Physiological Characterization of Common Fungi Associated with Cheese.

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ABSTRACT

A multivariate statistical method (PLS) was used for a physiological characterization of fungi associated with the cheese environment. The combined effects of pH, salt content, oxygen and carbon dioxide levels on growth and sporulation were studied. Significant factors affecting growth were salt content, level of carbon dioxide and temperature. Interactions between those factors were illustrated and showed that some fungi were especially sensitive to carbon dioxide levels and salt content, at low temperature. Sensitivity to the factors often was more pronounced in the early growth phase. Results may aid in eliminating unwanted fungal growth during cheese production.

Key Words: fungi, cheese, physiology, chemometrics

INTRODUCTION

FUNGAL STARTERS PROVIDE CHEESES WITH CHARACTERISTIC APpearance, consistency and flavors. Shelf-life may be prolonged due to the protective effect of the starters to inhibit growth of unwanted microorganisms. The fungi used in the production of mold fermented cheeses include *Penicillium camemberti* in the production of Camembert and Brie cheese, and *P. roqueforti* in the production of blue-veined cheeses. The yeast-like fungus *Geotrichum candidum* is used as a starter in the manufacture of Camembert cheese, and is important in the ripening of some semi-hard cheeses as part of the bacterial surface smear (Philipp and Pedersen, 1988).

Microorganisms other than cheese starters can colonize and grow well on cheeses. Fungi have a slower growth rate than bacteria and yeast, and they will often diminish in direct competition. However, on the cheese surface, especially during ripening, the water activity is lowered due to drying. Thus, a microenvironment is created which enhances growth of fungi and they may become dominant (Stadhouders, 1975).

Fungal contaminants on cheeses have been isolated and identified as species of *Penicillium* (Lund et al., 1995; Taniwaki and van Dender, 1992; Kivanc, 1990; Bullerman, 1980). Studies about the most dominant species have not always agreed, Lund et al., (1995) using the taxonomic system of Frisvad and Filtenborg (1989), found that *P. commune* (42%) was the most common contaminant on cheese. Other species such as *P. nalgiovense*, *P. verrucosum*, *P. solitum* and *P. roqueforti* were less frequently found but were included in the associated mycobiota of cheese. Several of these species produce mycotoxins.

Unwanted fungal growth on cheeses may be reduced by strict hygienic standards, by pure starter cultures, by control of cheese smear contamination and by packaging under modified atmospheric compositions, but the problem is common and recurring. In order to control growth of fungal contaminants, knowledge about the effects of environmental factors on fungal development and colonization is needed. The main environmental factors influencing fungal growth in and on cheeses are temperature, pH, salt content and concentration of oxygen and carbon dioxide (Stadhouders, 1975). Much work has been reported to characterize development of fungal starters, however, information on contaminant fungi is scarce or lacking.

Our objective was to investigate the combined effects of pH, salt content, temperature, oxygen and carbon dioxide on growth and sporulation of seven common fungi associated with cheese. A chemometric method (multivariate statistical method) was used for the physiological characterization of the fungi and for evaluating possible interaction effects of the tested factors on growth and sporulation.

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MATERIALS & METHODS

Microorganisms and preparation of inoculum

Organisms were obtained from the Culture Collection at the Department of Biotechnology, Technical University of Denmark and included *Geotrichum candidum* (no. 7644), *Penicillium camemberti* (no. 15441), *P. caseifulvum* (no. 15151), *P. commune* (no. 10253), *P. nalgiovense* (no. 12105), *P. roqueforti* (no. 12845) and *P. verrucosum* (no. 13045). The selected species represent common cheese starters or contaminants (Philipp and Pedersen, 1988; Lund et al., 1995).

Conidial suspensions were prepared by spreading dried spores on Czapek Yeast autolysate Agar (CYA, (Pitt, 1979)), modified in accordance with Samson et al. (1995). After growth for 7 days at 25°C, cultures were transferred to fresh media and reincubated for another 7 days. Then conidia were suspended in 1.5 mL spore solution containing 0.5% agar and 0.5% Tween 80 to a final concentration of 10^6 conidia per mL.

