The fungal problem in cheese industry

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Abstract

Mould growth on cheese represents both a quality and a food safety problem, and poses significant economic losses. Several mould genera may destroy cheese, however normally just a few fungal species dominate on a specific type of cheese. *Penicillium* is the major genus followed by *Aspergillus*. Cheese contaminating mould species may produce mycotoxins, and some of the toxins as ochratoxin A, cyclopiazonic acid and sterigmatocystin have been shown to be stable under normal processing conditions. The main mould contamination source is the environment in the production facilities. Visible mould growth on cheese in the plant should be avoided in order to prevent problem moulds to spread. For identification of the contamination source, identification at or below species level is necessary.

Introduction

Fungi are a major cause of spoilage in food since they have a great versality for growing substrates and conditions where other microorganisms are not able to grow [1]. Fungal spoilage of cheese is a problem and cause quality reduction due to visible or invisible defects such as off-odour and off-flavour. Some of the fungi growing on cheese may also produce mycotoxins, which lead to a food safety issue.

Despite a lot of work in the dairies to reduce mould growth, fungal spoilage of cheese has significant economic losses due to product losses and waste, reduction of the quality, additional work, and food safety issues if mycotoxins are produced.

Cheese can be divided into groups depending on the water water activity (a_w). In addition, there are cheeses ripened with moulds, like blue or white mould cheeses that are ripened with *P. roqueforti* and *P. camemberti* respectively. However, even if fungal contamination happens more easily on soft cheeses (high a_w), fungi are capable to grow on all sorts of cheeses.

Associated mycobiota on cheese

Fungi responsible for problems in cheese production are diverse and belong to several genera as *Acremonium, Alternaria, Aspergillus, Aureobasidium, Botrytis, Cladosporium, Epicoccum, Eurotium, Exophiala, Fusarium, Gliocladium, Lecanicillium, Mucor, Penicillum, Rhizopus, and Wallemia* [2]. However, the genus most frequently isolated from spoiled cheese is *Penicillium* followed by *Aspergillus* [2-6]. Different *Penicillium* species which are isolated from cheese is listed in Table 1. Due to a variety of cheese types with different characteristics, the large variety of different fungal genera that can spoil cheese is not surprising. Many studies show that even if there is a large variety of genera and species that occasionally are isolated from a specific type of cheese, there are still only a few

47 species that are regularly dominating [4, 6, 7]. A certain food product has their own associated my-
48 coflora [7] and different cheese types may therefore have their very specific mycoflora on visible
49 mouldy cheese.

50 *Penicillum* is the domination genus (91%) on spoiled hard, semi-hard and semi-soft cheeses from dif-
51 ferent countries [8]. The most frequently isolated species were reported to be *Penicillium commune*
52 (42%), *P. nalgiovense*, *P. verrucosum*, *P. solitum*, *P. roqueforti*, *P. crustosum*, *P. atramentosum*, *P.*
53 *chrysogenum*, and *P. echinulatum*. Later Kure et al. [9] also identified *P. commune* as one of the most
54 frequently occurring species on visible mouldy semi-hard cheese together with the closely related *P.*
55 *palitans*, and *P. roqueforti* [10]. Garnier et al. [3] identified *P. commune* and P. *bialowienzense* as the
56 most common filamentous fungi in various French dairy products, while *P. crustosum* and *P. solitum*
57 were the dominating *Penicillium* species associated with Italian hard cheese [11]. Also a new *Penicil-*
58 *lium* species named *P. gravinivasei* is isolated from Italian cheese [12].

59 Cheese is normally kept refrigerated, some are vacuum packaged or gas flushed, hence the cheese
60 spoiling mould species in those cases are dominated by psycrotorelant species that can grow at low
61 O_2 concentrations [13]. Both *Penicllium roqueforti* and *P. commune* meet these criteria which make
62 them among the dominating cheese spoilage moulds.

63 Blue and white mould cheeses are mainly ripened with *P. roqueforti* and *P. camemberti* respectively,
64 and often together with *Geotrichum candidum*. Even though the surface is covered by desired
65 moulds, there may be spoilage moulds too. Also on these cheese types different *Penicillium* species
66 are isolated [14]. Other genera isolated from mould ripened cheese include *Alternaria*, *Aspergillus,*
67 *Cladosporium, Mucor and Rhizopus* [15, 16]. Bekada et al. [16] isolated *M. racemosus* from Camem-
68 berti cheese and *Mucor* has been observed as so-called “cat-hair” defect on soft cheeses [17]. At
69 least six different *Mucor* species have been isolated from cheese, including *M. circinelloides*, *M. race-*
70 *mosus*, *M. hiemalis* and *M. plumbeus*.

