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THE INFLUENCE OF DIFFERENT AMOUNT OF STARTER CULTURE ON THE PROPERTIES OF YOGURTS OBTAINED FROM EWE'S MILK

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ABSTRACT

Ewe's milk was inoculated with 2%, 3% and 4% yogurt starter culture Y180 DVS (Ch. Hansen). The yogurts were stored at 4°C for 14 days. The raw milk and pasteurized milk were analyzed, also yogurt after 1, 7, 14 days of storage were estimated. Organoleptic evaluation and texture analyses were done in yogurts. It was observed that yogurts made with higher starter's addition contained more free fatty acids, the vitamin C and the ascorbic acid, but less nitrogen compounds. Amount of starter influenced the texture parameters.

Key words: ewe's milk, yogurt, starter culture, physico-chemical properties, vitamin C, texture

INTRODUCTION

The properties of yogurt such as: titratable acidity, pH, free fatty acids contents, aromatic compounds contents (acetaldehyde, diacetyl, ethanol) also sensory quality and nutritional value depend on the different factors: quality of milk used to the production, technology, additives and kind of yogurt starter culture and its activity. That is established that fermentative, aromatic, lipolytic and proteolytic ability of starters' bacteria vary [2, 9,11, 23]. Changes in yogurts caused by starters' culture microorganisms have many directions. As the results of these changes new substances are formed, that are often included in the metabolic cycle changes of bacteria or are used by other bacteria. The knowledge of starter culture composition and of the ratio between type of bacteria

allow to anticipate approximately quality of yogurt. Manufactures add usually 2–4 % yogurt starter culture depending on its activity. So appear question: to what extent different starter's amount influence keeping quality of the yogurts.

Therefore the objective of this study was to estimate the changes (especially changes of vitamin C content) during 14-days storage in yogurt produced from ewe's milk inoculated with different starter's amount.

MATERIALS AND METHODS

Yogurts were produced from ewe's milk obtained from Polish long-fleece sheep breaded in sheepyard of Academy of Agriculture in Cracow. Milk was sampled from cooling tank at morning milking five times during summer. All analyses were performed during two subsequent hours and then yogurts were produced.

Milk analyses

The dry solids content by drying method, the fat content by Gerber method, the total nitrogen contents by Kjeldahl method, the casein content by Kjeldahl method (after precipitation with sodium acetate and acetic acid), the non-protein nitrogen contents by Rowland method, the lactose amount by Bertrand method [6], the free fatty acids contents by Dole method [7], the acetaldehyde content by Lees and Jago method [15], the diacetyl content by Pien method [16] and the vitamin C and ascorbic acid contents by Tillmans-Sharp method [24] were determined. The milk density using densitometer, pH by pehameter, titratable acidity by Soxhlet-Henkel method [12] were measured. After pasteurization (93°C/5min) the vitamin C and the ascorbic acid contents were determined.

Yogurts production

Milk was pasteurized (93°C/5min), cooled to 45°C, divided into 3 parts. First milk sample was inoculated with 2% (A), second milk sample with 3% (B) and third milk sample with 4% (C) of yogurt starter culture Y180 DVS (Ch. Hansen). In order to add equally the yogurt starter culture, dvs (amount allowing to inoculate five liters of milk) was diluted in 10 ml of 0.1% peptone and then it was added in suitable proportion to milk samples. The samples were incubated at 45°C to obtain soft curd, to pH 4.6-4.7. Then yogurts were cooled to 4°C and stored in the same temperature during 14 days.

Yogurts analyses

After 1, 7, 14 days the free fatty acids content, the diacetyl and the acetaldehyde content, the soluble nitrogen content and ammonia nitrogen content, the vitamin C and the ascorbic acid contents, titratable acidity and pH were determined using the same methods like for the milk.

The texture profile of yogurts were measured using The Universal TA-XT2 Texture Analyser with an plastic cylinder (20 mm in diameter) that was inserted into each product to a depth of 20 mm at speed of 1 mm/s. The instrument was connected to a computer (fitted with a Texture Expert v. 1.05, programmed using the algorithm Fracture TPA) to measure the texture profiles of yogurts [17, 22]. The measurements recorded were: hardness, determined as the final strength required to reach a stable deformation (the maximum point of inflection on the curve during the first cycle of pressing); adhesiveness, calculated as the areas of a negative peak; cohesiveness, calculated as quotient field surfaces indicated by the curves of the second and first press; springiness, as a ratio of time measured from the start of the second and first cycles of pressing to reach maximum deformation during each cycle; gumminess, determined as a multiple of hardness and cohesiveness.

