Effects of transglutaminase treatment on the production of set skim milk yoghurt: microbiological aspects

Von H. Neve [1], P. Chr. Lorenzen [2], A. Mautner [2], E. Schlimme [2], K. J. Heller [1]

[1] Institute for Microbiology and
[2] Institute for Dairy Chemistry and Technology of the Federal Dairy Research Centre, P.O. Box 6069, D-24121 Kiel, Germany

1. Introduction

For the production of traditional yoghurt, blends of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* are used as starter cultures. Both bacterial components are homofermentative and produce lactic acid during growth, which results in a rapid pH decrease during milk fermentation and in the formation of the characteristic yoghurt gel due to the destabilisation of the milk casein complex (33, 34, 36). These acid gels differ significantly from enzymic-induced milk gels made by renneting (34). Since the first are less robust and stable than the latter gels, various strategies exist to improve the gel stability of set-style yoghurts (e.g., concentration of the total solids of milk by evaporation or addition of milk powder, increase of the protein content by ultrafiltration or addition of milk proteins and addition of stabilisers).

It has been shown recently that the yoghurt micro-structure can also be improved by treatment of the milk with transglutaminase (11, 18, 21). Transglutaminase (TGase; EC 2.3.2.13) induces the transfer of acyl groups between γ-carboxyamide groups of peptide-bound glutamine residues and the ε-amino groups of lysine residues, leading to the formation of intra- and /or intermolecular isopeptide bonds. This enzymic reaction results in the formation of cross-linked protein polymers (9, 22). It has been shown, that casein in particular is one of the best substrates for TGase in milk (3, 18, 23, 25, 27, 28, 29). TGases are widespread in nature and have been identified in various eukaryotes (e.g., mammalian tissues and organs, plants, fish) and also in prokaryotic microorganisms. Strains of *Streptoverticillium mobaraense* (now classified as *Streptomyces mobaraense*) are producing TGase extracellularly (16), and commercial preparations of microbial TGase (i.e., MTGase) are available. Benefits of using MTGase are their lower costs for extraction and purification and their Ca^{2+}-independent catalytic action (9, 22).

It has already been shown before that yoghurts made from MTGase-treated milk exhibit an improved micro-structure (11, 18, 21). Several advantageous effects of the addition of MTGase to milk for the production of set-style yoghurts are known, including an increase of gel strength, a decrease of whey syneresis, generation of a milder taste, and a smoother surface.

The enzymatic crosslinking of milk proteins by MTGase introduces new covalent bounds into the yoghurt gel. In contrast, in traditionally fermented yoghurt, the milk gel is mainly stabilised by noncovalent physical cross-links (i.e., electrostatic interaction, hydrogen bonding and hydrophobic bonds). Hence these different protein networks may affect the growth behaviour of the yoghurt starter bacteria, which are fastidious

microorganisms and - due to their auxotrophy for various amino acids - are dependent on the availability of various amino acids derived from the milk proteins. (4, 12-15, 17, 19, 31, 32). It is the objective of this short contribution to investigate the effect of an MTGase-treatment of milk on the fermentation behaviour of the yoghurt starter bacteria and their viability during storage at low temperature. The development of non-starter microorganisms during storage of MTGase-treated yoghurt was also studied.

Two experimental designs were included in our studies: In the first experimental setup, skim milk was pretreated for 2 h with MTGase, which was subsequently inactivated before inoculation with yoghurt starter bacteria. This setup mimicks conditions considered to be suitable for MTGase application for industrial yoghurt manufacture. In the second set of experiments, MTGase and yoghurt starter culture were added simultaneously to the skim milk and the inactivation step for the cross-linking enzyme was omitted to test a long-term incubation time.

2. Material and Methods

2.1 Preparation of set-style skim milk yoghurt with and without MTGase treatment

Raw milk was obtained from the experimental station of the Federal Dairy Research Centre.

In order to study the effect of a 2-h pretreatment of skim milk with MTGase on the growth of the yoghurt starter culture, skim milk (produced by separation of raw milk with a disk centrifuge [Westfalia separator, Oelde, Germany] at 45°C) was preheated at 92°C for 5 min. For the enzymatic treatment of a 4-l batch of skim milk with MTGase (Activa, Ajinomoto Europe Sales GmbH, Hamburg, Germany) (declared activity: 1000 U/g), the appropriate amount of enzyme was resuspended in 20 ml skim milk and added to the milk with an enzyme/substrate ratio of 1/2000 (wt/wt). After 2 h incubation at 40°C, the enzymatic reaction was stopped by a short heat treatment (80°C, 1 min). A 4-l control batch without MTGase treatment was subjected to the same temperatures. Both milk samples were inoculated (3% vol/vol) with a commercially available traditional thermophilic yoghurt starter culture consisting of *S. thermophilus* and *Lb. delbr.* subsp. *bulgaricus* pregrown overnight in skim milk. The inoculated milk samples were distributed into 150-ml plastic cups and sealed with aluminium lids. Milk fermentation was performed at 43±1°C. Fermentations were usually stopped by cooling the yoghurt samples down to 5°C, when a pH value of 4.68 was reached (approximately after 3 h). In one experiment, fermentation was not stopped at pH 4.68 but continued (fermentation time of 6.5 h) in order to ensure that both yoghurt starter strains had reached the stationary growth phase.