Experimental design

The experiment was carried out as a response surface design using a statistical program (MODDE version 3.0, UMETRI AB, Umeå, Sweden). A central composite circumscribed (CCC) design with 4 starpoints and 4 centerpoints (Box and Draper, 1987) was used. This design resulted in 12 media (Table 1) which were incubated under each of 12 atmospheric compositions (Table 2). The experiments were carried out at 10 and 20°C.

The factors and levels of each factor included levels of pH in the range (4–8) and salt content in the range (0-10% NaCl) adjusted in the media and levels of O_2 in the range (4–19%) and CO_2 in the range (1–25%) in balance with N_2 in the incubation atmosphere. The values of pH and NaCl were selected based on levels most common in cheese (Shaw, 1993; Tamime, 1993). Levels of oxygen and carbon dioxide were determined by the limits of the incubator. In the

Table 1–Experimental design of media[a]

Media no.	pH	% NaCl
1	5	5
2	5	0.25
3	7	5
4	7	0.25
5	4	0.5
6	8	0.5
7	6	10
8	6	0
9	6	0.5
10	6	0.5
11	6	0.5
12	6	0.5

[a]Each fungus was inoculated in 3-points on 12 media. The media were incubated under the various atmospheric compositions (see Table 2). Media no 9–12 represent centerpoints which were used for calculation of standard deviations.

Table 2–Experimental design of Controlled Atmosphere-conditions (CA-con) created in the incubators[a]

CA-con.	Oxygen	Carbon dioxide
1	4	5
2	16	5
3	4	25
4	16	25
5	4	15
6	19	15
7	10	1
8	10	25
9	10	15
10	10	15
11	10	15
12	10	15

[a]Together with Table 1 this design comprises the entire experimental setup which resulted in (12 × 12) coloumns × 4 rows (pH, salt content, levels of O_2 and CO_2) giving the *X* matrix in the PLS-analysis

package of several cheeses, however, levels of oxygen and carbon dioxide were within the tested values (Haasum and Nielsen, 1996).

Media

The medium had the following composition (g): casein, 100; lactate (90%), 8.3; lactose, 7.9; $CaCl_2 \cdot 2H_2O$, 7.3; $MgSO_4 \cdot 7H_2O$, 2.6; agar, 20; $FeSO_4 \cdot 7H_2O$, 0.025; $CuSO_4 \cdot 5H_2O$, 0.004; unsalted butter, 250 and water to a total weight of 1kg. To avoid problems with acid hydrolysis of the agar and Maillard reactions butter, casein, agar, $CaCl_2 \cdot 2H_2O$ and water (total weight 600 g) were autoclaved in a bottle. Lactate, lactose, $MgSO_4 \cdot 7H_2O$, NaCl, trace metal solutions and water (total weight 400g) were simultaneously autoclaved in another bottle in which pH was adjusted. After autoclaving, the two bottles were combined and the media were poured into 9 cm Petri dishes.

This medium was developed in our laboratory especially for growth of cheese-associated fungi. By the use of chemometric methods this medium was optimized and found to resemble cheese regarding performance of fungal growth (Hansen and Nielsen, 1997). The salt content of the media was adjusted by adding NaCl (w/v) in concentrations from 0–10%. Initial pH was adjusted by adding 1M HCl and 1M NaOH.

Inoculation and incubation conditions

All media were three points inoculated with conidial suspensions of the fungi and incubated in duplicate. The experiments were carried out in a Tri-Gas Incubator (Dual Chamber model 3319, Forma Scientific, Inc., OH) with external cooling and automatic control of O_2 (4–20%), CO_2 (0–25%), at a constant flow rate of 1.5 mL/s. The temperature was 10° or 20°C.

Growth examinations

All media were incubated in two incubators (A and B). Plates were taken from A, examined and reincubated after 7 or 10 days. The plates in incubator B were kept uninterrupted. After 14 days the experiment was terminated and plates from incubator A and B were examined. The colony diameter was measured and onset of sporulation was visually observed as color development. No significant difference between plates from the two incubators (A and B) was observed. Sporulation at 10°C was very slow, therefore, plates from incubator B were held in the incubator another week and sporulation was observed after 21 days.

