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The effects of environmental conditions on the lipolytic activity of strains of *Penicillium roqueforti*

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Abstract

The lipolytic activity of 30 strains of *Penicillium roqueforti* was investigated by agar diffusion tests on tributyrin (esterase activity) and olive oil agar (lipase activity), by titration of the free fatty acids (FFA) produced and by gas chromatographic analysis of the individual FFA released after growth at 25 or 10°C in butterfat emulsions containing 0, 2 or 7% NaCl. All strains investigated by the agar diffusion tests possessed esterase activity and 23 strains were also able to hydrolyse olive oil, but differences in esterase activity were seen. The agar diffusion tests and the titration of FFA showed that the amount of FFA released by a strain of *P. roqueforti* is determined by both esterase and lipase activity. A large release of FFA was only seen for strains with the ability to hydrolyse both short- and long-chained fatty acids, while strains with esterase activity produced smaller amounts of FFA. Between 7 and 14 days of incubation a steep increase in the release of FFA was observed both by the titration and by GC analysis, and then a decline from 14 to 21 days, probably caused by conversion of FFA to methyl ketones. Identical FFA profiles were found for two strains with different lipolytic activity. Long-chained fatty acids dominated the profile, while the short-chained fatty acids only were detected in small amounts and mainly in the end of incubation. Both strains were stimulated by NaCl in the emulsions.

Keywords: Lipolytic activity; *P. roqueforti*; Free fatty acids; NaCl; Cheese

1. Introduction

Lipolysis in blue cheese is, like proteolysis, more pronounced than in other cheeses (Fox and Law, 1991; Woo et al., 1984) and very important for the production of aroma compounds, e.g. methyl ketones, which are essential for the quality of blue cheese (Gallois and Langlois, 1990; Kinsella and Hwang, 1976; Anderson and Day, 1966). Apart from being precursors for the methyl ketones, the free fatty acids themselves also contribute to the flavour characteristics of blue-veined cheeses. The lipolysis in blue-veined cheeses is mainly caused by the lipases produced by the mould starter culture *Penicillium roqueforti* (Gripon, 1993; Coghill, 1979; Kinsella and Hwang, 1976), but the native milk lipase, which will be present in varying amounts in the

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cheese due to no or moderate heat treatment of the cheese milk, also contributes to the degradation of triglycerides, especially during the early stages of ripening. Yeasts, which can be present in high numbers (van den Tempel and Jakobsen, 1998; Gobbetti et al., 1997), could also have a positive effect by producing aroma compounds like esters. Gobbetti et al. (1997) found that lipolysis in Gorgonzola cheese depended less on the activity from *P. roqueforti* and more on the surface microflora, where yeasts were found at about 7.5 log CFU/g.

P. roqueforti produces two lipases extracellularly, an acidic and an alkaline lipase (Mase et al., 1995; Lamberet and Menassa, 1983b; Menassa and Lamberet, 1982; Lobyreva and Marchenko, 1980; Eitenmiller et al., 1970). The relative importance of the lipases in cheese has not been fully determined. The acidic lipase has a pH optimum which is close to the prevailing pH in blue cheese (Lamberet and Menassa, 1983b), but the alkaline lipase shows the highest activity against butterfat (Menassa and Lamberet, 1982). However, Lamberet and Menassa (1983a) investigated the lipolytic activity of seven French blue cheeses and found that six of these contained more acidic lipase activity than alkaline lipase activity. The specificity of the alkaline lipase against milk fat was determined by Kornacki et al. (1979) who found a marked increase of short chained fatty acids during incubation.

