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Enzymes influence on the physico-chemical characteristics during semi-hard paste cheese rippening

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Abstract

The main purpose of this research is the optimization of the maturation period using proteolytic and lipolytic enzymes, in order to obtain a final product with improved sensorial, physicochemical, nutritive and biological characteristics.

Keywords: lipolytic enzymes, proteolytic enzymes, dry substance, proteins

1. Introduction

The opportunity of the study lies in the fact that a better comprehension of maturation process leads to the optimization of this process and to an improved cheeses quality.

There are two distinguish phases in the cheeses maturation process: first phase, the first 10 days of maturation, is characterized by a slow modification of parameters values; the second phase, after 10 days of maturation, is characterized by a significant parameters changes. Thus, analyzing the cheeses composition, the maturation period can be estimated.

The physicochemical properties of experimental samples of Holland cheese were determined (from the processing phase until the end of maturation).

This study took into consideration three types of semi-hard cheeses:

A. Variant I – witness sample – the cheese was obtained using the classic production technology for the Holland cheese assortment;

B. Variant II – cheese with added lipolytic enzymes; aroma lipolytic enzymes were also added in milk before coagulation;

C. Variant III – cheese with added proteolytic enzymes; proteolytic enzymes (papain) were also added in milk before coagulation.

2. Materials and methods

The maturation parameters (temperature and humidity) were monitored, experimental cheese sample being ripening in the same conditions (table 1).

Table 1. The variation of temperature and relative humidity of air in the maturation room of experimental cheeses samples:

Crt. Nr.	Sample	The number of records	Mean value	
			Tempera-ture, °C	Relative humidity, %
1.	M_1, Bl_1, Bp_1	2	13.5	86
2.	M_2, Bl_2, Bp_2	6	16	84
3.	M_3, Bl_3, Bp_3	20	14.5	82
4.	M_4, Bl_4, Bp_4	20	15	82.5
5.	M_5, Bl_5, Bp_5	22	15	83

The samples were analyzed at certain time intervals from production (24 hours from production, 10 days, at 24-35-50 days of maturation):

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M_1 – the witness sample at 24 hours from production;
Bl_1 – the cheese sample with lipolytic enzymes 24 hours from production;
Bp_1 – the cheese sample with proteolytic enzymes at 24 hours from production;
M_2 – the witness sample at 10 days from production;
Bl_2 – the cheese sample with lipolytic enzymes at 10 days from production;
Bp_2 – the cheese sample with proteolytic enzymes at 10 days from production;
M_3 – the witness sample at 25 days of ripening;
Bl_3 – the cheese sample with lipolytic enzymes at 25 days of ripening;
Bp_3 – the cheese sample with proteolytic enzymes at 25 days of ripening;
M_4 – the witness sample at 35 days of ripening;
Bl_4 – the cheese sample with lipolytic enzymes at 35 days of ripening;
Bp_4 – the cheese sample with proteolytic enzymes at 35 days of ripening;
M_5 – the witness sample at 50 days of ripening;
Bl_5 – the cheese sample with lipolytic enzymes at 50 days of ripening;
Bp_5 – the cheese sample with proteolytic enzymes at 50 days of ripening.

The methodology used for cheese examination:

- Fat reported to dry substance, % minim	Acido-butirometric method	STAS 6344/88
- dry substance, % minimum	Drying closet method	SR ENISO 5534/2004
- Proteins, % minimum	Kjeldahl method	STAS 6355 – 89
- Sodium chloride, % maximum	Silvermetric titration method	STAS 6354 – 84
- Acidity, Thörner grades	Actual method by titration	SR ISO 1740/2004

3. Results and Discussion

The *dry substance content* in the investigated cheeses samples increases during the study. In all cases a slower increase was noticed between the time periods 25-35 day of maturation. The *water content* for all analyzed experimental cheese samples, registered a constant decrease during the study. The *fat content reported to dry substance from cheese* (G_{SU}), decrease in all cases, *the proteins content* for Holland cheeses assortment showed a decrease during maturation.

A pronounced acidification was noticed, in all cases, after 35 days of maturation. The *pH* value increases in the first phase of maturation, and at the end of the maturation period, the value of pH decrease for cheese samples with added enzymes and increase for witness sample.

In tables 2, 3 and 4 results of statistical analysis of physical-chemical parameters (dry substance content, humidity, fat, reported to dry substance content from cheese, proteins, titrable acidity, pH) are shown, in case of experimental variants with and without added enzymes at different stages of maturation (two way ANOVA).

*In the control version***,** statistically significant differences (***) were recorded between M_1-M_5, M_3-M_5 and M_1-M_5 samples, for the following parameters: dry matter content, moisture and NaCl. G_{SU} parameter showed distinct significant differences (**) between samples M_1-M_3 and M_1-M_5,, between samples M_3-M_5 are not significant differences (ns).

