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INFLUENCE OF CULTURE ACTIVITY ON AROMA COMPONENTS IN YOGHURTS PRODUCED FROM GOAT'S AND COW'S MILK

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In this paper the experiments on microbiological quality and aroma components of yoghurt samples produced from long life goat's and cow's milk, and also from milks with 2% milk powder addition, during 9 days of refrigerated storage are described. Milk fermentation was conducted at 42 °C for 6 h. During days 1, 3, and 9 of storage the changes in acidity (pH value and lactic acid percent), viable counts of streptococci and lactobacilli and aroma components (acetaldehyde and diacetyl) were determined. Lower pH values and smaller lactic acid concentrations were found in control yoghurt samples. Viable counts of streptococci decreased during storage (from 1.01×10^8 to 3.97×10^7 CFU ml^{-1}), whereas the viable counts of lactobacilli increased in all samples (from 6.95×10^6 to 2.32×10^7 CFU ml^{-1}). The increase in count of lactobacilli was the greatest in goat's milk yoghurt samples. On the ninth day of storage, ΔlogN between cow's and goat's milk yoghurt samples was 0.2. Acetaldehyde in yoghurts decreased during storage time from 5.47 mg kg^{-1} (on day 1) to 1.05 mg kg^{-1} (on day 9). Both control yoghurt samples had lower acetaldehyde concentration than the supplemented samples. During nine days of storage, a significant increase in diacetyl content of yoghurt samples (from 14.20 mg kg^{-1} on the first day to 18.65 mg kg^{-1} on day 9) was noticed. Goat's milk yoghurts, especially that manufactured without milk powder addition, had very soft consistency, but no syneresis was observed.

Keywords: goat's milk yoghurt, microbiology, acetaldehyde, diacetyl, storage

In recent years, consumption of yoghurt has increased considerably. Nutritive and health importance of yoghurt consumption includes yoghurts antibiotics influence and relief of gastrointestinal disorders (BUTTRISS, 1997; CHEN et al., 1999; MEYDANI & HA, 2000). Many authors investigated the influence of different kinds of milks (DAVE & SHAH, 1998) and starter cultures (GEORGALA et al., 1995; DAVE & SHAH, 1997) on yoghurt quality. It is well known that goat's milk has better digestibility in comparison with cow's milk, because of the smaller size of fat globules (ATTAIE & RICHTER, 2000), it has more easily hydrolysable triacylglycerols containing short-chain fatty acids, more essential amino acids (ALICHANIDIS & POLYCHRONIADOU, 1997; HELLIN et al., 1998), higher proportion of soluble minerals (PARK, 2000) and smaller size of casein micelles (JANDAL, 1996; URBIENÉ, et al., 1997). Although goat's milk is used for therapeutic purposes, largely due to its antiallergenic effect (PARK, 1994; SABBAH et al., 1997), it has not been thoroughly investigated yet. As the significance of goat breeding increases in Croatia (SINKOVIĆ, 2000) this subject is of interest to a segment of the dairy industry.

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At the beginning of the 20th century, investigations pointed out the importance of bacterial growth for aroma development in fermented products. It is established that acids and taste are the result of fermentation with different cultures and that aroma is different from taste. This is the reason for the careful selection and mixing of starter cultures with the aim to achieve the best taste and aroma in fermented products. Of the acidic nature of yoghurt, its characteristic aroma is very much appreciated by consumers. Of more than 100 chemical compounds isolated from this type of dairy products, only acetaldehyde, ethanol, acetone, diacetyl and 2-butanone have great influence on desirable taste (ULBERTH, 1991). Although acetaldehyde, diacetyl, acetone, acetoine and 2-butanone are very important for yoghurt aroma, acetaldehyde is recognised as the principal flavour component (ESTÉVEZ et al., 1988). Besides these compounds, lactic acid and other volatile acids contribute to the characteristic aroma, and improve product quality (RASH, 1990). Yoghurt aroma is different from the aroma of other fermented dairy products. This is mostly imputed to the almost 90% share of acetaldehyde among carbonyl compounds present (KROGER, 1976). Lactic acid prolongs durability of yoghurt, and the produced acetaldehyde and diacetyl are expressively responsible for taste. Because of the fact that yoghurt is produced in lots of countries, and from different kinds of milk, yoghurt taste can also vary (MARSHALL, 1984).