Marín et al. [18] studied the environmental factors, in particular the water content, that affect the growth of the mould species dominating on spoiled cheese. The results showed that decreased water content had an inhibitory effect on the growth of *M. circinelloides* and *M. racemosus,* while *Penicillium* and *Aspergillus* were more tolerant to water restriction. The environmental conditions influence the dynamics of fungal populations while growing on cheese, favouring certain fungal genera in different stages of the cheese making and ripening [18].

Contamination sources

Mould contamination of cheese can appear at different stages of the production. The milk used for cheese production is mostly pasteurised or heat treated before cheese making. Mould spores are in general not heat resistant and hence the milk do not consist an important source of contamination [2]. However, heat-resistant spores can occasionally cause spoilage in heat processed cheeses such as cream cheese [13]. Raw milk cheese is produced from unpasteurised milk and mould spores in the raw milk may thus later appear as visible moulds on the cheese [6].

The air in the production plant is contaminated with mould spores at different levels [6, 19], and the air represents one of the major contamination sources. Airborne spores can contaminate the curd in the vessels and vats. Mould spores that enter the surface of the cheese may be allowed to grow on the cheese later in the process if the growth conditions are suitable. The cheese will normally be immersed in brine for a certain period (Figure 1). As the brine can be a reservoir of moulds like *P. commune* [6] it needs to be rinsed regularly to remove mould spores. In some dairies, pressurised air is used to press the cheese out of the vats. The so called “dairy mould” *Geotrichum candidum* was isolated from pressurised air and was reported to be the major contamination source in that dairy [19].

The level of mould spores in the packaging room is particularly critical for vacuum packed cheese since this is the last step before ripening and refrigeration. Species as *P. commune*, *P. palitans* and other xerofilic fungi can grow at refrigerated temperature. As long as the package is closed there will

be no growth, but the spores will survive and may grow if the cheese is cut in to smaller pieces and kept without vacuum. Cheese that is ripened without packaging is sensitive for contamination from the air during ripening [20].

In one study, the contamination source of cheese was identified to the coating process where brushes whirled conidia in to the air and contaminated the next uncoated cheeses [21]. In another dairy, handling of mouldy cheese was shown to be the problem [21]. Despite high pressure in the packaging room located far away from the place where mouldy cheeses were handled, identical isolates could be traced from mouldy cheese and the air in the packaging room.

To prevent mould spores from visible mouldy cheese to spread in the production rooms, it is crucial to handle mouldy cheese extremely carefully. In some dairies cheeses with visibly mould growth is "cleaned" for surface moulds [20]. This allows the problem mould for that cheese type to spread in the air and contaminate new cheeses through the air and the smear. Although the air normally contains several different mould genera and species, only a low proportion of the airborne conidia constitutes the associated mould flora of the cheese produced in the plant [5]. However, if visible moulds from the cheese are allowed to spread to the air, the concentration of problem moulds in the air, and thus the production environment, increases and consequently also the mould contamination problem.

Methods for detection of moulds and preventative measures

To assess the mould level and the composition of the mycobiota, appropriate methods need to be used [22]. Both surface sampling and air sampling is necessary to do. DG18 (Dichloran Glycerol Agar) and MEA (Malt Extract Agar) are recommended as growth media for enumeration of fungal spores in air with both non-volumetric and volumetric sampling [23]. The air sampler has shown to give higher number of species and mean number of colony forming units (CFU)/plate than settle plates, however the two methods showed similar profiles of dominating fungal genera and species in air in a dry

cured meat processing plant [24]. Both methods can be used to monitor the mycobiota of the indoor air.

The level of mould spores in the processing sites, storage rooms and air– and other filtration systems is recommended sites to be monitored in order to know the normal level of spores and consequently be in position to discover changes that might predict mould contamination problems on the cheese if the level is higher than normal [23]. However, there might be rise in level of general number of mould spores without increase in the level of problem moulds, hence methods that monitor the level of problem moulds will be most suitable. To monitor the level of spoilage moulds specialised growth media should be used. Dichloran creatine medium (CREAD) has been proven as a suitable selective growth medium to monitor the level of problem moulds in dairies [25, 26].

