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FACTORS AFFECTING THE SYNERESIS AND COAGULATION IN ULTRA-FILTERED CHEESE

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Abstract

The use of ultrafiltration in the world as a method of the serum proteins utilization is a long tradition, although in our country it almost has no application.

During the tests were monitored three variants of ultra-filtered white brined cheese: control (M), M_1 variant with 0.6 g/L enzyme transglutaminase (TG) applied, and M_2 variant with 1.3 g/L TG enzyme. The application of microbiological enzyme transglutaminase was in order to determine its impact on the physical, technological and rheological features in the variants. The factors that affect coagulation and syneresis of the cheese weight, as well as their chemical composition were controlled in the milk laboratory at the Institute of Animal Science in Skopje, Macedonia. Actually, it was monitored how: amount of separated whey (syneresis) during coagulation, amount of rennet added and pH on the coagulation index, the occurrence of initial and complete coagulation at different temperature regime of curdling (31 and 34°C) and utilization of the milk components in retentate (concentrate), were depending on these factors.

In terms of the results it was found that the smallest amount of separated whey occurs after cutting the cheese in all three variants, with special emphasis on reduced syneresis in variants M_1 and M_2, where the enzyme is applied. In terms of environmental pH it was found that at higher pH of the environment there is a prolonged coagulation, while at pH lower than 6.51 the coagulation reduces by 12% compared to the previous (6.87), 16.44% for M_1 and 14.82% for M_2 variation in relation to the M variant. It was found that by increasing the amount of $CaCl_2$ and the curdling temperature, the coagulation accelerates too, and the TG enzyme is independent of the action calcium ions. The main goal of this research is determination of the opportunities for higher utilization of the serum-proteins and their incorporation in the protein complex, with various factors affecting the coagulation and syneresis of the cheese weight.

***Key words**: Ultra-filtered cheese, Coagulation, Syneresis.*

1. Introduction

Ultra-filtration is membrane filtration as a way of utilizing serum protein. It was first proposed by the French researchers Maubois, Masquot and Vassal [1], so in the literature this method is frequently called "MMV" procedure. The ultra-filtration enables separation of lactose, minerals, non-protein nitrogen and vitamins in permeate, while concentrating the fat and total protein in retentate. The increased interest in production of cheese type Feta by process of ultra-filtration is explained by a number of advantages, most of all by increasing the cheese production for 30%. This mode of production depends on the concentration ratio which increases the nutritious value due to incorporation of serum proteins, compared to the production of traditional Feta ([2] and [3]). The application of the transglutaminase enzyme, depending on the stage when added, causes positive effects on physical and sensory features of the final product. In our researches this enzyme was applied together with rennet and influenced the coagulation and syneresis during the production, which is also the goal of this research.

2. Materials and Methods

During the research was used cow's milk collected in Kumanovo region. By membrane filtration or ultra-filtration were produced the following experimental variants:

- *Experimental variant* 1 - M - production of white brined cheese by ultra-filtration.
- *Experimental variant* 2 - M_1 - production of ultra-filtrated white brined cheese by adding 0.6 g/L transglutaminase enzyme
- *Experimental variant* 3 - M_2 - production of ultra-filtrated white brined cheese by adding 1.3 g/L transglutaminase enzyme.

As additional raw materials during the production of white brined cheese from ultra-filtrated milk were used: adding mesophylic homoferementative starter culture type O-R704, composed of *Lactococcus lactis* supsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (Chr. Hansen, Denmark); $CaCl_2$ solution (0.02 - 0.04%); rennet: Chy Max 2080 IMCU/g, 0.02%, adding previously prepared 40% solution of $CaCl_2$, transglutaminase enzyme (TG) with microbiological origin and trade name "ACTIVA" MP, (Ajinomoto, Germany). In our research, as raw material was used concentrate - retentate of ultra-filtrated milk containing 13% proteins that served as a basis for the amount of applied transglutaminase enzyme (0.6g/L and 1.3 g/L). At all three variants were monitored the time from adding the rennet in the retentate to the starting and complete coagulation, expressed in seconds/minutes depending on: the pH value of milk, amount of added $CaCl_2$ (0.200 mg/L, 0.400 mg/L) and temperature of coagulation of milk (33 ^{0}C, 35 ^{0}C). Methodology of ultra-filtration for obtaining three experimental variants (M, M_1, M_2), was followed by identical technological operations up to the phase of curdling, i.e. when the transglutaminase enzyme was applied in two variants (M_1 and M_2) with various amounts of 0.6 g/L TG and 1.3 g/L TG enzyme. Further operations (curdling, salting, maturing) of the variants was conducted in a uniform manner of producing white brined cheese.