The aim of this paper was to investigate the influence of yoghurt culture activity on the major aroma components (acetaldehyde and diacetyl), acidity (pH value and lactic acid percent), microbiological, and sensory evaluation of cow's and goat's milk yoghurts manufactured with and without milk powder supplementation during 9 days of refrigerated storage.

1. Materials and methods

Commercially available goat's and cow's long-life milks with 3.2% fat content were used. Goat's and cow's milks were divided into two portions apiece and milk powder was added to either portions. Skim milk powder contained 0.05% milk fat, 4.0% moisture, and had a pH of 6.7. All milk samples were inoculated with 2% direct Vat Set (DVS) yoghurt culture and 150-ml aliquots were distributed in sterile buttercups. The YC-180 yoghurt culture (*Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus delbrueckii* subsp. *bulgaricus)* was obtained from Chr. Hansen A/S, Denmark. Inoculation with the DVS culture was done according to the producer's recommendations (2 g/10 l). Fermentation was conducted at 42 °C until firm coagulum was reached. Fermented milk was cooled with water and stored in refrigerator (at 8 °C) for nine days.

Acidity measurements, microbiological and sensory analyses, and determinations of acetaldehyde and diacetyl contents were conducted periodically (third, sixth and 9th day).

The pH values were measured using a pH-meter ("Knick", type 647-1). Titratable acidity (°SH) was determined by the Soxhlet-Henkel method and expressed as % of lactic acid for yoghurt samples (RAŠIĆ & KURMANN, 1978) and proteins were determined by the Kjeldahl method. Total solids (drying at 105 °C until constant mass) and ash content (at 550 °C) were analysed according to the National Standard (A.O.A.C., 1995). Lactose was analysed by the Luff-Schoorl method (VAJIĆ, 1963). Viable counts were determined by the standard microbiological methods on MRS agar (Biolife, Italy) with adjusted pH value to 5.4 by addition of glacial acetic acid under microaerophilic condition (Generbag, bioMérieux, France) for *Lactobacillus delbrueckii* and on M17 agar (Biolife, Italy) for *Streptococcus thermophilus*. Incubation was conducted at 37 °C for 48 h. The sensory properties of fermented beverages were evaluated by a panel of 5 sensory analysts, using a 20-point scoring system. Analysis of variance was performed to examine the statistical significance between samples and between sensory scores of the same sample during storage. The final scores were obtained by multiplication of the scores for each property (1–5) with the weighted factor (ISO, 1985). The acetaldehyde concentrations were determined by an enzymatic method, and the amount of diacetyl was measured by the modified Hill's colourimetric method (BOEHRINGER, 1989; HILL et al., 1954).

The experiment was repeated five times, and the results are presented as means.

2. Results

Chemical composition and acidity of cow's and goat's milk used for yoghurt production are presented in Table 1. Goat's milk had slightly higher levels of total solids, ash and proteins, whereas lactose content was higher in cow's milk. The pH of milks was almost identical but titratable acidity of goat's milk was 1.62 °SH higher, probably because of better buffering capacity of goat's milk induced by the high level of whey proteins, NPN and phosphate (PARK, 1994). Higher buffering capacity requires more lactic acid for changing the pH value. Moreover, the enhancement of the buffering capacity increases survival of live bacteria and their enzymatic activity in vivo and helps lactose maldigesters to readily consume dairy products (KAILASAPATHY et al., 1996).

