodium* sp.), Calopogônio (*Calopogonium mucunoides*), among others [18].

c. Araxá artisanal cheese

The *Araxá cheese* is a typical product from the micro-region comprising the municipalities of Araxá, Tapira, Pratinha, Conquista, Ibiá, Campos Altos, Perdizes, Pedrinópolis and Sacramento. These cheeses present semi-hard consistency, with a tendency to soft, and a butter-like nature. They have a thin crust and are yellowish without cracks and generally present cylindrical size of 13 to 15 cm diameter and weigh between 1.0 and 1.2 kg. The smell and taste are acid, pleasant and not spicy [28].

The local herd for milk production is composed mostly by mixed-breed animals, mainly Dutch, with around 476,000 animals, divided into roughly 7,000 farms, with an average of less than 60 heads per property. The herd is formed mostly by mestizo animals, resulting from crossings between European races, mainly Dutch. The average production is 2,400 liters of milk per lactation, with fat content above 3,0%. Few areas present unmodified vegetation cover, with around 88% of pasture lands [28]. Physical and environmental conditions such as altitude, soil and microclimate, provide special pastures, mainly *Capim gordura* (also known as *Capim meloso*), *Capim Jaraguá*, and *Macega* [28]. There are approximately 1,336 cheese producers in this region.

d. Other types of Minas artisanal cheese

This group encompasses cheeses named Parmesão da Mantiqueira (officially Queijo Artesanal Mantiqueira de Minas), Parmesão de Alagoa (officially Queijo Artesanal de Alagoa), Requeijão Moreno and Cabacinha. The region of Mantiqueira and the municipality of Alagoa were recently recognized as artisanal cheese producer regions in the state of Minas Gerais [8,9] (**Figure 1**). Despite

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being produced with raw milk, Queijo Artesanal Mantiqueira de Minas and Queijo Artesanal de Alagoa are different from the traditional Minas artisanal cheeses because they are made with commercial starter culture and go through a thermal process during production [29]. The Cabacinha cheese, a local version of the Italian Caciocavallo cheese, is produced in the Vale do Jequitinhonha, in the North of the state. The curd is cooked in boiling water, shaped like the porongo (or porungo) fruit (Lagenaria siceraria), tied in pairs by strings and hung to dry. The cheese can be smoked or filled with butter [30,31]. Due to its peculiar format, the Cabacinha cheese is also known as Porongo cheese (or Porungo cheese). The Requeijão Moreno cheese is mechanically pressed, with high salt content [32]. This cheese is produced in Jequitinhonha and Vale do Mucuri in the North of Minas Gerais.

e. Moldy artisanal cheeses

Production of moldy cheeses, similar to the French Brie cheese, is gaining importance in the state of Minas Gerais, particularly in Serra da Canastra and Serro. These cheeses present a white mold rind, are firm to the touch but creamy in the mouth, with striking aroma and taste that can be delicate or intense [33]. Fungi that proliferate in the surface of the cheese introduce their digestive enzymes into the mass, breaking down fat and proteins, turning it softer [34]. Even though Brazilian artisanal moldy cheeses do not have a specific legislation, they have gained a precious status in specialized stores and fine restaurants in several Brazilian big cities [35]. Little information is available on the production techniques of artisanal moldy cheeses in Brazil [36], but it is known that the fungi come from the ripening rooms, as producers do not add a specific mold to their product. The production conditions are not controlled and the contamination, in a way, occurs at random.

2.1.2. Cheeses from the State of São Paulo

Artisanal cheeses produced in the state of São Paulo are distinct from those of the Minas Gerais state. As producers in the state of São Paulo cannot rely on centuries of tradition, they invest in innovation, using European fine cheeses as models. However, little is known about the production processes, annual turnover, market and production practices. Even the "artisanal" concept in the state of São Paulo differs from that used in Minas Gerais state, as this nomenclature refers to cheeses produced in small producing properties, using a large array of technological processes and ingredients, some of them quite sophisticated. These cheeses may be produced with raw or pasteurized milk from different types of animals (cow, goat, buffalo, sheep), resulting in unique cheeses not found in other parts of the country.

