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INFLUENCE OF LYSOZYME ON THE EXTENT OF THE PROTEOLYTIC PHASE OF RENNET COAGULATION OF MILK

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Key words: lysozyme, rennet proteolysis of milk, $\varkappa$-casein solution, casein micelles.

In the present studies, the influence of lysozyme on the extent of rennet proteolysis of milk, of $\varkappa$-casein solution and casein micelles, suspended in artificial serum of milk, was examined.

The obtained results showed that lysozyme did not affect signicicantly the rennet hydrolysis of casein. At the highest level of lysozyme addition applied (2.0 mg/cm^3), a certain lowering in the amount of glycopeptide released from casein by rennet, was observed; it makes it possible to suppose that lysozyme decreased the quantity of hydrolized casein indispensable for a coagulation of this protein.

INTRODUCTION

In the process of casein coagulation under the effect of rennet, two phases are distinguished. The first phase has na enzymatic character and consists in splitting off the part of polypeptide chain called glycomacropeptide from the molecules of $\varkappa$-casein. The second phase although causatively connected with the first, is not dependent on the rennet and leads to the formation of curd, as a result of interactions between the micelles. This is explained by the fact that the micelles lose part of their hydration coating after disconnection of the glycomacropeptide and that their electrokinetic potential is lowered [2, 3, 4, 11].

The process of rennet coagulation of casein is affected by many factors which include: pH, temperature and addition of certain compounds. All these factors find application in various methods of casein isolation from milk [2, 11].

As shown by the experiment, the time of rennet coagulation of milk is shortened by cationic type compounds, including lysozyme. The addition of this enzyme to milk accelerates the rennet coagulation time, but it is not known which phase of the rennet coagulation of milk is stimulated by the presence of lysozyme. The aim of the present study was to determine the influence of lysozyme on the proteolysis of casein induced by rennet.

MATERIAL AND METHODS

The experimental part was conducted in three series. As a substrate in the first series, milk regenerated (in 0.01 M $CaCl_2$) from skim-milk powder was used while in the second series, 1% solution of $\varkappa$-casein (in phosphatecitrate buffer) was applied [15]. In both series the additives of lysozyme were: 0.5 and 2.0 mg/cm^3 respectively. Rennet in the form of a 1% solution was used in an amount ensuring that the time of coagulation at 37°C would be about 15 minutes in the control samples. 30 minutes after rennetting in the samples with lysozyme and in the control samples, TCA-soluble nitrogen compounds were determined as well as the amount of N-acetylo-D-galactoso-amine (NAcGal) connected with this fraction. In the samples with an addition of lysozyme amounting to 2 mg/cm^3, changes in the level of the above mentioned compounds after coagulation and during 45-minute holding of the curd, were determined. The quantity of soluble nitrogen compounds was determined by the spectrophotometric method, measuring the absorption of light at 280 nm [14].

The quantity of NAcGal connected with the soluble fraction of proteins was determined by the method of Kumar and Hansen [10].

The last series of the experiments was performed on the solution of casein micelles obtained by centrifugation of fresh milk (Beckman L-565, $107 \times \times 10^3$ g — 1 h, temp. 1-2°C), suspended in an artificial solution of milk salts [9]. The applied additives of lysozyme and rennet were the same as in the previous series of the experiments. 30 minutes after rennetting the level of 12% TCA-soluble nitrogen compounds was determined in the sample (the spectrophotometric method), and the content of sugar compounds by gas chromatography, using the methods given by Sinkinson and Wheelock [13].

RESULTS AND DISCUSSION

The results of the first series of the experiments, determining the gains in the quantities of soluble nitrogen compounds and the amount of NAcGal 30 minutes after milk rennetting, have not given an univocal answer as to the influence of lysozyme on the process of proteolysis. The gains in the content of soluble nitrogen compounds in the samples with lysozyme (0.5, 1.0 and 2.0 mg/cm^3) were: 33.0; 32.0 and 14.0%, respectively while in the control samples they amounted to about 21%. The quantity of NAcGal, released as a result of rennet's action in milk with the addition of lysozyme was equal to 40.0; 50.1 and 52.3% and in the control samples it averaged 46.2% (Tab. 1).

In the first series with a lysozyme addition equal to 2 mg/cm^3, the changes in the level of soluble nitrogen compounds and NAcGal after milk coagulation and during 45-minute incubation of the obtained curd, were investigated. The average gain in the quantity of soluble nitrogen 15, 30 and 60 minutes after rennetting amounted to 14.2; 19.6 and 28.6% in samples with the lysozyme addition and 8.0; 16.5 and 15.6 in the control samples.

