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Dynamics of Active and Titratable Acidity in Bieno Cheese

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ABSTRACT

Experimental research describes the autochthonous traditional production of bieno cheese and its importance in conditions of globalization and industrialization in food production.

The purpose of this research was to present the technology of three varieties of indigenous bieno cheeses (A, B, and C) in the Mariovo region, in the Republic of North Macedonia, and to monitor the dynamics of active and titratable acidity as important parameters in the technological process.

During the research, the changes of these parameters of the three variants (A, B, and C) of cheeses in different stages of maturation were monitored (in curd, cheese after dry ripening, cheese after dry salting, the 20th day of ripening, and the 45th day of ripening). The rheological properties of the cheese are greatly influenced by the active (pH) and titratable (^{0}SH) acidity. During the ripening of cheeses under the influence of lactic acid bacteria, the biggest changes occur in lactose, which transforms lactose into lactic acid and other organic acids that play an important role in the formation of sensory and rheological characteristics of cheeses.

The displayed technological parameters are of great importance for the bieno cheeses and can serve in the direction of standardization of this cheese and its protection with a designation of origin or a geographical indication based on the source technology.

***Key Words**: Bieno cheese, technology, active and titratable acidity*

INTRODUCTION

Cheese ripening is a complex process that involves the breakdown of the coagulum by proteolysis, lipolysis, and other enzyme-catalyzed reactions which, in turn, result in changes in taste and texture typical of different types of cheese. Enzymatic processes are responsible for the production of a significant number of ingredients, which as a result of their presence, concentration and ratio are characteristic of certain types of cheese (Woo & Lindsay, 1984).

Cheese maturation is a complex of interrelated complex biochemical processes in which the deepest changes undergo proteins, so the proteolysis of proteins during the maturation process has a significant impact on the formation of consistency, texture, appearance, taste, and aroma of cheeses, (Baltadzhieva, 1993).

Maturation in the broadest sense of the word begins in milk, continues during coagulation, and finally acquires the greatest dynamics during maturation. The changes of the ingredients in the different stages of maturation are not equal. The volume and depth of maturation are determined by the ripening conditions, pH, quantity, and type of microorganisms, as well as by the length of maturation (Mańćić, 1994). The influence of proteolytic enzymes on the ripening of bieno cheese is monitored in the research of Mateva, (2004) who found that the chemical composition of bieno cheese where genetic renin is used, on the 45th day of ripening is as follows: dry matter 59.67 %, fat 21.74%, protein 21.59%, salt 3.58%, yield 10.96%, acidity 191^{0}T and pH 5.24. Several parameters determine the formation of taste in cheese: starter cultures (Kieronczyk et al., 2004; Smit & Engels, 2005), non-starter

lactic acid bacteria (Beresford & Cogan, 2000).

The role of lactic acid bacteria in the development of texture and organoleptic characteristics depends on the enzyme potential that they retain in the process of cheese production. Peptides are the most important products of non-starter lactic acid bacteria enzymes in the cheese ripening process and are responsible for producing free amino acids and small peptides (Fox et al., 1998).

MATHERIALS AND METHODS

The production of Bieno cheese was made in three different locations in the Mariovo region, each of them owing to a special production technology (A, B, and C). The technological procedure includes: filtering the milk and transporting it to the duplicator where reheating to a temperature of 35-37°C has been performed. Then, a curdling with final coagulation of 45-60 minutes was performed. When the coagulum has become firm enough, it was processed for 2-5 min thus acquiring a grainy structure, and left to rest for 10-15 minutes. This was followed by beating again for 2-5 minutes and obtaining curds in the size of a grain of corn. There was a resting time in order to separate the whey, and at the end 2/3 of the whey was removed with a pump. In the next phase, the cheese mass was steamed for 30 minutes, then the curd was separated at the bottom of the cheese bathtub, followed by cutting, kneading, and shaping of the cheese dough. The shaped cheese dough in a form of a ball was left to drain, i.e. self-pressing for a period of 16-18 hours. The self-pressing was left up until the next day. Further on followed the phase of dry ripening of the cheese. The formed blocks are placed in wooden molds, on wooden shelves in a maturation chamber, where t = 26-280°C and the relative humidity is 90-95%, for a period of 5-6 days, during which the cheese blocks get a wax-yellow color. After the completion of the ripening period of the cheese blocks, they are cut along their entire length into 5-6 cm strips. Dry salting with coarse sea salt begins after 1-2 days, and subsequently they are placed in bins while 20-22% salt is added. The cheese is stored at t = 16-18°C in a room where it is further matured for up to 45 days and finally stored.