Table 1. Chemical composition (%) and acidity of milk for yoghurt production

Composition	Cow's milk	Goat's milk
Total solids	11.89	12.01
Ash	0.75	0.83
Lactose	4.09	3.92
Fat	3.20	3.20
Proteins	3.75	3.92
Acidity pH	6.60	6.59
°SH	6.06	7.68

Fermentation of all four milk samples (cow's and goat's, with and without milk powder supplementation) took 6 h. The samples were then stored refrigerated (8 °C) for 9 days. Goat's milk yoghurt had higher acidity than cow's milk yoghurt at each sampling time (Fig. 1). According to the literature (FELDHOFER et al., 1994; ALICHANIDIS & POLYCHRONIADOU, 1997), lactic acid production is faster and stronger in goat's milk than in cow's milk. Milk powder addition increased pH value, especially in goat's milk yoghurt. Lactic acid is responsible for the sharp, refreshing taste of all kinds of fermented milk, and although non-volatile, lactic acid is an excellent base for the typical taste and aroma of each fermented milk (RASH, 1990).

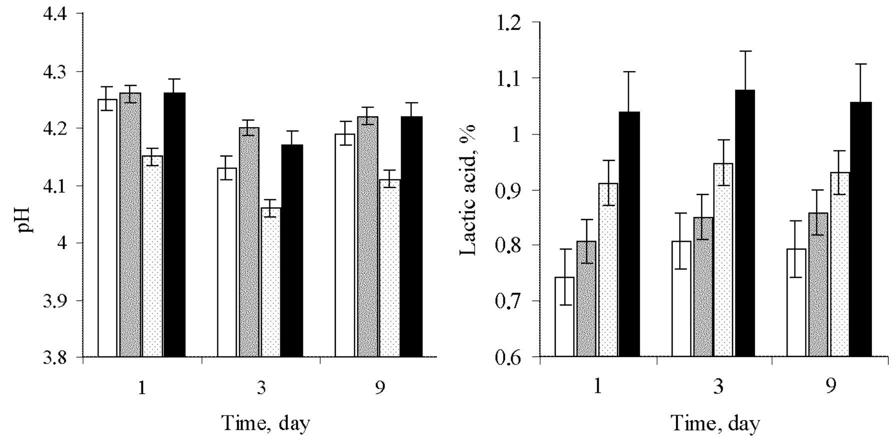

Fig. 1. Changes in pH and lactic acid quantity (%) of yoghurt samples during nine days of storage at 8 °C. □ cow's milk yoghurt; ▒ cow's milk yoghurt with milk powder supplementation; ░ goat's milk yoghurt; ■ goat's milk yoghurt with milk powder supplementation

The greatest decrease in pH value of all yoghurts was noticed between days 1 to 3 of storage, and pH became more stable afterwards. During the same period, an increase in the counts of lactobacilli, which produced more acid than did streptococci, was observed in all four yoghurts (Fig. 2). Changes in viable counts of lactobacilli during further storage (between days 3 to 9) were negligible.

In all products during storage, the viable counts of streptococci were higher than those of lactobacilli (Fig. 2). By using 30 commercial yoghurt cultures KNEIFEL and co-workers (1993) obtained higher viable counts of the streptococci (3.5×10^7–1.2×10^9 CFU ml^{-1}) than viable count of the lactobacilli (5.5×10^7 – 6.5×10^8 CFU ml^{-1}), at the end of fermentation. Lactobacilli decompose the proteins slowly and thus form free amino acids (valine, histidine, leucine), which stimulate the growth of streptococci. However, when the concentration of lactic acid reaches 1–1.5%, streptococci enter the stationary phase of growth, and lactobacilli become active, repressing streptococci and other microorganisms present (RAŠIĆ & KURMANN, 1978).