Table 1. Changes in the content of soluble nitrogen compounds and N-acetyl-D-galactos-amine (NAcGaL) in milk after incubation with lysozyme and rennet

Kind of sample		12-% TCA-soluble nitrogen compounds E_{280}			NAcGal content mg/g protein			Released NAcGal %
		before incubation	after incubation	increase	before incubation	after incubation	difference	
Control		0.105	0.127	0.022	1.43	0.77	0.66	46.2
With the addition of lysozyme · /mg/cm³/	0.5	0.108	0.141	0.035	1.50	0.90	0.60	40.0
	1.0	0.103	0.136	0.033	1.70	0.84	0.86	50.1
	2.0	0.114	0.130	0.016	1.71	0.81	0.90	52.3

[681]

Table 2. Changes in the content of soluble nitrogen compounds and N-acetyl-D-galactos-amine (NacGal) during incubation of milk with lysozyme and rennet

Kind of sample	12-% TCA-soluble nitrogen compounds E_{280}				NAcGal content (mg/g protein)			
	before incubation	after incubation			before incubation	after incubation		
		15*	30*	60*		15*	30*	60*
Control	0.115	0.124	0.134	0.133	1.70	1.10	0.86	0.88
With the addition of lysozyme (2 mg/cm³)	0.112	0.128	0.134	0.144	1.72	0.98	0.95	0.87

Table 3. Changes in the content of soluble nitrogen compounds and N-acetyl-D-galactos-amine (NacGal) in κ-casein after incubatipon with lysozyme and rennet

Kind of sample		12-% TCA-soluble nitrogen compounds E_{280}			Content of NacGal (mg/g protein)			NAcGal released
		before incubation	after incubation	increase	before incubation	after incubation	difference	%
Control		0.191	0.297	0.106	1.84	0.73	2.22	61.2
With the addition	0.5	0.202	0.289	0.087	1.66	0.76	0.89	54.1
of lysozyme	1.0	0.226	0.324	0.119	1.76	1.12	0.64	36.0
(mg/cm^3)	2.0	0.216	0.340	0.124	1.97	1.03	0.94	47.3

[061]

Table. 4 Changes in the content of soluble nitrogen compounds and N-acetyl-D-galactos-amine (NacGAl) during incubation of the solution of κ-casein with lysozyme and rennet

Kind of sample	12-% TCA-soluble nitrogen compounds E_{280}				Content of NAcGal (mg/g protein)				Released NAcGal %		
	before incubation	after incubation			before incubation	after incubation			after incubation		
		15*	30*	60*		15*	30*	60*	15*	30*	60*
Control	0.289	0.370	0.390	0.422	0.24	0.14	0.13	0.13	41.6	45.8	45.8
With the addition of lysozyme ($2m/cm^3$)	0.300	0.371	0.416	0.402	0.24	0.14	0.14	0.14	41.6	41.6	41.6

*) Time of incubation (min.)

The quantities of NAcGal connected with the soluble fraction of proteins in both types of samples were similar (Tab. 2).

The results of the second series of the experiments performed on the solution of $\varkappa$-casein showed that gains in values of absorbance in lysozyme-modified samples and in the control samples were similar. No distinct dependencies were observed between the amount of released NAcGal and concentration of lysozyme in the sample (Tab. 3). The analysis of results concerning changes in the quantities of soluble nitrogen compounds and free NAcGal during 60-minute incubation of samples with rennet has not revealed either any distinct influence of lysozyme on the proteolysis of $\varkappa$-casein (Tab. 4).

The studies of the last series of the experiment, conducted on the artificial milk serum have not showed any distinct effect of lysozyme on the level of glycopeptides released from casein by the rennet. The gains in the quantities of 12% TCA-soluble nitrogen compounds in samples with lysozyme addition were somewhat lower in the control samples (Tab. 5). Thus, no significant differences between the sugar content in glycopeptides released by the rennet from lysozyme-modified casein and non-modified one, were stated. The initial quantities of these compounds being similar, their gains after treatment were found to be of a similar level in samples with lysozyme and in the control samples (Tab. 6).

Analysis of all the results obtained in this study makes it possible to state that the applied additives of lysozyme did not affect significantly the hydrolysis of casein by rennet. This statement is opposed to the hypothesis of Bakri and Wolf [1] concerning destabilization of casein micelles by lysozyme by the hydrolysis of glycoside bonds of $\varkappa$-casein. In their studies on the interaction of cationic compounds and casein micelles, Green et al. [5, 6, 7, 8] revealed that the suspension of these micelles with lysozyme caused a reduction in their coagulation time with a simultaneous lowering in the level of 2% TCA-soluble nitrogen compounds and free carbohydrates. Pierr [12] stated that the addition of lysozyme to milk decreased considerably the amount of glycomacropeptide splitted off from casein by rennet.

