

CHANGES IN QUALITY OF FRESH CHEESE USING DRESSING WITH AND WITHOUT PROBIOTIC CULTURE DURING STORAGE

Erika Flimelová, Vladimíra Kňazovická, Margita Čanigová, Emília Benczová

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Abstract

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The aim of the study was to evaluate selected chemical, microbiological and sensorial quality parameters in cheeses without (C), with addition of probiotic culture (E1) and with addition of probiotic culture and inulin (E2). These samples were analysed during 15 days of storage at cooling temperature (7 ± 2 °C). No significant differences ($P > 0.05$) were observed in the physico-chemical parameters (content of dry matter, fat, sodium chloride and water activity) by comparing of analysed samples. Titratable acidity values in the samples increased proportionally by time of their storage. Higher increase of titratable acidity was recorded in E1 and E2 samples compared with C sample. Significant difference ($P < 0.05$) was noticed between samples E1 and E2 in the 1st day following the cheese production, whereas the highest values were found in E2 sample during the whole period of storage. Significant difference ($P < 0.01$) was recorded between the control sample and E2 sample in the 1st day following the cheese production. Results of titratable acidity were confirmed by pH values. In all samples, counts of lactic acid bacteria exceed value of 10^6 CFU g⁻¹ during whole period of storage. Number of microorganisms rose till 6th day of storage. After 15 days of storage, higher microbial numbers were detected in C cheese sample compared with cheese samples E1 and E2. Additions of probiotic culture as well as inulin were positively perceived by assessors till 10th day of storage. Overall, assessors described the samples as unsuitable for consumption after 15 days of storage in term of their taste and smell.

fresh cheese, probiotic culture, inulin

Milk and dairy products including cheeses are one from the product groups that are necessary for nutrition and development of human organism. These products support normal function of metabolism and provide all macro- (proteins, lipids, saccharides) and microelements (vitamins and enzymes) (Boza, Sanz Sampelayo, 1997; Ataro *et al.*, 2008).

Manufacturers try to produce cheeses enriched by various healthy additives including probiotic cultures. Cheese is dairy product and is potentially suitable transfer medium for probiotic microorganisms determined to the human gastrointestinal tract because of specific chemical and physical properties in comparison to fermented

milk (higher value of pH, lower value of titratable acidity, higher buffering capacity, higher lipid content, higher nutrient availability, lowest oxygen content and denser texture of matrix) (Boylston *et al.*, 2004; Karimi *et al.*, 2011).

According to Hutkins (2006), probiotics are microorganisms that can overcome the unfavourable environment in human stomach – i.e. influence of lower pH value, hydrochloric acid and proteolytic enzymes. Many previous and current authors have been focused on studying of various probiotic microbial cultures and strains and their survival in low pH and under the presence of bile (Millette *et al.*, 2008; Pisano *et al.*, 2008; Kimoto-Nira *et al.*, 2009). If probiotics have been administered in

sufficient amounts, they modulate the quantity and distribution of microorganisms in certain anatomic place of host thereby have a beneficial effect on his health (Schrezenmeier, de Vrese, 2001; Sanders, 2003; Buriti *et al.*, 2007). Frece *et al.* (2005) mentioned that probiotic bacteria possess anticarcinogenic effects on entire gastrointestinal tract, regenerate intestinal microbiota, stimulate immunity system and have an important role in prevention of gastritis caused by *Helicobacter pylori*.

Kolařová *et al.* (2012) found a different influence of probiotic and synbiotic on *Clostridium* sp. and *Escherichia coli* counts in human intestinal system. Consumption of probiotic resulted in decrease of *E. coli* counts and consumption of synbiotic result in increase of *E. coli* and *Clostridium* sp. counts in human intestinal system.

For support of probiotic culture growth, prebiotic preparations have increasingly been used, such as inulin, prebiotic crude fiber (Franck, 2002), which takes effect on intestinal microorganisms (Gibson *et al.*, 2004) and supports the growth of bifidobacteria (bifidogenic effect) (Meyer, Stasse-Wolthuis, 2009). Inulin is quite often used in food industry because of its nutritional and technological properties (Tungland, Meyer, 2002; Arcia *et al.*, 2011).