In order to identify the contamination source the fungi should be identified to at least species level. Sometimes it is necessary to identify below species level to track the mould strains in the production plants [27, 28]. Different molecular methods have been used for this purpose [21, 28, 29]. MLST (multilocus sequence typing) is frequently used in medical mycology for typing of moulds, however in food mycology, the application is limited [22]. At the present the whole genome sequencing (WGS) of moulds is not practical as a tool for problem solving in food plants. However, the development of WGS is fast and it is expected that WGS will become standard typing methods in a near future [22]. Using molecular typing methods to trace the spoilage mould in the production plants it was demonstrated that mould spores could be transported from room to room with staff or equipment [28]. Amplified fragment length polymorphism (AFLP) was found to be a useful method to identify cheese contaminating fungi below species level, and as the same strains were detected repeatedly over a period of more than a year it was proven that the problem causing strain was well established in the plant [28].

In order to reduce mould contamination of cheese its needed to work systematically with preventative measures and measures that inhibit or reduce the mould growth. Preventative measures include

146 regularly cleaning and disinfection of equipment, including conveyer belts and vats, and the produc-
147 tion environment. The air in the production facility should have as low level of mould spores as possi-
148 ble, hence preventive measures as strict zonal regulations of the plant, filtration of the air, high-pres-
149 sure air in rooms where the cheese is kept for a long period (during ripening) or just before packag-
150 ing, may be necessary.
151
152 Hazard Analysis Critical Control Point (HACCP) can be used to identify critical control point for myco-
153 toxigenic moulds in food production [30]. For quality reducing mould species the pre-requisites are
154 important. A systematic overview of pre-requisites and procedures that impact the level of moulds
155 spores in the production plants will help to identify critical routines and procedures. A HACCP case
156 study was used in a Greek dairy plant for incorporation of microbiological and molecular methods in
157 HACCP monitoring scheme of mould and yeast [31]. Implementation of a constant monitoring of the
158 air quality and the recognition, as a critical point, led to a lower fungal air load.
159
160 **Antifungal effects on mould growth**
161 After packaging, the surface of the cheese may contain non-visible mould spores that can grow if the
162 conditions allow it. Hard cheese is often vacuum packed, while some, especially grated cheese, is
163 packed with gas. Modified gas atmosphere packaging (MAP) can prevent mould spores form sporula-
164 tion since the majority of cheese spoilage moulds have an absolute requirement of oxygen [13].
165 Never the less a wide variety of mould species are able to grow under reduced O_2 partial pressure (as
166 low as 1%), and for some spoilage moulds 0% O_2 is necessary to prevent growth [32]. Most spoilage
167 moulds are sensitive to high levels of carbon dioxide. Van Long et al. [32] indicate that CO_2 levels
168 above 50% was necessary to achieve fungal growth inhibition and up to 90% carbon dioxide was
169 needed to inhibit growth. The sensitivity to carbon dioxide and the level of O_2 required for growth

vary among the spoilage species (unpublished data). Hence the packaging method needs to be thoroughly tested to determine the optimal gas for prevention of the spoilage mould for each specific product.

Toxin production

Secondary metabolites (mycotoxins) are low molecular weight metabolites produced by filamentous fungi. Mycotoxins are found in cheese primarily as a result of either indirect contamination, resulting from the manufacture of cheese from animals which have ingested contaminated feed or directly resulting from mould growth on cheese [33-35]. The cheese contaminating mould species may produce mycotoxins as ochratoxin A, citrinin, cyclopiazonic acid, patulin, roquefortin C, mycophenolic acid, PR-toxin, penicillic acid, isofumigaclavine A and B, and andrastin A-D [36]. Some of these toxins, as PR-toxin, are unstable in cheese matrix and is readily inactivated or converted to other compounds [37]. However, other toxins as ochratoxin A (OTA), cyclopiazonic acid and sterigmatocystin have been shown to be stable under normal processing conditions [36]. The risk of mycotoxins in cheese is increasing when toxigenic moulds are allowed to grow during manufacturing and storage.

