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Evaluation of the Proteolytic and Lipolytic Activity of Different *Penicillium roqueforti* Strains

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ABSTRACT

An attempt has been made to evaluate 11 strains of Penicillium roqueforti *for their proteolytic and lipolytic activity as well as their development of blue cheese flavour in an aseptic curd model.*

Aseptic curds made from either fresh cow's milk or recombined milk were individually inoculated with each examined strain of P. roqueforti *and incubated at 25°C for 21 days.*

Inoculation of aseptic curds with each of the tested strains showed remarkable variation in their proteolytic and lipolytic activity at the various stages of incubation. P. roqueforti *strain 6829 Wiesby and* P. roqueforti *strain IMI 173224 each showed higher proteolytic and lipolytic activities than the other examined strains.*

Water-soluble nitrogen, 12% TCA-soluble nitrogen, 5% PTA-soluble nitrogen and Total Volatile Fatty Acids, in aseptic curds individually inoculated with the 11 examined strains, all increased during incubation. The levels of these compounds were higher with the P. roqueforti *strains 6829 Wiesby and IMI 173224 than the others.*

Sensory evaluation showed that P. roqueforti *strain 6829 Wiesby and* P. roqueforti *strain IMI 173224 developed typical blue cheese flavour after 14 and 16 days of incubation, respectively.*

INTRODUCTION

Penicillium roqueforti cultures are selected strains of moulds essentially added either to cheese milk or curd in the manufacture of blue cheese to bring about casein breakdown and fat hydrolysis which leads to the

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development of the unique flavour of the final product. These changes are mainly due to the proteolytic and lipolytic action of the proteinases and lipases produced by *P. roqueforti.*

Several investigators have studied the proteolytic and lipolytic activities of different strains of *P. roqueforti.* They have shown that the examined strains have a wide range of both proteolytic and lipolytic activities. Most of these studies have shown that the use of *P. roqueforti* strains having moderate proteolytic and high lipolytic activities seemed to be most suitable for successful blue cheese manufacture (Niki *et al.*, 1966; Sato *et al.*, 1966; Lambert & Lenoir, 1972; Stepaniak & Habaj, 1972; Stepaniak *et al.*, 1974 and Mosashvili *et al.*, 1978). Therefore the present work was carried out to evaluate the proteolytic and lipolytic activities of 11 imported strains of *P. roqueforti* to select the most suitable strains for blue cheese making.

MATERIALS AND METHODS

Milk

Fresh cow's milk used in blue cheese curd making was obtained from the Misr Milk and Food Company, Mansoura, Egypt.

Low-heat, non-fat dry milk, produced in 1984 in the USA, was obtained from the Misr Milk and Food Company, Mansoura.

Butter oil

Anhydrous milk fat was obtained from the Misr Milk and Food Company, Mansoura, produced in the USA in 1985.

Rennet

A rennet powder, Chr. Hansen's Hala, Copenhagen, was used for making the aseptic curds.

Penicillium roqueforti strains

The following 11 strains of *P. roqueforti* were evaluated.

1.	*P. roqueforti*	6829 Wiesby Laboratorium Wiesby GmbH Co.
2.	Blue mould	Chr. Hansens Laboratorium A/S, Sankt Annae Plads 3DK—1250, Copenhagen K, Denmark.

3.	*P. roqueforti* (Danablu Schimmel)	Chr. Hansen's Laboratorium A/S, Sankt Annae Plads 3 DK—1250, Copenhagen K, Denmark.
4.	*P. roqueforti S.*	VEB Ostra-8020, Dresden, DDR.
5.	*P. roqueforti* PRB 15	Lactolaba, Italy.
6.	*P. roqueforti* Cepp	Cultura selezionata Ceppa, NG.N. Italy.
7.	*P. roqueforti* IMI 24313	On slant agar.
8.	*P. roqueforti* IMI 92261	On slant agar.
9.	*P. roqueforti* IMI 173224	Freeze dried ampoul.
10.	*P. roqueforti* IMI 148775	Freeze dried ampoul.
11.	*P. roqueforti* IMI 129207	Freeze dried vial.