Selecting a strain of *P. roqueforti* for production of blue cheese relies in most cases on experience rather than on a detailed knowledge of the characteristics of the individual strain. Investigations have shown differences in lipolytic as well as in proteolytic activity between strains of *P. roqueforti* (Larsen et al., 1998; López-Díaz et al., 1996; Farahat et al., 1990; Gallois and Langlois, 1990; Stepaniak et al., 1980) and thereby also differences in the formation of free fatty acids (FFA) and aroma compounds (Gallois and Langlois, 1990; Farahat et al., 1990). Differences between strains according to NaCl tolerance, germination and growth rate etc. under different environmental conditions has also been described (López-Díaz et al., 1996; Godinho and Fox, 1981a,b). The microenvironment in blue cheese is characterised by pronounced pH- and NaCl-gradients from the surface of the cheese to the core (Godinho and Fox, 1981b; Gobbetti et al., 1997) which slowly reaches equilibrium during the ripening period. These differences will affect the germination, growth and sporulation of *P. roqueforti* (Cuppers et al., 1997; Godinho and Fox, 1981a,b) and the enzymatic activity of the strains (Godinho and Fox, 1981c, 1982). The low ripening temperature used for blue cheese, approx. 10°C, also influences these parameters. So far, the effect of these conditions on the lipolytic activity of *P. roqueforti* has been scarcely investigated (Alford and Pierce, 1961; Godinho and Fox, 1981c).

The most commonly employed techniques for detecting lipolytic activity of micro-organisms are agar diffusion tests using different dyes and substrates (García-Lepe et al., 1997; Lima et al., 1991; Karnetová et al., 1984), titration of free fatty acids produced on different media (Rapp and Backhaus, 1992; Stepaniak et al., 1980; Castberg et al., 1975) or gas chromatographic (GC) analysis of the free fatty acids or their methyl esters (Alford and Pierce, 1961; Kornacki et al., 1979). Furthermore, GC has been used to study the triglyceride and fatty acid composition of blue cheeses during ripening (Alonso et al., 1987; Madkor et al., 1987; Anderson and Day, 1965). Spectrophotometric determinations on, e.g. *p*-nitrophenyl palmitate have also been applied (Gobbetti et al., 1997; Rapp and Backhaus, 1992).

The purpose of this work was to screen strains of *P. roqueforti* for lipolytic activity by agar diffusion tests on tributyrin and olive oil agar and furthermore to investigate the effect of NaCl, temperature and pH relevant to the environmental conditions found in the Danish blue cheese Danablu on lipolysis by *P. roqueforti* grown in butter fat emulsions.

2. Materials and methods

2.1. Fungi

Initially 30 strains of *P. roqueforti* were investigated for their lipolytic activity by agar diffusion on tributyrin and olive oil agar. The origins of the strains are shown in Table 1. The strains were maintained as stock cultures of dried conidia, produced as previously described (Larsen et al., 1998).

2.2. Agar diffusion test

For the initial screening of lipolytic activity of strains of *P. roqueforti* a standard preparation, as described below, of tributyrin and olive oil agar were

Table 1
Lipolytic activity of 30 strains of *P. roqueforti* measured by agar diffusion test on tributyrin agar (esterase activity) and olive oil agar (lipase activity) after 5 days of incubation at 25°C

Strain of *P. roqueforti*	Origin of the strain	Esterase activity[a]	Lipase
Mauri	Mauri[b]	5.0±0	−
PA	Visby[c]	6.0±0	−
roq 2	AJL[d]	6.5±0.5	+
roq 32	Visby	7.0±0	−
roq 4	AJL	7.0±0	−
fr 1	French blue cheese[e]	8.0±0	−
PJ	Visby	9.0±0	−
roq 19	AJL	9.2±0.2	+
roq 34	Visby	9.5±0	−
fr 7	French blue cheese	9.7±0.2	+
roq 11	AJL	9.8±0.2	−
roq 1	AJL	10.0±0	−
fr 4	French blue cheese	10.0±0	+
roq 18	AJL	10.2±0.2	+
PV	Visby	10.3±0.5	+
roq 8	AJL	10.3±0.5	+
fr 5	French blue cheese	10.7±0.5	+
roq 35	Visby	10.8±0.2	+
roq 5	AJL	11.0±0	+
roq 31	Visby	11.0±0	+
roq 14	AJL	11.3±0.5	−
roq 33	Visby	11.5±0.4	+
CSL	CSL[f]	12.0±0	+
fr 3	French blue cheese	12.0±0	+
roq 36	Visby	12.0±0.8	+
fr 6	French blue cheese	12.0±0.4	+
roq 37	Visby	12.2±0.5	+
fr 2	French blue cheese	12.2±0.2	+
roq 15	AJL	12.7±0.2	+
roq 38	Visby	13.8±0.2	+