The mycotoxin cyclopiazonic acid (CPA) has been found in samples of white mould cheese [38, 39] and other chees varieties [40]. In some of the samples, high concentrations of CPA were found in commercially available cheese. CPA is produced by certain *Penicillium* and *Aspergillus* species [13, 36]. In samples with high CPA level the toxin was only detected in the outer layer of the cheese.

Fontaine et al. [41] did not find aflatoxins in blue veined cheese, but 97.7% and 37.2% of the samples contained roquefortine C and mycophenolic acid, respectively. Pattono et al. [42] examined semi-hard cheese for the presence of OTA and patulin. They found OTA in both the rind and the inner part of the cheese, while patulin was found mainly in the rind. López-Díaz et al. [43] found roquefortine C in an artisanal blue cheese. Coton et al. [44] found OTA and citrinin in Comté cheese.

Aflatoxin M1 in cheese occurs due to the use of contaminated milk [45]. The presence vary due to the cheese type and the reduction of the level in cheese depends on different processing parameters as temperature, pH, pressing time etc. For instance, oaxaca cheese in Mexico City was surveyed for presence of aflatoxins and their hydroxylated metabolites [46]. Aflatoxin B_1 and aflatoxicol were most frequently detected in addition to eight other aflatoxin varieties, fortunately in relatively low levels. In a study of cheese in Serbia, however, Aflatoxin M_1 was detected in samples of white cheese and hard cheese, and 13 % of the samples exceeded the adopted limit of 0.25 μ g/kg in the European Union (Regulation 1881/2006) [47].

Conclusion

To summarise, mould growth on cheese represents both a quality and a food safety problem, and poses significant economic losses due to disposal of products and increased work load. Several mould genera may destroy cheese, however normally just a few fungal species dominate on a specific type of cheese. *Penicillium* is the major genus followed by *Aspergillus*. Cheese contaminating mould species may produce mycotoxins. Some toxins are fortunately unstable in cheese matrix and is readily inactivated or converted to other compounds. However, other toxins as ochratoxin A, cyclopiazonic acid and sterigmatocystin have been shown to be stable under normal processing conditions. The main source for mould contamination of cheese is the environment in the production facilities; hence the level of mould spores in the facilities is crucial. Visible mould growth on cheese in the plant should be avoided in order to prevent problem moulds to spread. In order to survey the level of fungal spores both surface sampling and air sampling is necessary. DG18 (Dichloran Glycerol Agar) and MEA (Malt Extract Agar) are recommended growth media for enumeration of airborne fungal spores with both non-volumetric and volumetric sampling. For identification of the contamination source,

identification at or below species level is necessary. Further work should focus on methods for monitoring of problem moulds and methods for identifying problem moulds below species level. The dairies need methods that can be implemented at sites.

Conflict of interest statement

Nothing declared

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Papers of special interest to read:

[48], [32], [35],[22],[2],[36],[8]

References

[1] Taniwaki MH: *Editorial overview: Food mycology*. 2018, Elsevier.

[2] Garnier L, Valence F, and Mounier J: *Diversity and Control of Spoilage Fungi in Dairy Products: An Update.* Microorganisms 2017. **5**(3): p. 42.

[3] Garnier L, Valence F, Pawtowski A, Auhustsinava-Galerne L, Frotté N, Baroncelli R, Deniel F, Coton E, and Mounier J: *Diversity of spoilage fungi associated with various French dairy products.* International Journal of Food Microbiology 2017. **241**: p. 191-197.

[4] Pamela A, Miriam H, Filomena E, Teresa CM, Antonio M, Antonio L, and Antonia S: *Fungal mycobiota and mycotoxin risk for traditional artisan Italian cave cheese.* Food Microbiology 2019. **78**: p. 62-72.

[5] Ropars J, Cruaud C, Lacoste S, and Dupont J: *A taxonomic and ecological overview of cheese fungi.* International Journal of Food Microbiology 2012. **155**(3): p. 199-210.

[6] Marín P, Palmero D, and Jurado M: *Occurrence of moulds associated with ovine raw milk and cheeses of the Spanish region of Castilla La Mancha.* International Journal of Dairy Technology 2015. **68**(4): p. 565-572.

[7] Filtenborg O, Frisvad JC, and Thrane U: *Moulds in food spoilage.* International Journal of Food Microbiology 1996. **33**: p. 85-102.