Data analysis

Growth and sporulation data were analyzed by partial least squares (projection on latent structures) (PLS) regression (Wold et al., 1984) using *SIMCA-P for Windows (version 2.1,* 1995, Umetri AB, Sweden). PLS is a multivariate method closely related to principal component analysis (PCA). In PLS two matrices *X* and *Y* are related to each other so that the information in *Y* has an influence on the bilinear decomposition of *X* into a low number of latent components (PLS-components). The modelling procedure is carried out in order to balance improvement in fit and increase in model variation (Höskuldsson, 1996). Two advantages of PLS compared with multiple linear regression are that results can be presented in objects and latent variable plots as in PCA and the number of variables can exceed the number of objects. PLS has been used for multivariate calibration but is also useful for evaluation of experimental designs (Martens and Næs, 1989). In this work the *X* matrix consisted of the CCC design data (Table 1 and 2) and resulted in 12 × 12 rows and 4 columns (pH, salt content and levels of O_2 and CO_2 in the incubation atmosphere). The *Y* matrix consisted of colony diameter measurements of the 7 fungi and resulted in 12 ×12 rows and 7 columns. The number of experiments carried out at each temperature was consequently 1008 (12 × 12 × 7). All variables were standardized to mean 0 and variance 1 prior to PLS-analysis.

RESULTS & DISCUSSION

GROWTH DATA AT 10° AND 20°C FOR ALL TESTED FUNGI WERE combined in a data matrix and PLS-analysis was performed. The growth responses after 7 days incubation were markedly different from responses after 10 and 14 days, which were similar. Therefore, the models were based on data after 7 and 14 days incubation.

Effects of the environmental factors on fungal growth.

First the data were analyzed using only linear terms (temperature, pH, salt content, O_2 and CO_2). After 7 and 14 days the first PLS-component explained 76.9 % and 82.4%, and the second 1.0% and 2.0% respectively, of the total variation in the growth data (R^2 = 0.78 and 0.84, predictive power of model, Q^2 = 0.77 and 0.84, respectively). However, by including cross-terms (quadratic and interaction) model predictions were improved (R^2 = 0.89 and 0.92 and Q^2 = 0.88 and 0.91, respectively). This model improvement emphasized the importance of a multifactor approach in the design. Interaction effect of environmental factors on growth would not be explained by traditional one factor trials.

Cross validation showed that two PLS-components should be used to obtain an optimal model for growth data of the 7 fungi. Loading plots of growth data for component 1 and 2, after 7 and 14 days were compared (Fig. 1 and Fig. 2). The most significant factors describing growth of fungi after 7 and 14 days were CO_2, NaCl content in the medium, and incubation temperature (Te). Initial pH of the media had some effect whereas the effect of O_2 was slight. The interaction terms between the most significant factors (NaCl*CO_2, NaCl*Te and Te*CO_2) exhibited a significant effect on fungal growth. All other cross-terms were situated in the center of the plot and were consequently not shown in the figures. The fungi were positioned in the plot according to physiological responses to tested factors

Effect of carbon dioxide and temperature

We found that *P. roqueforti*, *P. commune* and *P. camemberti* changed position in the plots (Fig. 1, 2). Positions of the factors were very similar, although, the interaction between temperature and carbon dioxide (Te*CO_2) was more pronounced in one (Fig. 1). These observations were confirmed by results shown in Tables 3 and 4 which show the inhibitory effects of temperature or carbon dioxide and their interactions on colony diameter after 7 (Table 3) or 14 days (Table 4). All fungi were more strongly inhibited by reducing temperatures from 20° to 10°C than increasing levels of CO_2 from 5 to 25%, irrespective of incubation time. After 7 days incubation at 10°C with increased levels of CO_2, growth of *G. candidum*, *P. roqueforti*, *P. verrucosum* and *P. nalgiovense* was completely inhibited. At 20°C, increasing levels of CO_2 only resulted in 47.3, 13.9, 49.5 and 62.3% inhibition, respectively. *P. commune* also showed great sensitivity to