For analysis of the long-term effect of active MTGase on fermentation activity of the yoghurt starter culture during fermentation and the microbial stability during storage at 6°C, MTGase (Activa-MP, declared activity: 100 U/g) was resuspended in 20 ml skim milk and added to 4 l of pasteurised skim milk (see above) at an enzyme/substrate ratio of 1/4000 (wt/wt). At the same time, the yoghurt starter culture (see above) was added. The milk samples were distributed into 150-ml plastic cups and sealed with aluminium lids. Fermentation at 43±1°C was stopped at a pH value of 4.68 by cooling the samples down to 5°C. In these experiments the heat inactivation step of the transglutaminase was omitted. Control batches without MTGase treatment were included following the same procedure. The yoghurt samples were stored for 6 weeks at 6°C. This experiment was performed twice.

2.2 Microbiological characterisation

All media were obtained from Merck (Darmstadt, Germany). Microbiological analysis was performed (i) with the yoghurt samples either during fermentation (in 30-min intervals) or during 6-week storage at 6°C (in 2-week intervals), (ii) with the freshly pasteurised skim milk samples, and (iii) with a freshly prepared MTGase solution (suspension of 20 g Activa-MP in 50 ml sterile double destilled water) following the German VDLUFA standards (1). All serial dilutions were done in ¼-strength Ringer solution. Colony-forming units of the thermophilic yoghurt starter bacteria were determined on lactose M17 agar plates (35) supplemented with 5% skim milk (for counting of *S. thermophilus*) and on Rogosa agar plates (selective for *Lb. delbr.* subsp. *bulgaricus*; [6]) after incubation at 43°C for 3 days. The Rogosa plates were incubated in anaerobic jars using the Anaerocult A kit (Merck). Determination of the mesophilic total cell counts was done on Plate Count (casein-peptone dextrose yeast extract) agar after a 3-day incubation at 30°C. Colony counts of yeasts and moulds were determined on YGC (yeast extract glucose chloramphenicol) agar (25°C, 3 days). The Most Probable Number (MPN) procedure was used to determine the number of coliforms in laurylsulfate-tryptose broth (30°C, 3 days). Samples were heated for 10 min at 80°C before determination of the numbers of aerobic spore formers (plated on Nutrient agar and incubated at 30°C for 3 days) and of the anaerobic spore formers (determined by the MPN procedure in DRCM [differential reinforced clostridial broth] after incubation at 37°C for 7 days in tubes sealed with parafin). Plate counts were done in duplicate and MPN numbers were determined in triplicate.

2.3 Chemical-technological characterisation

The methods used have been described before (i.e, analysis of gel strength of the yoghurt samples with a Stevens Texture Analyser, measurement of whey drainage [serum leakage] from 30-g yoghurt samples kept for 2 h at 6°C; determination of pH and titratable acidity [°SH-values according to the Soxhlet-Henkel method] of the yoghurt samples; [21]). All measurements were done in triplicate.

3. Results

Growth and activity of thermophilic yoghurt cultures in milk pretreated with MTGase for 2 h.

In order to assess the effect of cross-linking of milk proteins by MTGase on the growth behaviour of a traditional yoghurt starter culture (i.e., *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*), viable counts were determined during the fermentation period of MTGase-treated and untreated skim milk yoghurt samples. Figure 1 shows the growth curves of *S. thermophilus* and *Lb. delbr.* subsp. *bulgaricus* grown in untreated milk and in milk pretreated with MTGase. Transglutaminase was inactivated as described in Material & Methods before the culture was added. In principle, growth curves of the cultures in the enzyme-treated skim milk samples differed only slightly from those of the untreated controls. During the logarithmic growth phase, *S. thermophilus* revealed higher cell numbers when grown in MTGase-treated milk, hence entering the stationary phase earlier (i.e., after 125 min) than the culture growing in the untreated skim milk (Fig. 1). Comparably high *S. thermophilus* cell numbers were obtained in both milk samples (untreated sample: 8.1×10^8 CFU/g, MTGase-treated sample: 8.4×10^8 CFU/g). A different growth behaviour was documented for *Lb. delbr.* subsp. *bulgaricus*. As expected, the

