THE INFLUENCE OF CONTAMINATION YOGHURT, QUARK AND SEMI-HARD CHEESE BY YEASTS ON THEIR SENSORY PROPERTIES

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Summary. The aim of our research was to determine the influence of contamination yoghurt, quark and semi-hard cheese by yeasts on their sensory properties. Products were made under laboratory conditions. Pasteurized milk for the manufacture of the experimental yoghurt, quark or cheese was contaminated by adding the quantity of yeast cultures or their mixtures required to reach yeast count in the product ranging from 10^5 to 10^7 CFU/g. Lactose fermenting yeasts *Candida kefyr* and *Kluyveromyces marxianus* var. *marxianus* had a higher impact on the sensory properties of yoghurt than non-lactose fermenting yeasts such as *Saccharomyces cerevisiae* and *Debaryomyces hansenii*. The experimental yoghurt contaminated by *C. kefyr*, *K. marxianus* var. *marxianus, S. cerevisiae* and *D. hansenii* received the total sensory evaluation resp. by 9.0, 11.5, 4.4 and 6.5 scores lower than the control yoghurt. The experimental quark contaminated by the mixture of yeast cultures of species *D. hansenii* and *Trichosporon cutaneum* was evaluated by 4.7 scores lower compared to the control quark. Total sensory evaluation of experimental semi-hard cheeses contaminated by the mixture of lactose fermenting yeast (*C. kefyr*, *K. marxianus* var. *marxianus* and *K. marxianus* var. *lactis*) was up to 4.3 scores lower compared to control cheeses.

Key words: quark, yoghurt, semi-hard cheese, contamination, yeasts, sensory properties.

JOGURTO, VARŠKĖS IR PUSKIEČIO FERMENTINIO SŪRIO UŽTERŠTUMO MIELĖMIS ĮTAKA GAMINIŲ JUSLINĖMS SAVYBĖMS

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Santrauka. Norint nustatyti užterštumo mielėmis įtaką jogurto, varškės ir puskiečio fermentinio sūrio juslinėms savybėms, laboratorinėmis sąlygomis pagaminti mielių kultūromis užteršti pieno produktai. Į jų bandomajai gamybai skirtą pasterizuotą pieną buvo pilamas toks mielių kultūrų arba jų mišinių kiekis, kad mielių produkte būtų apytikriai 10^5–10^7 KSV/g. Laktozę fermentuojančios mielės *Candida kefyr* ir *Kluyveromyces marxianus* var. *marxianus* turėjo didesnę įtaką jogurto juslinėms savybėms, nei laktozės nefermentuojančios mielės *Saccharomyces cerevisiae ir Debaryomyces hansenii*. Bandomosios gamybos jogurto su *C. kefyr, K. marxianus* var. *marxianus, S. cerevisiae ir D. hansenii* bendras juslinis įvertinimas buvo atitinkamai 9,0; 11,5; 4,4 ir 6,5 balo mažesnis už kontrolinės gamybos jogurto. Bandomosios gamybos varškės, užterštos *D. hansenii* ir *Trichosporon cutaneum* rūšių mielių mišiniu, bendras juslinis įvertinimas buvo 4,7 balo mažesnis, negu kontrolinės gamybos varškės. Bandomosios gamybos sūrių, užterštų laktozę fermentuojančių rūšių mielių mišiniu (*C. kefyr*, *K. marxianus* var. *marxianus* ir *K. marxianus* var. *lactis*), bendras juslinis įvertinimas buvo iki 4,3 balo, o skonis ir kvapas – iki 4,1 balo mažesnis už kontrolinės gamybos sūrio.

Raktažodžiai: varškė, jogurtas, puskietis fermentinis sūris, užteršimas, mielės, juslinės savybės.

Introduction. Yeasts are undesirable microorganisms in almost all milk products except kefir. These microorganisms usually do not survive pasteurization but they can get into products during post-contamination from the equipment, hands and clothes of personnel, from the air or whey. Their growth conditions (low pH value, low moisture content, high salt concentration) are unsuitable for many bacteria species (Fleet, 1992; Savova, Nikolova, 2002). Cultured milk products create favourable conditions for their growth (Rohm, 1991; Viljoen et al., 2003). In cultured milk products yeasts multiply and can cause defects of flavour, odour and texture (Fleet, 1990; Rohm, 1991). The changes of cultured milk products caused by metabolytic activity of yeasts depend on degradation of lactose or compounds of its hydrolysis, secretion of lipolytic and proteolytic enzymes, assimilation of organic salts and the ability to multiply at low (5–10 °C) temperature during product storage (Jakobsen, Narvhus, 1996; Fleet, 1992).