Preparation of *P. roqueforti* inoculum

P. roqueforti spores were grown in a sterilized culture medium according to Pitt (1979) as follows:

malt extract	20 g
peptone	1 g
glucose	20 g
distilled water	1000 g

pH of the medium was adjusted to 5·4 and 20 ml of the medium were transferred into 100 ml conical flasks and autoclaved at 121°C for 8 min. The medium was inoculated with *P. roqueforti* spores and incubated at 25°C for 7 days without shaking (until a thick coat of green spores on the surface of the medium was completed). A loop of each mat of each strain was aseptically transferred to a one litre flask containing 100 ml of malt extract broth medium and incubated at 25°C for 7 days according to King and Clegg (1979).

Preparation of aseptic curd

Fresh cow's milk containing 4% fat or recombined milk of 4% fat and 10% non-fat solids was pasteurized at 71°C/15 s, then cooled to 30°C. The milk was acidified with lactic acid (80% v/v) to bring the pH of the milk to about 5·2. The milk was then renneted with 1% Hansen's rennet solution at a rate of 6 ml/10 kg milk. After complete coagulation, the curd was cut and drained using cheese cloth for 12 h without pressing until the curd contained about 60% moisture (King & Clegg, 1979).

The resultant curd from each type of milk was divided into nine equal parts (500 g). Each part was transferred into a one litre flask containing 20 g NaCl. The curd was well mixed with the salt and autoclaved for 8 min at

115°C. After cooling to room temperature, each flask was aseptically inoculated with *P. roqueforti* strain mycelium at a ratio of 0·5%, shaken well and incubated at 25°C for 21 days with occasional shaking. The same steps were carried out for each examined strain. All experiments were conducted in triplicate.

Samples of inoculated curd were taken periodically when fresh and after 3, 5, 7, 9, 11, 14, 16, 18 and 21 days of incubation at 25°C, respectively. These samples were organoleptically examined and chemically analysed.

Sensory evaluation

Each inoculated curd was organoleptically examined for its flavour intensity using a scale from zero to 8 as suggested by King and Clegg (1979).

Chemical analysis

Samples of inoculated curds were periodically analysed for total N, water-soluble nitrogen (WSN) and 12% TCA-soluble nitrogen as described by Ling (1963). The 5% PTA-soluble nitrogen was determined by Stadhouders (1959) method. The pH of cheese curd samples was measured by the method described by Godinho and Fox (1982) using a Scott Geräte (SGH) pH meter GG 811. Total Volatile Fatty Acids were determined according to Kosikowski (1978).

RESULTS AND DISCUSSION

Changes in pH

Table 1 shows the changes in pH values of aseptic curd made from either fresh cow's milk or recombined milk inoculated with different strains of *P. roqueforti* and incubated at 25°C for 21 days.

Results show that the pHs of aseptic curds made from fresh cow's milk or recombined milk without mould (fresh curd) were nearly the same (5·15 and 5·25, respectively).

The pH changed little during the first 3 days of incubation. Between the 7th and 21st days, the pHs of all aseptic curds, made from either fresh milk or recombined milk individually inoculated with the tested strains of *P. roqueforti*, increased but at somewhat different rates. Generally the pH increased rapidly at this stage of incubation with all examined strains. These results clearly indicated that the type of strain had a remarkable effect on the

TABLE 1
Changes in pH of Aseptic Curd Inoculated with Different *P. roqueforti* Strains during Incubation at 25°C

P. roqueforti *strain*	*Type of curd*	*Incubation period (days)*				
		0	*3*	*7*	*14*	*21*
German Wiesby, 6829	F	5·15	5·25	5·80	6·30	6·66
	R	5·25	5·35	5·60	6·23	6·56
Danish, I	F	5·15	5·35	5·50	5·80	6·32
	R	5·25	5·33	5·49	5·73	6·12
Danish, II	F	5·15	5·30	5·50	5·80	6·20
	R	5·25	5·43	5·50	5·67	6·15
German, S	F	5·15	5·25	5·60	5·80	6·50
	R	5·25	5·30	5·56	5·67	6·40
Italian PRB 15	F	5·15	5·27	5·50	5·95	6·10
	R	5·25	5·26	5·98	5·90	5·91
Italian Cepp	F	5·15	5·20	5·40	6·00	6·15
	R	5·25	5·27	5·43	6·15	6·16
IMI 24313	F	5·15	5·20	5·30	5·40	5·50
	R	5·25	5·28	5·35	5·35	5·44
IMI 92261	F	5·15	5·30	5·55	5·80	6·30
	R	5·25	5·27	5·70	5·86	6·25
IMI 173224	F	5·15	5·32	5·80	6·10	6·25
	R	5·25	5·29	5·56	6·58	6·18
IMI 148775	F	5·15	5·27	5·55	5·70	5·80
	R	5·25	5·33	5·51	5·73	5·49
IMI 129207	F	5·15	5·30	5·50	5·70	5·90
	R	5·25	5·34	5·46	5·65	5·82

