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ORIGINAL ARTICLE

Suitability of FTIR to distinguish pure cultures of problematic mould species from closely related species in the meat industry

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Abstract

Aims: The aim of the study was to apply Fourier Transform Infrared spectroscopy (FTIR) as a rapid screening method for moulds in a specific food production environment (cured meat) and to evaluate whether the method was sufficiently accurate to distinguish *Penicillium* species that constitute a hazard for the food quality and safety (*Penicillium solitum* and *Penicillium nordicum*) from closely related species.

Methods and Results: FTIR was applied to classify the indigenous mycobiota of two production sites for dried and cured meat products in Norway. Results showed that FTIR was suitable to analyse large amounts of data. While correct classification rates varied depending on the species, overall results indicated that FTIR was able to distinguish the undesired mould species *P. solitum* and *P. nordicum* from other species and may hence present an option for rapid screening of large numbers of samples to identify changes in mould composition on site.

Conclusions: FTIR presents a potential method for detecting changes in levels of undesired fungi in meat-processing environments.

Significance and Impact of the study: This is the first study that applies FTIR to a specific food production environment and it increases the knowledge on both possibilities and limitations of the method in classification of fungi.

Introduction

Growth of moulds and yeasts is one among the main causes for microbiological decay of food. Each year, the food industry has to recall or destroy vast amounts of food because of mould contamination. The meat industry, in particular, regularly experiences problems with the growth of *Penicillium* spp. and *Aspergillus* spp. on their products, especially cured and dried meats, that require prolonged storage during production (Lopez-Diaz *et al.* 2001; Comi *et al.* 2004; Sorensen *et al.* 2008; Asefa *et al.* 2009; Sonjak *et al.* 2011). In order to reduce the losses due to mould contamination, the major strategy is to prevent mould from entering and spreading in production environments by regular cleaning and disinfection routines. Moulds may enter the facilities via many routes —raw materials, personnel, equipment or inlet air. As moulds are easily spread through the air, the complete eradication of moulds from the production environments has proven to be practically impossible. Second in line are early detection methods in order to both determine contamination sources and to quickly take measures in cases of contamination. As various mould species show great variations in metabolite production (Frisvad *et al.* 2004), certain species may be more problematic for the product than others as they produce mycotoxins or other spoiling components. Also, each product has an associated spoilage mycobiota. The focus of potential detection methods must thus be to spot and identify the specific problematic organisms for the product in question.

Several methods have been used to identify various moulds in the laboratory. The classical polyphasic approach (Frisvad and Samson 2004) is still used, but is both time-consuming and dependent on the expertise of the analysing personnel. DNA sequencing has proven efficient in identifying moulds; however, analyses are costly, and partial sequencing might not be sufficient to identify all moulds to the species level (Rico-Munoz *et al.* 2019). Several spectroscopy-based methods have been introduced, and even if many of them have proven useful in the laboratory, applications in the industry have not yet been broadly explored.

Fourier transform infrared spectroscopy (FTIR) has been frequently mentioned as a possible method for early detection and identification of moulds and several studies have proven the potential of this method to identify problematic moulds (Shapaval *et al.* 2010, 2013). However, with few exceptions (Fischer *et al.* 2006), previous studies have focused on general applications and commonly included a wide range of distantly related mould genera (Shapaval *et al.* 2010). So far, the method has not been applied to a defined mycobiota belonging to a specific processing environment. It is of interest to investigate whether the method can separate moulds that pose quality and safety problems on the product from sporadically appearing airborne moulds and whether the method can distinguish closely related species. The set of moulds that were used in this study are mainly isolates from food production environments of dry cured meats. For the industry, the most important species to identify and remove are the dominant species on the products and the toxin-producing species. Some of these are closely related to relatively harmless variants, thus making classification complicated and important. In this case, *Penicillium solitum* and *Penicillium nordicum* were identified as most important organisms as they were dominating on the products from two distinct production facilities (Schirmer *et al.* 2018). The aim of this study was to investigate the usefulness of FTIR to detect the specific spoilage mould species *P. solitum* and *P. nordicum* and to distinguish them from closely related moulds species typically found in the production environments of dried and cured meat products. The main challenge was to evaluate the efficacy of the method within a range of closely related mould species within the genus *Penicillium*.