All laboratory tests and analyzes were conducted in the Milk Laboratory at the Sts. Cyril and Methodius University, Institute of Animal Science in Skopje, while the variants of white brined cheese made by ultra-filtration, were produced at the dairy "Zdravje Radovo" Kumanovo.

3. Results and Discussion

3.1 Chemical composition of cow's milk-raw material for ultra-filtration

Table 1 presents the results of the cow's milk chemical composition, used as raw material for production of ultra-filtrated white brined cheese. These values, compared to the chemical composition of cow's milk during the production of white brined cheese produced in classic industrial way are higher in total protein content and the ratio of protein/fat. Permeate is a whey separated during the ultra-filtration, consisted of lactose, minerals and part of non protein nitrogen.

3.2 Chemical composition of permeate

Permeate is a whey separated during the ultra-filtration, consisted of lactose, minerals and part of non protein nitrogen. In Table 2 is shown chemical composition of permeate i.e. whey separated during the ultra-filtration process. Comparatively, the values for the content of dry substance in permeate with the same value in the whey obtained by industrial mode of production of white brined cheese has large deviations. These differences show the workability of certain parameters during ultra-filtration, thus reducing losses in permeate.

Table 1. Chemical composition of cow milk

Values	Investigated parameters (%)							
	Dry matter	Fats	Total proteins	Total nitrogen	Lactose	P/F*	Acidity (^{0}SH)	Acidity pH
X (n=5)	11.93	3.37	3.14	0.49	4.49	0.93	6.32	6.43
min	11.42	3.12	2.98	0.47	4.27	0.86	6.00	6.24
max	12.25	3.50	3.31	0.52	4.60	1.06	6.80	6.60
Sd	0.31	0.16	0.15	0.02	0.13	0.08	0.33	0.14
CV	2.56	4.69	4.73	4.73	2.98	8.41	5.30	2.22

*Note: P/F - relation protein / fats

Table 2. Chemical composition of permeate (whey by ultra-filtration)

Values	Investigated parameters (%)						
	Dry matter	Total proteins	Total nitrogen	Lactose	Fats	Acidity	
						pH	(°T)
x (n=5)	5.53	0.49	0.08	4.46	0.31	6.48	11.10
min	5.07	0.35	0.05	4.21	0.06	6.29	10.20
max	5.85	0.66	0.10	4.60	0.56	6.65	11.70
Sd	0.34	0.11	0.02	0.17	0.18	0.15	0.59
CV	6.18	22.95	22.95	3.88	56.77	2.28	5.29

Table 2 represents the chemical composition of permeate i.e. whey separated during the ultra-filtration process. Comparatively, the values for the content of dry substance in permeate with the same value in the whey obtained by industrial mode of production of white brined cheese has large deviations. These differences show the workability of certain parameters during ultra-filtration, thus reducing losses in permeate.

The results in table 2 show that the average content of dry substance in the obtained permeate has lower value compared to whey obtained by industrial production method of white brined cheese. In terms of the total protein content in permeate it is lower compared to whey, which shows the fact of reduced losses and full utilization of the total proteins in retentate regarding fat. In terms of using the milk fat permeate, compared to whey obtained by industrial mode of production, no significant differences are monitored, which explains the fact that the losses of fat are usually not reduced by the process of ultra-filtration.

3.3 Factors affecting the syneresis at ultra-filtrated cheese

Table 3 shows the amount of separated whey expressed in ml at three different variants in three different stages after the curdling of the received retentate. Notable differences are visible, compared to variants where transglutaminase enzyme was applied.