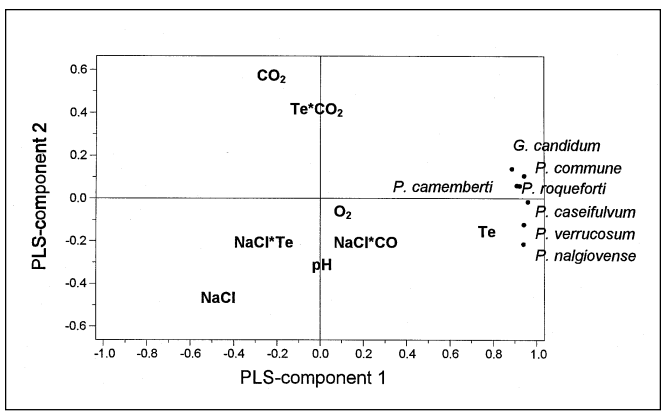

Fig. 1—Relationship of pH, NaCl, O_2, CO_2, temperature (Te) and cross-terms on fungal growth after 7 days incubation, using partial least square (PLS) regression. Fungi located close to each other in the plot respond in a similar way to changes in tested factors. Factors located far from the origin have a significant effect on fungal growth. If a factor is located opposite a fungus there exists a direct negative correlation between level of the factor and growth response of the fungus.

Table 3—Colony diameter[a] and inhibition of growth (%) as affected by increased CO_2 levels (5–25%) or decreased temperature (20–10°C) after 7 days of incubation

Species	*G. candidum*			*P. camemberti*			*P. caseifulvum*		
Temperature	**5% CO_2**	**25% CO_2**	**% inhibition**	**5% CO_2**	**25% CO_2**	**% inhibition**	**5% CO_2**	**25% CO_2**	**% inhibition**
20°C	20.5[b] ± 2.9[c]	10.8 ± 1.0	47.3	17.0 ± 0.8	6.0 ± 3.4	64.7	26.8 ± 1.0	15.3 ± 1.9	43.7
10°C	4.3 ± 1.0	0.0	100.0	5.0 ± 0	1.5 ± 1.7	70.0	10.0 ± 0.0	4.3 ± 0.5	57.0
% inhibition	79.0	100.0	—	70.6	75.0	—	62.7	71.9	—
Species	*P. commune*			*P. nalgiovense*			*P. roqueforti*		
20°C	22.0 ± 0.8	15.0 ± 1.4	31.8	22.0 ± 0.0	8.3 ± 0.5	62.3	32.5 ± 3.1	28.0 ± 4.6	13.9
10°C	6.3 ± 1.0	0.5 ± 1.0	92.1	5.3 ± 0.5	0.0	100.0	3 ± 0	0	100
% inhibition	71.4	96.7	—	75.9	100.0	—	90.8	100	—
Species	*P. verrucosum*								
20°C	18.8 ± 1.0	9.5 ± 0.6	49.5						
10°C	6.0 ± 0.8	0.0	100.0						
% inhibition	68.1	100.0	—						

[a]Colony diameters measured on medium 9–12 (pH = 6, NaCl = 0.5%; centerpoints, Table 1) under condition 2 and 4 (O_2 = 16%, Table 2)
[b]Mean of four colony diameters (mm).
[c]Standard deviation (four replicates) within experiments. Centerpoints (Table 1) were used for calculation of standard deviations.

Table 4–Colony diameter[a] and inhibition of growth (%) as affected by increased CO_2 levels (5-25%) or decreased temperature (20-10 °C) after 14 days of incubation

Species	*G. candidum*			*P. camemberti*			*P. caseifulvum*		
Temperature	**5% CO_2**	**25% CO_2**	**% inhibition**	**5% CO_2**	**25% CO_2**	**% inhibition**	**5% CO_2**	**25% CO_2**	**% inhibition**
20 °C	43.0 ± 3.6	24.5 ± 5.7	43.0	34.0 ± 0.8	19.3 ± 2.5	43.2	51.0 ± 2.6	32.0 ± 3.9	37.3
10 °C	11.5 ± 1.9	7.5 ± 0.6	53.3	15.0 ± 0.0	8.0 ± 0.0	46.7	24.5 ± 0.6	14.0 ± 0.0	42.9
% inhibition	73.3	69.4	--	55.9	58.5	--	52.0	56.3	--
Species	*P. commune*			*P. nalgiovense*			*P. roqueforti*		
20 °C	43.0 ± 1.6	34.3 ± 2.2	20.2	43.3 ± 2.6	22.0 ± 2.0	49.2	59.8 ± 2.7	60.0 ± 4.1	-0.3
10 °C	18.5 ± 1.3	10.0 ± 0.8	46.0	15.0 ± 0.0	3.8 ± 0.5	74.7	20.8 ± 1.0	14.0 ± 0.0	32.7
% inhibition	57.0	70.9	--	65.4	82.7	--	65.2	76.7	--
Species	*P. verrucosum*								
20 °C	39.3 ± 3.4	19.5 ± 2.5	50.4						
10 °C	16.5 ± 0.6	5.0 ± 0.0	69.7						
% inhibition	58.0	74.4							