Aiming at strengthening the artisanal cheese sector in the state of Sao Paulo, and removing these cheeses from clandestinity, a group of local producers created in 2017 the São Paulo Association of Artisanal Cheese (APQA - *Associação Paulista do Queijo Artesanal*). Currently, the APQA affiliates around 80 cheese producers from across the state [37]. APQA includes not only producers with more than 20 years of history in cheese production, but also new producers starting ventures in the cheese sector.

For the purpose of this review, artisanal cheeses produced in the state of São Paulo were divided into two groups:

a. Cheeses from the Paulista Artisanal Cheese Path (Caminho do Queijo Artesanal Paulista)

Currently, the *Paulista Artisanal Cheese Path* (Caminho do Queijo Artesanal Paulista) comprises 10 cheese producers from the municipalities of Joanópolis, Amparo, Porangaba, Itapetininga, São João da Boa Vista, São José do Rio Pardo, Pardinho, Cabreúva, Bofete and Porto Feliz, in the state of São Paulo. These producers use raw or pasteurized milk from cow, buffalo, goat and sheep, and cure the cheeses in ripening chambers or subterranean caves, and some add spices [38]. For instance, over 150 cheese varieties are produced in these dairies, highlighting the potential for innovation in cheese production in the state of São Paulo. The tropical climate during hot and rainy summers favors the growth of protein-rich pastures, such as *Capim elefante* (*Pennisetum purpureum*) and *Capim Tanzânia* (*Panicum maximum* cv. Tanzania), while in dry and relatively cold winter, equally rich oat (Avena sp), azevém (*Lolium multiflorum* Lam), sugarcane (*Saccarum officinarum*) and silage are used [39,40]. The cheese producers from the *Paulista artisanal Cheese Path* are also part of the APQA.

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b. Porungo cheese

The *Porungo cheese*, also called *porongo*, *cabacinha, cabaça, porunguinho, nozinho, cabecinha, enforcado* or *pescocinho*, is produced in the southwest of the São Paulo state, mainly in the municipalities of Angatuba, Buri and Campina do Monte Alegre [30]. This cheese is similar to the *Porungo cheese* produced in the state of Minas Gerais. It is manufactured from raw milk coagulated with commercial rennet and added of fermented whey that contains the autochthonous microbiota of milk, responsible for the peculiar sensory characteristics of the cheese. The sensorial characteristics of *Porungo cheese* resemble the mozzarella cheese. *Porungo cheese* producers are not part of the *Paulista Artisanal Cheese Path* or even affiliated to APQA, but commercialization of this product has great economic importance and is a source of income for numerous small producers that sell the cheeses informally directly to consumers or in free markets of the region [30].

2.2 Cheeses from the Northeast (states of Alagoas, Pernambuco, Ceará, Rio Grande do Norte, Paraíba, Bahia and Sergipe)

a. Coalho cheese

Coalho cheese is the most typical artisanal cheese produced in the Northeast region of Brazil, widely consumed by the local population and throughout the country. The most relevant producers are located in Sobral and Quixadá, in the state of Ceará; Riacho das Almas and Garanhuns, in the state of Pernambuco; Nossa Senhora da Glória, in the state of Sergipe; Juazeiro, Feira de Santana, Antas, Irecê and Chapada Diamantina National Park, in the state of Bahia; Seridó, in the state of Rio Grande do Norte; Batalha in the state of Alagoas and Riachão do Jacuípe, in the state of Maranhão; and around 50 municipalities in the state of Paraíba (**Figure 1**).