In three experimental variants (M, M_1 and M_2), could be a smallest amount of separated whey after cutting cheese mass compared to the other two phases of measurement. In other words, it is notable that the smallest amount of separated whey occurs after cutting the curd cheese in three variants, with special emphasis on reduced syneresis at M_1 and M_2 variants where the enzyme was applied. The reduced syneresis and the small amount of separated whey is also explained by the fact that during the technological process is used temperature regime of 70-76 ^{0}C, which can lead to partial denaturation of the serum proteins, which directly affect the reduction of syneresis. Our results are in accordance with the studies of several authors

Table 3. Amount of separated whey (mL) at three variants (M_1, M_2 and M_3)

Variant by ultrafiltration	Amount of separated whey (mL) at different time intervals			Whey/mL milk/1000mL (%)
	After curd cutting (1,30 ^{h})	Before dry salting (20 ^{h})	After dry salting (24 ^{h})	
M	14	39	43	9.6
	15	39	41	9.5
	18	53	51	12.2
M_1	6	34	35	7.5
	8	25	38	7.1
	12	30	40	8.2
M_2	2	16	37	6.1
	3	15	35	5.8
	5	11	32	5.3

like Tratnik [4], which consider that ultra-filtrated milk needs more time for separation of the whey due to the presence of concentrated whey proteins in the retentate that have the ability to bind water, as well as the presence of globular fat in the curd cheese that prolongs the syneresis. The transglutaminase enzyme visibly reduces the syneresis and allows separation of whey in smaller amounts over a longer time interval [5]. According to Pearce and associate collaborators [6], the smaller syneresis of gel containing denatured serum proteins explains the impact of aggregate β-lg and k-casein. According to Gustaw and associate collaborators [7], while examining the impact of TG enzyme on the mechanical features of the cheese types Feta and Danbo made from ultra-filtrated milk, it was found that the syneresis decreased by 9.3 - 6.1% at Feta cheese, while at Danbo cheese it significantly decreased from 17.6% to 6.3.

3.4 Impact of the amount of added rennet and pH on the coagulation

In table 4 can be seen the dynamics of coagulation depending on pH of the environment and the amount of added rennet. For curdling is used the rennet Chy Max richness 2080 IMCU/g, added to the amount of 0.10% and 0.20%.

From the results in Table 4 it can be determined that at higher pH of the environment there is a prolonged coagulation, or in other words, if for pH 6.87 is taken with index 100%, it is evident that at pH lower than 6.51, the coagulation is reduced by 12% compared to the previous. At the same time, at the variants M_1 and M_2 (applied with enzyme), regarding the same index, the coagulation is decreased of 16,44% at M_1 and 14.82% at M_2 variation in terms of M variant. From the results in Table 4 it is also noticeable that if adding small amounts of rennet of 0.10%, under the same environmental conditions, complete coagulation occurs in almost the same time interval as while adding twice as much rennet. These data verify the economic impact of adding the enzyme transglutaminase. At M_1 variant there is faster coagulation for 14.97% and 10.98% for M_2 variant while using a smaller amount of rennet (0.10%). According to the authors Kuraishi and Sakamoto [5], the coagulum which is derived from milk previously treated with transglutaminase enzyme contains more stable covalent (ε-(γ-Glu) Lys) links that have the ability to retain molecules of water, so the whey remains incorporated in the coagulum. This research is also in accordance with our researches, i.e. the variants treated with TG enzyme, which affects aggressively the curdling and shortens the time of coagulation. According to Holmes and associate collaborators, 1977, the amount of rennet retained in curd cheese after separation of the whey, affects the speed of proteolysis. Or, the amount of retained rennet is inversely proportional to the pH value of the cheese mass after separation of the whey. This research is confirmed in case of rennet of animal origin, while at microbiological rennet's there is no change in pH value [8].

3.5 Impact of the amount of added $CaCl_2$ and curdling temperature on the coagulation

Table 5 presents the results for the impact of the amount of added $CaCl_2$ in different temperature regime on the coagulation time of the retentate.

Studies of the impact of $CaCl_2$ on the time of curdling (complete coagulation) of concentrate in data shown in Table 5, confirm that there is a linear connection between the amount of added $CaCl_2$ and time of curdling. Taken into consideration and comparing the three variants (M, M_1 and M_2), although there is a difference in the last two variants in terms of added enzyme, no significant difference in terms of increasing the amount of calcium ions exists. In fact, this type of microbiological enzyme applied in two variants M_1 and M_2 is independent regarding the action of calcium ions, unlike transglutaminase of animal origin.