Five panelists using a 5-scores system with significance coefficients (0.2 for consistency; 0.2 for appearance and for color; 0.6 for taste and for flavour) judged sensory qualities of the yogurts [14]. Statistical analyses were carried out using computer's program Statgraphic 5.2.

RESULTS AND DISCUSSION

The dry solids content, the non-fat dry solids content, the fat content, the lactose content, the total nitrogen content, the non-protein nitrogen contents, the free fatty acids contents in milk as well as its density, the titratable acidity, and the pH (Table 1) were similar to those indicated in the literature [1, 3, 4]. The diacetyl presence was not found in milk, acetaldehyde content was 0.12 mg/dm^3. The vitamin C content (3.31 mg/100 g) was higher than this reported by Tamime and Robinson [23] – 1 mg/100 g and lower than it is given by Steron [21] and

Alichanidis and Polychroniadou [1] (4.5 mg/100 g and 5 mg/100 g respectively). The ascorbic acid content in milk constituted 68.3% of the total vitamin C content. After pasteurization the losses of vitamin C and ascorbic acid were 38.5% and 35.8% respectively. Renner reports that losses of this vitamin in cow's milk can range from 10% to 25%. Perhaps kind of milk (its chemical composition) has some influence on the vitamin C losses during pasteurization. According to Gawęcki [8] and Renner [19] the changes of vitamin C content are influenced by: light, oxygen, presence of Cu, Fe ions, temperature and time of pasteurization. In our study sheep's milk was pasteurized in glass bottles what could influenced the higher losses of vitamin C and ascorbic acid.

Table 1. The properties of ewe's milk

Properties	x	s
Dry solids [%]	16.18	0.18
Non-fat dry solids [%]	11.62	0.17
Fat [%]	4.66	0.16
Total nitrogen [Nx6.38] [%]	5.20	0.14
Casein [%]	4.25	0.10
Non-protein nitrogen [% N]	0.06	0.001
Lactose [%]	4.59	0.25
Free fatty acids [μ Eq/g]	1.68	0.09
Acetaldehyde [mg/dm^3]	0.12	0.007
Diacetyl [mg/dm^3]	0	0
Vitamin C [mg/100g]	3.31	0.08
Ascorbic acid [mg/100g]	2.26	0.07
pH	6.63	0.01
Titratable acidity [° SH]	8.8	023
Density [g/cm^3]	1.035	0.0002

The averages of estimated parameters for fresh yogurts with 2%, 3% and 4% starter's addition are presented in the Table 2. As seen, with increasing of the amount of starter culture increased also titratable acidity, the free fatty acids contents, the ascorbic acid and the vitamin C contents, but differences were significant ($p \leq 0.01$) only for vitamin C. Soluble nitrogen and ammonia nitrogen contents in the sample C were slightly lower than those of sample A and B, differences between averages are significant only for soluble nitrogen. Soluble nitrogen compounds are for bacteria substrates in many metabolic changes. Probably the higher addition of starters culture caused the increase of intake those compounds by bacteria [23]. The titratable acidity of yogurt samples ranged from 43.3 to 46.1°SH. In previous researches [3, 4] fresh yogurts had lower titratable acidity. The effect of the different amount of starter culture on adhesiveness, springiness and cohesiveness of the yogurts samples was statistically significant ($p \leq 0.01$ or ≤ 0.05). Probably these differences were results of changes rates in yogurts depending on the number of bacteria. In previous work Bonczar at al. [4] found that texture parameters depended on the composition of the yogurt starter culture. Organoleptic evaluation indicated slightly advantage of yogurt with 3% addition of starter over the yogurts with 2% and 4% starter's addition.

