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Application of a Coagulating Preparation Obtained with Rhizomucor Miehei N in Cheese-Making

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Abstract: Studies were carried out to determine the suitability of a coagulating preparation, Rhizomucor proteinase, obtained from Rhizomucor miehei for production of Camembert, Edam and Cheddar cheeses. While analyzing the cheese-making process, it was found that the obtained preparation could be used in the production of high quality cheeses without the need for changing the established technological parameters. In cheeses produced with Rhizomucor proteinase, proteolysis and lipolysis was more intense than in renin cheeses - which has a beneficial effect on cheese sensory properties. Lower utilization of milk nitrogen compounds and fat encourages further studies into the application of the obtained preparation in combination with other coagulating enzymes.

Key words: Rhizomucor – proteinase, coagulating preparation, cheeses, proteolysis, lipolysis

Introduction

In the 1960s, the production of cheese increased considerably and the number of livestock decreased. This caused a deficit in rennin, i.e. preparations obtained from young calves' abomasum used for milk protein coagulation.

Numerous studies showed that fungi synthesize coagulating enzymes which can be used in the cheese production (Garg and Bhavdish, 1994; Sternberg, 1976). Several companies started industrial production of coagulating preparations with the use of these fungi i.e. Rhizomucor pusillus – "Meito", "Emporase", Cryphonectria parositica – "Suparen", Rhizomucor miehei – "Fromase", "Rennilase", "Marzyme". Some of the above preparations are used in cheese production in Poland.

The quality of microbial preparations has been improved over the years, but still some of its properties have diverged from rennin properties. Therefore, further research aims at obtaining new preparations with better technological properties.

A coagulating preparation Rhizomucor proteinase (Kolakowski *et al.*, 1997) was obtained from Rhizomucor miehei N in the Chair of Food Biotechnology, University of Warmia and Mazury in Olsztyn.

The aim of the study was to determine the suitability of the obtained preparation in the production of ripening cheeses.

Materials and Methods

The experimental cheeses were produced in the following specialist dairies: the Dairy Factory in Gniezno – Camembert cheese, the Dairy Factory in Ilawa – Edam cheese and the Dairy Factory in Chorzele – Cheddar cheese.

Experimental preparation in the amount of 1.5 g /100 l was added to milk to obtain curd ready for cutting in the required time.

Cheeses produced in these dairies with the use of a commercial rennin liquid preparation constituted the control. Both the experimental and control cheeses ripened in the respective dairies.

Milk components utilization in cheese making technology was determined based on:

- the content of total nitrogen in milk and whey after curd cutting,
- the content of fat in milk and whey after curd cutting.

The process of cheese ripening was monitored based on:

- the changes in water and fat content and pH,
- protein degradation based on the changes in the content of free amino acids, non-protein nitrogen, peptide nitrogen, nitrogen soluble at pH 4.6, amino acid nitrogen,
- cheese proteins were separated in Sephadex G-100 gel ,
- fat lipolysis by measuring the content of free fatty acids and volatile fatty acids.

The sensory evaluation was carried out by a panel of experts in compliance with the quality norm established for a given cheese.

Results and Discussion

In the production of Camembert, Edam and Cheddar cheeses, the curd obtained with Rhizomucor proteinase was slightly less firm than that obtained with a rennin preparation.

The amount of milk nitrogen compounds and fat in whey

Table 1: The content of nitrogen compounds and fat in whey after cheese manufacture

Type of cheese	Preparation for milk coagulation			
	Rennet	Rhizomucor-proteinase	Rennet	Rhizomucor-proteinase
	Utilization of nitrogen compounds %		Utilization of fat %	
Camembert	73.2	72.2	82.6	80.4
Edam	73.4	72.2	88.2	84.3
Cheddar	75.4	73.6	85.7	80.4

obtained during coagulation by Rhizomucor proteinase preparation was higher than that obtained in the coagulation with rennin and depended on the type of cheese (Table 1).

The transfer of milk nitrogen compounds to whey in the production of Camembert, Edam and Cheddar cheeses with the use of the experimental preparation was 3.7, 4.5 and 7.3%, respectively, higher than that observed for renin.

Previous studies also indicated greater amounts of nitrogen compounds transferred from milk to whey in the milk protein coagulation with commercial microbial preparations used in the cheese-making (Poznański *et al.*, 1974; Reps, 1979).

It seems that the milk component utilization in cheese production can be increased by applying the experimental preparation in combination with another coagulating preparation of lower proteolytic activity.

The changes in the content of water and fat and in pH in all the cheeses complied with respective quality norms.

While analyzing the protein degradation process during Camembert cheese ripening, the nitrogen content of nitrogen compounds soluble at pH 4.6 was at a similar level in both the control and the experimental cheeses after the 7-day ripening period. On the other hand, after the two-week ripening period, the content of nitrogen compounds in cheese produced with Rhizomucor proteinase was 32.2%, while in the renin cheese it was only 22.8%. The content of non-protein nitrogen (N-NPN) in the experimental cheeses increased more intensely than in the control cheeses. After the 7-day ripening period, the N-NPN content was 10.9% in the experimental cheeses and 7.8% in the control cheeses. The longer the ripening period, the greater the difference in the N-NPN content in cheeses was observed.