[8] Lund F, Filtenborg O, and Frisvad JC: *Associated mycoflora of cheese.* Food Microbiology 1995. **12**: p. 173-180.

[9] Kure CF, Wasteson Y, Brendehaug J, and Skaar I: *Mould contaminants on Jarlsberg and Norvegia cheese blocks from four factories.* International Journal of Food Microbiology 2001. **70**(1): p. 21-27.

[10] Kure CF and Skaar I: *Mould growth on the Norwegian semi-hard cheeses Norvegia and Jarlsberg.* International Journal of Food Microbiology 2000. **62**(1): p. 133-137.

[11] Decontardi S, Mauro A, Lima N, and Battilani P: *Survey of Penicillia associated with Italian grana cheese.* International Journal of Food Microbiology 2017. **246**: p. 25-31.

[12] Anelli P, Peterson SW, Haidukowski M, Logrieco AF, Moretti A, Epifani F, and Susca A: *Penicillium gravinicasei, a new species isolated from cave cheese in Apulia, Italy.* International Journal of Food Microbiology 2018. **282**: p. 66-70.

[13] Pitt JI, Hocking, A. D.: *Fungi and Food Spoilage*. 3rd ed. 2009, Boston, MA: Springer 519.

[14] Belén Flórez A, Álvarez-Martín P, López-Díaz TM, and Mayo B: *Morphotypic and molecular identification of filamentous fungi from Spanish blue-veined Cabrales cheese, and typing of Penicillium roqueforti and Geotrichum candidum isolates.* International Dairy Journal 2007. **17**(4): p. 350-357.

[15] Moubasher AH, Abdel-Kader MIA, and El-Kady IA: *Toxigenic fungi isolated from roquefort cheese.* Mycopathologia 1979. **66**(3): p. 187-190.

[16] Bekada AMA, Benakriche, B., Hamadi, K., Bensoltane, A.: *Modelling of Effect of Water Activity, pH and Temperature on the Growth Rate of Mucor racemosus Isolated from Soft Camembert Cheese.* World Journal of Agricultural Sceince 2008. **4**(6): p. 4.

[17] Bärtschi C, Berthier J, and Valla G: *Evolution of the surface fungal flora of Reblochon cheese.* Lait 1994. **74**(2): p. 105-114.

[18] Marín P, Palmero D, and Jurado M: *Effect of solute and matric potential on growth rate of fungal species isolated from cheese.* International Dairy Journal 2014. **36**(2): p. 89-94.

[19] Kure CF, Skaar I, and Brendehaug J: *Mould contamination in production of semi-hard cheese.* International Journal of Food Microbiology 2004. **93**(1): p. 41-49.

[20] Barrios MJ, Medina LM, Lopez MC, and Jordano R: *Fungal biota isolated from Spanish cheese.* Journal of Food Safety 1998. **18**(2): p. 151-157.

[21] Lund F, Nielsen AB, and Skouboe P: *Distribution of Penicillium commune isolates in cheese dairies mapped using secondary metabolite profiles, morphotypes, RAPD and AFLP fingerprinting.* Food Microbiology 2003. **20**(6): p. 725-734.

[22] Rico-Munoz E, Samson RA, and Houbraken J: *Mould spoilage of foods and beverages: Using the right methodology.* Food Microbiology 2018.

[23] Samson RA, Houbraken, J., Thrane, U., Frisvad, J.C., Andersen, B.: *Food and indoor fungi*. CBS Laboratory Manual Series. 2010, Utrecht, The Netherlands: CBS-KNAW Fungal Bioderversity Centre.

[24] Asefa DT, Langsrud S, Gjerde RO, Kure CF, Sidhu MS, Nesbakken T, and Skaar I: *The performance of SAS-super-180 air sampler and settle plates for assessing viable fungal particles in the air of dry-cured meat production facility.* Food Control 2009. **20**(11): p. 997-1001.

[25] Kure CF, Borch E, Karlsson I, Homleid JP, and Langsrud S: *Use of the selective agar medium CREAD for monitoring the level of airborne spoilage moulds in cheese production.* International Journal of Food Microbiology 2008. **122**(1): p. 29-34.

[26] Lund F: *Differentiating Penicillium species by detection of indole matabolites using a filter paper method.* Letters in Applied Microbiology 1995. **20**: p. 228-231.