Table 2. The properties of fresh yogurts depending on amount of starter culture

Properties	Amount of starter culture		
	2%	3%	4%
1. Organoleptic evaluation [scores]	4.93 ± 0.06	5.00 ± 0.00	4.91 ± 0.06
2. Titratable acidity [°SH]	43.33 ± 2.47	44.80 ± 1.01	46.13 ± 0.35
3. pH	4.69 ± 0.04	4.57 ± 0.14	4.66 ± 0.06
4. Free fatty acids [μEq/g]	4.23 ± 0.95	4.97 ± 0.94	5.33 ± 0.67
5. Soluble nitrogen [%]	0.102 ± 0.004	0.102 ± 0.008	0.086 ± 0.003
6. Ammonia nitrogen [%]	0.049 ± 0.001	0.044 ± 0.007	0.042 ± 0.011
7. Acetaldehyde [mg/dm^3]	1.37 ± 0.38	1.23 ± 0.37	1.33 ± 0.37
8. Diacetyl [mg/dm^3]	0.37 ± 0.07	0.37 ± 0.04	0.47 ± 0.12
9. Vitamin C [mg/100g]	1.36 ± 0.00 A	1.93 ± 0.11 AB	2.03 ± 0.01 B
10. Ascorbic acid [mg/100 g]	1.26 ± 0.05	1.48 ± 0.11	1.54 ± 0.14
11. Hardness TPA [G]	73.77 ± 23.80	38.21 ± 8.52	96.91 ± 11.61
12. Adhesiveness TPA [G.s]	-662.2 ± 80.7 A	-821.3 ± 49.5 B	-1475.9 ± 42.8 AB
13. Springiness TPA	0.96 ± 0.004 a	0.90 ± 0.049	0.84 ± 0.037 a
14. CohesivenessTPA	0.48 ± 0.003 a	0.40 ± 0.024	0.37 ± 0.009 a
15. Guminess TPA [G]	35.7 ± 12.13	15.66 ± 5.00	40.34 ± 4.56

The averages of yogurts estimated parameters depending on the amount of starter culture and on the storage time are presented in the Table 3. According to the two factorial analysis of variance, starter's amount influenced significant the titratable acidity, the pH, the free fatty acid contents, the soluble nitrogen contents, the vitamin C and the ascorbic acid contents, and the all texture parameters. While storage time had significant influence on the sensory quality, the titratable acidity, the pH, the diacetyl and the acetaldehyde contents, the free fatty acids contents, the vitamin C and ascorbic acid contents. The effect of the storage period on the sensory quality, the titratable acidity, the pH and the diacetyl contents was compatible with that observed by the other authors [2, 9]. But some otherwise changed acetaldehyde content. As seen from Table 3, acetaldehyde content increased during all storage period. Our data differed from those reported by Beshkova [2] and Georgala [9] who found that this flavour compound increased in the beginning and then decreased during storage. However, Kang et al., quoted by Tamime and Robinson [23], received also an increase of acetaldehyde content during storage time. Perhaps those results' differences are caused by different yogurt starter's composition.

Table 3. The lowest squared means of yogurts' parameters depending on the amount of starter culture's addition (2, 3, 4%) and on the storage time (1, 7, 14 days)

Parametres of yogurt	x ± s	Addition of starter culture				Storage days			
		2%	3%	4%	Statistically	1	7	14	Statistically
		A	B	C	significant	D	E	F	significant
Organoleptic evaluation [score]	4.85 ± 0.02	4.88	4.88	4.79	-	4.95	4.80	4.80	D-E,F*
Titratable acidity [°SH]	50.91 ± 0.57	49.4	50.5	52.8	A-C*	44.1	50.5	58.2	D-E,F**;E-F**
pH	4.56 ± 0.15	4.61	4.56	4.50	A-C**	4.70	4.54	4.44	D-E,F**,E-F*
Diacetyl [mg/dm^3]	0.59 ± 0.09	0.60	0.58	0.58	-	0.40	0.99	0.37	E-D,F*
Acetaldehyde [mg/dm^3]	2.52 ± 0.19	2.30	2.38	2.88	-	1.32	1.66	4.58	F-D,E*
Free fatty acids [µEq/cm^3]	5.79 ± 0.19	5.20	5.80	6.37	A-C*	4.85	5.83	6.69	D-E*;D-F**
Soluble nitrogen [% N]	0.094 ± 0.003	0.103	0.094	0.085	A-C*	0.099	0.098	0.085	-
Ammonia nitrogen [% N]	0.050 ± 0.002	0.051	0.052	0.049	-	0.045	0.051	0.055	-
Vitamin C [mg/100g]	1.66 ± 0.03	1.36	1.70	1.92	A-B,C**; B-C*	1.77	1.66	1.55	D-F*
Ascorbic acid [mg/100g]	1.34 ± 0.02	1.20	1.41	1.40	A-B,C**	1.43	1.32	1.26	D-F*
Hardness TPA [G]	81.95 ± 6.35	72.30	54.95	118.61	C-A,B**	70.18	80.88	94.79	-
Adhesiveness TPA [G.s]	-1027 ± 87	-684	-1032	-1365	A-C**	-1008	-854	-1220	-
Springiness TPA	0.88 ± 0.02	0.94	0.88	0.83	A-C*	0.90	0.89	0.85	-
Cohesiveness TPA	0.39 ± 0.01	0.45	0.38	0.36	A-C**; A-B*	0.42	0.39	0.38	-
Guminess TPA [G]	32.44 ± 2.81	35.73	19.77	41.86	A-B*; B-C**	30.84	32.52	33.96	-