[a] The results for esterase activity are expressed as mm clearing zone, and for lipase activity as positive or negative (+: activity, −: no activity). The strains are listed in order of increasing esterase activity. The mean value and standard deviation are reported for triple determinations, carried out on two different occasions.
[b] Mauri Laboratories, Dorset, England.
[c] Laboratorium Visby, Tønder, Denmark.
[d] Alfred Jørgensen Laboratory A/S, Copenhagen, Denmark.
[e] Isolates from French blue cheeses. The remaining strains are commercial cultures for production of Danablu.
[f] Centro Sperimentale Del Latte, Milan, Italy.

used. The method of agar diffusion in tributyrin agar was modified after Lima et al. (1991). Cheese agar was prepared according to Larsen et al. (1998), inoculated with 1 ml of a conidial suspension (10^7 conidia/ml) of *P. roqueforti* and incubated at 25°C for 5 days. At the end of incubation, agar plugs with a diameter of 10 mm were transferred to test tubes with tributyrin and olive oil agar and placed with the mould growth upwards. After 3 days of incubation at 25°C the depth of the clearing zone (tributyrin) or the colour intensity (olive oil) was measured. All tests were repeated on two separate occasions in triplicate and the mean value and standard deviation reported for the tributyrin test.

The tributyrin medium was prepared from 0.5% bactopeptone (Difco, Detroit, MI, USA), 0.3% yeast extract (Difco) and 1.2% bacto agar (Difco), pH 7.0, and autoclaved at 121°C for 20 min. After cooling to 60°C, 0.1% filter sterilised tributyrin (Sigma, St. Louis, MO, USA) was added. The substrate was then emulsified on an Ultra-Turrax (Junke and Kunkel IKA Labortechnic, Staufen, Germany) for 5 min at 13 500 rpm and distributed in 10 ml portions into sterile tubes.

Screening for lipase activity, when carried out on tributyrin is not considered a real lipase assay (Jensen, 1983), and therefore an additional agar diffusion test on olive oil agar with nile blue as indicator was used. For the olive oil agar 90 ml 0.1 M Tris-HCl buffer, pH 7.0, 1.8 g gum arabic (Sigma) and 5 ml 1 M NaN_3 (Sigma) were mixed with 20 ml olive oil and 100 mg Nile Blue (Sigma) on an Ultra-Turrax at 13 500 rpm for 10 min. Then 0.5 ml of this emulsion was mixed in a preheated sterile tube with 3.75 ml of 8.5 g agarose (type HSB, B&B, Rødovre, Denmark) dissolved in 850 ml 0.1 M Tris-HCl buffer, pH 7.0.

2.3. Lipolysis of butter fat emulsions

The butter fat emulsion used as a model system to investigate the lipolytic activity of strains of *P. roqueforti* was prepared in Czapek yeast extract broth (CYA, Difco) prepared with phosphate buffer, 0.2 M. The CYA broth was added to 0, 2 or 7% NaCl (w/v) and gum arabic (Sigma) 10% (w/v). Melted butter fat, 10% (w/v), was added slowly during homogenisation on an Ultra-Turrax at a speed of 24 000 rpm for 4×1 min with 1 min intervals. The emulsions were autoclaved at 121°C for 15 min and pH adjusted to 5.5 using sterile HCl or NaOH. The assay mixture for the titration experiments contained 0.9 ml emulsion and 0.1 ml conidial suspension (10^7 conidia/ml) of *P. roqueforti*. Control emulsions were added to 0.1 ml sterile 50 mM phosphate buffer, pH 6.0. Incubation was at 25°C for 7, 14 and 20 days. Each experiment was carried out