Coalho cheese is a firm, lightweight yellowish white fresh cheese, prepared with raw milk of cow, buffalo, goat or sheep and rennet, presenting 35.0% to 60.0% fat content. It has a slightly salty and acidic flavor and elastic texture and it is used for preparation of the popular "roasted cheese" as it does not melt when heated. This cheese has great economic importance for the region, impacting significantly the income of milk suppliers, especially those who lack access to milk processing plants [41,42]. It is estimated that about 40% to 50% of milk production in the Northeast region is destined to the production of *Coalho cheese* [42,43]. In this region, the use of genetically modified animals, such as the F1 hybrid (Dutch/Zebu), is common. These animals present profitable characteristics such as high resistance and productivity [43].

b. ***Manteiga cheese (Sertão Cheese)***

Also called *butter cheese*, this product is soft and has a fat content ranging between 25% and 55%. The taste is light, slightly acidic, and salty, and the color is light yellow [44,45]. Its production consists of coagulating whole or skimmed cow's milk, draining the curd obtained by acidification, melting and addition of butter or vegetable oil to the melted curd, cooking at 85°C for 15 min and pressing. The butter used in the manufacture (*Manteiga de Garrafa*, *Manteiga da Terra* or *Manteiga do Sertão*) is artisanal as well.

c. Other artisanal cheeses of this region

Other not so well characterized cheeses produced in this region are *Dom Ariano*, *Dom Manelito* and *Cariri cheeses*, in the state of Paraíba, and *Galego cheese, Requeijão Pernambucano* and *Flor de Mandacaru*, in the state of Pernambuco.

2.3. Cheeses from the South (states of Santa Catarina, Rio Grande do Sul and Paraná)

The South region of Brazil is characterized by subtropical climate with temperature ranging between 0ºC (occasionally below 0ºC) and 32ºC [46]. The arrival of European immigrants, mainly Italian and German, to this region in the 19th and 20th centuries had strong influence on the cheese-

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making culture [47]. The most prominent artisanal cheeses produced in this region are the *Serrano*, *Colonial* and *Kochkäse cheeses.*

a. Serrano cheese

The *Serrano cheese* is the main type of cheese produced in Serrana, in the state of Santa Catarina, and in Campos de Cima da Serra, in the state of Rio Grande do Sul [48,49]. *Serrano cheese* is a semi-fat cheese of medium moisture, made with raw cow milk. Its color is yellowish or straw yellow. It has a compact mass and elastic consistency, tending to the greasiness, and may contain small mechanical and/or propionic eyes, lacking a standard for shape, weight, moisture and salt content [50,51,52]. The milk comes from mixed breeds, mainly Charolais, Dutch, Devon, Norman, Angus and Hereford, fed on pasture [49].

Serrano cheese production in the state of Santa Catarina is widespread, with approximately 2,000 producers and 1,600 tons of cheese traded every year, and total gross sales around R$ 21 million (U$ 3,8 million). In Rio Grande do Sul, there are around 1,500 producers that trade 800 tons of *Serrano cheese* per year, with sales of approximately R$ 10 million (U$ 1,8 million) [46]. In March 2020, the INPI granted the Geographical Indication "*Campos de Cima da Serra*", in terms of "*Origin Appellation*" to Serrano cheese produced in this location [53].

b. Colonial cheese

Traditionally made from cow's raw milk, production of *Colonial cheese* had to change to pasteurized milk due to legal requirements. The cheese curd is heated to 30°C and can be added with spices or whole vegetable products. They must ripen for a minimum period of 10 days, and ripening can be done in wine or vinegar [54]. The cheeses present square and round shapes, and the weight of each piece varies from 1.0 to 1.2 kg [55]. Artisanal *Colonial cheese* is produced by many rural families in the South of Brazil, especially in Santa Catarina [56]. The cattle used in milk production belong to Dutch and Jersey breeds, fed on pastures with the addition of corn, sweet potato leaves and forage [57].

c. Kochkäse cheese

This cheese is made from raw or pasteurized milk and the mass is cooked. It is produced in the Itajaí Valley, mainly in the cities of Indaial, Timbó, Pomerode, Blumenau, Caminhos do Príncipe, and Joinville, in the state of Santa Catarina. This cheese has a mild flavor and is light yellow [58]. Milk comes from Jersey breed, fed with *Capim gramão novo*, *Capim gordura, Catamão branco* and silage. Many families produce *kochkäse* for their own consumption and commercialization, but current health and safety standards have forced some producers to stop producing this cheese [59].