Inulin is an indigestible carbohydrate built up from β -(2,1)-linked-fructosyl residues mostly ending with a glucose residue and it is present as storage carbohydrate in large number of plants (Meyer *et al.*, 2011) as garlic (*Allium sativum*), asparagus root (*Asparagus officinalis*), Jerusalem artichoke (*Helianthus tuberosus*), dahlia tubers (*Dahlia* sp.) or chicory root (*Cichorium intybus*) (Rocha *et al.*, 2006). Presence of inulin stimulates growth and activity of non-pathogenic bacteria, especially of bifidobacteria and lactobacilli (Roberfroid, Slavin, 2000) and inhibits the harmful bacteria (e.g. clostridia) (Shanahan, 2000).

The aim of this study was testing of fresh cheese with addition of probiotic culture, of inulin and of both compared with control cheese without them. Selected physico-chemical, microbiological and sensorial properties in these cheeses were evaluated.

MATERIAL AND METHODS

Cheeses were made and assessed in Department of Evaluation and Processing of Animal Products, Slovak University of Agriculture in Nitra. Half-fat milk with fat content of 1.5% obtained from trade network was used for cheese production. Cheeses were made using mesophilic lactic bacteria (1%; Laktoflora, Czech Republic), calcium chloride (2ml of 40% solution per 1L of milk; Reachem, Slovak Republic) and rennet (Milase, CSK, Food Enrichment, Netherlands) in the doses resulted in coagulation of milk during five hours at 31 °C. After, coagulation, cutting, heating up and drying of curd were followed, respectively. Whey was removed and obtained cheese grain was incorporated into the sour cream with fat content of 10% and added

sodium chloride (2%). This product was marked as control sample (C). Cheese grain incorporated into the sour cream, sodium chloride and probiotic culture (0.1%) – *Lactobacillus acidophilus* (Laktoflora, Czech Republic) was marked as experimental sample 1 (E1). Cheese grain incorporated into the sour cream, sodium chloride, probiotic culture and inulin (1%) was marked as experimental sample 2 (E2). Final products were packed and stored at 7 ± 2 °C during 15 days. Followed physico-chemical properties of these three cheese samples: titratable acidity, active acidity (pH) using pH-meter (Gryf 209L, Czech Republic), water activity using FA-st lab (GBX, France), content of sodium chloride, fat content and dry matter content were analysed. The analyses were performed according to STN 57 0107 (2001). Content of sodium chloride was determined by direct argentometric titration from cheese filtrate. Fat content was determined by acidobutyrometric (operating) method according to van Gulik. Dry matter determination was made by gravimetric method – drying of cheese sample at 102 ± 1 °C to constant weight.

Enumeration of lactic acid bacteria was carried out by cultivation in MRS agar at 37 ± 1 °C for 72 h under the anaerobic conditions for sample 1 and 2, and by cultivation in M17 agar at 22 ± 1 °C for 5 days under aerobic conditions for control sample (FIL-IDF, 1997a).

Sensorial properties – colour, smell, taste, texture, grain uniformity and general appearance were assessed according to standard of IDF (FIL-IDF, 1997b). Sensorial analysis was performed by six-member committee of assessors who evaluated selected parameters of produced cheeses by five point scale.

Evaluation (consisted of physico-chemical, microbiological and sensorial parameters) was carried out in the 1st, 6th, 10th and 15th day following the cheese production. Production of control and two experimental cheeses together with above mentioned analyses were repeated six times. Obtained results were processed by variation-statistical methods in ANOVA.