Materials and Methods

Strain selection

The species to be tested were selected after comprehensive sampling in 2014 and 2015 of two producers of Norwegian cured meat products, including hams, salami type sausages, dried lamb ribs and dried mutton legs. Target organisms were *Penicillium* spp. Besides isolates from samplings, additional strains were added from the Norwegian Veterinary Institute's strain collection. All *Penicillium* isolates were identified morphologically using a polyphasic approach (Frisvad and Samson 2004) including macroscopic and microscopic inspection and by partial sequencing of the ITS and β-tubulin genes as described by Schirmer *et al.* (2018). These methods are henceforth referred to as traditional identification methods. A total of 76 *Penicillium* strains comprising 10 different species and 25 *Aspergillus* strains that were not further identified to species level were used for initial analyses. *Aspergillus* spp. were included in the study since they are frequently observed in the meat production environment and some species may be mistaken for *Penicillium* sp. at first sight. By having the *Aspergillus*-group in the FTIR classification, the risk of misclassification of these strains is reduced, and most non-*Penicillium* will automatically be classified in this group.

Additional sampling of the same two sites was carried out in 2016, and 38 *Penicillium* isolates of the same 10 species and six *Aspergillus* isolates were used as a blind test to evaluate the model created with the previous isolates.

FTIR analysis

All analyses were carried out on pure cultures which was ensured by pre-cultivation on malt extract agar (MEA) for 5 days at 20°C. All isolates were then cultivated on yeast extract sucrose (YES) agar (Samson *et al.* 2004a) for 48 h at 25°C. YES agar was chosen because the selected fungi produced less spores than on MEA and 48 h of incubation time was chosen to minimize the risk of spore formation in the liquid media. One loop full (1 μl size) of mycelium was transferred to the wells of a 96-well microtiter plate (MTP, EnzyScreen BV, Heemstede, Netherlands) containing 800 μl of malt extract broth (MEB) (Samson *et al.* 2004a) and incubated for 48 h at 25°C with shaking at 180 rev min^{-1}. Two replicate plates were used for each experiment and each isolate was transferred to three wells on each of these plates, resulting in six measurements per isolate per experiment. For the vast majority of isolates, these were cultured three times, and each of these cultures were sampled six times each for cultivation in two microtiter plates (3 + 3 wells). Every sample (3 × 6 = 18) was used to create one spot on the FTIR plates. In order to avoid cross-contamination within the plates, empty rows were employed between samples from different isolates.

After incubation, the mycelium was washed three times with 800 μl of H_2O. Finally, the mycelium was dissolved

in 50–100 μl dH_2O and sonicated with a tip-sonicator (Qsonica LLC) for 30 s at 50% efficiency.

From each of these homogeneous cell suspensions, 8 μl was transferred in triplicate to an IR-light transparent Silicon 384-spot plate (Bruker Optik GmbH, Ettlingen, Germany). Plates were dried for 30–40 min at room temperature to form films suitable for FTIR analyses. FTIR measurements were performed using a High-Throughput Screening eXTension (HTS-XT) unit coupled to a Tensor 27 spectrometer (both Bruker Optik GmbH). The spectra were recorded in the region between 4000 and 500 cm^{-1} with a spectral resolution of 6 cm^{-1} and an aperture of 5·0 mm. For each spectrum, 64 scans were averaged. A schematic overview of the workflow is presented in Fig. 1.

Data analysis

Spectra were pre-processed in two steps starting with computing smoothed first derivatives by Savitzky–Golay filtering (Savitsky and Golay 1964) with third-order polynomial and nine-point sliding window. A model-based correction of the baseline and differences in intensity by basic extended multiplicative signal correction (Martens and Stark 1991) was carried out and non-informative regions of the spectra were removed. Regions between 3000–2800 cm^{-1} and 1800–900 cm^{-1} were kept for analyses. Examples of the characteristic spectra of *P. solitum* and *P. Nordicum* are shown in Fig. 2. Wavelength regions 3000–2800 and 1800–900 were optimized in Shapaval *et al.* (2013) and reused here without further optimization.

For each scanning, the sum of squared deviation between average spectra and single spectra was calculated and all samples with values >0·00035 were removed from the analyses. These were mainly spectra with weak signals due to small sample volumes deposited on the silicon plate. To be accepted for classification, at least three valid replicates were required.