It is known that a condition of the coagulation of rennet-modified micelles is the hydrolysis of a defined quantity of $\varkappa$-casein. On the basis of the experiments of Green and Pierr [5, 6, 7, 8, 12] and on the basis of the results obtained in the

Table 5. Changes in the content of 12-% TCA-soluble nitrogen compounds, released by the rennet from lysozyme—modified casein micelles

Kind of sample	12-% TCA—soluble nitrogen compounds E_{280}		
	before incubation	after incubation	increase
Control	0.214	0.294	0.080
With the addition 0.5	0.216	0.256	0.040
of lysozyme 1.0	0.214	0.282	0.068
(mg/cm^3) 2.0	0.202	0.276	0.074

Table 6. Content of sugar released by rennet's action from casein micelles, modified by lysozyme

Kind of sample	Content of sugars (mg/100 cm^3)											
	before treatment				after treatment				content of released sugars			
	D-man-nose	G-galac-tose	NAcGal	NANA	D-man-nose	D-galac tose	NAcGal	NANA	D-man-nose	D-galac-tose	NAcGal	NANA
Control	traces	0.12	traces	0.09	0.09	4.43	5.39	4.03	0.09	4.31	5.39	3.93
With the addition	traces	0.11	traces	0.10	0.10	4.55	5.58	4.12	0.11	4.44	5.58	4.02
of lysozyme	traces	0.13	traces	0.09	0.10	4.36	5.43	3.94	0.10	4.23	5.43	3.84
0.5	traces	0.10	traces	0.10	0.10	4.24	5.12	4.03	0.11	4.14	5.12	3.93
(mg/cm^3) 1.0												
2.0												

present work, it may be stated that introduction of lysozyme to milk decrease the quantity of hydrolysed casein indespensable for a coagulation of this protein by rennet. Splitting off the highly-charged glycomacropeptide from casein by rennet lowers the charge of micelles to a certain critical value at which a modification of micellar structures and their coagulation takes place. The probable partial neutralization of micellar charge by lysozyme causes that the quantity of spllited glycomacropeptide necessary for reducing the micellar charge is lower. Hence, the modification of micellar structures by rennet in lysozyme-treated milk is easier.

This is also an explanation of the shorter time of rennet coagulation of lysozyme-treated milk. The obtained results are also a valuable information on lysozyme's effect on cow milk casein.

CONCLUSIONS

The applied additions of lysozyme (0.5; 1.0; 2.0 mg/cm^3) did not affect significantly the degree of hydrolysis of casein by rennet but only decreased the amount of glycomacropeptide spllited off from casein indispensable for coagulation of this protein.

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WPŁYW LIZOZYMU NA ZAKRES PROTEOLITYCZNEJ FAZY PODPUSZCZKOWEGO KRZEPNIĘCIA MLEKA

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Streszczenie

W pracy prześledzono wpływ dodatku lizozymu (0,5; 1,0; i 2,0 mg/cm^3) na proteolizę podpuszczkową mleka. Doświadczenie wykonano w 3 seriach, w których substrat do badań stanowiły kolejno: mleko regenerowane z odtłuszczonego proszku mlecznego, 1% roztwór κ-kazeiny oraz roztwór miceli kazeinowych wyizolowanych z mleka przez wirowanie. Miarą stopnia proteolizy było oznaczenie przyrostu ilości związków azotowych rozpuszczalnych w 12% KTO oraz ilości N-acetylo-D-galaktozaminy (NAcGal) związanej z tą frakcją po inkubacji prób z podpuszczką.

Wyniki doświadczeń przeprowadzonych na mleku nie dały jednoznacznej odpowiedzi co do wpływu lizozymu na process proteolizy (tab. 1 i 2). Nie stwierdzono również istotnych różnic w ilości uwalnianych związków azotowych pomiędzy próbkami z lizozymem i próbkami kontrolnymi, w doświadczeniach przeprowadzonych na roztworze κ-kazeiny (tab. 3 i 4) oraz na micelach kazeinowych zdyspergowanych w sztucznym serum mleka (tab. 5, 6).

Na podstawie uzyskanych w pracy wyników można stwierdzić, że lizozym nie wpływał w istotny sposób na hydrolizę kazeiny przez podpuszczkę. Zaobserwowane obniżenie poziomu uwalnianych glikopeptydów w próbkach z wyższym dodatkiem lizozymu (2 mg/cm^3) potwierdza wysuwaną przez niektórych autorów hipotezę, że lizozym obniża jedynie niezbędną ilość uwalnianego z kazeiny glikomakropeptydu, konieczną do spowodowania koagulacji tego białka pod wpływem podpuszczki.