RESULTS AND DISCUSSION

Average values of dry matter, fat, sodium chloride and water activity in evaluated cheese samples in the 1st and 15th of the storage at 5 °C are recorded in Tab. I. No significant differences ($P > 0.05$) were observed between analysed samples (Tab. II). Slight increase of dry matter in E2 sample could be due to addition of prebiotic compound – inulin. The most results obtained in the present study are similar to those reported by Viana *et al.* (2002). According to Araújo *et al.* (2010), inulin is utilised as a partial substitute for fat, and may be employed in amounts that produce a final fat content of 1/3 of that normally found in the foodstuff. Araújo *et al.* (2010) evaluated the physico-chemical parameters in cottage cheese with addition of inulin and they found significant

I: Average values of selected physico-chemical parameters in fresh cheeses in the 1st and 15th day of storage (n = 6 for every sample)

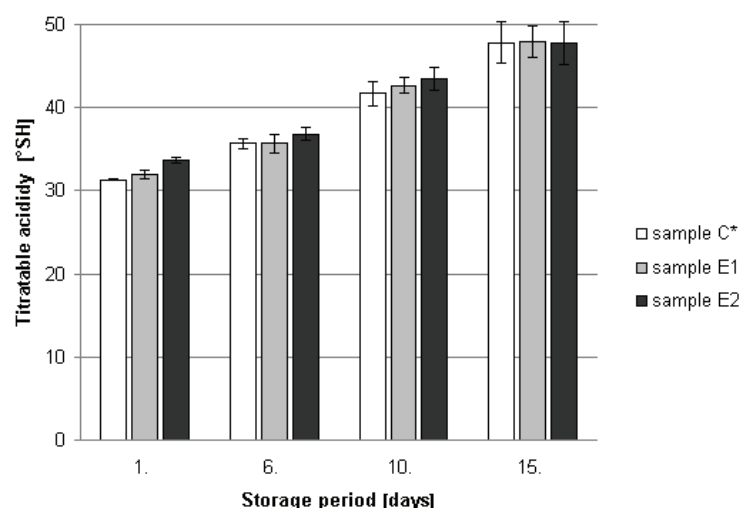
Parameter	Cheese sample	1 st day		15 th day	
		mean $\pm$ sd	cv [%]	mean $\pm$ sd	cv [%]
Dry matter [g.100g ⁻¹]	C	27.8 $\pm$ 0.23	1.98	27.1 $\pm$ 0.32	2.91
	E1	27.6 $\pm$ 0.32	2.88	26.7 $\pm$ 0.34	3.12
	E2	27.9 $\pm$ 0.26	2.24	27.3 $\pm$ 0.22	1.99
Fat [g.100g ⁻¹]	C	9.00 $\pm$ 0.40	10.97	9.17 $\pm$ 0.83	2.23
	E1	8.71 $\pm$ 0.57	15.98	8.96 $\pm$ 0.16	4.47
	E2	8.63 $\pm$ 0.74	20.96	9.17 $\pm$ 0.05	1.41
NaCl [g.100g ⁻¹]	C	1.52 $\pm$ 0.05	7.49	1.54 $\pm$ 0.03	4.29
	E1	1.51 $\pm$ 0.03	4.60	1.53 $\pm$ 0.02	3.35
	E2	1.50 $\pm$ 0.02	3.43	1.51 $\pm$ 0.02	2.75
Water activity	C	0.98 $\pm$ 0.00	0.44	0.98 $\pm$ 0.00	0.25
	E1	0.98 $\pm$ 0.00	0.56	0.98 $\pm$ 0.00	0.23
	E2	0.98 $\pm$ 0.00	0.64	0.98 $\pm$ 0.00	0.21

C – control sample (without addition of probiotic culture and inulin), E1 – experimental sample 1 (with probiotic culture), E2 – experimental sample 2 (with probiotic culture and inulin), s. d. – standard deviation, cv – coefficient of variation

II: Statistic comparison (*p*-value) between evaluated parameters in the cheeses after the 1st and 15th day following cheese production

Experimental group	Dry matter	Fat [g.100g ⁻¹]	NaCl	Water activity
C₁ : C₁₅	0.105*	0.694*	0.695*	0.719*
E1₁ : E1₁₅	0.090*	0.681*	0.589*	0.628*
E2₁ : E2₁₅	0.079*	0.481*	0.590*	0.904*