Similarly, the content of amino acid nitrogen in the experimental cheeses was higher than that in the control renin cheeses, while the content of peptide nitrogen was at a similar level in these cheeses (Table 2).

The obtained results explicitly indicate that the protein degradation process is more intense in cheese produced with Rhizomucor proteinase and thus ripens faster.

In the experimental Edam cheese, protein degradation was also more intense. After the 4 week ripening period of the experimental cheeses, the nitrogen content in nitrogen compounds soluble in pH 4.6 and N-NPN was 36.0% and 19.7%, while in the control cheeses it was 20.4% and 8.3%, respectively. After the 8-week ripening period, the content of the above forms of nitrogen compounds in the experimental cheeses increased to 41.9% and 28.4% and in the control cheeses to 25.4% and 23.2%, respectively. Peptides and amino acids were also released more intensely in the experimental cheeses.

Similar to the Edam cheeses, higher contents of the analyzed nitrogen compounds were recorded for the experimental Cheddar cheeses. After the 24-week ripening period, the nitrogen content in the nitrogen compounds soluble at pH 4.6, the N-NPN and the amino acids was 31.0%, 26.2% and 12.6% in the experimental cheeses and 26.7%, 20.3% and 10.9% in the rennin cheeses, respectively.

The content of free amino acids increased during cheese ripening and this increase was more intense in the experimental cheeses (Table 3).

For example, the content of free amino acids was 54.1, 81.6 and 128 mg/100g of cheese in the control Edam cheese, and 79.0, 105.4 and 197.40 mg/100g of cheese in the experimental cheeses after the 4, 6 and 12-week ripening periods, respectively.

This more intense protein degradation process in the experimental cheeses was confirmed by the cheese protein separation in Sephadex G-100 gel.

Based on the chromatographic separation, the experimental cheese protein separation produced a larger number of fractions. Throughout the entire ripening period, the highest content of nitrogen in the control cheeses was found in high-molecular weight fractions (i.e. over 100 kDa) while in the experimental cheeses it was in the medium- and low-molecular weight fractions (Table 4).

Based on the results, the protein degradation process is more intense during the ripening of the cheeses produced with Rhizomucor proteinase. It should also be mentioned here that the majority of the commercial coagulating preparations used in the cheese-making produces more intense protein degradation than rennin (Poznański *et al.*, 1974; Reps, 1979).

Fat lipolysis considerably affects cheese sensory properties. Greater amounts of free fatty acids were recorded for the experimental cheeses (Table 5).

Table 2: The content of protein breakdown products in cheeses during ripening

Type of cheese	Nitrogen	Preparation used for milk coagulation						
		Rennet						
		Time of cheese ripening /weeks/						
		1	2	4	6	8	16	24
		In % of total N						
Camembert	N-soluble in pH 4,6	15.04	22.81					
	Non protein nitrogen	7.82	16.37					
	Peptides N	3.70	5.21					
	N-amino acid	5.24	6.28					
Edam	N-soluble in pH 4,6			20.37	20.29	2539		
	Non protein nitrogen			8.26	10.32	23.20		
	Peptides N			2.17	2.88	3.65		
	N-amino acid			4.11	4.52	5.42		
Cheddar	N-soluble in pH 4,6					11.47	19.42	26.72
	Non protein nitrogen					7.80	13.29	20.25
	Peptides N					2.57	2.98	6.37
	N-amino acid					4.88	6.09	10.93
Type of cheese	Nitrogen	Preparation used for milk coagulation						
		Rhizomucor-proteinase						
		Time of cheese ripening /weeks/						
		1	2	4	6	8	16	24
		In % of total N						
Camembert	N-soluble in pH 4,6	17.81	32.21					
	Non protein nitrogen	10.90	24.22					
	Peptides N	3.80	5.53					
	N-amino acid	6.28	7.03					
Edam	N-soluble in pH 4,6			36.01	38.84	41.90		
	Non protein nitrogen			19.70	25.57	28.42		
	Peptides N			6.78	11.24	13.26		
	N-amino acid			6.82	6.09	9.38		
Cheddar	N-soluble in pH 4,6					14.33	25.84	31.00
	Non protein nitrogen					10.83	17.39	26.17
	Peptides N					3.24	5.77	7.47
	N-amino acid					6.81	12.30	12.63

Table 3: The content of free amino acids in cheeses during ripening

Type of cheese	Preparation used for milk coagulation													
	Rennet							Rhizomucor-proteinase						
	Time of cheese ripening /weeks/													
	1	2	4	6	8	16	24	1	2	4	6	8	16	24
	Free amino acids (mg / 100g cheese)													
Camembert	8.65	32.00						12.17	33.94					
Edam			54.10	81.65	128.18					79.02	105.37	197.40		
Cheddar					66.21	112.30	136.15					51.81	138.09	188.18