[27] Lund F and Skouboe P: *Identification of Penicillium caseifulvum and P. commune isolates related to specific cheese and rye bread factories using RAPD fingerprinting.* Journal of food Mycology 1998. **1**(3): p. 131-139.

[28] Kure CF, Skaar I, Holst-Jensen A, and Abeln ECA: *The use of AFLP to relate cheese-contaminating Penicillium strains to specific points in the production plants.* International Journal of Food Microbiology 2003. **83**(2): p. 195-204.

[29] Kure CF, Abeln ECA, Holst-Jensen A, and Skaar I: *Differentiation of Penicillium commune and Penicillium palitans isolates from cheese and indoor environments of cheese factories using M13 fingerprinting.* Food Microbiology 2002. **19**(2): p. 151-157.

[30] Asefa DT, Kure CF, Gjerde RO, Langsrud S, Omer MK, Nesbakken T, and Skaar I: *A HACCP plan for mycotoxigenic hazards associated with dry-cured meat production processes.* Food Control 2011. **22**(6): p. 831-837.

[31] Beletsiotis E, Ghikas D, and Kalantzi K: *Incorporation of microbiological and molecular methods in HACCP monitoring scheme of molds and yeasts in a Greek dairy plant: A case study.* Procedia Food Science 2011. **1**: p. 1051-1059.

[32] Nguyen Van Long N, Joly C, and Dantigny P: *Active packaging with antifungal activities.* International Journal of Food Microbiology 2016. **220**: p. 73-90.

[33] Bullermann LB: *Public health significance of molds and mycotoxins in fermented dairy products.* Journal of Dairy Science 1981. **64**: p. 2439-2452.

[34] Dobson ADW: *Chapter 23 - Mycotoxins in Cheese*, in *Cheese (Fourth Edition)*, P.L.H. McSweeney, P.F. Fox, P.D. Cotter, and D.W. Everett, Editors. 2017, Academic Press: San Diego. p. 595-601.

[35] Sengun I, Yaman D, and Gonul S: *Mycotoxins and mould contamination in cheese: a review.* World Mycotoxin Journal 2008. **1**(3): p. 291-298.

[36] Benkerroum N: *Mycotoxins in dairy products: A review.* International Dairy Journal 2016. **62**: p. 63-75.

[37] Chang SC, Lu KL, and Yeh SF: *Secondary metabolites resulting from degradation of PR toxin by Penicillium roqueforti.* Applied and Environmental Microbiology 1993. **59**(4): p. 981-986.

[38] Ansari P and Häubl G: *Determination of cyclopiazonic acid in white mould cheese by liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) using a novel internal standard.* Food Chemistry 2016. **211**: p. 978-982.

[39] Le Bars J: *Cyclopiazonic acid production by Penicillium camemberti Thom and natural occurrence of this mycotoxin in cheese.* Applied and Environmental Microbiology 1979. **38**(6): p. 1052.

[40] Finoli C, Vecchio A, Galli A, and Franzetti L: *Production of cyclopiazonic acid by molds isolated from Taleggio cheese.* Journal of Food Protection 1999. **62**(10): p. 1198-1202.

[41] Fontaine K, Passeró E, Vallone L, Hymery N, Coton M, Jany J-L, Mounier J, and Coton E: *Occurrence of roquefortine C, mycophenolic acid and aflatoxin M1 mycotoxins in blue-veined cheeses.* Food Control 2015. **47**: p. 634-640.

[42] Pattono D, Grosso A, Stocco PP, Pazzi M, and Zeppa G: *Survey of the presence of patulin and ochratoxin A in traditional semi-hard cheeses.* Food Control 2013. **33**(1): p. 54-57.

[43] Lopez-Diaz TM, Roman-Blanco C, Garcia-Arias MT, Garcia-Fernandez MC, and Garcia-Lopez ML: *Mycotoxins in two Spanish cheese varieties.* Int.J.Food Microbiol. 1996. **30**(3): p. 391-395.

[44] Coton M, Auffret A, Poirier E, Debaets S, Coton E, and Dantigny P: *Production and migration of ochratoxin A and citrinin in Comté cheese by an isolate of Penicillium verrucosum selected among Penicillium spp. mycotoxin producers in YES medium.* Food Microbiology 2019. **82**: p. 551-559.

