Blue Cheeses

Subjects: Food Science & Technology
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Blue cheeses are those whose matrix is veined with a blue, blue-grey, or blue-green colour due to the development of *Penicillium roqueforti*. There are more than 45 varieties of blue cheese produced worldwide, with some distinct features, although the manufacture process is similar. In addition to *P. roqueforti*, complex microbial populations interact and succeed throughout the manufacturing and ripening at the cheese's surface (the rind) and interior (matrix). The microbiota of blue cheeses is made up of a vast array of both prokaryotic and eukaryotic microorganisms. Proteolysis is the most complex and important primary biochemical process involved in blue-veined cheeses during ripening, with P. roqueforti being considered the main proteolytic agent. Lipolysis is also strong, originating aroma compounds characteristic of blue-veined cheeses. In addition, several bioactive compounds are produced during ripening. The biochemical activities, mainly of microbial origin, are responsible for the sensory characteristics of these very appreciated cheese varieties worldwide.

blue cheeses | Penicillium roqueforti | mycotoxins | proteolysis | lipolysis | cheese microbiota

1. Introduction

Blue cheeses (blue-veined cheeses) are characterized by the presence of blue veins in the interior due to the development of the fungus *Penicillium roqueforti* (naturally present or added as secondary culture). The first blue cheese described was Gorgonzola (Italy, ninth century), followed by Roquefort (eleventh century), although there are some reports that place the French cheese in the eight century [1]. The other varieties were first described from the 17th century onwards [2].

Blue cheeses are semi-hard cheeses, with a very variable weight depending on the type (from 0.3 to more than 10 kg), a fresh dry matter of 50–60%, a fat content of 30–40%, a protein content of 20–30%, and a variable NaCl content (most commonly, 3–4%) [1].

Manufacture of blue cheese has as differential points the following: addition of *P. roqueforti* spores (optional); addition of hetero-fermentative lactic cultures (*Leuconostoc* spp.); cutting of the curd into small pieces (to create an open paste); dry or, less frequently, brine-salting; piercing (allows air to enter, which activates the *P. roqueforti* growth and the blue veins development; optional) and ripening at around 10 °C, and 85–95% humidity (for some varieties, in natural caves), for at least 1–2 months) [1][2][3][4].

2. Types of Blue Cheese

There are more than 45 varieties of blue cheese produced worldwide (https://en.wikipedia.org/wiki/List_of_blue_cheeses, accessed on 1 May 2023). Of them, the best-known are made in Europe (Roquefort, Cabrales, Stilton, Gorgonzola, and Danablu), all of which have been granted with Protected Designation of Origin (PDO) or Protected Geographical Indication (PGI). Apart from these, Spain produces other varieties such as Valdeon (PGI), Picón-Bejes Tresviso (PDO), and Gamonedo (PDO), all of them in the Picos de Europa mountain range (in the North) and France, the Bleu d'Auvergne (PDO), or the Bleu de Bresse, among 14 other varieties.

Blue cheeses may be made with raw or pasteurised milk (on some occasions, thermised Danablu) coming from cow, ewe or, occasionally, goat, or a mixture of them, although worldwide, cow's milk is most usual. For instance, in France most blue cheeses are made with cow's milk, except Roquefort, and in Spain and Greece, it is usual to add sheep´s milk to the cow´s milk. As for the use of raw milk, in some varieties it is mandatory according to the PDO (i.e., Cabrales or Roquefort) [1][2][3][4].

3. Microbiology

Blue cheese microbiota is complex, particularly when raw milk is used. Lactic acid bacteria (LAB) and fungi dominate the process. Among the first, apart from *Lactococcus* spp., *Leuconostoc* is usually present, which favors an open texture. Among the fungi, apart from *P. roqueforti*, different yeasts are usually present. All of them contribute to the characteristics of the final product.

3.1. Ecological Factors

The ecological factors that influence the microbiota of cheese are variable to some degree depending on the blue cheese variety. The pH increases during ripening, from 4.7–5.0 (first days of manufacture) to 6.0–7.0 at the end of ripening (the pH in the interior rises more rapidly than on the surface). This increase is due to the degradation of lactic acid by non-LAB moulds (*P. roqueforti*) and yeasts and proteolysis. Water activity decreases rapidly during the first week and slowly in the rest of the manufacture, ending in 0.91–0.94. As for the sodium chloride content, the range in the final product is 2–5% (most commonly, 3–4%) [1][2]. pH and salt in humidity gradients and low temperature are the physicochemical coordinates driving the development of secondary microbiotas and guiding the enzyme activities required for proper maturation.