The operations of this production technology are the same as for the produced Bieno cheese A, B and C, however, there are differences in the used rennet. Bieno cheese from the production series A is produced in industrial conditions using chymosin - cheese powder, CHY-MAX with an intensity of 2080 imcv/g, while the production series B, C are produced in two different individual households with an indigenous technology applied, using microbiological rennet - "Kvasko" with an intensity of 1: 5000.

During the experimental research, active and titratable acidity tests were performed on three varieties of bieno cheese (A, B, and C) at different stages of ripening, as follows:

- In curd
- In cheese after ripening for 5-6 days
- In cheese after dry salting
- In cheese after the 20th day in brine
- In cheese after the 45th day in brine

In cheese were conducted the following analysis:

Active acidity in the cheese with a digital pH meter (testo 206);

Titrable acidity (^{0}SH) - by the method of Soxhlet Henkel (Caric et al. 2000)**;**

RESULTS AND DISCUSSION

The rheological properties of the cheese depend on its chemical composition. The content of water, proteins, active (pH), and titratable acidity (^{0}SH) are very important.

Titratable acidity is an important parameter that gives us the value for the total amount of acids found in the product. During the fermentation process in bieno cheese, there is a constant increase in titratable acidity, and the reason for this is

the breakdown of lactose by lactic acid bacteria to lactic acid, and also as a reason for the increase in titratable acidity can be the breakdown of some protein components and salts.

Active acidity (pH) is defined as the negative decadic logarithm of the hydrogen ion concentration. According to Baltadzieva (1993) this important parameter in cheese production has a control function and determines buffer capacity because by increasing the concentration of hydrogen or hydroxide ions to some extent, the active acidity remains unchanged.

During ripening, the largest changes occur in milk sugar under the action of lactic acid bacteria, which transforms milk sugar into lactic acid and other organic acids that play an important role in the formation of sensory and rheological characteristics of cheeses (Fox & Cogan, 1990; Fox et al., 1993). According to the authors, about 98% of lactose is transformed very quickly, which, of course, depends on the salt content in the aqueous phase of the cheese. The initial acidity of the cheese, to a large extent, affects the further course of biochemical processes during cheese ripening. That is why the acidity of the cheeses is used as one of the indicators for the way of conducting the technological process during the making of the cheese and for the achieved degree of ripening. During ripening, complex biochemical changes occur that lead to the formation of sensory as well as rheological characteristics of the cheese.

In the research, the dynamics of active and titratable acidity during the different stages of ripening of bieno cheese are monitored, which are shown in Table (1) and Table (2).

From the obtained results it can be concluded that in the first stage of ripening in the three varieties of bieno cheeses, variant A, B, and C the active (pH) acidity ranges from: 6.21 ± 0.10; 6.38 ± 0.05 and 6.55 ± 0.03. At this stage of ripening of bieno cheese, there are statistically significant differences in the values of active acidity and their initial acidity, which has a great impact on the further technological process. The lowest pH values are present in variant A, the highest in variant C and the highest values in variant B.

In the second stage of maturation, after dry maturation, the values for the active acidity of the three variants are as follows: 5.48 ± 0.10; 5.61 ± 0.05 and 5.58 ± 0.14. At this stage, there are no significant differences in the values for the active acidity. In the third stage of maturation, after dry salting, the following values of active (pH) acidity appear 5.36 ± 0.03; 5.48 ± 0.09 and 5.46 ± 0.02. There are statistically significant differences at the level of $p < 0.05$ as a result of the influence of the different percentages of salt, during the manual addition of salt, ie during the dry salting.