2.4 Artisanal Cheeses from the North

a. Marajó cheese

The *Marajó cheese* is the most famous artisanal cheese from the North of Brazil. It is an unripen cheese produced for over 200 years in the Marajó archipelago, in the state of Pará, using buffalo milk or a mix of buffalo and cow milk. The buffalo herd in this region is the largest in the country, around 800 thousand animals, and among these, 450 thousand in state of Pará state [60]. The buffalo herd is made up of the *Carabao, Baio, Mediterraneo, Múrrah, Jaffarabadi* and crossbred breeds [61]. The main milk and buffalo cheese producers are in the municipalities of Soure and Cachoeira do Arari, in the state of Pará [62]. There are 60 cheese producers in the Marajó Island, which make 60 to 100 kg of cheese per day and this activity is very important for the local economy [63].

The Marajó Island has a rainy tropical climate and an average temperature of 27ºC. The rainiest months are January to June and the less rainy ones are September to November [64]. The climate contributes to the presence of native pastures such as *Capim canarana verdadeira (Echinochloa polystachya)* and *Capim quicuio (Brachiaria humidicola),* which are used to feed the herd [61].

There are two types of Marajó cheese: one is butter-type, made with whole milk and added butter, and the other is cream-type, made with skimmed milk and cream from skimmed milk. For

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manufacturing, the curd is drained and washed with water or milk. The product has a light-yellow color and presents slightly acidic and salty flavor and semi-hard consistency [30,65].

b. Other artisanal cheeses from this region

The Manteiga and Coalho cheeses, produced in the Northeast region of the country, are also manufactured in Manaus and surroundings, in the Amazonas state, following the same cheese making techniques [30].

2.5. Artisanal Cheeses from the Central-West

a. Caipira cheese

Caipira cheese is manufactured in Mato Grosso do Sul state, traditionally recognized as a cattle production state. Nevertheless, in 1980, rural families started producing cheese as an option of income. The majority of producers are located in São Gabriel do Oeste, Corguinho, Rochedo, Jaraguari, Terenos, Ribas do Rio Pardo, Aquidauana, Bandeirantes, Camapuã, Santa Rita do Pardo and Sidrolândia, besides Campo Grande, the state's capital [66]. *Caipira cheese* is made with raw, fresh whole cow milk, following historical and cultural tradition of the region of production. The product must be manufactured in the original rural property and must be submitted to 60 days of ripening. The climate of this region is tropical, characterized by hot and rainy weather. The main breeds of cattle used for milk production are Dutch, Swiss-Parda, Schwyz, Jersey, Guernsey, Ayrshire or crossbred, and the feed is based on a mixture of silage, hay, chopped green grass, added with energy and protein concentrates, minerals and vitamins [67].

Unlike Minas artisanal cheese, the sensorial and physicochemical characteristics of *Caipira cheese* are not well defined, so the producers belonging to the Association of Artisanal Cheese Producers in Mato Grosso do Sul (AQUEIJART; *Associação dos Produtores de Queijo Artesanal de Mato Grosso do Sul*) have recently partnered with the State Agency for Animal and Plant Sanitary Defense (AGRAER; *Agência Estadual de Defesa Sanitária Animal e Vegetal*) and State Secretariat for the Environment, Economic Development, Production and Agriculture (SEMAGRO; *Secretaria de Estado de Meio Ambiente, Desenvolvimento Econômico, Produção e Agricultura Familiar*) in order to establish quality and production parameters, by means of technical-scientific and microbiological studies [68].

3. Microbiological Safety of Brazilian Artisanal Cheeses

The microbial communities in cheeses manufactured with raw milk play an important role during ripening. Besides determining the sensorial and physicochemical properties of the final products, they modulate the growth of pathogens [69]. High humidity and fast ripening cheeses are at greater risk of harboring pathogens, in comparison to lower moisture and slower ripening varieties [70]. In the beginning of ripening, there is a higher prevalence of lactic acid bacteria, mainly *Lactococcus*, *Streptococcus* and *Lactobacillus*, responsible for the fermentation of sugars and development of the unique sensorial characteristics of artisanal cheeses (aroma, flavor and texture). Their capability to produce organic acids from sugars during ripening causes pH drop and lowers the oxy-reduction potential. In addition, production of hydrogen peroxide, carbon dioxide, acetaldehyde, diacetyl and bacteriocins inhibit the growth of pathogens [71,72].