[a]Colony diameters measured on medium 9–12 (pH = 6, NaCl = 0.5%; centerpoints, Table 1) under condition 2 and 4 (O_2 = 16%, Table 2).
[b]Mean of four colony diameters (mm).
[c]Standard deviation (four replicates) within experiments. Centerpoints (Table 1) were used for calculation of standard deviations.

elevated CO_2 at the low temperature after 7 days incubation. Inhibition at 10°C was 92.1% and 31.8% at 20°C (Table 4). *P. camemberti* and *P. caseifulvum* were only slightly more inhibited by elevated CO_2 at 10° compared to 20°C.

After 14 days incubation the inhibitory effects of both factors (temperature and CO_2) were less pronounced than those after 7 days. The fungi which showed marked sensitivity to CO_2 at decreased temperature after 7 days incubation adapted very well to the environmental stress after 14 days.

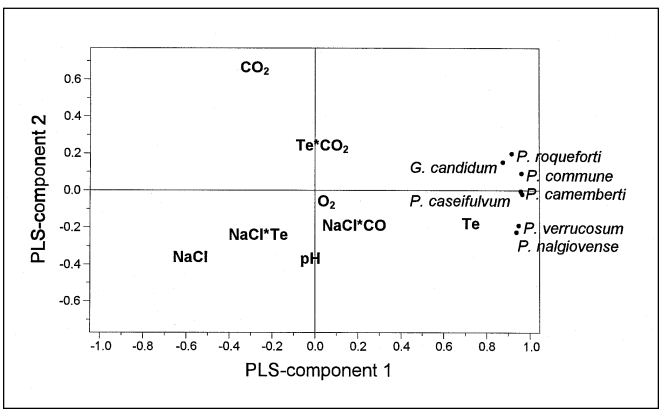

Fig. 2—Relationship of pH, NaCl content, O_2, CO_2, temperature (Te) and cross-terms on radial growth after 14 days incubation, using partial least square (PLS) regression.

The sharp effect of carbon dioxide after 7 days incubation on growth of *P. verrucosum* and *P. nalgiovense* was somewhat expected. The effect on growth of *P. roqueforti* and *G. candidum* was, however, unexpected since both fungi are very resistant to elevated carbon dioxide and reduced oxygen (Magan and Lacey, 1984; Wells and Spalding, 1975). Our results indicate that these two fungi, along with *P. commune* were more sensitive in the lag or early growth phase to elevated levels of CO_2.

These findings confirmed results by Magan and Lacey (1984) that the lag time of *P. roqueforti* was greatly increased at low a_w (0.85) in combination with reduced oxygen or increased CO_2. Also the effect was more pronounced at 14° compared to 23°C. They concluded that fungi could tolerate decreased O_2 and increased CO_2 concentrations, but growth initiation was delayed, especially at low a_w, and could be further inhibited at low pH or temperature. Our results demonstrated that decreased temperature had a significant effect on growth initiation but pH (4–8) showed no significant effect on either growth or growth initiation on the fungi.

P. commune is the most widespread and most frequently occurring contaminant on hard, semi-hard and semi-soft (Hocking 1994; Lund et al., 1995). On the basis of antigenic characterization (Polonelli et al., 1987), the fungus was characterized as the wild-type ancestor of *P. camemberti*. These two closely related species showed distinct differences in responses to the factors tested. *P. camemberti* seemed to be better adapted to growth at 10°C and did not show the

same reduced sensitivity to elevated CO_2 when the temperature was increased to 20°C.