The impact of yeasts reveals itself both during the spoilage process of cheeses and during the maturation process of some cheese varieties. There are several mechanisms by which yeast growth is thought to influence the maturation process and the final quality of cheese. Fermentation of residual lactose within the curd by species such as *Kluyveromyces marxianus* produces secondary (flavour) metabolites as well as carbon dioxide gas that opens up the curd texture. The activity of extracellular proteases and lipases produced by some yeast species, such as *Candida lipolytica*, could alter curd flavour and texture. And the release of autolytic products by yeasts can influence the flavour, and encourage the growth of bacteria (Fleet, 1992). In some cases, the high proteolytic and lipolytic activity of yeasts does not allow

the cheese surface to form: the yeasts may produce substances that stimulate surface growth of both lactic acid bacteria and aerobic bacteria (Welthagen, Viljoen, 1998). They may ferment residual lactose, metabolise the lactates, and influence flavour formation by producing volatile acids and carbonyl compounds (Fleet, 1992).

Yeasts can cause not only defects of milk and other food products, but also different diseases in people and animals, e.g., candidosis, mycosis and gastroenteritis (Lugauskas et al., 1997).

The aim of our research was to determine the influence of contamination yoghurt, quark and semi-hard cheese by some yeasts on their sensory properties.

Materials and methods. Milk products were made under laboratory conditions. Yoghurt was made from pasteurized homogenised milk with fat content of 2.5 % using traditional technology. The samples of product for determining yeast count and coliform count were taken during the manufacturing process at the following selected points: milk mix (milk mixed with starter), yoghurt shortly after manufacture and yoghurt after 7 days of storage at refrigeration temperature. Four experimental yoghurt variants were made with addition of yeasts: two variants with lactose fermenting yeasts, i. e. *Candida kefyr* – E1, *Kluyveromyces marxianus* var. *marxianus* – E2 and two variants with lactose non-fermenting yeasts, i. e. *Saccharomyces cerevisiae* – E3, *Debaryomyces hansenii* – E4. Control yoghurt (C) was made without addition of yeasts.

Quark was manufactured from pasteurized milk using traditional technology. During manufacture of the experimental quark (E), 1% of the suspension of two yeast cultures (*D. hansenii* and *Tr. cutaneum*, in ratio 1:1) from the quantity of milk was added into the pasteurized milk. Control quark (C) was made without addition of yeasts. The moisture content of quark was 64.5–65.0 %; the fat content was 18 %.

Semi-hard cheese was made using Lithuanian cheese technology. Control cheeses (C) and two variants of experimental cheeses (E1, E2) were manufactured. Suspensions of yeast cultures (0.003 and 0.3 % from the quantity of milk) mentioned above in the ratio of 1:1:1 were added into the pasteurized milk of experimental cheeses E1 and E2 to receive low and high yeast count in pasteurized milk. The moisture content of ripened cheese was 50–52 %; the fat content was 30 % FIDM; and the pH was 5.4–5.5.

The cultures of yeasts – *Candida kefyr*, *Kluyveromyces marxianus* var. *marxianus, Saccharomyces cerevisiae, Debaryomyces hansenii, Trichosporon cutaneum* and *Kluyveromyces marxianus* var. *lactis* – used in the manufacture of yoghurt, quark and cheeses were isolated from milk products. Yeast cultures were grown for 2–3 days at 25±1 ^{o}C on the slants tubes of yeast glucose chloramphenicol agar (*Liofilchem Diagnostici*, 610070). Colonies of yeasts were washed from the slants with peptone–saline solution. The turbidity of suspensions in the peptone–saline solution was determined by McFarland standard No 3. Pasteurized milk for the manufacture of experimental yoghurt, quark or cheese was contaminated by adding the suspensions of selected yeast cultures with cell contents ranging from 10^7 to 10^8 CFU/ml.