F, fresh cow's milk; R, recombined milk.

pH of both types of curd, but the type of milk used in preparing the curd did not affect the pH.

The increase in the pH of aseptic curd could be explained on the basis that deamination of amino acids and metabolism of organic acids leads to the accumulation of NH_4^+ with a rise in pH (El-Soda *et al.*, 1977; Godinho & Fox, 1982).

Protein breakdown

Protein breakdown was measured by estimation of nitrogenous compounds soluble in water (WSN), in 12% trichloroacetic acid (TCA-soluble N) and in 5% phosphotungstic (PTA-soluble N). The nitrogen in each fraction is expressed as a percentage of total nitrogen (TN). These ripening indices were

chosen for assessment of casein degradation in aseptic curds inoculated with eleven *P. roqueforti* strains during incubation at 25°C for 21 days.

Formation of WSN compounds

Table 2 shows the changes in the levels of WSN in aseptic curd made from either fresh cow's milk or recombined milk inoculated with various *P. roqueforti* strains and incubated at 25°C for 21 days.

The formation of water-soluble nitrogen (WSN) compounds in both types of aseptic curd individually inoculated with the eleven examined strains of *P. roqueforti* increased gradually up to the 14th day of incubation, then these compounds intensively increased with progressive incubation reaching their maximum concentrations at the end of incubation time.

Aseptic curds inoculated with *P. roqueforti* strain 6829 Wiesby and *P.*

TABLE 2

Changes in Water-Soluble N (% of TN) of Aseptic Curd Inoculated with Different Strains of *P. roqueforti* during Incubation at 25°C

P. roqueforti *strain*	*Type of curd*	*Incubation period (days)*									
		0	*3*	*5*	*7*	*9*	*11*	*14*	*16*	*18*	*21*
German Wiesby, 6829	F	8·93	10·9	15·1	22·1	32·6	55·96	58·1	63·4	67·1	73·0
	R	8·92	15·3	25·8	35·2	48·8	51·89	52·3	62·4	70·6	75·3
Danish, I	F	8·93	12·4	14·9	22·1	27·8	38·71	47·7	48·2	50·9	54·5
	R	8·92	14·2	19·2	19·1	26·6	32·97	35·7	43·4	50·0	56·5
Danish, II	F	8·93	12·6	13·2	25·8	26·5	34·91	45·9	47·6	50·1	53·3
	R	8·92	16·3	21·7	25·3	28·9	31·40	37·9	54·0	56·5	59·6
German, S	F	8·93	14·8	16·0	27·2	31·5	39·71	46·3	48·2	56·0	61·8
	R	8·92	15·1	25·9	27·6	39·2	41·49	51·4	59·3	59·9	64·5
Italian PRB 15	F	8·93	13·0	21·2	27·4	34·2	43·94	46·5	51·6	54·0	55·3
	R	8·92	17·6	19·1	22·5	32·5	33·74	35·4	50·0	52·8	57·2
Italian Cepp	F	8·93	11·8	20·0	27·7	31·0	33·15	39·1	41·6	45·5	50·7
	R	8·92	11·7	22·4	27·2	32·8	38·06	46·6	50·4	53·8	56·3
IMI 24313	F	9·13	13·4	20·31	28·4	33·5	38·31	47·5	50·3	52·34	55·9
	R	8·92	15·0	18·33	23·6	25·8	37·05	40·4	50·9	53·7	58·0
IMI 92261	F	9·13	13·6	17·60	24·1	29·6	37·32	46·4	51·7	57·0	58·0
	R	8·92	11·7	15·44	30·0	33·5	48·32	52·7	59·0	61·9	64·1
IMI 173224	F	9·13	16·0	23·27	35·7	47·8	52·99	59·0	67·3	70·9	72·6
	R	8·92	13·2	26·89	32·6	37·6	48·31	53·6	64·1	68·8	71·4
IMI 148775	F	9·13	13·6	20·03	25·1	38·1	47·76	50·6	58·2	60·5	63·4
	R	8·92	12·1	15·31	16·7	23·9	29·59	44·9	47·2	53·0	63·2
IMI 129207	F	9·13	13·8	25·54	28·0	38·7	54·75	57·1	60·8	66·7	68·8
	R	8·92	16·1	26·0	26·3	42·0	50·59	56·2	62·9	66·3	67·5