lactobacilli revealed a long logarithmic phase and entered the stationary phase late during fermentation. Maximal viable *Lactobacillus* counts obtained were 2.7×10^8 CFU/g (untreated sample) and 2.3×10^8 CFU/g (MTGase-treated sample). In MTGase-treated skim milk, a minor decrease in growth rate was found for the lactobacilli after a fermentation time of $1^1/_2$ h. This observation correlated well with the concomitant occurence of a minor decrease in pH-value (maximal △pH of 0.14 at 150-min fermentation time) in the fermentation profile of the enzyme-treated yoghurt samples (Fig. 2).

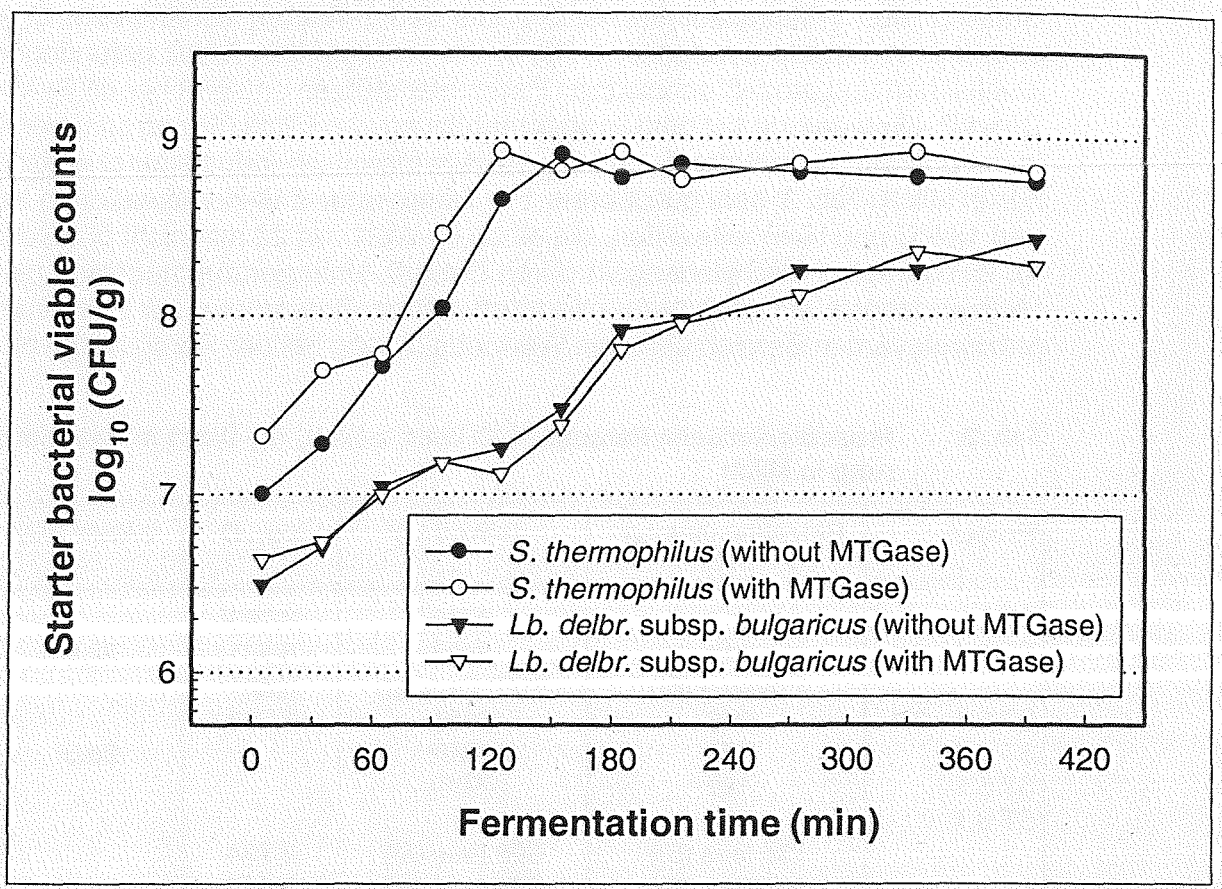

Fig. 1. Growth of a yoghurt starter culture (*Streptococcus thermophilus* and *Lb. delbr.* subsp. *bulgaricus*) in skim milk pretreated with MTGase for 2 h and in non-treated skim milk (control). MTGase was heat-inactivated before inoculation with the starter culture. The data are from one representative experiment, in which the yoghurt fermentation was not terminated at pH 4.68.

