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The fungal problem in cheese industry

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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 such 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.

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Introduction

Fungi are a major cause of spoilage in food since they have a great versatility 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 activity (a_w). In addition, there are cheeses ripened with moulds, like blue or white mould cheeses that are ripened with *Penicillium roqueforti* and *Penicillium 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*, *Penicillium*, *Rhizopus*, and *Wallemia* [2*]. However, the genus most frequently isolated from spoiled cheese is *Penicillium* followed by *Aspergillus* [2*,3–6]. Different *Penicillium* species which are isolated from cheese is listed in [Table 1](#). Because of 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 species that are regularly dominating [4,6,7]. A certain food product has their own associated mycoflora [7] and different cheese types may therefore have their very specific mycoflora on visible mouldy cheese.

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

Cheese is normally kept refrigerated, some are vacuum packaged or gas flushed, hence the cheese spoiling mould species in those cases are dominated by psychrotolerant species that can grow at low O₂ concentrations [13]. Both *P. roqueforti* and *P. commune* meet these criteria which make them among the dominating cheese spoilage moulds.

Table 1

Penicillium species on cheese by country

Species	Country	References
<i>Penicillium atramentosum</i>	Norway, France	[8*]
<i>P. bialowienzense</i>	France	[3]
<i>P. brevicompactum</i>	France	[2*,3,13]
<i>P. chrysogenum</i>	Denmark, USA, Spain	[6,8*]
<i>P. commune</i>	Denmark, Greece, France, UK, Australia, Germany, Azores, USA, Belgium, Japan, New Zealand, Czech Republic, Netherlands, Norway, Spain	[3,6,8*,9,10]
<i>P. crustosum</i>	Denmark, Italy, France, Azores, UK, Norway	[8*,9,11]
<i>P. echinulatum</i>	Australia, South Africa,	[8*]
<i>P. expansum</i>	Norway	[9,10,13]
<i>P. gravinivasei</i>	Italy	[12]
<i>P. palitans</i>	Norway	[9,10]
<i>P. nalgiovense</i>	Denmark, Greece, Slovakia	[8*]
<i>P. roqueforti</i>	Denmark, Germany, Australia, Greece, Malt, Costa Rica, Norway	[8*,9,10]
<i>P. solitum</i>	Denmark, Greece, Italy, Norway, Spain,	[6,8*,9,10,11]
<i>P. verrucosum</i>	Denmark, Greece, Spain,	[8*]
<i>P. viridicatum</i>	Australia	[48]