On the 20th day of ripening of the bieno cheese in the three variants, the active (pH) acidity is as follows: 5.30 ± 0.07; 5.44 ± 0.06 and 5.40 ± 0.02. At this stage, statistically, significant differences also occur at the level of $p < 0.05$.

On the 45th day of ripening the bieno cheese in the three variants, the active (pH) acidity gradually decreases at A to 5.24 ± 0.02; in B at 5.41 ± 0.07 and in C at 5.32 ± 0.06. There are no statistically significant differences between the three varieties of bieno cheeses.

Table 1: Dynamics of active acidity in bieno cheese

		Bieno cheese A					Bieno cheese B					Bieno cheese C				
		$\bar{x}$	min	max	Sd	Cv	$\bar{x}$	min	max	Sd	Cv	$\bar{x}$	min	max	Sd	Cv
pH	**Curd**	6,21±0,10	6,02	6,38	0,14	2,20	6,38±0,05	6,26	0,22	0,07	1,14	6,55±0,03	6,50	6,58	0,04	0,55
	After dry ripening	5,48±0,07	5,34	5,57	0,09	1,67	5,61±0,05	5,55	0,32	0,06	1,06	5,58±0,14	5,42	5,76	0,17	3,02
	After dry salting	5,36±0,03	5,31	5,41	0,04	0,69	5,48±0,09	5,40	0,46	0,11	2,03	5,46±0,02	5,43	5,49	0,03	0,47
	20th day of ripening	5,30±0,07	5,20	5,39	0,08	1,59	5,44±0,06	5,36	0,56	0,09	1,63	5,40±0,02	5,38	5,43	0,02	0,40
	45th day of ripening	5,24±0,02	5,21	5,29	0,03	0,60	5,41±0,07	5,30	0,09	0,10	1,84	5,32±0,06	5,22	5,40	0,08	1,45

Table 2: Dynamics of titratable acidity in bieno cheese

		Bieno cheese A					Bieno cheese B					Bieno cheese C				
		$\bar{x}$	min	max	Sd	Cv	$\bar{x}$	min	max	Sd	Cv	$\bar{x}$	min	max	Sd	Cv
°SH	**Curd**	20,04±0,51	19,20	20,60	0,61	3,04	17,17±0,83	15,46	0,13	1,11	6,47	18,33±0,76	16,56	19,80	1,16	6,35
	After dry ripening	49,94±0,97	48,28	51,28	1,23	2,46	45,72±0,62	44,64	0,14	0,81	1,77	47,63±0,46	46,52	48,60	0,74	1,55
	After dry salting	61,45±1,55	58,96	64,56	2,12	3,45	58,04±1,73	55,60	0,15	2,30	3,96	60,02±0,42	59,56	60,60	0,49	0,82
	20th day of ripening	64,83±0,37	64,45	65,40	0,43	0,67	63,68±0,54	62,60	0,17	0,73	1,15	63,22±0,42	62,60	63,80	0,50	0,80
	45th day of ripening	67,70±0,28	67,40	68,40	0,41	0,60	65,42±0,08	65,28	10,15	0,10	0,15	66,30±0,36	65,40	66,60	0,51	0,76

$F_{0,05}$=3,88 за p<0,05
$F_{0,01}$=6,93 за p<0,01

Bieno cheese in all three variants shows a tendency to decrease the active acidity, which is most pronounced in variant A. In variants A and C there are more intense dynamics of active acidity resulting in more intense biochemical processes that affect the sensory and rheological characteristics of the cheese.

The dynamics of titratable acidity during different stages of ripening of bieno cheese are shown in Table 2.

The titratable acidity in the initial stage of ripening in the three varieties of bieno cheeses has the following values: 20.04 ± 0.51 SH^0; 17.17 ± 0.83 SH^0 and 18.33 ± 0.76 SH^0. At this stage, statistically significant differences in the titratable acidity values are established. The increase in acidity in the first period of maturation is due to the active breakdown of lactose by lactic acid microorganisms, the partial breakdown of proteins, and some salts (Gruev, 1995; Fox, 1993).