in triplicate. The assay mixture for the GC analysis contained 10 g emulsion and 1 ml conidial suspension (10^7 conidia/ml) of *P. roqueforti*. Control emulsions were added to 1 ml sterile 50 mM phosphate buffer, pH 6.0. Incubation was at 10°C for 7, 14 and 21 days. Each experiment was carried out on two different occasions.

2.4. Analysis of free fatty acids by titration

The strains were grown in butterfat emulsions, pH 5.5, at 25°C with two different concentrations of NaCl to asses lipolysis by titration of FFA. The amount of free fatty acids was determined according to Sørensen and Samuelsen (1996).

2.5. Analysis of free fatty acids by GC

To investigate the effect of NaCl on the production of individual FFA two strains of *P. roqueforti* with different lipolytic activities were grown in butterfat emulsions, pH 5.5, with 0, 2 or 7% NaCl added. This experiment was carried out at 10°C, the ripening temperature normally used for Danablu. FFA were extracted and the composition analysed after 7, 14 and 21 days of incubation. The method for extracting lipid was according to Bligh and Dyer (1959), modified by using equal amounts of methanol (Merck) and chloroform (Merck) in the first step. After the extraction the lipid containing chloroform phase was mixed with water free sodium sulphate (Merck). The chloroform in this lipid extract was evaporated under vacuum and the lipid transferred to tubes with 3×5 ml pentane (Aldrich-Chimie, Steinheim, Germany) and stored at -40°C before separation of the FFA. Separation of the free fatty acids were done according to Kaluzny et al. (1985) using pentane instead of hexane. Heptadecanoic acid (Sigma) was used as internal standard. The samples were evaporated to dryness at ambient temperature and dissolved in 200 μl pentane prior to GC analysis. The samples were analysed on a HP 6890A GC (Hewlett Packard, Birkerød, Denmark) equipped with a flame ionisation detector. The column used was a HP INNOWax, 30 m $\times$ 0.25 mm $\times$ 0.25 μm (HP part no. 19091N-133). The GC-conditions were as follows: injector temperature 250°C, FID temperature 300°C, initial temperature 120°C for 1 min, increasing to 240°C at 10°C/min with a final hold for 17 min. Helium was used as carrier gas at a flow rate of 3 ml/min and injection (2 μl) was in the split-less mode. The FFA were identified by their relative retention times determined by GC of the pure FFA. The fat content of the samples was determined by weighing.

3. Results and discussion

All 30 strains investigated were able to degrade tributyrin, but differences in activity were observed, as can be seen from Table 1. In the test on olive oil agar seven strains did not show any activity. The remaining 23 strains were all lipolytic against olive oil, but the colouring intensity and the diameter of the coloured zone of the indicator varied, and the reaction were recorded as positive or negative according to the colour intensity of the zone (Table 1). The ability of some strains to degrade tributyrin, but not olive oil, which contains a high amount of triolein, corresponds with the findings that *P. roqueforti* lipases show higher activity against short chain fatty acids (Menassa and Lamberet, 1982; Lamberet and Menassa, 1983b; Cerning et al., 1987; Kornacki et al., 1979) while triolein is hydrolysed at a much lower rate (Lamberet and Menassa, 1983b).