The classification was carried out using the HotPLS method, described by Liland *et al.* (2014). The method uses a phylogenetic tree and creates a new classification model at each node as it moves down the tree from division, class, order, family, genus, subgenus, section and

Figure 1 Schematic work flow for FTIR analysis.

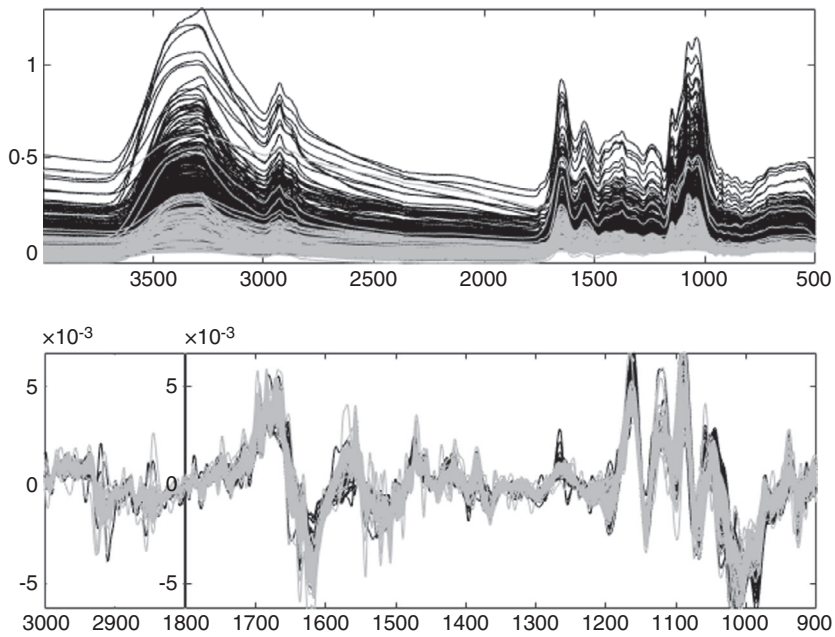

Figure 2 Example FTIR spectra for *Penicilliumsolitum* (black) and *Penicilliumnordicum* (gray), raw spectra from HTS-XT (top) and pre-processed spectra cut to informative regions (bottom).

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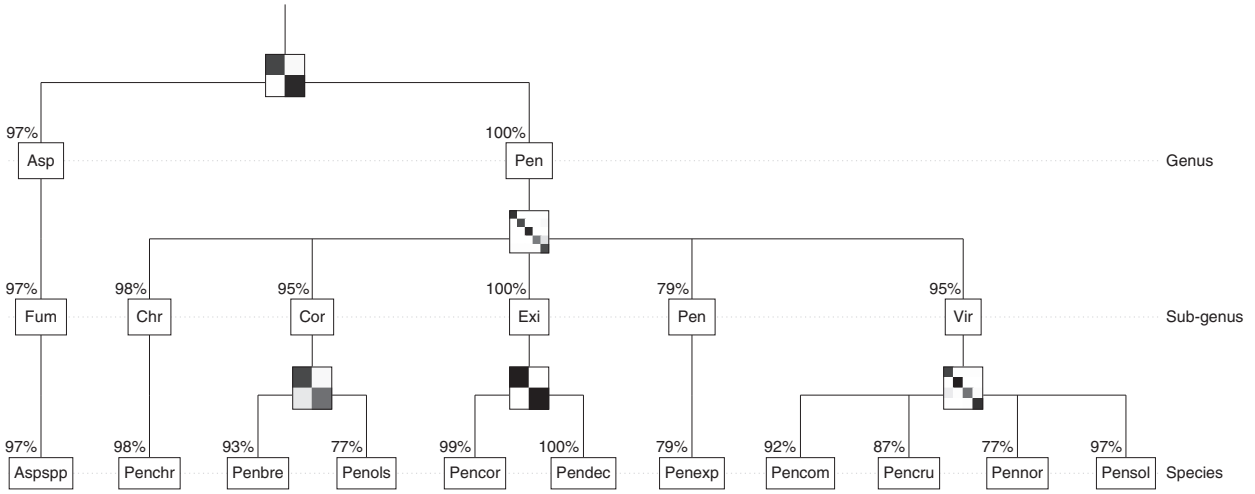