Several studies have evaluated the effect of ripening on pathogens in Brazilian artisanal cheeses. Dores et al. [73] evaluated the effect of the ripening temperature (8ºC and 25ºC) on the counts of pathogenic and indicator bacteria in *Canastra cheeses*. They observed that ripening at 25ºC for 22 days was sufficient to decrease the counts of total coliforms, *Escherichia coli* and *Staphylococcus aureus* to the levels required by the legislation (< 1000 CFU.g^{-1} for *E. coli* and coagulase positive staphylococci), while refrigeration at 8°C required 64 days to reach these levels. None of the tested cheeses presented *Listeria monocytogenes* or *Salmonella* spp. Lower values of Aw, pH and sodium chloride were detected in cheeses ripened at 25°C, suggesting that these characteristics may have had a positive effect on the control of pathogens. In a similar study conducted by Martins et al. [74] with *Serro cheese* ripened at room temperature (25 ± 4°C) and under refrigeration (8 ± 1°C), the authors

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observed that ripening at room temperature for 17 days was the ideal condition to reduce the counts of *S. aureus* to safe limits (< 1000 CFU.g^{-1}). *L. monocytogenes* was not detected in the 256 tested cheese samples, but *Salmonella,* present in one initial sample, was no longer detected after 22 days.

Mata et al. [75] evaluated the effect of "*pingo*" collected in the Serro region on the survival of *Listeria* during ripening of artisanal cheeses produced in laboratory conditions. Cheeses were prepared with raw milk experimentally contaminated with *L. innocua* ATCC 33090 (10 CFU.mL^{-1}). Results indicated that *L. innocua* was not eliminated even after 60 days at 30 °C, evidencing that the physicochemical changes and activity of the competitive microbiota during ripening are not enough to guarantee the absence of *L. monocytogenes* in the final product.

Campagnollo et al. [76] conducted a quantitative risk assessment of *L. monocytogenes* in semi-hard artisanal cheeses prepared with raw milk experimentally contaminated with 6 log CFU.mL^{-1} of *L. monocytogenes* and ripened up to 22 days at 22 ± 2°C. The authors concluded that these cheeses presented lower risk of listeriosis than a soft cheese produced with pasteurized milk containing 1 log CFU.mL^{-1} of *L. monocytogenes,* observing that the longer the ripening time the lower the risk. This study reinforced that mitigation of listeriosis risk is related to the cheese ripening period, when pH decreases, sodium chloride concentration increases, Aw decreases and interactions with lactic acid bacteria control the survival of *L. monocytogenes*. Recently, Campos et al. [77] confirmed that 22 days of ripening are sufficient to maintain the populations of hygiene indicator microorganisms (total coliforms, coagulase-positive *Staphylococcus* and *E. coli*) in *Canastra cheese* samples in accordance with the levels established by the current regulations.

Even though there are some reports of outbreaks due to consumption of raw milk and raw milk cheeses around the world, accurate information on this issue is lacking in Brazil. Data from the National Health Surveillance Agency of the Brazilian Ministry of Health indicate that milk and dairy products were responsible for 2.75% of the foodborne outbreaks reported in the 2000-2018 period [78,79]. Even considering that the type of dairy product associated with the reported outbreaks is unknown and that the number of outbreaks is underreported, the relevance of artisanal cheeses prepared with raw milk as causes of foodborne diseases cannot be ignored. Studies conducted in other countries, like the Microbiological Risk Assessment of Raw Milk Cheese published in 2009 by the Food Standards Australia/New Zealand based on 84 outbreaks attributed to cheese consumption in 1973-2006 in Switzerland, USA, Sweden, Canada, France, Brazil, United Kingdom, Spain, Malta, Scotland, England and Finland [80] concluded that 69% of the outbreaks were associated to raw milk cheeses, while 7.2% were caused by cheeses with no information about heat treatment. Among the outbreaks, two were attributed to cheeses produced in Minas Gerais state, Brazil. However, none of these cheeses fit the category of artisanal cheeses, as defined by the Brazilian regulatory standards [81,82,83].