Based on the results from the initial screenings nine strains of *P. roqueforti* were selected for further studies. Comparing the results in Tables 1 and 2 the ranking of the strains is not very similar. However, it can be seen that the strains PA and roq 1 had low activity towards tributyrin, none towards olive oil and also produced low amounts of FFA, while the remaining seven strains hydrolysed both tributyrin and olive oil (Table 1) and produced high amounts of FFA (Table 2). This showed that the amount of FFA released is determined by both esterase and lipase activity, and that a large release of FFA is dependant on the ability of the strain to hydrolyse both short- and long-chained fatty acids. Therefore both esterase and lipase activity tests should be applied to achieve an adequate description of the lipolytic activity of strains of *P. roqueforti*.

All nine strains examined by titration of FFA (Table 2), independent of NaCl concentration, produced a steep rise in FFA production from 7 to 14 days of incubation, and then either a slower increase or a plateau in the production of FFA from 14 to 20

Table 2
The total amount of free fatty acids released (μmole FFA/100 g fat) after 7, 14 and 20 days of incubation at 25°C of nine strains of *P. roqueforti* in butterfat emulsions, at pH 5.5 with 0 or 7% NaCl added, the results are an average of two replications, controls subtracted

NaCl % (w/v)	Inc. time (days)	PA	roq 2	roq 19	roq 1	roq 18	PV	roq 5	CSL	roq 15
0	7	146.32	325.41	351.94	156.61	308.06	368.78	256.02	209.59	394.79
0	14	582.14	832.65	1077.55	329.08	946.94	1004.08	1053.06	692.86	1175.51
0	20	824.49	1004.34	1194.13	338.78	1652.81	1041.33	1304.85	1059.69	1170.41
7	7	nd[a]	394.79	454.75	169.28	368.78	429.49	308.06	193.77	238.67
7	14	487.24	1085.72	1224.49	566.33	1322.45	930.61	767.35	851.02	995.92
7	20	807.14	1107.14	1312.75	616.32	1328.57	1206.12	798.72	1162.50	1202.04

[a] Not determined.

days of incubation was seen. Only small differences were observed in the production of FFA after 7 days of incubation between the emulsions containing 0 and 7% NaCl, but after 14 and 20 days of incubation four of the strains, CSL, roq 19, roq 2 and especially roq 1, showed a higher production of FFA when the emulsion contained 7% NaCl. Godinho and Fox (1982) found that accumulation of FFA in experimental blue cheeses seemed to have an optimum at 4 to 6% NaCl. This could explain the observations made in this study that these strains produced higher amounts of FFA in emulsions containing 7% NaCl. The strain PA seemed to be rather salt tolerant, releasing very similar amounts of FFA at the two NaCl concentrations used, while the strain roq 5 clearly was inhibited by 7% NaCl in the emulsion. The remaining strains, PV, roq 15 and roq 18, differed from 14 to 20 days of incubation. Growth of the nine strains was, in general, slower at 7% NaCl, but did not correlate to the production of FFA at any of the NaCl concentrations used (results not shown). The stagnation in FFA production seen from 14 to 20 days of incubation for several of the strains (Table 2) is probably caused by the conversion of FFA to methyl ketones (Godinho and Fox, 1982). After 20 days of incubation the nine strains could be divided into three different groups according to the amount of FFA released: PA and roq 1 released low amounts of FFA, roq 2, PV, roq 5, CSL and roq 15 medium amounts and roq 19 and roq 18 released high amounts of FFA.

The effect of NaCl on the production of individual FFA by two strains of *P. roqueforti*, PA and roq 15, releasing different amounts of FFA (Table 2), is shown in Table 3 (PA) and Table 4 (roq 15). The long chained fatty acids (C12–C18:2) dominated the FFA released from the emulsions. The short chained fatty acids (C4–C10) could only be detected in low amounts and mainly at the end of incubation. This result differs from the observation of Kornacki et al.