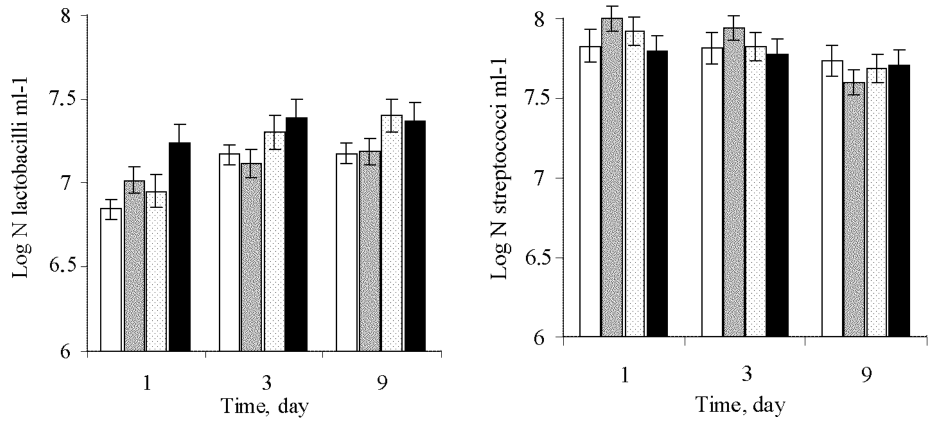

Fig. 2. Changes in viable cell counts of lactobacilli and streptococci (log N ml^{-1}) in yoghurt samples during nine days of storage at 8 °C. □ cow's milk yoghurt; ▒ cow's milk yoghurt with milk powder supplementation; ░ goat's milk yoghurt; ■ goat's milk yoghurt with milk powder supplementation

Viable counts of streptococci decreased (from 1.01×10^8 to 3.97×10^7 CFU ml^{-1}), whereas those of lactobacilli increased in all samples (from 6.95×10^6 to 2.32×10^7 CFU ml^{-1}). The increase in lactobacillus count was higher in goat's milk yoghurt and on days 9 of storage ΔlogN between goat's and cow's milk yoghurts was approximately 0.2 log cycle.

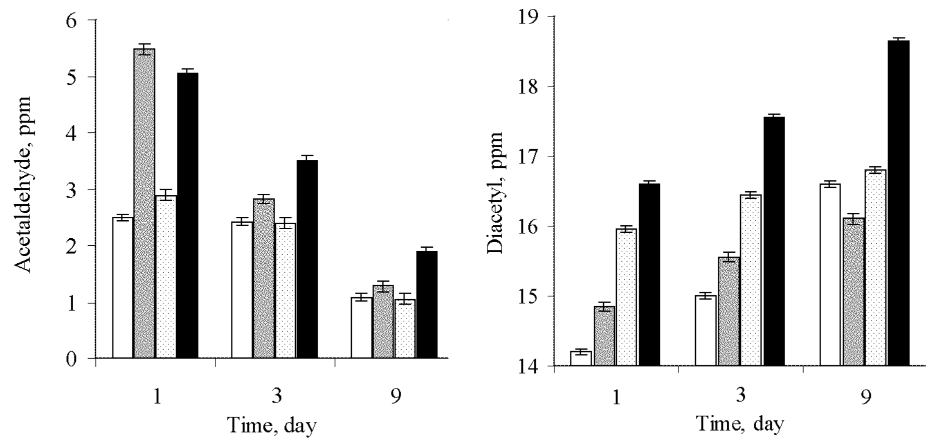

Fig. 3. Acetaldehyde and diacetyl contents (mg kg^{-1}) of yoghurt samples during nine days of storage at 8 °C. □ cow's milk yoghurt; ▒ cow's milk yoghurt with milk powder supplementation; ░ goat's milk yoghurt; ■ goat's milk yoghurt with milk powder supplementation

A lot of components at different concentrations were isolated from yoghurt, and some of these have a great influence on yoghurt's aroma. Acetaldehyde is responsible for the pleasant aroma of yoghurt. At the beginning of storage, the acetaldehyde concentration of yoghurt supplemented with milk powder was almost twice as high as that of controls (Fig. 3). The reason of this may be the higher viable counts of lactobacilli in these samples (Fig. 2), because *Lactobacillus delbrueckii* subsp. *bulgaricus* is known to be responsible for production of acetaldehyde in yoghurt (GEORGALA et al., 1995).