[45] Campagnollo FB, Ganev KC, Khaneghah AM, Portela JB, Cruz AG, Granato D, Corassin CH, Oliveira CAF, and Sant'Ana AS: *The occurrence and effect of unit operations for dairy products processing on the fate of aflatoxin M1: A review.* Food Control 2016. **68**: p. 310-329.

[46] Carvajal-Moreno M, Vargas-Ortiz M, Hernández-Camarillo E, Ruiz-Velasco S, and Rojo-Callejas F: *Presence of unreported carcinogens, Aflatoxins and their hydroxylated metabolites, in industrialized Oaxaca cheese from Mexico City.* Food and Chemical Toxicology 2019. **124**: p. 128-138.

[47] Škrbić B, Antić I, and Živančev J: *Presence of aflatoxin M1 in white and hard cheese samples from Serbia.* Food Control 2015. **50**: p. 111-117.

[48] Hymery N, Vasseur V, Coton M, Mounier J, Jany J-L, Barbier G, and Coton E: *Filamentous Fungi and Mycotoxins in Cheese: A Review.* Comprehensive Reviews in Food Science and Food Safety 2014. **13**(4): p. 437-456.

[49] Hocking AD and Faedo M: *Fungi causing thread mould spoilage of vacuum packaged Cheddar cheese during maturation.* International Journal of Food Microbiology 1992. **16**(2): p. 123-130.

385 Table 1

386 *Penicillium* species on cheese by country.

Species	Country	References
Penicillium atramentosum	Norway, France	[8]
P. bialowienzense	France	[3]
P. brevicompactum	France	[2, 3, 13]
P. chrysogenum	Denmark, USA, Spain	[6, 8]
P. commune	Denmark, Greece, France, UK, Australia, Germany, Azores, USA, Belgium, Japan, New Zealand, Czech Republic, Netherlands, Norway, Spain	[3, 6, 8-10]
P. crustosum	Denmark, Italy, France, Azores, UK, Norway	[8, 9, 11]
P. echinulatum	Australia, South Africa,	[8]
P. expansum	Norway	[9, 10, 13]
P. gravinivasei	Italy	[12]
P. palitans	Norway	[9, 10]
P. nalgiovense	Denmark, Greece, Slovakia	[8]
P. roqueforti	Denmark, germany, Autralia, Greece, Malt, Costa Rica, Norway	[8-10]
P. solitum	Denmark, Greece, Italy, Norway, Spain,	[6, 8-11]
P. verrucosum	Denmark, Greece, Spain,	[8]
P. viridicatum	Australia	[49]

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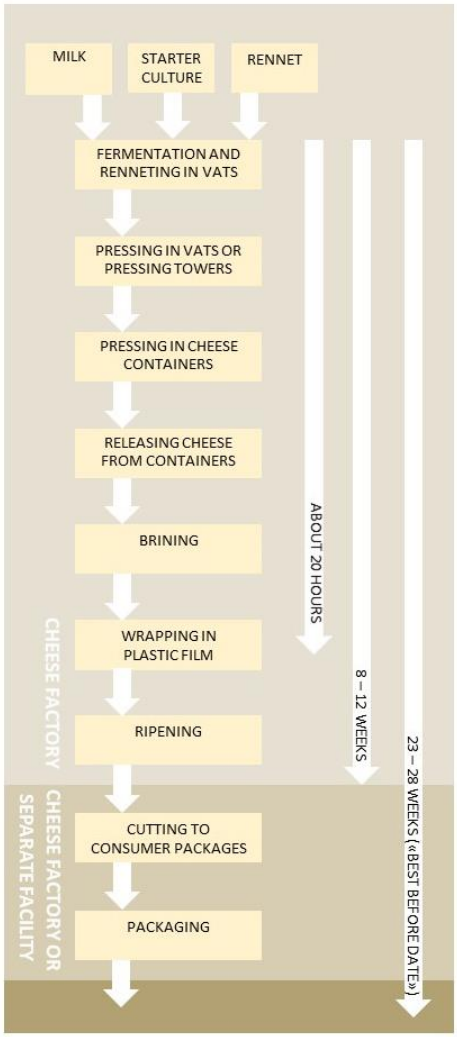

Figure 1. Flow diagram semi-hard cheese production.