Figure 3 HotPLS tree showing how samples are classified hierarchically on genus level, sub-genus level, and lastly on species level. Percentages indicate percentage correctly classified, corresponding to Table 1. Checkerboards indicate where moulds have ended up in classification. See for instance lower left checkerboard, where lower left gray patch indicates that some *Penicilliumolsoni* (Penols) have been mis-classified as *Penicilliumbrevicompactum* (Penbre).

species (Fig. 3). The phylogeny of moulds is constantly updated as new knowledge is gained about existing species and new strains are discovered. For identification of mould species by FTIR, the optimal choice of phylogeny would be the one that best reflects the metabolic differences in different branches such that these can easily be differentiated in the spectra. Using proper pre-processing and hierarchical multivariate classification models though HotPLS, we leverage large regions of the spectra to create reproducible models that are robust against spectral noise, sample concentrations, dry film condition, etc. The specific phylogeny underlying the HotPLS is adapted to the current set of moulds. In particular, the taxonomic level *section* has been included between genus and species instead of sub-genus (Liland *et al.* 2014), as some of the included species are very closely related.

For cross-validation, the dataset was split into six parts according to MTP replication. Each of these parts was held out once when building a hierarchical classification model and their classification accumulated. Majority voting across technical replicates was applied for the final classification while all scans are displayed in the table.

An alternative strategy was also tested to emulate the occurrence of a new isolate, not previously included in the database. Here, cross-validation was performed removing one isolate at the time, that is, all biological replicates and scannings corresponding to an isolate each time. For this strategy, a less strict filtering of the samples was performed using a cut-off of 0·001 instead. The reasoning is that majority voting among the many replicates will dominate the inclusion of noisy samples and that more isolates will be accepted for analysis.

In addition to the isolates used to build and cross-validate the HotPLS classification, a separate, unlabelled, test set based on isolates from the sampling in 2016 has been prepared and classified. This means that we have a true validation, where all choices of pre-processing, filtering, model complexities and strategies had to be chosen before classification and before the taxonomic classification was revealed. This removed any possibilities of tampering with the results and results in a realistic situation, comparable to future use of the model on new samples. The complexity of all the models was chosen automatically using MTP replication as cross-validation segments.

Results

Among the *Penicillium* isolates from 2014 and 2015, 1230 out of 1340 successful screenings (91·8%) showed compliance between the FTIR classification and the traditional classification methods on species level, and 1267 (94·6%) were classified within the same section of closely related species according to Samson *et al.* (2004b). For the *Aspergillus* isolates, 195 out of 202 screenings (96·5%) were classified identically by both methods on the genus level (Table 1). The overall compliance between the methods varied from 76·7 to 100% depending on the species.

A total of 296 successful screenings were obtained from 15 isolates of *P. solitum* of which 287 (97·0%) classified the isolate in compliance with the traditional method. Three of the screenings classified the isolate as species within the same section as *P. solitum* (*P. nordicum*), whereas six classified the isolate as species from other taxonomic sections (*P. chrysogenum* or *P. brevicompactum*).

Table 1 Classification of mould strains by traditional identification methods versus Hot PLS classification of FTIR spectra

		Identified by FTIR												
	# isolates	# scannings	*Aspergillus sp.*	*P. chrysogenum*	*P. brevicompactum*	*P. olsonii*	*P. corylophilum*	*P. decumbens*	*P. expansum*	*P. commune*	*P. crustosum*	*P. nordicum*	*P. solitum*	Compliance %
Identified by morphology and DNA seq.														
Aspergillus sp.	**14**	202	**195**	2	0	1	0	1	1	0	2	0	0	96·5
*P. chrysogenum**	10	167	1	**164**	0	0	0	0	0	1	0	1	0	98·2
P. brevicompactum†	10	171	0	0	**159**	7	0	0	0	1	0	1	3	93·0
P. olsonii†	5	86	0	0	12	**65**	0	0	1	5	2	0	0	76·7
P. corylophilum‡	5	116	0	0	0	0	**115**	0	0	1	0	0	0	99·1
P. decumbens‡	6	111	0	0	0	0	0	**111**	0	0	0	0	0	100
P. expansum§	5	112	2	0	0	0	0	0	**88**	22	0	0	0	78·6
P. commune¶	5	86	0	0	1	2	0	0	1	**79**	1	1	1	91·9
P. crustosum¶	5	120	2	0	1	0	6	0	7	0	**104**	0	0	86·7
P. nordicum¶	6	75	0	2	1	2	0	0	0	9	1	**58**	2	77·3
P. solitum¶	15	296	0	5	1	0	0	0	0	0	0	3	**287**	97·0

Bold types indicate spectra that were identified as the same species by both methods; Grey shades indicate taxonomic sections **Chrysogena*, †*Coronata*, ‡*Viridicata* Ser. *Viridicata*, §*Penicillium*, ¶*Viridicata* Ser. *Verrucosa*, *Camemberti*, *Solita*.