P. caseifulvum was isolated from blue cheeses and it displayed the greatest resistance to elevated CO_2 after 7 days growth at 10°C (Table 3). As the fungus may compete with *P. roqueforti* in and especially on blue-cheeses, it is very important to make sure that conditions favor growth of the starter and not the contaminant i.e. careful piercing of the cheese to allow escape of CO_2. Blue cheeses are kept at 10–12°C during ripening (Shaw, 1993), and our results showed that this temperature was more beneficial for the contaminants than for the starter. The starter of blue cheese, *P. roqueforti*, was more sensitive to elevated levels of carbon dioxide after 7 days at 10°C than the two blue cheese contaminants, *G. candidum* and *P. caseifulvum* (Table 3). At 20°C *P. roqueforti* was more resistant than all other fungi to elevated carbon dioxide (Table 3). Thus, by increasing temperature the first days of ripening the starter may gain an advantage.

Effect of salt content and pH

Salt content and to a lesser extend pH in the media had a significant effect on growth of the fungi. The physiological characteristics of growth as affected by NaCl and pH were shown by contour plot of 3 representative fungi: *P. roqueforti*, *P. camemberti* and *P. verrucosum* after 7 (Fig 3A, B and C) and 14 days incubation (Fig. 4A, B and C).

The positions of *G. candidum* and *P. roqueforti* (Fig 1 and 2) indicate that NaCl and pH had a stronger inhibitory effect than CO_2 on growth . *P. roqueforti* was inhibited by NaCl and pH (Fig. 3A and 4A) the effect was especially pronounced after 7 days of incubation. This indicates that lag or early growth phase of *P. roqueforti* not only showed greater sensitivity to CO_2 and temperature but also to NaCl concentrations. Furthermore, *P. roqueforti* showed great sensitivity to pH at NaCl concentrations < 4%.

P. camemberti is well adapted for growth on the cheese surface and was only moderately affected by NaCl concentrations. *P. commune* and *P. caseifulvum* showed much the same response to NaCl concentrations as *P. camemberti*. However *P. commune* showed some sensitivity to high pH (> 6.5) at low NaCl (< 2%) after 14 days incubation.

P. verrucosum is a contaminant on stored grain (Frisvad, 1995) and is well adapted for growth on cheeses as well (Lund et al., 1995). In the PLS plot, the fungus was grouped with *P. nalgiovense*, which is used as a commercial starter in the production of sausage and has a high NaCl tolerance (Philipp and Pedersen, 1988).

In 1913 Thom and Currie showed that *P. roqueforti* was more capable than 25 other species to grow under elevated carbon dioxide, and reduced oxygen. They concluded that this ability enabled *P. roqueforti* to colonize several cheeses (Gorgonzola and Stilton) as an almost pure culture, even though it was not added as starter. Pure fungal starter cultures are used in most cheese making but contamination of blue-veined cheeses occurs especially by *G. candidum*, *P. camemberti*, and *P. verrucosum* (de Boer and Kuik, 1987). *G. candidum* and *P. camemberti* do not pose health hazards and are used as fungal starters in other cheese products. However, *G. candidum* may represent an unwanted and very competitive contaminant inside the cheese as it responded in much the same way to the tested factors as *P. roqueforti* (Fig 1, 2). *P. verrucosum* is a potential producer of mycotoxins (Frisvad, 1995; Fukal, 1990) and efforts should be made to eliminate this species from cheeses.

Blue cheeses are heavily salted with mean concentrations for mature cheeses ranging from 3-5%. As the cheeses are normally salted by surface application of dry salt, the salt content throughout the cheeses differs markedly. It has been reported that growth of *P. roqueforti* is absent or low in the outer regions of blue cheese due to localized high salt concentrations (Morris, 1964). This gives an op-