Table 3
Amount of individual FFA released by the *P. roqueforti* strain PA at pH 5.5, 10°C, the results are reported as mg FFA/100 g fat, controls subtracted, mean value±standard deviation

	0% NaCl			2% NaCl			7% NaCl		
	7 days	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days
C4	0	0	0	0	0	1.9±0.4	0	0	0
C6	1.3±0	1.05±0.4	0	0	0.8±0	1.3±0	0	1.5±0	3.3±0
C8	0	2.04±0.05	3.5±0	0	1.9±0	0.7±0.02	0	1.9±0	1.5±0.1
C10	0	1.1±0.1	3.8±1.4	0	0	2.8±0	0	0.1±0	0
C12	0	1.8±0	10.1±2	0	0	6.2±0	0	1.5±0	0.3±0
C14	0	11.8±1.05	31.9±11	4.5±0	14±1.1	11.4±1.8	3±0	17.3±1.2	10.5±1.8
C16	7.4±0	24.4±4.5	41.5±16.4	24.1±0	61.5±1.4	45.7±0.6	21.5±2.6	78.2±1.8	55.6±1.5
C18	0	13.4±5	34.1±8.4	1.9±0	27.4±5.4	17.2±0.3	10.9±0.8	34.5±3.5	20.7±0.9
C18:1	11.1±0	29.9±0.05	39.3±21	28±16.8	84.2±1.9	61.3±3	14.2±0.8	94.4±1.7	71.2±7.6
C18:2	12.6±8.7	21.2±5.4	59.5±14.3	11.4±1.8	12.2±0.8	16.5±1.1	13.1±0.6	24±0.9	21.9±0.2
Total	32.4±8.7	106.7±13.5	223.7±28.9	69.9±18.6	201.9±8.4	165±6.5	62.6±3.3	253.4±3.1	184.9±3.6

Table 4
Amount of individual FFA released by the *P. roqueforti* strain roq 15 at pH 5.5, 10°C, the results are reported as mg FFA/100 g fat, controls subtracted, mean value±standard deviation

	0% NaCl			2% NaCl			7% NaCl		
	7 days	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days
C4	1.3±0	7.1±0	10.6±1.8	0	10.2±0	7.1±2.2	0	1.5±0	0
C6	1.7±0	2.6±0.07	14.7±5.2	0	3.1±0.04	9.2±0.9	0	2±0	14.2±1.04
C8	2.3±0	10.9±0.01	13.5±4.9	0.51±0	4.5±0.2	16.2±3.3	0	1.7±0.02	2.3±0.5
C10	4.0±0	21.1±0.3	32.05±11.2	0.13±0	21±4.6	38.2±3.6	0	0.5±0	1.8±0.3
C12	4.5±0	25.8±0.3	43.6±12.8	1.8±0	26.1±3.5	48.7±2.9	0	2±0	4.7±0.3
C14	1.3±0.1	72.8±3.1	108.6±28.4	19.8±0	85.3±3	125.6±8.6	1.1±0.6	13±0.1	27.4±2.7
C16	22.4±9.6	197.1±5.7	289.3±74.8	25.6±0.2	286.5±64.9	346.2±27.3	20.2±0.3	67.7±1.8	153.4±22.9
C18	27.7±6.4	55.8±3.6	92.5±20.9	24.1±6.5	94.6±23.6	98±5.1	12.3±1.7	29.2±2	59.6±18.7
C18:1	28.6±8.1	305.5±11.7	237.5±77.2	33.2±3.9	439.1±74.1	552.6±43.5	23.2±3.3	101.6±0.7	192±3.2
C18:2	21.2±4	40.4±0	51.6±0	17.6±1.2	39±2.7	52.8±4	20.8±11.6	23.6±1.7	50.8±17.2
Total	114.9±7.4	739.1±24.3	893.9±82.8	122.7±9.5	1009.3±160.4	1294.6±91.2	77.6±7.3	242.8±6.3	506.2±62.5

(1979) who found an increase in short chained fatty acids and a simultaneous decrease in long chained fatty acids by *P. roqueforti* lipase in milk fat emulsions. However, results obtained from blue cheeses (Anderson and Day, 1965; Madkor et al., 1987) where a combined effect of milk lipase, lipolytic yeast (van den Tempel and Jakobsen, 1998) and the *P. roqueforti* strain is seen, corresponds to the observations made in the present investigation and supports that *P. roqueforti* is the major agent in lipolysis. Several researchers have also found that the level of FFA in blue cheeses increases during ripening (Gobbetti et al., 1997; Contarini and Toppino, 1995; Gonzalez de Llano et al., 1992; Alonso et al., 1987; Madkor et al., 1987; Vanbelle et al., 1978).