For *P. nordicum,* 58 out of 75 successful screenings, distributed on 10 isolates, gave results complying with the traditional method. In all, 12 classified the isolate as *Penicillium* species within the same section while 5 classified the isolate as species from other taxonomic sections (*P. chrysogenum*, *P. brevicompactum* or *P. olsonii*).

It is notable that less than 3% of the spectra of the commonly found *P. brevicompactum* misclassified the isolate as one of the important spoilage organisms *P. nordicum* or *P. solitum*, indicating that it is possible to distinguish predominating air moulds from the problematic spoilage moulds.

To determine how many of the actual isolates were classified in compliance with the traditional identification method, all screenings for each isolate were collected and a majority voting strategy was employed to identify each isolate based on all screenings. Results are shown in Table 2. By this method, 98·9% of the isolates were classified correctly by genus, 97·8% by section and 94·4% by species level.

Results showed that all 15 *P. solitum* isolates were classified correctly, whereas 6 out of 7 *P. nordicum* isolates were classified correctly. One *P. nordicum* isolate was wrongly classified as *P. commune*. All 10 isolates of the common environmental species *P. brevicompactum* were classified as *P. brevicompactum* (9) or the closely related *P. olsonii* (1).

Strains isolated in 2016 were used to test the model that was developed based on isolates from 2014 and 2015 (Table 3). The full dataset with all known samples was used to train a hierarchical classification model. The classification based on morphology and DNA sequencing was then revealed and the results are summarized in Table 3.

As before, all screenings for each isolate were collected and a majority voting strategy was employed to identify each isolate based on all screenings. Results are shown in Table 4.

Classification success varied greatly between species, and for the selected problematic species, correct classification rates were higher, 100% for *P. nordicum* and 80% for *P. solitum*. It is also notable that no problematic moulds were classified as typical environmental moulds; however, some *P. brevicompactum* and *P. olsonii* were falsely classified as *P. solitum*, potentially giving rise to false-positive results.

A total of 1829 screenings were performed (1493 for *Penicillium* spp. and 336 for *Aspergillus* spp.). For *Penicillium* species, 153 screenings of *Penicillium* (10·2%) were discarded due to poor spectra; the same was the case for 134 screenings of *Aspergillus* (39·9%).

Discussion

The aim of the study was to assess whether FTIR could be used as a rapid method for detection of problematic

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Table 2 Collective classification of selected isolates by use of all single screenings using a majority voting system for each set of samples corresponding to one isolate

			Identified by FTIR										
	# isolates	*Aspergillus sp.*	*P. chrysogenum*	*P. brevicompactum*	*P. olsonii*	*P. corylophilum*	*P. decumbens*	*P. expansum*	*P. commune*	*P. crustosum*	*P.nordicum*	*P.solitum*	Compliance %
Identified by morphology and DNA seq.													
Aspergillus sp.	16*	15	0	0	0	0	0	0	0	1	0	0	93·8
P. chrysogenum	10	0	10	0	0	0	0	0	0	0	0	0	100
P. brevicompactum	10	0	0	9	1	0	0	0	0	0	0	0	90·0
P. olsonii	5	0	0	1	4	0	0	0	0	0	0	0	80·0
P. corylophilum	5	0	0	0	0	5	0	0	0	0	0	0	100
P. decumbens	6	0	0	0	0	0	6	0	0	0	0	0	100
P. expansum	5	0	0	0	0	0	0	4	1	0	0	0	80·0
P. commune	5	0	0	0	0	0	0	0	5	0	0	0	100
P. crustosum	5	0	0	0	0	0	0	0	0	5	0	0	10
P. nordicum	7*	0	0	0	0	0	0	0	1	0	6	0	85·7
P. solitum	15	0	0	0	0	0	0	0	0	0	0	15	100

*Nine *Aspergillus* and three *P. nordicum* isolates did not yield any spectra that fulfilled the selection criteria and were hence omitted from the table. Grey shades indicate closely related species (taxonomic sections).