3.2. Blue Cheese Microbiota

Blue cheese can still be manufactured in different sizes and shapes using milk from different mammal species (or mixtures) and following different manufacturing and ripening technologies [5]. The type of milk and the technological processing greatly influence the cheese microbiota during the manufacturing and ripening stages, and thus its final sensory attributes. Most traditional blue cheeses (e.g., Roquefort, Gorgonzola, Cabrales, Gamonedo, etc.) are still manufactured with raw milk following traditional artisan technologies. Therefore, the large diversity in microbial populations coupled with different aroma and taste profiles is not surprising. For standardization, strains of mesophilic LAB species (*Lactococcus lactis*, *Lc. cremoris*, and *Leuconostoc* spp.) are currently being added as starters for either raw milk-made and pasteurized milk-made cheeses [1]. As an exception, Gorgonzola starters are mixes of mesophilic (as above) and thermophilic (*Streptococcus thermophilus* and *Lactobacillus delbrueckii*) LAB species [6]. Traditionally, cheeses were not inoculated either with *P. roqueforti* spores. They became naturally contaminated with this fungus from manufacturing and ripening environments. However, adding commercial spores is common practice at both artisan and industrial manufacturing scales. Other factors affecting the microbiota are the salting of the cheeses by applying coarse salt onto the surface or by milling and mixing the curd with salt prior to moulding [1], or brine immersion, as well as the ripening of blue cheese at low temperatures (8–12 °C) and high relative humidity (>90%). Finally, cheeses are frequently pierced to facilitate the entry of air to allow a uniform development of *P. roqueforti* into the cheese matrix, which contributes to the typical visual aspect at cutting.

3.2.1. Microbial Techniques

Culturing methods have been amply used for the characterization of the diversity and succession of the microbial populations throughout cheese manufacturing and ripening before the advent of molecular culture-independent techniques in food microbiology. Among the latter techniques, the temporal temperature gradient electrophoresis (TTGE) and denaturing gradient gel electrophoresis (DGGE) and others have been widely applied to study the spatial and temporal evolution of prokaryotic and eukaryotic communities in several kinds of blue cheese, including Bleu d´Auvergne, Cabrales, Gorgonzola, and Stilton. More recently, the application of high-throughput sequencing (HTS) techniques has broadened the bacterial and fungal biotypes detected at both the surface and the interior of many cheese varieties [7][8].

3.2.2. Microbial Diversity and Succession in Blue Cheeses

The manufacture of most traditional blue cheeses from raw milk assures a high microbial diversity; even higher if pre-maturation processes are employed [5]. As in many other cheese types, the microbial analysis of blue cheeses has been addressed to the search for and selection of acidifying (LAB) and maturing (*P. roqueforti*) cultures [9][10][11][12][13][14]. As a result of artisan-like manufacture, where uncontrolled environmental conditions are common, large microbial differences between batches, producers, and seasons have been reported [10][15][16].

- Bacterial populations

Lc. lactis and *Lc. cremoris*, which reach cell densities up to 10^9 cfu/g of cheese take over the acidification of the curd; their populations decline slowly during ripening. Other LAB involved include several lactobacilli species such as *Lactiplantibacillus plantarum*, *Lacticaseibacillus paracasei*, and other mesophilic homo- and hetero-fermentative species (e.g., *Levilactobacillus brevis*, *Latilactobacillus curvatus*) develop slowly, although they may surpass the lactococci after 15–30 days of ripening. Dextran-producing *Leuconostoc* (*Leuc. mesenteroides*, *Leuc. citreum*, and *Leuc. pseudomesenteroides*) are frequently found in lower numbers. Among other LAB populations, counts on *Enterococcus*- and streptococci/micrococci-selective media are usually high; these include *E. faecalis*, *E. faecium*, and *Streptococcus* and *Staphylococcus* species. More recently, the use of new culturing techniques has allowed for the recovery—as part of the dominant microbiota—of new bacterial species such as *Tetragenococcus* spp., *Staphylococcus equorum*, and species of *Brevibacterium* and *Corynebacterium* genera [unpublished data].