Yeast counts in products were determined by pour plate techniques using yeast glucose chloramphenicol agar by incubating for 5 days at 25±1 ^{o}C. If it was necessary, yeast colonies were confirmed by microscopic examination.

Coliform count was determined by the MPN method, using brilliant-green bile lactose broth by incubating at 30±1 ^{o}C. Titratable acidity was determined by titration with 0.1 mol/l NaOH and the active acidity was determined by using an *Orion* potentiometer.

Sensory properties of yoghurt, quark and cheese were evaluated by a group of 5 experts from KTU Food Institute. Taste, flavour, texture and other properties of these products were evaluated, respectively, by 20 (yoghurt, quark) and 100 (cheese) scores system. Each property of yoghurt and quark (taste, flavour, texture, appearance) was evaluated by maximum 5 scores: 1 score – a property is very weakly expressed, not typical; 2 scores – a property is weakly expressed, not typical; 3 scores – a property is satisfactorily expressed; 4 scores – a property is strongly expressed, typical for product; 5 scores – a property is very strongly expressed, typical for product [9]. Cheese sensory properties: taste and flavour, texture, colour, holeyness, appearance and packing were evaluated by maximum 45; 25; 5; 10; 10 and 5 scores, respectively [10]. Yoghurt, quark and cheeses in each experimental group were made five times in parallel with controls.

Results and discussion. The results of microbiological examination of yoghurt are presented in the Table 1.

Table 1. **Contamination of yoghurt during its manufacture and storage**

Yoghurt	Yeast count, CFU/ml (g)		
	Milk mix	Yoghurt shortly after manufacture	Yoghurt (after storage for 7 days at 6°C)
C (control)	<10	<100	<100
E1 (*C. kefyr*)	$5.4 \cdot 10^6$	$1.1 \cdot 10^7$	$3.7 \cdot 10^7$
E2 (*K. marxianus* var. *marxianus*)	$7.6 \cdot 10^6$	$1.7 \cdot 10^7$	$5.6 \cdot 10^7$
E3 (*S. cerevisiae*)	$1.2 \cdot 10^6$	$1.1 \cdot 10^7$	$9.8 \cdot 10^7$
E4 (*D. hansenii)*	$1.7 \cdot 10^6$	$1.3 \cdot 10^7$	$9.5 \cdot 10^7$

Coliforms were found neither in control samples nor in experimental yoghurts. Yeasts were not found in the control samples of yoghurt, but 1.2–7.6 mln CFU of yeasts were found in the 1 ml of milk mix intended for the manufacture of the experimental samples of yoghurt as these microorganisms were added to the pasteurized milk for special contamination. Incubation temperature of yoghurt (42 ^{o}C) was not favourable for the growth of yeasts (optimal growth temperature of yeasts is 25–30 ^{o}C), but during the culturing stage the lactose fermenting yeast count increased 2.0–2.2 times. During the storage of yoghurt the yeast count increased about 3.3–3.4 times. The titratable and active acidity shortly after the manufacture of yoghurt and after 7 days of storage were close to each other.

During culturing of yoghurt with lactose non-fermenting yeasts their count increased by 9 times (*S. cerevisiae*) and 8 times (*D. hansenii*) and during the storage of yoghurt the yeast count increased by 9 times (*S. cerevisiae*) and 7 times (*D. hansenii*).

The impact of contamination by yeasts on the sensory properties of yoghurt was determined immediately after manufacture. Data are presented in Table 2.

Table 2. **Sensory evaluation of yoghurt**

Yogurt	Evaluation, scores				
	Taste	Flavour	Appearance	Texture	Total
C (control)	5.0±0.1	5.0±0.1	4.6±0.1	4.6±0.1	19.2±0.1
E1 (*C. kefyr*)	2.0±0.2	2.8±0.1	2.8±0.3	2.6±0.3	10.2±0.2
E2 (*K. marxianus* var. *marxianus*)	1.8±0.3	2.0±0.2	2.0±0.5	1.9±0.3	7.7±0.3
E3 (*S. cerevisiae*)	2.6 ±0.2	4.0±0.3	3.6±0.2	4.6±0.2	14.8±0.2
E4 (*D. hansenii)*	3.2±0,2	2.8±0.7	3.4±0.2	3.3±0.5	12.7±0.4