F, fresh cow's milk; R, recombined milk.

roqueforti strain IMI 173224 had the highest concentrations of soluble nitrogenous compounds at each stage of the incubation period compared with the other examined strains.

It was also noticed that the behaviour of each strain in both types of aseptic curd was almost the same.

Formation of 12% TCA and 5% PTA-soluble nitrogen

Changes in 12% TCA-soluble nitrogen as a percentage of TN in all aseptic curds inoculated with the eleven examined strains of *P. roqueforti* and incubated at 25°C for 21 days are presented in Table 3.

The levels of 12% TCA-soluble nitrogen in both types of aseptic curd inoculated with the different strains intensively increased during the incubation period reaching their maximum concentration after 21 days. *P.*

TABLE 3

Changes in 12% TCA-Soluble N (% of TN) of Aseptic Curd Inoculated with Different Strains of *P. roqueforti* during Incubation at 25°C

P. roqueforti *strain*	*Type of curd*	*Incubation period (days)*									
		1	*3*	*5*	*7*	*9*	*11*	*14*	*16*	*18*	*21*
German Wiesby, 6829	F	6·19	9·95	10·8	12·47	25·5	40·71	42·0	45·5	48·6	52·3
	R	5·89	6·04	16·1	17·60	29·2	38·53	39·5	44·9	51·0	52·9
Danish, I	F	6·19	10·4	11·0	15·53	18·7	25·83	29·3	26·5	38·7	38·4
	R	5·89	6·05	9·98	14·90	18·8	19·83	23·5	27·1	28·0	30·3
Danish, II	F	6·19	9·91	9·91	19·20	21·4	25·10	33·6	36·9	38·6	39·9
	R	5·89	7·40	8·95	16·37	19·4	21·85	26·6	26·6	30·0	31·4
German, S	F	6·19	10·5	10·8	15·45	20·6	21·52	30·0	34·5	39·7	44.4
	R	5·89	7·10	14·7	17·87	25·2	36·88	38·2	44·5	45·8	46·7
Italian PRB 15	F	6·19	9·91	16·8	16·82	22·5	30·54	32·9	37·1	40·0	40·7
	R	5·89	6·29	8·90	10·90	19·1	22·69	25·8	26·3	28·0	36·5
Italian Cepp	F	6·19	9·08	13·5	14·52	15·9	18·89	25·0	28·1	28·7	31·1
	R	5·89	6·75	14·8	17·73	18·1	19·88	21.0	22·0	25·5	36·3
IMI 24313	F	7·53	7·98	8·98	14·63	20·1	22·84	28·4	31·5	36·0	39·6
	R	5·89	6·15	11·4	12·26	13·1	25·74	31·1	38·4	39·9	41·1
IMI 92261	F	7·53	8·38	12·3	14·26	14·6	27·36	32·3	35·1	38·5	40·8
	R	5·89	8·02	11·4	19·93	21·6	27·61	36·0	39·7	40·5	41·8
IMI 173224	F	7·53	9·30	13·8	19·99	31·0	35·37	40·6	45·7	49·7	52·4
	R	5·89	6·93	14·9	25·39	25·4	35·68	38·5	46·3	50·1	51·3
IMI 148775	F	7·53	9·42	12·2	17·11	27·6	36·12	41·3	45·5	46·5	50·0
	R	5·89	5·83	8·48	8·48	19·1	21·96	32·7	33·9	39·4	49·5
IMI 129207	F	7·53	9·16	12·1	16·36	27·0	32·33	35·4	42·1	50·2	51·5
	R	5·89	12·8	18·7	18·89	31·2	35·65	40·2	44·1	47·9	49·7