In addition to the bacteria recovered in culture, the DGGE and TTGE techniques allow for the detection of unconventional bacterial types beyond those recovered via culturing, including among other LAB species *Lc. garvieae* and *Lc. Raffinolactis*; *St. thermophilus* [15][16]; and others from the genera *Sphingobacterium*, *Mycetocola*, *Brevundimonas*, etc. [17].

- Yeast and moulds

P. roqueforti is the pivotal ripening agent of blue cheeses and is responsible for the visual aspect as well as the texture, taste, and aroma profiles [18]. Nonetheless, particularly in cheeses made from raw milk, a large number of yeasts species can grow accompanying *P. roqueforti* and other fungi; all together, they compose the blue cheese microbiota. Starting from small numbers in milk, yeasts reach majority populations during ripening (up to 10^8 ufc/g) [19]. Both *P. roqueforti* and yeasts possess potent proteolytic and lipolytic systems that help transform the milk components into flavour compounds. Indeed, selected yeast strains have been proposed as adjuncts and maturing cultures for certain blue cheeses [9][11]. *Geotrichum candidum* (teleomorph state of *Galactomyces candidus*) is among the dominant yeast species in the surface and interior of the cheeses. *G. candidum* produces several enzymes for the breakdown of proteins and fats, resulting in key aroma compounds [20]. Besides *G. candidum*, *Debaryomyces hansenii*, *Kluyveromyces lactis*, *Pichia* spp., *Rhodotorula* spp., *Zygosaccharomyces* spp., and *Saccharomyces* spp. have all been isolated and identified at different numbers from distinct varieties. The microbiota of blue cheeses deserves further characterization, as it may represent a source of new species [21].

Blue-veined cheeses belong to a category of specialty cheeses that are well distinguished from all others by their visual, taste, and aroma profiles. The overall blue cheese quality is thought to result from the concerted action of all members of the microbiota, which, as revealed by the use of state-of-the-art culturing and culture-independent molecular techniques, is formed by an impressive diversity of bacterial and fungal species.

3.3. Lactic Cultures

In the production of blue-veined cheeses, natural acidification made by lactic acid bacteria (LAB) has been substituted by the deliberate addition of selected starter cultures. These primary LAB cultures must be able to lower the pH of the milk and survive phage attack. Thus, the main commercially available starter mixtures for blue cheese contain a mixture of strains belonging to the *Lactococcus* genus. Most of the blue cheese types require a mesophilic starter culture, which usually contains strains of *Lc. lactis*, lactis subspecies, and *Lc. cremoris*. These bacteria also contribute to the organoleptic properties of the cheese, generating flavor compounds, either directly by cellular metabolism or indirectly by the release of enzymes. Strains of *Lc. lactis*, subsp. *lactis* biovar *diacetylactis*, are frequently included in mesophilic starter cultures, as this microorganism is able to catabolize citrate to carbon dioxide and the flavor compound diacetyl, which gives the cheese a distinct buttery flavor [22].

Strains of *Leuc. mesenteroides* subsp. *cremoris* are also added due to their ability to produce flavor (diacetyl) but mostly because of its CO_2 production, which breaks the structure of the curd, helping the development of the *Penicillium* mould inside the cheese [23].

In those blue cheeses in which the heating of milk and curd is a part of the cheesemaking process, a mixture of mesophilic and thermophilic starters can be added. These starters contain mixtures of the strains mentioned and small amounts of *St. thermophilus* and *Lb. delbruecki* subsp. *bulgaricus* [1].

3.4. Penicillium roqueforti and Other Adjunct Cultures

P. roqueforti is the most important microorganism involved in the manufacture of blue cheese. It belongs to the subgenus *Penicillium* (characterized by ter-/quaterverticillata conidiophorus); colonies grow rapidly (40–70 mm diameter in 7 d, a characteristic feature), plane or lightly radially sulcate, low, and velutinous; conidiophorus walls are very rough [24]. According to Frisvad and Filtenborg [25], there are two varieties, with *P. roqueforti* var. *roqueforti* being the one used in cheese manufacture. In addition, using molecular tools [26] proposed three species that were confirmed later [27]: *P. roqueforti*, *P. carneum*, and *P. paneum*.