Table 4: Chromatograms of cheese protein gel filtration on Sephodex G-100

Type of cheese		Preparation used for milk coagulation														
		Rennet								Rhizomucor-proteinase						
		Molecular weight /kDa/														
		>100	49	30	26	16	7-8	5-6	4	>100	83	60-65	42	31	22-25	6-7	4
		N of peak / N eluted in %Camembert															
Camembert	2	69.96	11.65			6.92	9.60	1.87		45.45		18.38			20.15	14.93	1.09
Edam	4	84.51					14.89	0.60		8.30	14.84				26.58	47.24	2.94
	8	76.72		7.31			14.94	1.04		5.67			10.29		26.78		57.26
Cheddar	16	67.29		10.92				20.60	1.19	23.93		13.24			20.07	41.52	1.24
	24	57.01			17.90			24.33	0.76	22.40				24.23		51.18	2.19

Table 5: The content of free fatty acids in cheeses during ripening

Type of cheese	Preparation used for milk coagulation													
	Rennet							Rhizomucor-proteinase						
	Time of cheese ripening /weeks/													
	1	2	4	6	8	16	24	1	2	4	6	8	16	24
	Free fatty acids (mg / 100 g cheese)													
Camembert	301.49	436.48						484.00	629.28					
Edam			97.06	115.98	256.53					208.39	226.26	361.92		
Cheddar					381.90	442.62	450.10					441.58	517.47	1178.14

After the 2-week ripening period, the content of free fatty acids in the Camembert cheese was 630 and 437 mg/100g of cheese in the experimental and the control cheeses, respectively. Moreover, a greater content of free unsaturated fatty acids was observed in the experimental cheeses. Precursors of methylketones which influence the mould cheese flavour and odour dominated among the detected free fatty acids.

In the Camembert cheeses after a 7-day ripening period, the C_4/C_2, C_3/C_2 and LKT/C_2 free acid ratios were at a similar level. After two-week ripening period, the above ratios changed and were 0.44, 0.21 and 9.32 in the experimental cheese, respectively and 0.17, 0.65 and 17.9 in the control cheese, respectively (Table 6).

In the Edam cheeses, fat lipolysis was less intense than in the Camembert cheeses. After 4, 6 and 8 weeks of ripening fat lipolysis was more intense in the experimental cheeses.

Table 6: The correlation between selected free volatile fatty acids in cheeses during ripening

Type of cheese	Preparation used for milk coagulation	C_2 / C_4			C_3 / C_2			VFA / C_2			VFA / mg / 100g cheese		
		Time of cheese ripening /weeks/											
Camembert	Rennet	1		2	1		2	1		2	1		2
		0.35		0.17	0.28		0.65	14.04		17.89	9.97		14.31
	Rhizomucor-proteinase	0.40		0.44	0.25		0.21	12.76		9.32	12.12		16.21
		Time of cheese ripening /weeks/											
Edam	Rennet	4	6	8	4	6	8	4	6	8	4	6	8
		0.82	1.60	0.79	0.23	0.07	0.14	5.99	3.27	6.35	9.22	13.17	18.11
	Rhizomucor-proteinase	0.35	1.15	0.95	0.52	0.22	0.25	19.75	5.11	5.92	8.69	13.60	14.22
		Time of cheese ripening /weeks/											
Cheddar	Rennet	8	16	24	8	16	24	8	16	24	8	16	24
	Rhizomucor-proteinase	0.94	1.40	1.34	0.13	0.08	0.03	5.61	4.10	3.33	14.75	16.21	16.74
		1.28	1.19	2.05	0.12	0.09	0.38	3.59	4.86	3.12	7.80	15.81	28.71

In the control and experimental Cheddar cheeses, fat lipolysis was similar until week 16 of ripening. The content of free fatty acids increased intensely until week 24 of ripening and was 450.1 mg/100 g of cheese in the control cheese, while it was 1178.1 mg/100 g of cheese in the experimental cheese.

Based on the report from the panel of experts, both control and experimental cheeses had high sensory values. At the same time, an evaluation team noted that the experimental cheeses are more ripened and exhibit a richer flavour and odour bouquet. Therefore, Rhizomucor proteinase can be utilized in the cheese-making to accelerate the cheese ripening process.

The obtained results explicitly indicate that the Rhizomucor proteinase preparation can be successfully applied to cheese production.

Conclusion: While analyzing the making and ripening processes of Camembert, Edam and Cheddar cheeses, it was found that with the use of Rhizomucor proteinase high quality ripening cheeses can be obtained without the need for changing the established technological parameters.

The utilization of milk nitrogen compounds and fat in milk coagulation by Rhizomucor proteinase was lower than in the coagulation by rennin.

Based on the content of free amino acids, nitrogen in nitrogen compounds soluble in pH 4.6, non-protein nitrogen, amino acid nitrogen and peptide nitrogen in cheeses, protein degradation was more intense in the cheeses produced with the use of Rhizomucor proteinase.

The more intense proteolysis in cheeses with Rhizomucor proteinase was confirmed by protein separation on Sephadex G-100 gel.

The higher content of free and volatile fatty acids in cheeses with Rhizomucor proteinase indicates a more intense fat lipolysis than in rennin cheeses.

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