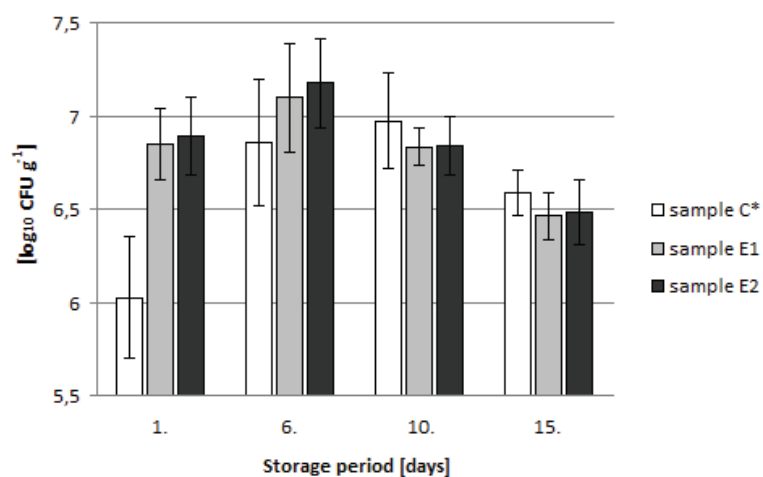
C₁ – control sample after the 1st day following cheese production, C₁₅ – control sample after the 15th day following cheese production, E1₁ – experimental sample 1 after the 1st day following cheese production, E1₁₅ – experimental sample 1 after the 15th day following cheese production, E2₁ – experimental sample 2 after the 1st day following cheese production, E2₁₅ – experimental sample 2 after the 15th day following cheese production, * – *P* > 0.05

1: Titratable acidity in cheese samples stored at 7 $\pm$ 2 °C during 15 days

* Explanatory descriptions of samples C, E1 and E2 are placed under the Table I

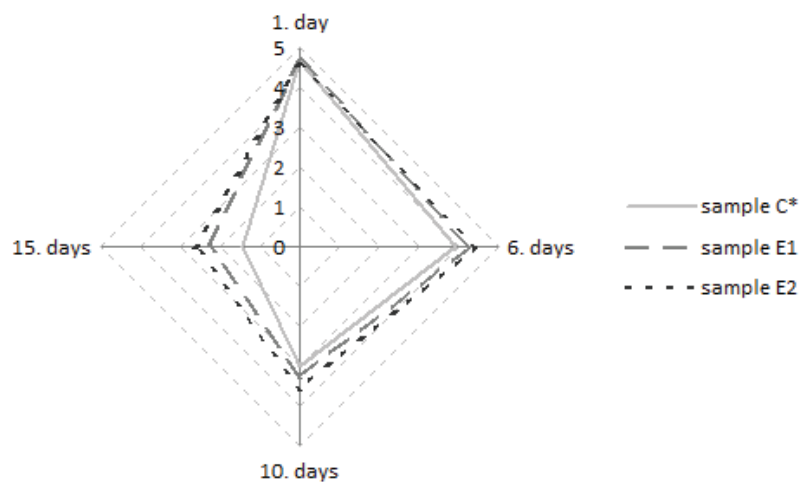
differences (*P* < 0.05) compared with control cheese in content of water, fat and total nitrogen. However, Araújo *et al.* (2010) applied higher amount of inulin into the cheese comparing with our experiment.

Titratable acidity values in cheese samples increased proportionally with time of its storage, what is visualized in Fig. 1. The higher increase of titratable acidity was recorded in E1 and E2 samples,