Artisanal *Coalho cheese* was incriminated in 55 foodborne disease outbreaks occurred in the Amazonas state between 2005 and 2009. According to the Department of Epidemiological and Environmental Surveillance of Manaus, Amazonas, 14 (25%) of these outbreaks were due to coagulase positive *S. aureus*, four (8%) due to *Bacillus cereus*, two (4%) due to *Salmonella* spp and one due to *Clostridium perfringens*. In one outbreak, both coagulase-positive *Staphylococcus* and *B. cereus* were found. The etiological agent was not determined in the remaining outbreaks [84].

Besides *L. monocytogenes*, other pathogens that have been associated to outbreaks caused by cheeses manufactured with unpasteurized milk in other parts of the world are Shiga Toxin-producing *E. coli* [85], *Salmonella* Muenster [86], *Salmonella* Typhi [80], *Brucella* spp. [80,87], *Campylobacter* [88] and *Clostridium jejuni* [89]. These etiological agents have caused outbreaks of different intensity and severity, showing that they deserve the attention of those responsible for the safety of artisanal cheeses.

Several studies have evaluated the occurrence of pathogenic microorganisms in different types of Brazilian artisanal cheeses, summarized in Table 2.

Table 2. Summary of studies on pathogenic bacteria in Brazilian artisanal cheeses.

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Type of cheese	Number of tested samples	Results highlights	Reference
Coagulase positive *Staphylococcus* (CPS)			
Canastra	10	Seven samples presented counts of *S. aureus* above 4.8 log CFU.g-1 with 5 days of ripening. Nine *S. aureus* isolates were positive for at least one of SEA, SEB, SEC, SED and TSST-1	Borelli et al., 2006 [90]
Serro	100	Counts of CPS were lower than 3 log CFU.g-1 after 15 days of ripening in the rainy season and 45 days of ripening in the dry season*. Some CPS isolates produced SEB, SEC and TSST-1	Cardoso et al., 2013 [91]
Araxá	30	28 samples presented CPS counts above 3 log CFU.g-1* (no information about ripening time)	Souza et al., 2015 [92]
Serro	53	40 samples presented CPS counts above 3 log CFU.g-1* (no information about ripening time). None of the isolates produced SEA, SEB, SEC, SED and SEE nor had SE related genes	Andretta et al., 2019 [93]
Canastra	78	33 samples presented CPS counts above 3 log CFU.g-1* after 22 days of ripening	Campos et al., 2020 [77]
Listeria spp* and *L. monocytogenes			
Coalho (Manaus, AM)	58	*Listeria* sp. was detected in 2 samples	Ramos and Costa, 2003 [94]
Canastra	32	*Listeria* sp. was not detected	Dores et al., 2013 [73]
Serro (17 days of ripening)	256	*L. monocytogenes* was not detected	Martins et al., 2015 [74]
Coalho (Pernambuco)	60	*Listeria grayi* detected in 2 samples	Aragão et al., 2019 [95]