Concentrations of acetaldehyde in cow's and goat's milk yoghurts were 2.50 mg kg^{-1} and 2.89 mg kg^{-1} initially, dropping to 1.08 mg kg^{-1} and 1.88 mg kg^{-1}, respectively after 9 days of storage. Similar changes in acetaldehyde content of supplemented cow's and goat's milk yoghurt samples were observed. The initial value of 5.47 mg kg^{-1} for supplemented cow's milk yoghurt and 5.03 mg kg^{-1} for supplemented goat's milk yoghurt decreased on days 9 of storage to 1.28 mg kg^{-1} and 1.88 mg kg^{-1}, respectively. Similarly, the concentrations of acetaldehyde in yoghurts during storage in refrigerator decreased as was described by MCGREGOR and WHITE (1987) and LAYE and co-workers (1993). Concentration of acetaldehyde in our experiments was quite low, probably because yoghurt culture YC-180 gives tender, creamy products with low quantity of acetaldehyde. According to ULBERTH (1991) concentration of acetaldehyde in yoghurt can be from 1.25 to 40 mg kg^{-1}, and optimal yoghurt aroma is obtained with an acetaldehyde concentration of 23–41 mg kg^{-1} (KNEIFEL et al., 1992). However, classification based only on this component is not adequate. For example, a characteristic product with less than 8.4 mg kg^{-1} is described as a medium aromatic one. Nevertheless, some yoghurts with low concentration of acetaldehyde can still have typical aroma. The lack of acetaldehyde can probably be substituted for by a relatively high concentration of diacetyl. According to ULBERTH (1991), concentration of diacetyl in yoghurt is between 0.16 and 5 mg kg^{-1}. The presence of diacetyl contributes to the delicate, full flavour and aroma of yoghurt and is especially important if acetaldehyde is low, because it can enhance yoghurt flavour (MARSHALL, 1984).

Diacetyl and acetoin are the result of the metabolic activity of *Streptococcus thermophilus* and are mostly very low, rarely reaching 0.5 mg kg^{-1} (MARSHALL, 1984). However, in our study initial concentration of diacetyl was 14.20 mg kg^{-1} in cow's milk yoghurt and 15.95 mg kg^{-1} in goat's milk yoghurt. During storage, concentrations of diacetyl increased to 16.60 mg kg^{-1} and 16.80 mg kg^{-1}, respectively. In supplemented cow's and goat's milk yoghurts, initial concentration of diacetyl increased from 14.85 mg kg^{-1} to 16.10 mg kg^{-1} and from 16.60 mg kg^{-1} to 18.65 mg kg^{-1}, respectively. It is well established that the time and temperature of storage significantly influence the changes in diacetyl concentration.

Sensory properties of goat's milk yoghurt samples were poorer than those of cow's milk yoghurt samples (Table 2).

Table 2. Sensory scores of cow's (C) and goat's (G) milk yoghurts without and with milk powder (m) addition during nine days of storage at 8 °C

Time of storage	Properties	C	Cm	G	Gm
1st day	General appearance	1.0	0.9	1.0	1.0
	Colour	1.0	1.0	1.0	1.0
	Flavour	2.0	2.0	2.0	2.0
	Consistency	3.7	3.9	1.6	2.7
	Taste	10.4	11.4	12.0	12.0
	Total	18.1 ± 0.8	19.2 ± 0.4	17.6 ± 0.3	18.7 ± 0.3
3rd day	General appearance	0.9	1.0	0.8	0.8
	Colour	1.0	1.0	1.0	1.0
	Flavour	2.0	2.0	2.0	2.0
	Consistency	4.0	4.0	1.9	2.7
	Taste	11.8	12.0	12.0	12.0
	Total	19.7 ± 0.5	20.0 ± 0.3	17.7 ± 0.3	18.5 ± 0.7
6th day	General appearance	0.9	1.0	0.9	0.9
	Colour	1.0	1.0	1.0	1.0
	Flavour	2.0	2.0	2.0	2.0
	Consistency	3.9	3.8	2.0	2.9
	Taste	12.0	12.0	12.0	12.0
	Total	19.8 ± 0.4	19.8 ± 0.4	17.9 ± 0.3	18.8 ± 0.5
9th day	General appearance	0.7	0.9	0.7	0.7
	Colour	1.0	1.0	1.0	1.0
	Flavour	2.0	2.0	2.0	2.0
	Consistency	4.0	4.0	1.9	2.8
	Taste	12.0	12.0	10.8	10.8
	Total	19.7 ± 0.3	19.9 ± 0.3	16.4 ± 0.5	18.3 ± 0.8