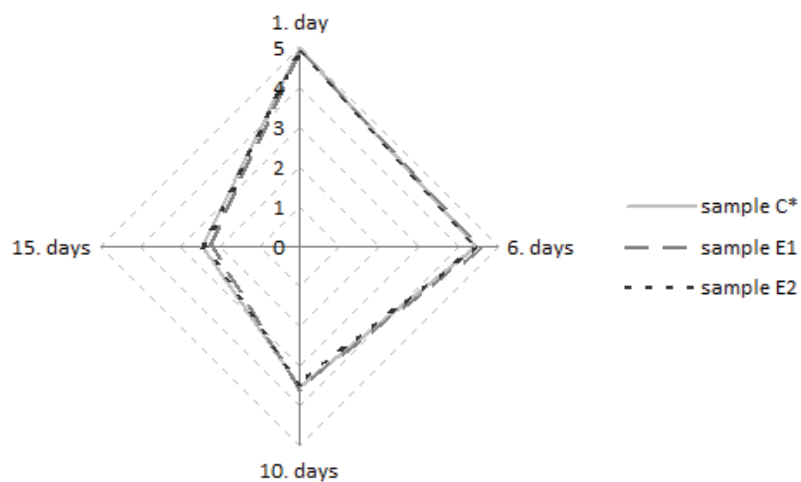
2: Counts of lactic acid bacteria in cheese samples stored at 7 ± 2 °C during 15 days

* Explanatory descriptions of samples C, E1 and E2 are placed under the Table I



3: Sensorial evaluation of smell overall acceptance in cheeses stored at 7 ± 2 °C during 15 days (1 – dislike extremely; 5 – like extremely)

* Explanatory descriptions of samples C, E1 and E2 are placed under the Table I



4: Sensorial evaluation of taste overall acceptance in cheeses stored at 7 ± 2 °C during 15 days (1 – dislike extremely; 5 – like extremely)

* Explanatory descriptions of samples C, E1 and E2 are placed under the Table I

what could have been connected with activity of microorganisms contained in probiotic culture. Increasing acidity resulted mainly from activity of *Lactobacillus acidophilus* as a part of culture, what corresponds with the results of Vinderola *et al.* (2002). Significant difference ($P < 0.05$) was found between the E1 and E2 samples and significant difference ($P < 0.01$) was found between the C and E2 samples in the 1st day following the cheese production. The highest values of titratable acidity were in E2 sample, which contains addition of probiotic culture and inulin, too. According to Araújo *et al.* (2010), inulin supports the growth of lactic acid bacteria.

Results of titratable acidity were confirmed by pH values that ranged from 5.86 to 5.88 in the 1st day following the cheese production. After 15 days of storage, the pH values in all samples had decreased by the value of 0.4. Araújo *et al.* (2010) found lower pH value (4.75) in the cottage cheese with addition of inulin and probiotic culture and pH value of 4.82 was detected in their control sample. These facts are probably due to the higher amount of probiotic culture and inulin and to different technology of cheese production comparing with samples in our experiments. Stijepić *et al.* (2011) researched the physico-chemical properties of yoghurt with addition of inulin and they found decrease of pH value from 4.49 to 4.28 in the sample of yoghurt with 1% inulin and less marked decrease of pH value from 4.52 to 4.42 in the control sample.

If the precious cultures of lactic acid bacteria are parts of fermented milk products in excessive numbers, they have a beneficial effect on human organism (Čanigová *et al.*, 2010). According to Araújo *et al.* (2010) as well as Staruch and Mati (2010), various reports published that the count of probiotic cells in a foodstuff should be approximately 10^6 CFU.g⁻¹, and in order to obtain a beneficial effect, a daily ingestion of 10^8 – 10^9 CFU for each portion is recommended. Obtained results indicate that all samples achieved the bacterial counts exceeded the value of 10^6 CFU g⁻¹ during the whole period of storage. Number of microorganisms increased till 6th day of storage as it is illustrated in the Fig. 2. After 15th day of storage, higher microbial counts were found in control sample compared with E1 and E2 samples. Reduction of microbial counts may be related to intensive production of lactic acid. According to Juillard *et al.* (1987), lactic acid is a key product of probiotic cultures and simultaneously the main inhibitor of microbial growth. This microbial reduction may be result from storage at 7 ± 2 °C, because evaluated probiotic microorganisms are

thermophilic organisms and their counts can be reduced under the long-time storage.