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Canastra	78	*L. monocytogenes* detected in 1 sample.	Campos et al., 2020 [77]
Pathogenic *Escherichia coli*			
Raw milk cheeses from Paraná, São Paulo, Minas Gerais, Mato Grosso do Sul and Bahia	147	*E. coli* was found in 28 cheeses. One among 39 *E. coli* isolates was positive for *eae* gene, and negative for *bpf* and *efa1/lifA* genes, being classified as atypical EPEC (aEPEC).	Campos et al., 2018 [96]
Serrano (Santa Catarina)	109	22 among 109 *E. coli* isolates presented the *eae* (EPEC), *st* and *lt* (ETEC) or *aggR* (EAEC) genes	Parussolo et al., 2019 [97]
***Salmonella* spp**			
Coalho (Pernambuco)	127	*Salmonella* spp. was detected in 7 samples	Duarte et al., 2005 [98]
Serro	100	*Salmonella* spp. was not detected	Cardoso et al., 2013 [91]
Coalho (artisanal and non-artisanal samples (Northeast)	104	*Salmonella* spp. was detected in 1 artisanal cheese sample	Sousa et al., 2014 [99]
Serro	256	*Salmonella* spp. was detected in 1 sample	Martins et al., 2015 [74]
Minas artisanal (Montes Claros, MG)	18	*Salmonella* spp. was detected in 2 samples	Pinto et al., 2016 [100]
Canastra	78	*Salmonella* spp. was not detected	Campos et al., 2020 [77]
***Brucella* spp**			
Raw milk cheese (São Paulo)	38	*Brucella* spp. was detected in 5 samples	Kobayashi et al., 2017 [101]
Minas artisanal (4 and 8 days of ripening) (Serro, MG)	55	*Brucella* spp was detected in 17 samples	Silva et al., 2018 [102]

* Counts of CPS must be lower than 3 log CFU.g-1 according to Minas Gerais (2008) [103].

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The major concerns in dairy products made with unpasteurized milk are *Brucella* spp. and *Staphylococcus aureus. Brucella* spp causes brucellosis, a zoonosis that can be transmitted from animals to humans and vice versa. In cattle, this disease can cause abortion and congenital related problems, while in humans it is usually manifested as a general infection. This pathogen can be present in the mammary gland of infected animals and eliminated through milk. Brucellosis presents economic losses due to the reproductive problems caused by the disease in cattle [104]. Another important zoonotic disease is tuberculosis caused by *Mycobacterium bovis*, which contributes to the development of nodular lesions in tissues or organs of the animal, and causes abortion, fever, drop in milk production and weight reduction, which can lead to the death or slaughter of animals. In humans, the disease can be transmitted through the consumption of unpasteurized milk and dairy products, the consumption of uncooked meat or by contact with the infected animal and may present symptoms such as fever, weight loss, lung problems, cough, shortness of breath, among others [105].

The Brazilian regulations, by means of the National Program for the Control and Eradication of Brucellosis and Animal Tuberculosis, set the requirements for the control of *Brucella* spp, *Mycobacterium bovis* and pathogens in dairy products manufactured with raw milk, establishing that dairies must be certified as brucellosis and tuberculosis free [106].

The most relevant microbial pathogen in unpasteurized milk is *S. aureus*, as it causes mastitis, an infectious process that affects the mammary gland and causes changes in the secretion and composition of milk, resulting in great economic losses in milk production. *S. aureus* in contaminated milk can be transmitted to dairy products, and cause staphylococcal gastroenteritis or intoxication, through production of enterotoxins [107,108,109].

Other relevant, but less frequent pathogens in artisanal cheeses are *Salmonella* and *Listeria monocytogenes.* These pathogens may originate from raw materials (milk) or from the factory environment, especially from the processing area, including equipment, personnel or cross contamination between finished products and raw materials [110,111,112]. The occurrence of *Salmonella* and *L. monocytogenes* in cheeses with higher humidity is more common, suggesting that lower water content could be less favorable to their survival. Also, low pH values, low water activity (aw) and the presence of lactic acid bacteria that have antimicrobial activity may decrease or eradicate the presence of these pathogenic microorganisms, and probably because of that, the studies conducted so far indicate that prevalence of *Salmonella spp* and *L. monocytogenes* in Brazilian artisanal cheeses is low (Table 2).

4. Good Hygiene and Manufacturing Practices

Brazilian regulations on artisanal cheese production indicate that the good hygiene and manufacturing practices must start at the farm level (cattle feeding, vaccination and milking) [83,103,113,114,115,116,117]. Brazil has extensive legislation on hygiene and manufacturing practices for artisanal cheeses. Over the years, there have been major changes such as allowing marketing of raw milk cheeses provided they are ripen for 60 days, through a federal resolution [113]. Years later, through a state normative, Minas artisanal cheeses obtained the permission to market their cheeses with less than 60 days of ripening, like the Canastra cheese manufactured with 22 days of ripening [26]. One of the most important changes in the legislation of artisanal products of animal origin occurred in 2018 with the creation of the ARTE Seal (ARTE for artisanal in Portuguese: *artesanal*), where through this law, interstate marketing of this type of product would be allowed provided it was inspected by State or Federal Agencies [116].