Protein content showed statistically significant differences (ns) only in the first 25 days of ripening, and between samples M_3-M_5 and M_1-M_5 distinct differences were significant (**). Between samples M_1-M_3, the acidity and pH parameters recorded very significant differences (***). For the acidity and pH parameters were recorded distinct significant differences (**) between M_3-M_5 samples and very significant differences (***) in M_1-M_5 samples.

*In the experimental version of cheeses with lipolytic enzymes***,** significant differences were observed between samples Bl_1 – Bl_3, Bl_3 – Bl_5 and Bl_1 – Bl_5 (***) regarding the following parameters: dry matter content, moisture, NaCl, and acidity. In case of the fat content on dry weight and pH, statistically significant differences (*) between Bl_1 – Bl_3 and Bl_3 – Bl_5 samples were observed and not significant differences (ns) between Bl_3 – Bl_5 samples. The protein content showed statistically significant differences (*) between samples Bl_1 – Bl_3 and distinctly significant (**) between Bl_3 – Bl_5 and Bl_1 – Bl_5 samples.

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Table 2. Statistical analysis of physicochemical parameters of cheese witness sample (without added enzymes)

Nr crt	*Parameters analyzed*	M_1-M_3	*t*	*p*	Significance of differences	M_3-M_5	*t*	*p*	Significance of differences	M_1-M_5	*t*	*p*	Significance of differences
1.	Dry substance, %	-10.06 ± 0.4905	20.51	p<0.0001	***	-3.260 ± 0.3692	8.829	0.0009	***	-13.32 ± 0.3352	39.74	p<0.0001	***
2.	Humidity, %	9.960 ± 0.5006	19.90	p<0.0001	***	3.260 ± 0.3692	8.829	0.0009	***	13.22 ± 0.3498	37.80	p<0.0001	***
3.	NaCl, %	-1.000 ± 0.03697	27.05	p<0.0001	***	-0.4000 ± 0.03697	10.82	0.0004	***	-1.400 ± 0.03266	42.87	p<0.0001	***
4.	Fat in dry substance, %	2.150 ± 0.3476	6.185	0.0035	**	0.7700 ± 0.3563	2.161	0.0968	ns	2.920 ± 0.3757	7.773	0.0015	**
5.	Proteins, %	1.110 ± 0.5381	2.063	0.1081	ns	2.360 ± 0.3571	6.608	0.0027	**	3.470 ± 0.4936	7.030	0.0022	**
6.	Titrable acidity, °T	-75.00 ± 8.165	9.186	0.0008	***	-65.00 ± 10.41	6.245	0.0034	**	-140.0 ± 10.41	13.45	0.0002	***
7.	pH	-0.4100 ±0.04082	10.04	0.0006	***	-0.2200 ± 0.04509	4.879	0.0082	**	-0.6300 ± 0.04509	13.97	0.0002	***

ns – ($p > 0.05$); * – ($0.01 < p < 0.05$); ** – ($0.001 < p < 0.01$); *** – ($p < 0.001$).

Table 3. Statistical analysis of physicochemical parameters of cheese sample with added lipolytic enzymes

Nr. crt.	*Parameters analyzed*	Bl_1-Bl_3	*t*	*p*	Significance of differences	Bl_3-Bl_5	*t*	*p*	Significance of differences	Bl_1-Bl_5	*t*	*p*	Significance of differences
1.	Dry substance, %	-6.360 ± 0.4334	14.67	0.0001	***	-5.470 ± 0.3379	16.19	p<0.0001	***	-11.83 ± 0.3049	38.80	p<0.0001	***
2.	Humidity, %	6.360 ± 0.4334	14.67	0.0001	***	5.470 ± 0.3379	16.19	p<0.0001	***	11.83 ± 0.3049	38.80	p<0.0001	***
3.	NaCl, %	-1.080 ± 0.03266	33.07	p<0.0001	***	-0.3000 ± 0.02887	10.39	0.0005	***	-1.380 ± 0.02887	47.80	p<0.0001	***
4.	Fat in dry substance, %	1.780 ± 0.5811	3.063	0.0376	*	0.7700 ± 0.3064	2.513	0.0659	ns	2.550 ± 0.5876	4.340	0.0123	*
5.	Proteins, %	1.720 ± 0.4500	3.823	0.0187	*	2.000 ± 0.3511	5.696	0.0047	**	3.720 ± 0.4500	8.267	0.0012	**
6.	Titrable acidity, °T	-105.0 ± 10.41	10.09	0.0005	***	-100.0 ± 10.41	9.608	0.0007	***	-205.0 ± 8.165	25.11	p<0.0001	***
7.	pH	-0.1600 ± 0.04082	3.919	0.0173	*	-0.02000 ± 0.04509	0.4435	0.6803	ns	-0.1800 ± 0.04509	3.992	0.0162	*

ns – ($p > 0.05$); * – ($0.01 < p < 0.05$); ** – ($0.001 < p < 0.01$); *** – ($p < 0.001$).