Table 4. Index of coagulation time in correlation with added rennet and pH

Factors		Amount of added rennet			
		0.20%		0.10%	
Variants	pH	Time (sec.)	Coagulation index (%)	Time (sec.)	Coagulation index (%)
M	6.87	1500	100	1570	100
	6.51	1320	88	1450	92.35
M_1	6.87	1460	100	1470	100
	6.51	1220	83.56	1250	85.03
M_2	6.87	1080	100	1112	100

Table 5. Time of coagulation (complete coagulation), with different amount of added $CaCl_2$ and temperature of curdling on retentate

Variants	Factors		Complete coagulation	
	added $CaCl_2$ (%)	T° of curdling 31°C / 34°C	Time (sec.)	Coagulation index (%)
M	0.20	31	1370	100
	0.40	34	1056	77,08
M_1	0.20	31	1160	100
	0.40	34	1030	88,79
M_2	0.20	31	990	100
	0.40	34	854	86,26

Therefore, we believe that by increasing the amount of added $CaCl_2$ and raising the temperature of curdling, there are no significant differences in the variant M, where the same is not used. Our studies are in accordance with the authors de Wit and Klarenbeek [9], who followed the reversible variations as a result of serum proteins under temperature to 60°C. These changes are as a result of hydrophobic interaction, whose intensity increases with increasing the temperature itself. The obtained reaction can be intermolecular or intramolecular, when it comes to association or disassociation of the proteins of the whey. This phenomenon is often referred to as phase of "pre denaturation" caused by partial loss of three-dimensional structure and changes in the hydration. But the effect of polymerization of the protein with acting of the transglutaminase depends on the structure of the macromolecule of each protein substrate [10].

3.6 Chemical composition of retentate

The results of the chemical composition of obtained retentate can be seen in Table 6. In our research, the concentration of milk is 1:4, until reaching the dry substance of retentate 22-24%, measured by refractometer during the technological process.

From the results in Table 6, it can be seen that the total protein content ranged from 12.90 to 14.27%, with deviations of 1.37%, while the milk fat content from 13 to 15%, with deviations of 2%. In terms of concentration of retentate and its impact on the speed of formation of curd cheese, there are different opinions of various authors. The increased level of concentration of milk, a number of authors [11 and 12], confirmed it as a result of the reduction of coagulation time. Despite these authors, Garnot and the associate collaborators [13], consider that the increasing of the concentration of proteins results with prolonged coagulation. On the other hand, Lusicano and associate collaborators [14], consider that the coagulation time does not change until the protein content reaches up to 7%, but any increase of that protein content in the concentrate results with shortened coagulation. The author Puđa [15] refers to this opinion, stating that with any increase of the concentration of protein over 6%, comes to shortening the time between the initial coagulation and formation of curd cheese to certain solidity. The same found that with each increase of the concentration ratio of over 1:4 increased the content of fat in it over 10%, which is in accordance with our research, for average content of milk fat in retentate which is 13.98%.

Table 6. Chemical composition of retentate

Values	Investigated parameters (%)					
	Dry matter	Total proteins	Total nitrogen	Fats	Acidity	
					(°T)	pH
x (n=5)	24.00	13.60	2.13	13.98	14.88	6.69
min	24.00	12.90	2.02	13.00	13.60	6.60
max	24.00	14.27	2.24	15.00	16.00	6.76
Sd	0.00	0.62	0.10	0.73	1.04	0.06
CV	0.00	4.53	4.53	5.22	6.96	0.96

4. Conclusions

- The reduced syneresis in ultra-filtrated variants is as a result of the concentration of the and incorporation of milk serum proteins with casein in the milk, with special emphasis at variants M_1 and M_2 due to the additional application of the transglutaminase enzyme, which directly affects this process. In our researches, reduction of syneresis at variants M, M_1 and M_2 are with a value: 10.4% (M), 7.6% (M_1) and 5.7% (M_2).
- Time index of complete coagulation at variants differs as a result of variety of technological production process, i.e. at ultra-filtrated cheese it is reduced for a period of 20 min. on average, depending on factors that condition it (temperature, pH and amount of added $CaCl_2$. Time index of complete coagulation is equal to the ratio of the concentration during ultra-filtration, i.e. 1:4.

5. References

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