Table 3 FTIR classification of mould strains isolated in 2016 using the database based on strains from 2014, 2015 and historical isolates

	Identified by FTIR													
	# isolates	# scannings	*Aspergillus sp.*	*P. chrysogenum*	*P. brevicompactum*	*P. olsonii*	*P. corylophilum*	*P. decumbens*	*P. expansum*	*P. commune*	*P. crustosum*	*P. nordicum*	*P. solitum*	Compliance %
Identified by morphology and DNA seq.														
Aspergillus sp.	**6**	18	**18**	0	0	0	0	0	0	0	0	0	0	100
P. chrysogenum	2	11	0	**11**	0	0	0	0	0	0	0	0	0	100
P. brevicompactum	8	43	0	0	**16**	17	0	0	0	0	0	0	10	37·2
P. olsonii	5	6	0	0	0	**0**	0	0	0	0	0	0	6	0
P. corylophilum	2	11	0	0	0	0	**11**	0	0	0	0	0	0	100
P. decumbens	1	6	0	0	0	0	0	**6**	0	0	0	0	0	100
P. expansum	1	6	0	0	0	0	0	0	**0**	0	6	0	0	0
P. commune	2	7	0	0	0	0	0	0	0	**4**	3	0	0	57·1
P. crustosum	1	6	0	0	0	0	0	0	0	6	**0**	0	0	0
P. nordicum	6	15	0	0	0	0	0	0	0	0	0	**15**	0	100
P. solitum	10	59	0	0	0	0	0	0	0	0	0	11	**48**	81·4

Bold types indicate screenings that were identified by traditional methods and FTIR; Grey shades indicate closely related species (taxonomic sections).

Table 4 Collective classification of selected isolates from 2016 by use of all successful single screenings

	Identified by FTIR												
	# isolates	*Aspergillus sp.*	*P. chrysogenum*	*P. brevicompactum*	*P. olsonii*	*P. corylophilum*	*P. decumbens*	*P. expansum*	*P. commune*	*P. crustosum*	*P. nordicum*	*P. solitum*	Compliance %
Identified by morphology and DNA seq.													
Aspergillus sp.	**6***	**3**	0	0	0	0	0	0	0	0	0	0	100
P. chrysogenum	2	0	**2**	0	0	0	0	0	0	0	0	0	100
P. brevicompactum	8	0	0	**3**	3	0	0	0	0	0	0	2	37·5
P. olsonii	5*	0	0	0	**0**	0	0	0	0	0	0	1	0
P. corylophilum	2	0	0	0	0	**2**	0	0	0	0	0	0	100
P. decumbens	1	0	0	0	0	0	**1**	0	0	0	0	0	100
P. expansum	1	0	0	0	0	0	0	**0**	0	1	0	0	0
P. commune	2	0	0	0	0	0	0	0	**1**	1	0	0	50·0
P. crustosum	1	0	0	0	0	0	0	0	1	**0**	0	0	0
P. nordicum	6	0	0	0	0	0	0	0	0	0	**6**	0	100
P. solitum	10	0	0	0	0	0	0	0	0	0	2	**8**	80·0

*Three *Aspergillus* and four *P. olsonii* isolates did not yield any spectra that fulfilled the selection criteria and were hence omitted from the table. Grey shades indicate closely related species (taxonomic sections).

moulds in the production of dried and cured meat products. This study shows that FTIR has a potential as a monitoring method for spoilage moulds. Previous studies have shown the potential of the method to identify moulds; however, these studies have focused on the general applicability of the method and not specifically on one type of sampling environment. The mycobiota in the production of dried and cured meat products is composed of numerous mould species that are closely related, yet influence the quality and safety of the product in very different ways. While previous studies have shown that FTIR manages to separate various genera, and also certain species within these genera (Shapaval *et al.* 2010, 2013; Lecellier *et al.* 2014), no comprehensive study has addressed the challenge of separating and identifying numerous closely related species within the same environment.