Addition of milk powder improved sensory properties, especially in cow's milk yoghurt. There were more aroma components (acetaldehyde and diacetyl) (Fig. 3) and lactic acid in supplemented samples, which had a higher pH than control samples (Fig. 1). All yoghurt samples had maximal scores for colour and flavour during the whole 9 days of storage.

Syneresis, although notably lower in supplemented samples than in controls, was observed during storage of cow's milk yoghurts. The reason for this is the heat treatment of milk before fermentation. After supplementation with milk powder, milk was heated to 90 °C in order to obtain better homogenisation. Heating the milk at such a high temperature is considered sufficient to affect casein micellar aggregation and to induce interaction between denaturated whey proteins and casein. Complex gel structure is the result of the combination of heating and acid induced protein reactions. This gel structure gives a characteristic texture to yoghurt. Insufficient heating will result in weak-bodied yoghurt, while excessive heating will lower gel strength and result in grainy textured yoghurt with a tendency towards syneresis. According to KAILASAPATHY and SUPRIADI (1998), good quality yoghurt can be made from skim milk fortified with skim milk powder and whey protein concentrate using a lower heat treatment than commonly practised in the manufacture of yoghurt (78 °C for 25 min).

During the storage of goat's milk yoghurt samples no syneresis was noticed. Goat's milk yoghurt usually does not excrete the whey (FELDHOFER et al., 1994). In our samples, the homogeneous structure of curd was disturbed, most obviously on day 9 of storage, and especially in the supplemented goat's milk yoghurt, which had a soft consistency and a characteristic taste of goat's milk. Its other sensory characteristics were similar to cow's milk yoghurt. On day 1, goat's milk yoghurts, especially those without milk powder addition, had a very soft consistency. During storage, consistency improved (got firmer) until day 6. On day 9, disintegration of curd was noticed. On day 1, aroma and acidity were not expressed enough in cow's milk yoghurt samples, especially in the controls. During storage, taste of both cow's milk yoghurts improved. On day 9, no strange taste or aroma was noticed.

Sensory evaluation of yoghurts showed that differences between samples were statistically highly significant ($P<0.01$). Differences in sensory scores of the samples during storage were not statistically significant ($P>0.05$).

3. Conclusions

Goat's milk had slightly higher levels of total solids, ash and proteins, whereas lactose content was higher in cow's milk. The pH values of milks were almost identical even though titratable acidity of goat's milk was 1.62 °SH higher. Goat's milk yoghurt samples had higher acidity than did cow's milk yoghurts at each sampling time. Milk powder addition increased pH value and the lactic acid concentration in both types of yoghurt, especially in goat's milk yoghurt. A considerable decrease in pH value of yoghurts between days 1 to 3 of storage was observed, then pH became stable. During the same period, an increase in the viable count of lactobacilli was also noticed. During storage, the viable counts of streptococci were higher than those of lactobacilli. The viable counts of streptococci decreased during storage (from 1.01×10^8 to 3.97×10^7 CFU ml^{-1}), while the viable count of the lactobacilli increased in all samples (from 6.95×10^6 to 2.32×10^7 CFU ml^{-1}). The increase in counts of lactobacilli was especially noticeable in goat's milk yoghurt samples and on the ninth day of storage, ΔlogN between goat's and cow's milk yoghurt samples was ca. 0.2. During the storage of yoghurt, acetaldehyde concentration decreased, whereas diacetyl noticeably increased. Sensory properties of goat's milk yoghurt samples were found to be worse than those of cow's milk yoghurt samples. Milk powder addition improved sensory properties, especially of cow's milk yoghurt samples. Goat's milk yoghurts, especially that made without milk powder addition, had very soft consistency but no syneresis was observed.

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