F, fresh cow's milk, R, recombined milk.

roqueforti strains differed markedly in their proteolytic activity as shown in Table 3. On the other hand, *P. roqueforti* strain 6829 Wiesby and *P. roqueforti* strain IMI 173224 showed the highest levels of 12% TCA-soluble nitrogen in both types of curd compared with the other tested strains. *Penicillium roqueforti* Danish I and IMI 24313 produced the lowest levels of 12% TCA-soluble nitrogen, indicating lower proteolytic activity than the other tested strains of *P. roqueforti.* The increased formation of 12% TCA-soluble N during the incubation at 25°C could be attributed to the proteinases produced by *P. roqueforti.* The results agree with those obtained by Gripon and Lebars (1978). PTA-soluble N results showed a trend similar to 12% TCA-soluble nitrogen (Table 4).

Fat hydrolysis

Total Volatile Fatty Acids (VFA)

The accumulation of Volatile Fatty Acids (VFA) was taken as a

TABLE 4

Changes in 5% PTA-Soluble N (% of TN) of Aseptic Curd Inoculated with Different Strains of *P. roqueforti* during Incubation at 25°C

P. roqueforti *strain*	*Type of curd*	*Incubation period (days)*									
		0	*3*	*5*	*7*	*9*	*11*	*14*	*16*	*18*	*21*
German Wiesby, 6829	F	0·19	0·90	1·70	2·68	4·41	7·82	11·4	13·0	14·0	14·4
	R	0·25	3·75	6·56	7·20	8·60	10·4	10·8	12·8	16·5	16·9
Danish, I	F	0·19	0·89	1·34	2·39	2·62	3·94	5·20	6·38	6·61	7·77
	R	0·25	1·70	2·66	3·62	5·62	7·11	7·45	7·82	8·43	10·0
Danish, II	F	0·19	1·35	1·65	1·71	3·48	4·09	5·03	7·38	8·93	10·1
	R	0·25	2·90	2·90	3·29	3·98	4·36	5·09	7·90	8·51	11·1
German, S	F	0·19	2·49	3·73	4·06	10·20	12·0	12·2	21·5	13·3	13·5
	R	0·25	3·65	4·86	5·59	11·86	12·2	12·5	12·7	14·3	14·5
Italian PRB 15	F	0·19	2·25	5·28	6·53	9·02	10·2	11·4	12·1	12·5	12·6
	R	0·25	1·08	3·40	4·60	4·90	6·98	8·30	10·5	12·7	14·5
Italian Cepp	F	0·19	0·91	1·75	2·54	5·37	6·29	7·44	8·79	9·43	11·5
	R	0·25	2·19	5·40	6·76	10·69	12·0	13·2	14·1	15·0	15·7
IMI 24313	F	0·18	4·07	5·43	7·55	7·96	9·49	11·6	12·5	12·7	13·9
	R	0·25	0·73	3·15	3·95	4·58	5·43	6·33	8·27	11·1	14·0
IMI 92261	F	0·18	2·82	3·62	4·34	4·58	6·24	9·60	13·0	14·7	15·3
	R	0·25	1·22	2·92	4·60	5·93	11·5	13·6	13·9	15·0	16·3
IMI 173224	F	0·18	3·18	6·74	7·58	9·13	12·0	12·9	14·6	15·0	16·0
	R	0·25	2·63	4·78	5·36	6·88	10·2	33·1	16·1	16·8	16·8
IMI 148775	F	0·18	2·76	3·56	5·33	7·71	9·44	10·4	10·7	11·9	12·2
	R	0·25	1·21	2·42	2·66	3·60	6·00	10·3	10·7	11·6	12·5
IMI 129207	F	0·10	3·16	4·88	5·63	6·30	9·09	11·1	12·3	13·0	13·6
	R	0·25	2·66	4·36	5·02	8·84	11·0	12·3	12·6	13·3	14·8

F, fresh cow's milk; R, recombined milk.

measurement for assessment of lipolytic action of *P. roqueforti* strains on the fat of aseptic curds made from either fresh cow's milk or recombined milk and incubated at 25°C for 21 days.