Table 4. Statistical analysis of physicochemical parameters of cheese sample with added proteolytic enzymes

Nr. crt.	*Parameters analyzed*	Bp_1-Bp_3	*t*	*p*	Significance of differences	Bp_3-Bp_5	*t*	*p*	Significance of differences	Bp_1-Bp_5	*t*	*p*	Significance of differences
1.	Dry substance, %	-6.580 ± 0.5222	12.60	0.0002	***	-6.960 ± 0.5298	13.14	0.0002	***	-13.54 ± 0.2455	55.15	p<0.0001	***
2.	Humidity, %	6.580 ± 0.5222	12.60	0.0002	***	6.960 ± 0.5298	13.14	0.0002	***	13.54 ± 0.2455	55.15	p<0.0001	***
3.	NaCl, %	-0.4000 ± 0.03697	10.82	0.0004	***	-0.3800 ± 0.02887	13.16	0.0002	***	-0.7800 ± 0.03367	23.17	p<0.0001	***
4.	Fat in dry substance, %	2.190 ± 0.4759	4.602	0.0100	*	2.000 ± 0.3919	5.103	0.0070	**	4.190 ± 0.4759	8.805	0.0009	***
5.	Proteins, %	2.560 ± 0.3927	6.519	0.0029	**	2.200 ± 0.2972	7.402	0.0018	**	4.760 ± 0.3279	14.52	0.0001	***
6.	Titrable acidity, °T	-85.00 ± 10.41	8.167	0.0012	**	-80.00 ± 8.165	9.798	0.0006	***	-165.0 ± 10.41	15.85	p<0.0001	***
7.	pH	-0.3900 ± 0.04082	9.553	0.0007	***	-0.07000 ± 0.04082	1.715	0.1616	ns	-0.4600 ± 0.04082	11.27	0.0004	***

ns – ($p > 0.05$); * – ($0.01 < p < 0.05$); ** – ($0.001 < p < 0.01$); *** – ($p < 0.001$).

In the experimental version of cheeses with proteolytic enzymes, significant differences were observed between $Bp_1 – Bp_3$, $Bp_3 – Bp_5$ şi $Bp_1 – Bp_5$ (***) samples, for the following parameters: dry matter content, moisture and NaCl. G_{SU} content presented significant differences (*) between samples $Bp_1 – Bp_3$, distinctly significant differences (**) between samples $Bp_3 – Bp_5$ and very significant differences (***) between $Bp_1 – Bp_5$. The protein content showed statistically significant differences (**) between samples $Bp_1 – Bp_3$, $Bp_3 – Bp_5$ and very significant (***) between samples $Bp_1 – Bp_5$. The acidity parameter showed distinct statistically significant differences (**) between samples $Bp_1 – Bp_3$ and very significant differences (***) between $Bp_3 – Bp_5$ and $Bp_1 – Bp_5$, the pH showed statistically significant differences (ns) only between $Bp_3 – Bp_5$ samples and between $Bp_1 – Bp_3$ and $Bp_1 – Bp_5$ samples significant differences (***) can be observed.

4. Conclusion

- The cheeses maturation starts in the processing tank, so the lactic fermentation phase is rapidly taking place during the preparation for coagulation and during milk coagulation. By adding in the pasteurized milk the selected cultures of lactic bacteria, the maturation process can be directed in order to obtain final products with constant and uniforms characteristics, thus preventing the influence of microbiological daily variations of collected milk on the quality of cheeses. After the obtaining of curd, the aim of all further operations is to bring the clot in a compact mass. In this way, the optimal conditions for specific lactic bacteria growth are assured, in order to have the required enzymatic activity for the transformation of cheeses main compounds.
- After the analyses of sensorial, physicochemical and biochemical parameters of cheese samples, differences between samples were observed. Thus, if regarding the humidity, dry substance and fat reported to S.U. contents, all values are according to STAS (for all samples), the aroma and flavour principles are not fully expressed for the samples with no added enzymes. So, the general cheeses composition was improved by the added enzymes.
- Some of the *advantages* of enzymes use are:
  - Obtaining an improved taste and aroma in a shorter time;
  - Improvement of biological value by accumulation of essential amino acids in a concentration that can cover the daily quantity for human;
  - Relatively low costs with enzymes reported to liter of raw milk;
  - Reduction of costs for exploitation and maintenance of maturation spaces (rooms);
  - Smaller spaces immobilized for maturation;
  - Time and manual work economy in maturation phase.

As disadvantages:

- Supramaturation risk;
- The possibility of a non-uniform repartition of enzymes in cheese;
- The possibility of bitter taste and unspecific aroma appearance.

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