Lactose fermenting yeasts *K. marxianus* var. *marxianus* and *C. kefyr* had a more pronounced influence on the sensory properties of yoghurt than lactose non-fermenting yeasts *S. cerevisiae* and *D. hansenii.* Samples E1 and E2 were bubbly and had a strong yeast taste and flavour. Total sensory scores of yoghurts with lactose fermenting yeasts were lower by 9.0 and 11.5 points, respectively, than the control sample, and the total sensory evaluation with lactose non-fermenting yeasts was lower by 4.4 and 6.5 scores. The most intensive impact of contamination by yeasts was shown for product taste and flavour.

Control (C) and experimental (E) quark were produced to determine the impact of contamination by yeasts on the sensory properties of the product. During the technological process, physico-chemical (pH, titratable acidity) and microbiological properties (yeast count, coliform count) were determined. The data are presented in Tables 3 and 4.

Table 3. **Physico-chemical properties of quark during the technological process**

Criterion	Quark	Points of the technological process					
		Pasteurized milk	Milk mix	Milk clot	Quark after spontaneous pressing	Cooled quark	Final product
pH	C	6.35±0.03	5.8±0.03	3.93±0.04	3.93±0.03	4.04±0.05	4.04± 0.05
	E*	6.35±0.03	5.8±0.03	3.59±0.02	3.62±0.03	3.68±0.05	3.68±0.05
Titratable acidity, ^{o}T	C	17±0.00	27±0.00	112±1.00	148±1.00	168±1.00	168±1.00
	E*	17±0.00	27±0.00	118±1.00	160±1.00	170±1.00	170±1.00
* Experimental quark, contaminated with *D. hansenii* and *Tr. cutaneum*							

Table 4. **Contamination of quark by yeasts during the technological process**

Criterion	Quark	Points of the technological process						
		Pasteurized milk	Milk mix	Milk clot	Quark after spontaneous pressing	Cooled quark	Final product	Quark (after storage for 3 days at 6 °C)
Yeast count, CFU/ml (g)	C	<10	<10	<100	<100	<100	<100	<100
	E*	<10	$3.2\cdot10^{7}$	$3.4\cdot10^{7}$	$3.2\cdot10^{7}$	$3.8\cdot10^{7}$	$4.2\cdot10^{7}$	$6.5\cdot10^{7}$
* Experimental quark, contaminated with *D. hansenii* and *Tr. cutaneum*								

The active and titratable acidity, fat and moisture content of control and experimental quark were very close. There were no coliforms found in the mixed milk and at other points in the technological process of either control or experimental quark. No yeasts were found in control quark. Yeast counts in the experimental quark after pressing and in the final product were high. After 3 days of storage at 6–8 °C yeast count increased by 1.5 times. The sensory evaluation data are presented in Table 5.

Table 5. **Sensory evaluation of quark**

Quark	Evaluation, scores				
	Taste	Flavour	Appearance (colour)	Texture	Total
C (control)	4.2±0.5	4.3±0.1	2.7±0.1	4.2±0.6	15.5±1.7
E*	1.8±0.0	2.6±0.2	2.6±0.0	3.8±0.6	10.8±1.0
* Experimental quark, contaminated with *D. hansenii* and *Tr. cutaneum*					

D. hansenii and *Tr. cutaneum* deteriorated the taste and flavour of experimental quark by 2.4 and 1.7 scores, respectively. These yeasts had a lower impact on quark texture and appearance. Total sensory scores of the experimental quark were lower than those of the control quark by 4.7 scores.

Three yeast strains – *K. marxianus* var. *lactis*, *K. marxianus* var. *marxianus*, *C. kefyr* – capable of fermenting lactose under anaerobic conditions were chosen for use in cheese manufacture. Data are presented in Figure 1.