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

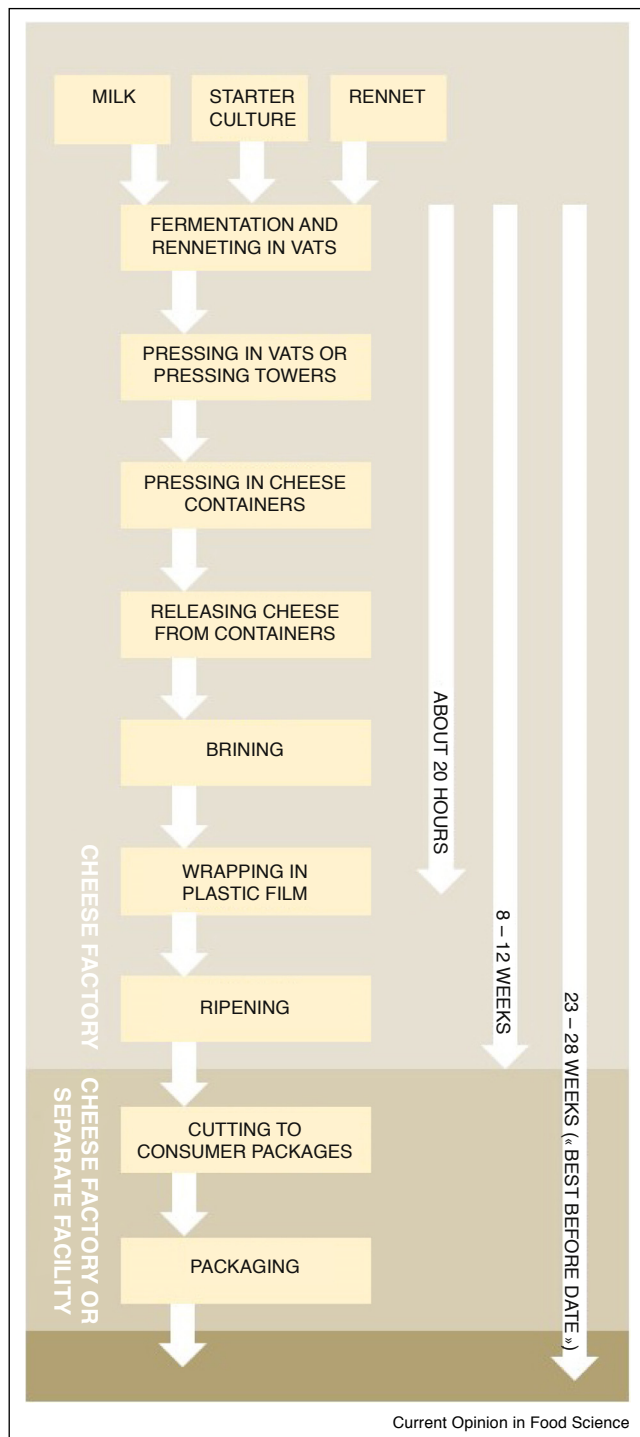
The air in the production plant is contaminated with mould spores at different levels [6,19], and the air represents one of the major contamination sources. Airborne spores can contaminate the curd in the vessels and vats. Mould spores that enter the surface of the cheese may be allowed to grow on the 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' *G. 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 xerophilic 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 into 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

Figure 1



Flow diagram semi-hard cheese production.

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 are 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 a 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 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, it is needed to work systematically with preventative measures and measures that inhibit or reduce the mould

growth. Preventative measures include regularly cleaning and disinfection of equipment, including conveyer belts and vats, and the production environment. The air in the production facility should have as low level of mould spores as possible, hence preventative measures as strict zonal regulations of the plant, filtration of the air, high-pressure air in rooms where the cheese is kept for a long period (during ripening) or just before packaging, may be necessary.

Hazard Analysis Critical Control Point (HACCP) can be used to identify critical control point for mycotoxigenic moulds in food production [30]. For quality reducing mould species the pre-requisites are important. A systematic overview of pre-requisites and procedures that impact the level of moulds spores in the production plants will help to identify critical routines and procedures. A HACCP case study was used in a Greek dairy plant for incorporation of microbiological and molecular methods in HACCP monitoring scheme of mould and yeast [31]. Implementation of a constant monitoring of the air quality and the recognition, as a critical point, led to a lower fungal air load.

Antifungal effects on mould growth

After packaging, the surface of the cheese may contain non-visible mould spores that can grow if the conditions allow it. Hard cheese is often vacuum packed, while some, especially grated cheese, is packed with gas. Modified gas atmosphere packaging (MAP) can prevent mould spores from sporulation since the majority of cheese spoilage moulds have an absolute requirement of oxygen [13]. Nevertheless, a wide variety of mould species are able to grow under reduced O₂ partial pressure (as low as 1%), and for some spoilage moulds 0% O₂ is necessary to prevent growth [32^{*}]. Most spoilage moulds are sensitive to high levels of carbon dioxide. Van Long *et al.* [32^{*}] indicate that CO₂ levels above 50% was necessary to achieve fungal growth inhibition and up to 90% carbon dioxide was needed to inhibit growth. The sensitivity to carbon dioxide and the level of O₂ 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 [33,34,35^{*}]. 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 cheese 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 varies due to the cheese type and the reduction of the level in cheese depends on different processing parameters as temperature, pH, pressing time and so on. For instance, oaxaca cheese in Mexico City was surveyed for presence of aflatoxins and their hydroxylated metabolites [46]. Aflatoxin B₁ 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 M₁ was detected in samples of white cheese and hard cheese, and 13% of the samples exceeded the adopted limit of 0.25 µg/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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