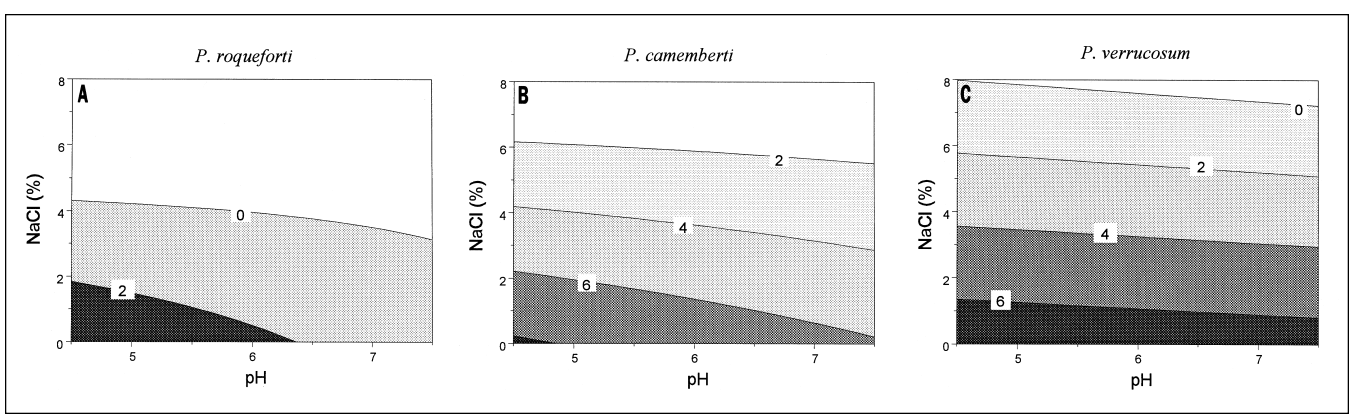

Fig. 3A, B and C—Contour plot showing growth (colony diameter, mm) as affected by pH and NaCl after 7 days of incubation. Constants O_2: 16%, CO_2: 1% at 10 °C.

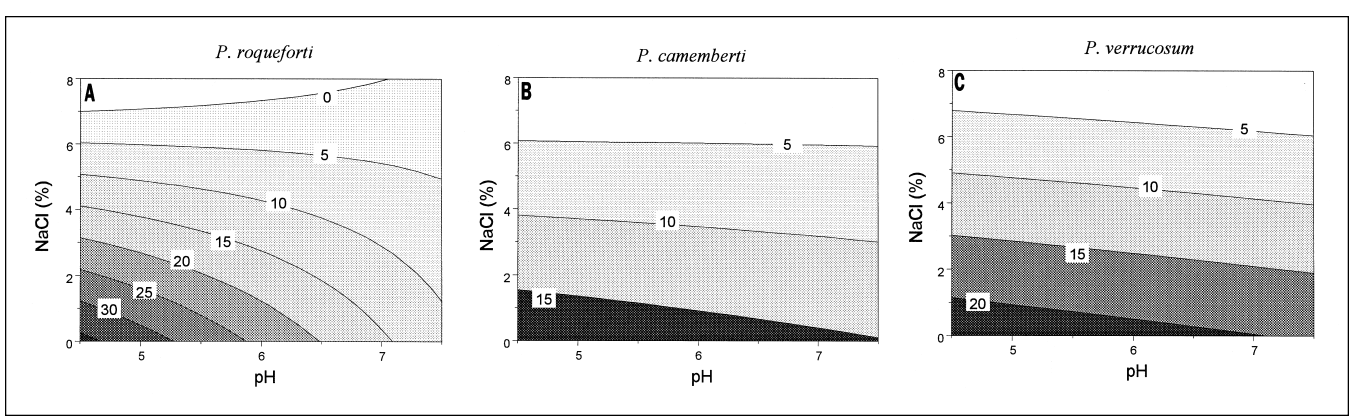

Fig. 4A, B and C—Contour plot showing growth (colony diameter, mm) as affected by pH and NaCl after 14 days of incubation. Constants O_2: 16%, CO_2: 1% at 10°C.

Table 5–Time until sporulation (21 days) as affected by NaCl contents under two different atmospheric compositions (ca-condition 2 and 4; oxygen: 16%; Incubation temp, 10°C

	P. roqueforti		*P. commune*		*P. caseifulvum*		*P. nalgiovense*		*P. verrucosum*	
NaCl (%)	**5% CO_2**	**25% CO_2**	**5% CO_2**	**25% CO_2**	**5% CO_2**	**25% CO_2**	**5% CO_2**	**25% CO_2**	**5% CO_2**	**25% CO_2**
0	10	13	13	21	21	21	13	ND	13	ND
0.25	10	13	13	21	21	21	13	ND	13	ND
0.5	10	13	13	21	21	21	13	ND	13	ND
5	ND[a]	ND	21	ND	21	ND	21	ND	21	ND
10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a] Not detected. Sporulation not detected within the time of incubation, 21days.

portunity for growth of more salt resistant fungi such as *P. verrucosum*. The NaCl content in the media has a great effect on a_w but it was not possible to confirm whether the salt acted as a specific inhibitor or retarded growth by decreasing a_w.