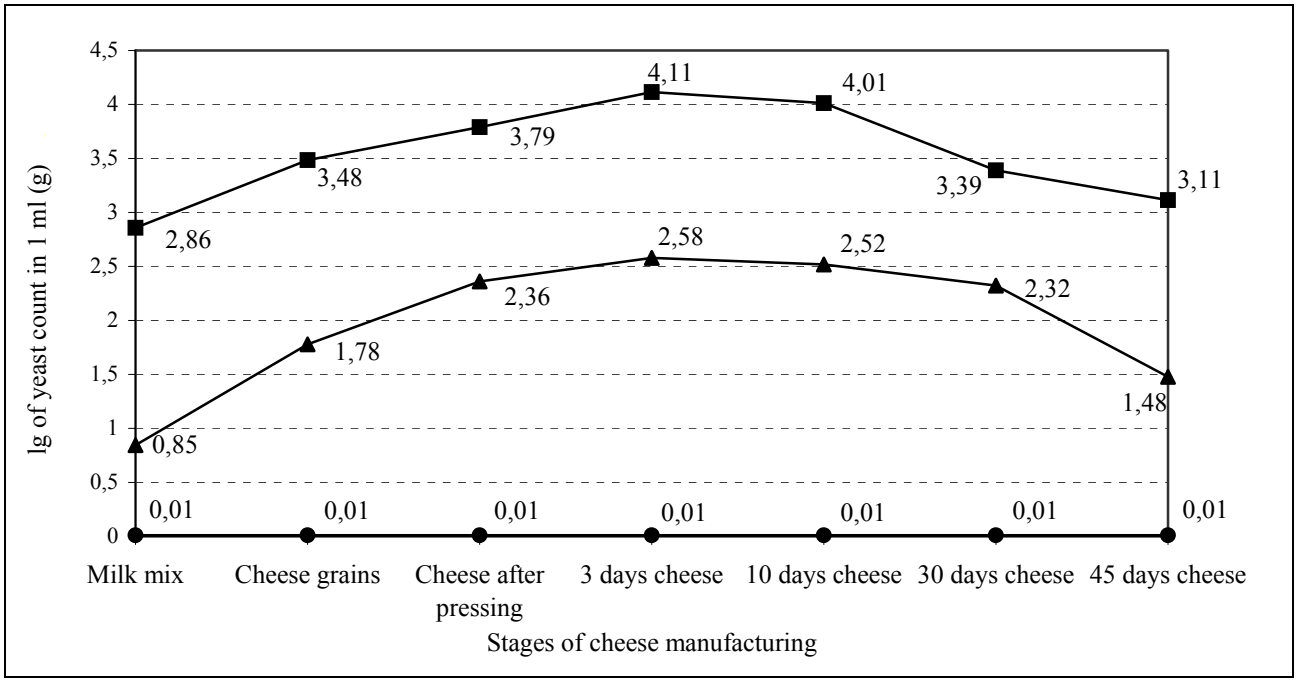

Fig. 1. **Growth of yeasts during the manufacture of Lithuanian cheese:**

● – control cheese;

▲ – experimental cheese (E1), contaminated with *K. marxianus* var. *lactis*, *K. marxianus* var. *marxianus*, *C. kefyr* (0.003 % from the quantity of milk);

■ – experimental cheese (E2), contaminated with *K. marxianus* var. *lactis*, *K. marxianus* var. *marxianus*, *C. kefyr* (0.3 % from the quantity of milk)

According to Fig.1, yeast count increased from the beginning of the technological process, reached the maximum in cheeses of 3–10 days of age and then decreased until the end of ripening. Coliform counts in experimental and control cheeses were negligible.

Moisture content of the control and experimental cheeses after pressing showed little difference. The pH value of experimental cheeses was by 0.5 pH unit lower than that of the control product. The lactic acid formation process was probably activated by the metabolites excreted by yeasts.

The presence of yeasts in cheese influenced the taste and flavour of experimental cheeses contaminated by yeasts of three species, *K. marxianus* var. *lactis*,

K. marxianus var. *marxianus* and *C. kefyr*. Taking into account the maximum number of yeasts in 3-day cheeses – $3.8 \cdot 10^2$ and $1.3 \cdot 10^4$ CFU/g in E1 and E2, respectively, the taste and flavour scores of experimental cheeses were lower than those of the control cheese by 1.4 and 4.1 scores (the total scores by 1.3 and 4.3), respectively (Table 6).