At the beginning of maturation, the titratable acidity in the three varieties is 20.04 ± 0.51 SH^0; 17.17 ± 0.83 SH^0, and 18.33 ± 0.76 SH^0.

In the second phase after the dry maturation, the titratable acidity increases in variant A to 49.94 ± 0.97 SH^0, in variant B to 45.72 ± 0.62 SH^0, and in variant C to 47.63 ± 0.46. SH^0.

In the third stage of maturation, after dry salting, the titratable acidity in variant A increases to 61.45 ± 1.55 SH^0, in variant B to 55.58.04 ± 1.73 SH^0, and in variant C to 60.02 ± 0, 42 SH^0. At this stage of maturation, statistically, significant differences are found at the level of p <0.05.

On the 20th day of ripening of the cheeses in brine, the three bieno cheeses (variants A, B, and C) show a slight increase in the titratable acidity values, as follows: 64.83 ± 0.37 SH^0; 63.68 ± 0.54 SH^0 and 63.22 ± 0.42 SH^0.

On the 45th day of ripening in brine, the following values of titratable acidity were ascertained: 67.70 ± 0.28 SH^0, 65.42 ± 0.08 SH^0, and 66.30 ± 0.36 SH^0.

From the beginning to the end of maturation, when monitoring the titratable acidity, an increase of the titratable acidity is determined in variant A by 47.66 degrees SH^0, in variant B by 48.25 degrees SH^0 and in variant C for 47.97 degrees SH^0. In all stages of ripening of bieno cheeses, there are statistically significant differences in the values of titratable acidity, ie different dynamics of titratable acidity which is an indicator of the different intensity of biochemical processes.

By comparing the obtained results to the results of other authors, it can be concluded that in Kapac-Parkacheva et al. 1974, pH values range from 5.28 to 5.65, which means they are similar to the values obtained from this research. Mateva, (2008), working on research in bieno cheese also gets results close to these results, while Radevska et al., (2003) get values for active acidity in the range of pH 4.98 to 5.10, and higher titratable acid values in the range 87.3 to 91.8 SH^0.

Muir et al., (1995) determine the pH values of mature gouda 5.44 and pH 5.26 in Edam cheese that is close to the results of this research of bieno cheese, while Joosten, (1988), notes a tendency to increase in pH

during maturation to 5.5 during the six-month maturation of gouda.

In the active acidity tests Scott, 1986 observed a slight increase in pH of 5.15 after pressing, 5.20 after dry salting, and 5.40 after eight weeks of maturation, which correlated with the results of this study on active acidity in varieties of bieno cheeses.

Mihajlov (2003), examining the active acidity of cheddar in different stages of maturation, finds that on the 45th day the active acidity shows similar results with these results, obtained from the same period of maturity. Similar examinations were obtained from Dusica Santa and Sonja Srbinovska (2014) during the examinations of Galichnik kashkaval in different periods of its ripening, where a decrease in the active acidity and an increase in the titratable acidity were determined.

CONCLUSION

During the ripening of cheeses under the influence of lactic acid bacteria, the biggest changes occur in lactose, which transforms lactose into lactic acid and other organic acids that play an important role in the formation of sensory and rheological characteristics of cheeses.

During the ripening of the three variants of bieno cheeses (A, B, and C) the dynamics of the active and titratable acidity was monitored and a tendency of decrease of the active acidity was ascertained, which is mostly expressed in variant A. Variant A and variant C have the most intense dynamics of active acidity resulting in more intense biochemical processes affecting the sensory and rheological characteristics of variants A and C. Regarding the dynamics of titratable acidity, there is a tendency of increased growth, most pronounced in variant A. In all stages of ripening of bieno cheeses, there are statistically significant differences in the values of titratable acidity, ie different dynamics of titratable acidity which is an indicator of the different intensity of biochemical processes.

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