Table 5 shows the changes in Volatile Free Fatty Acids of aseptic curds inoculated with *P. roqueforti* strains during incubation at 25°C.

Results indicate that the VFAs in all examined strains of *P. roqueforti* increased gradually during incubation in both types of curd and reached their maxima after 14 days of incubation, while after 16 days of incubation the concentrations of VFAs decreased slightly until the end of the incubation period for all examined strains. In addition, VFAs produced by *P. roqueforti* strain 6829 Wiesby and *P. roqueforti* IMI 173224 were higher than those produced by the other examined strains in both types of aseptic curd at each incubation period. They showed the highest levels of Total Volatile Fatty Acids at the end of the 14th day of incubation.

The decreased levels of Free Volatile Fatty Acids could be attributed to

TABLE 5

Changes in Total Volatile Fatty Acid Content (ml 0·1 n NaOH/100 g) of Aseptic Curd Inoculated with Industrial Strains of *P. roqueforti* during Incubation at 25°C

P. roqueforti *strain*	*Type of curd*	*Incubation period (days)*									
		0	*3*	*5*	*7*	*9*	*11*	*14*	*16*	*18*	*21*
German Wiesby, 6829	F	12·0	14·5	18·7	31·8	35·0	38·1	38·5	37·8	36·0	35·5
	R	15·2	17·5	21·7	28·4	34·2	37·9	40·2	39·8	36·5	35·8
Danish, I	F	12·0	12·8	13·8	17·5	23·2	28·0	31·8	32·8	31·2	30·7
	R	15·2	15·9	16·8	20·9	26·9	31·4	34·0	32·5	32·0	30·34
Danish, II	F	12·0	13·0	14·5	20·1	28·2	31·6	33·2	34·0	34·1	33·8
	R	15·2	16·5	17·3	23·2	31·5	34·0	36·4	37·2	35·4	33·2
German S	F	12·0	13·0	14·1	19·2	25·8	28·4	31·2	31·7	31·0	29·8
	R	15·2	16·0	17·2	21·3	28·9	31·4	34·5	35·0	34·1	32·0
Italian PRB 15	F	12·0	12·9	14·2	15·5	21·9	25·0	28·0	31·2	30·9	29·5
	R	15·2	15·8	17·0	18·9	24·5	28·5	32·0	34·0	30·7	30·2
Italian Cepp	F	12·0	13·1	14·2	16·9	22·6	26·5	31·1	32·1	31·7	30·4
	R	15·2	16·0	17·2	19·8	25·0	29·0	34·2	35·0	32·0	31·9
IMI 24313	F	12·2	13·5	14·6	18·2	28·2	31·5	32·9	33·0	32·0	31·4
	R	15·2	16·0	17·0	21·4	30·2	34·0	35·5	35·0	34·0	32·4
IMI 92261	F	12·2	12·8	13·6	15·6	21·6	24·0	28·9	32·2	30·4	30·1
	R	15·2	15·7	16·4	18·3	24·0	26·3	31·0	33·2	31·5	30·2
IMI 173224	F	12·2	13·9	18·0	24·2	31·1	35·4	37·0	37·0	36·5	36·0
	R	15·2	16·8	20·9	26·5	33·4	38·2	40·0	38·8	37·0	36·2
IMI 148775	F	12·2	16·4	17·1	21·8	28·4	33·6	35·4	37·0	36·1	34·2
	R	15·2	18·0	19·8	24·7	32·0	36·2	38·2	39·0	36·9	34·0
IMI 129207	F	12·2	13·0	14·2	16·0	21·0	24·3	28·7	31·6	30·5	29·2
	R	15·2	15·8	16·8	18·6	24·2	27·0	31·2	33·4	32·0	30·7

F, Fresh cow's milk; R, recombined milk.

their conversion to carbonyl compounds (Harte & Stein, 1977; King & Clegg, 1979; Godinho & Fox, 1981).