In terms of sensorial analysis, no differences were found between the evaluated samples in the 1st day following cheese production. Colour, texture and grain uniformity were convenient with minimal changes (max. 0.6 points) during the whole period of storage. Differences were recorded in the smell (Fig. 3) and taste (Fig. 4) in all three samples during the storage period. Taste and smell of analysed three samples were perceived by assessors positively till 10th day of storage. After 15 days of storage, changes in taste were marked. Smell of cheeses was undesirable and it was described as foreign or fruity smell. Assessors perceived these cheese samples as unsuitable foodstuff for consumption after 15 day of storage. Deterioration of taste and smell in the samples during the storage was probably due to the decomposition of milk components (fats and proteins) by activity of microorganisms contained in starter or other microorganisms contaminating the milk.

This type of cheese should be eaten within 10 days of production, if it is stored under the tested conditions. The recommendation has its base on results of sensorial analysis and mainly on counts of microorganisms that have beneficial effects on the human organism.

CONCLUSIONS

Fresh cheeses with dressing produced in our experiments and stored in the cold without vacuum packaging were positively perceived by assessors till 10th day of storage. Storage period of 10 days was optimal in terms of probiotics' counts as well as acceptable acidity of the product. Probiotic culture added into the dressing did not influence the cheese properties negatively.

Cheese with addition of probiotic culture could have beneficial effects on human body. Microorganisms with probiotic properties affect the balance of gastrointestinal tract and act in the benefit of host. Routine consumption of probiotic products and sufficiently high amounts of probiotic bacteria in these products are necessary requirements for positive effects manifestation. Prebiotics (e. g. crude fiber) are employed for growth support and survival of probiotics and therefore enrichment by these both components is important for products. These foodstuffs are more attractive for consumer, because many consumers take care of their health and healthy life style.

SUMMARY

Information about beneficial effects of probiotics on human intestinal tract has been known. In this study, the centre of interest was a technology of fresh cheese (using rennet and mesophilic lactic bacteria) with addition of dressing (consisted of sour cream and sodium chloride) and with additions of probiotic culture and inulin and their possible influence on cheese quality. In this experiment, 3 followed samples of fresh cheeses with dressing were produced: control sample (C) (without

probiotic culture and inulin), experimental sample 1 (E1) (with probiotic culture) and experimental sample 2 (E2) (with probiotic culture and inulin). Produced cheeses were stored at 7 ± 2 °C during 15 days. Physico-chemical (content of dry matter, fat, titratable acidity, pH, sodium chloride and water activity), microbiological (counts of lactic acid bacteria) and sensorial properties (colour, smell, taste, texture, grain uniformity and general appearance) were evaluated in the 1st, 6th, 10th and 15th day of storage. Addition of probiotic culture as well probiotic culture in combination with inulin did not have significant negative influence on overall quality of the cheese samples in comparison with the control sample without probiotic culture and inulin. In term of sensorial analysis, smell and taste were convenient in all 3 samples till 10th day following the cheese production. Contents of dry matter, fat, sodium chloride as well as water activity were statistically non-significant ($P > 0.05$) changed in all 3 samples during the storage. Titratable acidity values in the samples increased proportionally by time of their storage. Higher increase of titratable acidity was recorded in E1 and E2 samples compared with C sample. In all samples, counts of lactic acid bacteria exceed value of 10^6 CFU g⁻¹ during whole period of storage. Number of microorganisms rose till 6th day of storage. After 15 days of storage, higher microbial numbers were detected in C cheese sample compared with cheese samples E1 and E2. In the 15th day of storage, all 3 samples were unsuitable for consummation according to six-member committee of assessors.

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Address

Ing. Erika Flimelová, Ing. Vladimíra Kňazovická, PhD., doc. Ing. Margita Čanigová, CSc., RNDr. Emília Benczová, Department of Evaluation and Processing of Animal Products, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic, e-mail: erikaflimelova@gmail.com, vladimira.knazovicka@gmail.com, margita.canigova@uniag.sk, emilia.benczova@uniag.sk