* statistically significant difference between averages in the columns A, B, C, and D, E, F ($p \leq 0.05$)
** statistically high significant difference between averages in the columns A, B, C and D,E, F ($p \leq 0.01$)
x ± s - average ± standard error

Table 4. The changes of vitamin C and of ascorbic acid contents in raw milk, pasteurized milk and yogurts

Products	Starter's addition	Vitamin C x ± δ	% of raw milk content	% of pasteurized milk content	Ascorbic acid x ± δ	% of raw milk content	% of pasteurized milk content
Raw milk	-	3.31 ± 0.08	100.0	162.3	2.26 ± 0.07	100.0	155.9
Pasteurized milk	-	2.04 ± 0.19	61.6	100.0	1.45 ± 0.13	64.2	100.0
Yogurt (1-day)	2%	1.36 ± 0.00	41.1	66.7	1.26 ± 0.05	55.8	86.9
	3%	1.93 ± 0.11	58.3	94.6	1.48 ± 0.11	65.5	105.0
	4%	2.03 ± 0.01	61.3	99.5	1.54 ± 0.14	68.1	106.2
Yogurt (7-day)	2%	1.36 ± 0.00	41.1	66.7	1.19 ± 0.003	52.7	82.1
	3%	1.76 ± 0.20	53.2	86.3	1.41 ± 0.05	62.4	97.2
	4%	1.93 ± 0.11	58.3	94.6	1.36 ± 0.05	60.2	93.8
Yogurt (14-day)	2%	1.36 ± 0.00	41.1	66.7	1.16 ± 0.11	51.3	80.0
	3%	1.47 ± 0.11	44.4	72.1	1.33 ± 0.03	58.8	91.7
	4%	1.81 ± 0.16	54.7	88.7	1.30 ± 0.06	57.5	89.7

The vitamin C and the ascorbic acid contents in yogurts decreased during storage period. In the literature is only little information about vitamin C and ascorbic acid contents in ewe's milk and yogurts from this milk. Therefore in the Table 4 are presented the results of analyses of the raw milk, the pasteurized milk and the yogurts concerning those vitamin form contents. As seen, yogurts possessed from 41.1 to 61.3% of vitamin C content found in raw milk and from 66.7 to 99.5% found in pasteurized milk. The ascorbic acid content ranged from 51.3 to 68.1% and from 82.1 to 106.2% of the contents in raw milk and pasteurized milk respectively. The higher vitamin C and ascorbic acid contents were found in the yogurts with higher starter's addition. According to the authors quoted by Borek-Wojciechowska [5] fermentative processes can contribute to the stabilization of vitamin C in milk. During fermentation pH decreased, vitamin C is more stable in acid environment than in approximated to alkali. In comparison with other food products, milk and milk products are not considered as good source of vitamin C. Nevertheless its need to be noted that ewe's milk contains fourfold more vitamin C than cow's milk and threefold more than goat's milk [10, 21].

CONCLUSIONS

1. Amount of starter culture had significant influence on the titratable acidity, pH, the free fatty acids contents, the total nitrogen contents, the vitamin C and the ascorbic acid contents as well as the texture parameters (adhesiveness, springiness, cohesiveness) of fresh yogurts.
2. During 14 days of storage time, with increasing of the starters amount increased: the titratable acidity, the free fatty acids contents, the vitamin C and the ascorbic acid contents, hardness, adhesiveness and gumminess but decreased: pH, the soluble nitrogen contents and springiness.
3. The vitamin C and the ascorbic acid contents decreased in milk after pasteurization and in yogurts during storage period, but increased with increasing amount of starter culture.

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