Effect of environmental factors on sporulation.

Five fungi (*P. roqueforti*, *P. caseifulvum*, *P. commune*, *P. nalgiovense* and *P. verrucosum*) developed colored conidia, and onset of sporulation was recorded for these species by visual observations. When data from 10° and 20°C were combined, the only significant factor affecting sporulation was temperature. Consequently PLS-analysis were carried out separately at each temperature. As 10°C is the most relevant temperature for cheese production and storage, sporulation data at that temperature are presented.

When PLS-analysis was carried out on sporulation data after 7, 10, 14 or 21 days of incubation at 10°C, 3 significant PLS-components were found. The first component explained 46.3%, the second 16.7% and the third 5.6% respectively, of the total variation in sporulation data ($R^2 = 0.69$, predictive power of the model $Q^2 = 0.63$). As illustrated (Fig. 5), the most significant factors affecting conidia formation of the fungi were CO_2, NaCl, the quadratic terms NaCl*NaCl and CO_2*CO_2 and the cross-products term NaCl*CO_2. The factors pH and O_2 had minor effects on conidia formation. The fungi separated into two groups (Fig. 5), *P. caseifulvum*, *P. roqueforti* and *P. commune* forming the first group which showed greatest sensitivity to NaCl (Fig. 5). The second group consisted of *P. verrucosum* and *P. nalgiovense* which were very sensitive to elevated CO_2 (Table 5). An interaction effect of NaCl and CO_2 on time until conidia formation was also found.

Parameters affecting sporulation of *P. roqueforti* have been studied (Godinho and Fox, 1981), as proper sporulation is very important for the typical bluish-green color of blue-veined cheeses. Sporulation of cheese contaminants is also of great concern as sporulation is the key factor in their proliferation and dispersal.

CONCLUSIONS

THE USE OF CHEMOMETRIC METHODS HELPED PROVIDE KNOWLEDGE about the niche determinants controlling fungal growth in specific habitats. Temperature was a very critical factor in controlling fungal growth on cheese. Low temperature (10°C) may be very important in the production of Camembert cheese and hard cheeses as contaminating mycobiota typically are inhibited at low temperatures. In the production of blue cheeses it may, however, be useful to increase temperature as *P. roqueforti* showed optimal growth and resistance to CO_2 at 20°C. Sensitivity to salt varied but tolerance was more pronounced among the contaminating fungi which may develop on the surface of blue or hard cheeses under conditions of drying. Efforts should be made to control and maintain a humid atmosphere in ripening rooms.

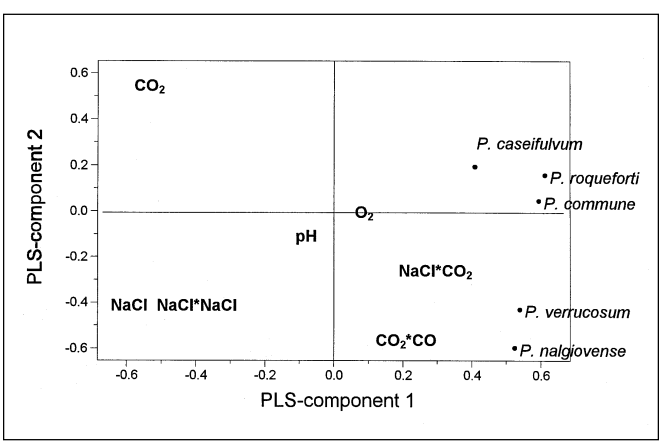

Fig. 5—Relationship of pH, NaCl content, O_2, CO_2 and temperature (Te) on time until sporulation after 21days incubation, using partial least square (PLS) regression.

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