The milking process is a critical control point. Before milking, udders must be washed with water containing 2-3 ppm of free chlorine and disinfected properly by pre-dipping with chlorinated solution (50–100 mg/L). After milking, the udders must be disinfected with iodine solution at 20–30 mg / L or another recommended disinfectant [118]. Animals with mastitis should be milked last and their milk cannot be destined for cheese production. Milk intended for cheese production should be filtered immediately after milking and stored in production tanks [118].

Cheese production sites must be well structured and maintained. The quality and safety of the water must be controlled, and corrals and milking rooms must be well-finished, with easy disposal

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of water and organic waste. Walls must be painted with washable paint and floors must be sturdy, waterproof and from non-slippery material. Walls, floors, utensils and equipment should be subjected to cleaning and disinfection with chlorinated solution, before and after milking. The cleaning of milk reception and raw material storage areas must be monitored, and manufacturing areas must be adequately ventilated [119].

Workers at the cheese production sites must keep updated health certificates and wear clean and appropriate clothing, rubber boots, masks and hat. Workers with health problems (cuts, wounds, cold, among others) cannot be present in the cheese manufacturing area. Hand wash with disinfectants is mandatory, before and after milking. Bad practices, such as smoking, sneezing, coughing, scratching the head, etc, and visitors must be avoided [118,119].

5. Animal health protection

The Brazilian Ministry of Agriculture, Livestock and Food Supply and state agencies of animal health protection require vaccination for brucellosis by means of the National Program for the Control and Eradication of Brucellosis and Animal Tuberculosis [120]. Vaccination against brucellosis is mandatory for females in ages between three and eight months. Vaccination against foot-and-mouth disease must be applied directly by the health authorities of each state. The vaccine against symptomatic anthrax must be applied to all animals over three months of age and needs to be repeated every six months until two years of age. Another important vaccination is against rabies that should be applied annually, especially in outbreak regions when much of the herd can be affected by the disease [120]. There are some vaccines that can be used to control some animal diseases such as botulism, clostridium disease, leptospirosis, and infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), mastitis, campylobacteriosis and colibacillosis [121].

An effective feeding system is also relevant for animal health protection. It must provide energy, protein, minerals and vitamins to meet the nutritional needs of each category of animal and at each stage of the life cycle of animals in the herd [122]. The planning of a balanced diet is an indispensable strategy for a positive impact on the economy and production in the livestock sector [123].

6. Perspectives

The challenges to assure absence of pathogens in Brazilian artisanal cheeses are no different than those in similar cheeses produced elsewhere: they are attributed to the use of unpasteurized milk and to disruptions in the production/trade chain regarding the failure in good hygiene and good manufacturing practices. The good hygiene practices must be adopted at all stages, from milking and manufacturing up to consumption, in order to guarantee microbiological safety and avoid public health problems. For the effective application of these practices, the proper training of cheese producers and food handlers is mandatory.

Brazil is a country with continental dimensions and hence with a great diversity of climate, vegetation, topography and culture that directly reflects the diversity of cheeses produced in the country, as indicated in this review. The popularity of artisanal cheeses in the national market has been growing. Flavor wise, these cheeses have already proven their attributes and value in international contests. The regulation for artisanal cheese production is numerous, and producers, particularly the small ones, consider it too rigorous and sometimes confusing and not well accepted or understood. The recent improvements in the regulations, at local, state and national levels, that revised old and obsolete laws, have contributed to combat clandestinity, bringing significant economic turn over for producers. The role of consumers demanding better quality and safety is also important. These actions, alongside with technical qualification of producers and development of research incentive projects will contribute to elevate Brazilian artisanal cheeses to worldwide recognition.

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