Flavour intensity

Table 6 shows that the curd model inoculated with *P. roqueforti* strains at different stages of incubation revealed a significant influence of strain type on the development of typical blue cheese flavour. *P. roqueforti* strain 6829 Wiesby and IMI 173224 developed typical blue cheese flavour in both types of aseptic curd made from either fresh cow's milk or recombined milk after 7 and 14 days of incubation at 25°C, respectively.

Both types of aseptic curd separately inoculated with the other strains showed satisfactory blue cheese flavour after 14 and 16 days of incubation. This was more pronounced with *P. roqueforti* S and Danish blue mould II than the other tested strains. The score points of flavour intensity of both

TABLE 6

Development of Blue Cheese Flavour (score out of 8 points) in Aseptic Curd Inoculated with Different Strains of *P. roqueforti* During Incubation at 25°C

P. roqueforti *strain*	*Type of curd*	*Incubation period (days)*							
		3	*5*	*7*	*11*	*14*	*16*	*18*	*21*
German Wiesby, 6829	F	3·5	5·0	6·4	7·3	7·7	7·5	6·2	5·0
	R	3·2	4·8	6·2	6·9	7·7	7·4	6·0	4·2
Danish, I	F	3·0	4·5	5·9	6·2	7·0	7·3	6·5	4·2
	R	2·9	4·2	5·8	6·4	6·8	7·1	6·3	4·3
Danish, II	F	3·2	4·6	5·8	6·6	7·0	7·3	6·3	4·2
	R	2·9	4·4	5·9	6·5	6·9	7·3	6·3	4·4
German, S	F	3·2	4·6	5·7	6·3	7·1	7·5	5·2	4·7
	R	3·0	4·5	5·8	6·4	6·9	7·5	5·0	4·3
Italian PRB 15	F	3·2	3·9	4·2	5·9	6·0	6·9	5·9	4·6
	R	2·8	3·9	4·0	5·8	6·2	6·6	5·6	4·3
Italian Cepp	F	2·5	3·8	4·3	6·2	6·5	6·8	6·0	5·2
	R	2·7	3·8	4·2	6·1	6·4	6·8	5·7	4·5
IMI 24313	F	2·5	3·6	4·9	5·3	6·5	6·7	5·5	4·3
	R	2·5	3·5	4·8	5·2	6·3	6·5	5·5	4·2
IMI 92261	F	2·8	3·6	4·9	5·7	6·8	6·8	5·4	4·5
	R	2·7	3·5	4·8	5·3	6·4	6·6	5·3	4·4
IMI 173224	F	3·2	4·8	6·2	6·7	7·2	7·5	5·8	4·8
	R	3·0	4·5	6·0	6·5	6·9	7·2	5·5	4·5
IMI 148775	F	2·9	3·8	4·8	5·3	6·2	6·8	5·5	4·5
	R	2·6	3·4	4·5	4·9	5·8	6·5	5·2	4·1
IMI 129207	F	3·2	3·9	4·8	5·2	6·2	6·9	5·8	4·9
	R	2·6	3·5	4·6	4·9	5·8	6·5	5·3	4·2

F, fresh cow's milk; R, recombined milk.

types of aseptic curd inoculated with different *P. roqueforti* strains increased gradually during incubation up to 16 days, then decreased gradually during incubation. The formation of typical blue cheese flavour developed by both *P. roqueforti* 6829 Wiesby and IMI 173224 may be due to the formation of soluble nitrogenous compounds and free fatty acids as well as methyl ketones resulting from the action of proteinases and lipases produced by *P. roqueforti.*

Prolongation of the incubation period resulted in atypical blue cheese flavour. This could be attributed to the intensive proteolysis suffered by the examined strains. Thus, the type of strain had a significant influence on the development of blue cheese flavour.

In addition, the type of milk used for preparing the curd did not significantly affect the flavour intensity.

In conclusion, *P. roqueforti* strain 6829 Wiesby and *P. roqueforti* strain IMI 173224 could be recommended for use in the production of blue cheese.

The behaviour of the above mentioned strains of *P. roqueforti* in commercial cheese curd is the subject of further investigation.

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