The two strains PA and roq 15 had, on the whole, the same FFA-profile and were both stimulated by NaCl, but their apparent conversion of FFA to methyl ketones are different.

The difference in total release of FFA between the two strains was clearly seen in the emulsions containing 0 and 2% NaCl, even after 7 days incubation (Tables 3 and 4). But with 7% NaCl in the emulsions, after 7 and 14 days of incubation, the PA strain produced almost the same amount of FFA as the strain roq 15. After 21 days the FFA level fell slightly in the PA sample while the roq 15 continued to increase. The same tendency of decreasing amounts of FFA was seen for PA at 2% NaCl and could be due to conversion of FFA to aroma compounds, but the differences from day 14 to day 21 are very small, especially with 7% NaCl, and could also be caused by variations in growth in the duplicate samples and variations in the extraction procedure. Like in the titration experiment the difference in FFA content after 21 days of incubation was not pronounced between the emulsions with 0 and 7% NaCl, but after both 7 and 14 days of incubation the latter contained more FFA than the emulsion containing 0%, and was equivalent to the emulsion with 2% NaCl. After 21 days of incubation the 0% emulsion contained the highest amount of FFA. The small differences in the amount of FFA released by PA at the three NaCl concentrations used, corresponds to the results observed by titration (Table 2) indicating a high tolerance of this strain towards NaCl. In general, the amounts of FFA produced by PA is very small compared to roq 15 and the increase during incubation is not as pronounced. This apparent standstill in the FFA production by PA could be an effect of the low temperature applied, delaying growth and metabolic activity and would maybe change after prolonged incubation.

Concerning roq 15 the content of FFA in the emulsions continued to rise throughout the incubation period. Like in the titration experiment there is a steep increase in FFA production from 7 to 14 days of incubation and then a levelling off between 14 and 21 days which could be due to the formation of aroma compounds. With 7% NaCl in the emulsion the production of FFA was inhibited compared to the

results from the titration assay, but the decrease in FFA production from 14 to 21 days was not as pronounced as in the other emulsions. This is probably due to a delay in growth caused by the combined action of the incubation temperature and the high NaCl content in the emulsion. Supporting this theory is also the very low amount of FFA after 7 days of incubation compared to the emulsions with 0 and 2% NaCl. The largest amount of FFA produced by roq 15 was seen in the emulsion with 2% NaCl, probably caused by a stimulation of the growth, and thereby possibly the production of lipases, by low concentrations of NaCl. This growth stimulating effect has been pointed out by both López-Díaz et al. (1996) and Godinho and Fox (1981a), (1981b).

The use of these strains in Danablu would give cheeses with very different flavour characteristics. Based on the results obtained at 10°C it seems likely that roq 15 would dominate the lipolysis early in the ripening period, while the lipolysis caused by PA would be delayed. This could give the native milk lipase and the non-starter micro-organisms an opportunity to influence the taste and aroma of the final product. The present study has not looked into the formation of aroma compounds by the two strains, e.g. methyl ketones, lactones, secondary alcohols etc., whose amounts are likely to be rather different because of the varying amounts of FFA produced, but this, combined with a more appropriate cheese like media and combinations of a wider range of environmental conditions, would be a suitable research area in the future. Furthermore, studies on the interactions between *P. roqueforti*, the native milk lipase and lipolytic yeast and their individual and combined effect on lipolysis in blue cheese are also needed.

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