The FTIR method showed good results for *P. solitum* (100% in the initial model, 80% for unknown samples) and *P. nordicum* (85·7 and 100%, respectively). Both of these species have previously been shown to be major spoilage organisms for dried cured meat products in Norway (Schirmer *et al.* 2018). FTIR managed to 'identify' the major spoilage moulds and separate them from typically airborne moulds like *P. brevicompactum* that do not pose any problem for the product. This shows the potential for this method to be used as a monitoring method for spoilage moulds. Some false positives were detected (*Penicillium* species that were incorrectly identified as *P. solitum* or *P. nordicum*); however, very few false negatives were detected in that very few *P. solitum* or *P. nordicum* isolates were incorrectly classified.

Screenings that gave incorrect classifications were distributed between various isolates; there were no indications that particular isolates within a species were more prone to misclassification than others. Misclassifications rather seem to be random and the result of technological variations between screenings.

Results of the study showed an overall compliance of FTIR with the traditional methods of 94·4%. This is lower than reported by Lecellier *et al.* (2014) (98·6%), but comparable to results obtained by Shapaval *et al.* (2013) (93·4%). However, both these studies included various genera and fewer closely related species and may hence pose a smaller challenge for the analyses.

For identification of single isolates, FTIR alone may not be sufficiently accurate. However, our results indicate that this method would be sufficient to identify relative increases or decreases in prevalence of specific spoilage organisms in an environment, even when allowing for 5% misclassifications, as long as sufficiently large numbers of air samples are taken.

Traditional identification of food-associated fungi is time-consuming and dependent on expertise. Partial

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sequencing of ITS rDNA is commonly used as an alternative for identification of fungi (Schoch *et al.* 2012). However, the resolution is insufficient for species identification of *Penicillium* and other genera (Samson *et al.* 2004a).

Dried and cured meat products are generally stored at the production facility for prolonged time during production. It is hence not crucial that the results of every analysis are ready the day after sampling. However, in order to monitor the production environment and quickly implement preventive measures if levels of spoilage mould should increase, it is preferable to use time-efficient methods.

As for other methods, including the polyphasic approach based on morphological properties (Frisvad and Samson 2004) and partial DNA sequencing, a pure culture of the mould is required. This commonly takes at least 5 days to obtain. Thereafter, a 2-day period of incubation in liquid medium is needed to acquire sufficient mycelium for analyses.

If a pure culture is present on the product, one might skip the purification step, or at least do it separately for confirmation. In this case, the time from sampling to result may be reduced to 2 days; however, it is not possible to ascertain the purity of a culture on the product without culturing on solid growth media. A potential application of FTIR may be in combination with traditional air sampling. Air sampling on solid media is routinely carried out to monitor total mould counts in the production environment and may yield pure cultures on agar plates. From these, material may be picked for FTIR analyses and give a result within 2 days.

One drawback of the FTIR method is the relatively high amount of failed screenings (15·7% of all screenings). It was noticeable that the number of failed screenings varied greatly from species to species, with highest failure rates for *Aspergillus* spp. (39·9%), *P. nordicum* (38·0%) and *P. solitum* (16·9%), while failure rates were negligible for other species such as *P. crustosum* (0%), *P. corylophilum* (0·9%) or *P. brevicompactum* (2·8%): This indicates that the method may be vulnerable to varying amounts of mycelium produced by various species in liquid medium under the selected conditions, and optimal conditions may have to be identified for each species individually. Also, in order to obtain enough successful screenings, several technical replicates are necessary. All experiments were carried out by the same person and it was noticeable that the number of failed screenings was highest in the first runs, indicating that training of the laboratory personnel increased the quality of the spectra.

In conclusion, the described method is capable of processing large amounts of samples in a relatively short amount of time. The results are sufficiently accurate to detect periodical increases in the occurrence of specific problematic moulds; however, the method is not by itself suitable for identifying single isolates. For this purpose, additional methods need to be applied. In order for FTIR to be implemented in the industry, there is still work to be done in the automation of the method and growth conditions for liquid cultures need to be standardized and adapted to specific environments depending on the species to be detected.

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Author contributions

Bjørn C. Schirmer: Writing of manuscript, experimental design, sample collection, traditional strain identification, supervision of master student. Kristian Hovde Liland: Data analysis, method development and selection, contributions to manuscript writing. Lene Øverby: Sample collection, FTIR analysis. Ida Skaar: Project management, experimental design, sample selection, traditional strain identification, contributions to manuscript writing. Cathrine F. Kure: Experimental design, supervision of master student, contributions to manuscript writing.

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