P. roqueforti appears to have the lowest oxygen requirements for growth of any *Penicillium* [24], which, together with other physiological features such as salt stimulation [28][29] and an ability to grow at low temperatures [30], would explain its presence (even natural) in the interior of blue cheese. On the other hand, this species, like many other *Penicillium* spp., produces several mycotoxins.

The unique characteristics of these cheeses are due, to a great degree, on the growth of *P. roqueforti*. The flavour of the final product is mainly due to the lipolytic and proteolytic activities of this fungus [30]. *Penicillium* is the main fungus responsible for the degradation of lipids in this variety of cheese [6][31], with a wide variety of volatile and nonvolatile aroma compounds being produced primarily by *P. roqueforti*. This species is also considered the main origin of the enzymes responsible for proteolysis in blue cheese [31]. In addition, the appearance of the cheese is defined by the blue veins produced by *P. roqueforti* in the interior of the paste. Finally, this species participates in the consumption of lactic acid and neutralization of the cheese [32].

For many years, manufacture of blue cheeses has been carried out in a completely natural way. However, nowadays, the manufacture of these varieties under controlled conditions and the use of selected *P. roqueforti* strains are common practices in the cheese industry and considered necessary to obtain a product with the desired characteristics. For the selection of the strains several technological properties are evaluated: proteolytic and lipolytic activities, colour, germination and growth rate at the ripening temperatures, salt tolerance, and mycotoxigenicity. The proteolytic activity of the strain is extremely important for texture development, whereas the lipolytic ability is essential for aroma development [1]. If proteolysis is not enough, the cheese will be dry and hard, while, if it is in excess, it may be too soft. Additionally, high lipolysis is linked to a more intense flavor. This is considered by the companies offering strains with different properties. Spore suspensions (min. 10^{10}/mL) of *P. roqueforti* may be added to the milk, to the curd, or during moulding.

Looking at other adjunct starters, yeasts, are part of the natural microbiota of cheeses and may play a role in the manufacture of blue cheeses. Among the list of species found in this variety (more than 20), those that could be used as potential adjunct cultures are *D. hansenii*, *Yarrowia lipolytica*, and *Sac. cerevisiae*, with the first one being the most frequently isolated species in blue cheese [1]. *D. hansenii* has been found in the labs, together with other yeasts such as *Y. lipolytica*, in several Spanish blue cheeses (Valdeón artisanal cheese [33]; Cabrales [9]). As for *Y. lipolytica*, it looks a good candidate to be used in blue cheese manufacture according to its ability to grow and compete with other naturally occurring yeasts such as *D. hansenii* and *S. cerevisiae*, compatibility with and possible stimulation of LAB when co-inoculated and remarkable lipolytic and proteolytic activities [1].

3.5. Potential Mycotoxin Production

P. roqueforti may produce a range of mycotoxins (toxic secondary metabolites) such as PR-toxin, mycophenolic acid and roquefotines, among others [24][34], and some of them have been found in commercial blue cheeses at very low concentrations. Considering this fact, the relatively low toxicity of the mycotoxins and the instability of some of them (PR-toxin and penicillic acid) means that even a large consumption of blue cheese does not pose a risk to the health of the consumer [1][35][36][37]. Nevertheless, a selection of strains to be used in cheese manufacture should include a mycotoxin evaluation to ensure the use of those with the lowest mycotoxigenicity.

4. Conclusions

The sensory characteristics of blue cheeses and, ultimately, the essence of these varieties are based on complex biochemical reactions due, to a large extent, to a diverse microbiota in which fungi and bacteria participate in an active way. Although there have been studies on the microbiology and biochemistry of blue cheeses in recent years, further research is needed, in particular in the characterization of artisanal cheeses. This would allow researchers to keep the global diversity of existing blue cheeses which enriches the broad list of cheese varieties available to the consumer. In addition, more research is needed to elucidate the role of bioactive compounds generated during ripening, such as GABA or bioactive peptides, on the functionality of these varieties.

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