Table 6. **Sensory evaluation of cheese**

Cheese	Evaluation, scores						
	Taste and flavour	Texture	Colour	Holes	Appearance	Package	Total
C (control)	39.7±0.2	23.7±0.1	5.0±0.0	8.0±0.0	10.0±0.0	5.0±0.0	91.4±0.2
E1*	38.3±0.2	23.8±0.1	5.0±0.0	8.0±0.0	10.0±0.0	5.0±0.0	90.1±0.2
E2**	35.6±0.3	23.5±0.1	5.0±0.0	8.0±0.0	10.0±0.0	5.0±0.0	87.1±0.3

* Experimental cheese, contaminated with *K. marxianus* var. *lactis*, *K. marxianus* var. *marxianus*, *C. kefyr* (0,003 % from the quantity of milk);
** Experimental cheese, contaminated with *K. marxianus* var. *lactis*, *K. marxianus* var. *marxianus*, *C. kefyr* (0,3 % from the quantity of milk)

Conclusions

1. Lactose fermenting yeasts *Candida kefyr* and *Klyveromyces marxianus* var. *marxianus* had a higher impact on the sensory properties of yoghurt than non-lactose fermenting yeasts *Saccharomyces cerevisiae* and *Debaryomyces hansenii*. Total sensory evaluation of yoghurt with lactose fermenting yeasts (respectively, $1.1 \cdot 10^7$ CFU/g and $1.7 \cdot 10^7$ CFU/g) was lower by 9.0 and 11.5 scores than the control sample (without yeasts). Total sensory evaluation of yoghurt with lactose non-fermenting yeasts (respectively, $1.1 \cdot 10^7$ CFU/g and $1.3 \cdot 10^7$ CFU/g) was lower, respectively, by 4.4 and 6.5 scores than the control sample.

2. Quark contaminated by yeasts of species *Debaryomyces hansenii* and *Trichosporon cutaneum* (yeast count $4.2 \cdot 10^7$ CFU/g of final product) was evaluated for 4.7 scores lower (2.4 scores lower for taste) than the control quark. Yeasts of these species had a lower impact on texture and appearance.

3. Yeasts of lactose fermenting species – *Kluyveromyces marxianus* var. *lactis*, *Kluyveromyces marxianus* var. *marxianus*, *Candida kefyr* – caused defects of taste and flavour in semi-hard cheese. Taste and flavour evaluation of the experimental cheeses contaminated by these yeasts (the maximum number of yeasts in 3-day cheeses was $3.8 \cdot 10^2$ and $1.3 \cdot 10^4$ CFU/g) was lower by 1.4 and 4.1 scores (total evaluation by 1.3 and 4.3 scores), respectively, than the control cheeses.

References

1. Jakobsen M., Narvhus J. Yeasts and their possible beneficial and negative effects on the quality of dairy products. International Dairy Journal. 1996. Vol. 6. P. 755–768.

2. Fleet G. H. Spoilage Yeasts. Critical Reviews in Biotechnology. 1992. Vol. 12. P.1–44.

3. Fleet G. H. Yeasts in dairy products – a review. Journal of Applied Bacteriology. 1990. Vol. 68. P. 199–211.

4. Lugauskas A. ir kt. Mikrobiologiniai medžiagų pažeidimai. Vilnius, 1997. 469 p.

5. Rohm H. Zur Bedeutung von Hefen und Schimmelpilzen für die Milchwirtschaft. Deutsche Milchwirtschaft. 1991. Vol. 14. P. 404–406.

6. Savova I., Nikolova M. Isolation and taxonomic study of yeast strains from Bulgarian dairy products. Journal of Culture Collections. 2002. Vol. 3. P. 59–65.

7. Viljoen B. C., Lourens-Hattingh A., Ikalafeng B., Peter G. Temperature abuse initiating yeast growth in yoghurt. Food Research International. 2003. Vol. 36. P. 193–197.

8. Welthagen J. J., Viljoen B. C. Yeast profile in Gouda cheese during processing and ripening. International Journal of Food Microbiology. 1998. Vol. 41. P. 185–194.

9. IDF Standard 99C:1997 Sensory evaluation of dairy products by scoring – reference method. International Dairy Federation. Brussels, Belgium.

10. Dukštas J., Kačerauskis D., Liutkevičius A., Ramanauskas R. Pieno perdirbimo technologija. Vilnius, 1994. 392 p.

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