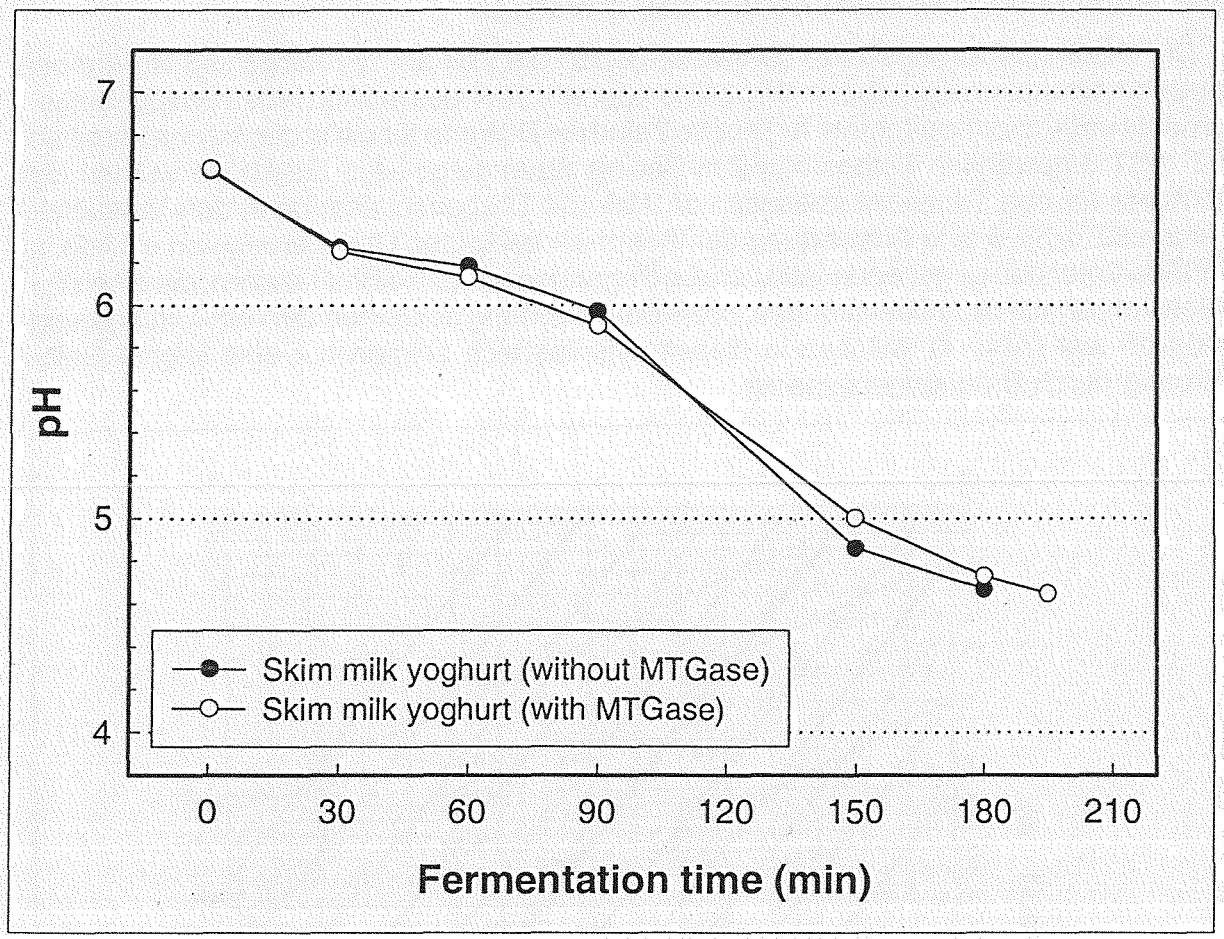

Fig. 2. Decrease of pH in skim milk preincubated with MTGase for 2 h and in untreated skim milk (control) during fermentation with a yoghurt starter culture. MTGase was inactivated before the yoghurt starter culture was added.

Microbiological analysis of set skim milk yoghurt treated with non-inactivated MTGase

Many chemical-technological studies have been performed with MTGase-treated yoghurt in which the cross-linking enzyme has been inactivated before addition of the thermophilic starter cultures. The scope of thè experiment shown here was to analyse the effects of a non-inactivated enzyme thus still active during fermention and subsequent storage of the yoghurt samples. Transglutaminase was added concomitantly with the yoghurt starter culture.

The fermentation profiles of the yoghurt cultures during growth in untreated and MTGase-treated milk (Fig. 3) illustrate that the concomitant use of the starter culture and of the enzyme preparation did not result in a decrease of the acidification activity in the enzyme-treated milk at the end of fermentation.

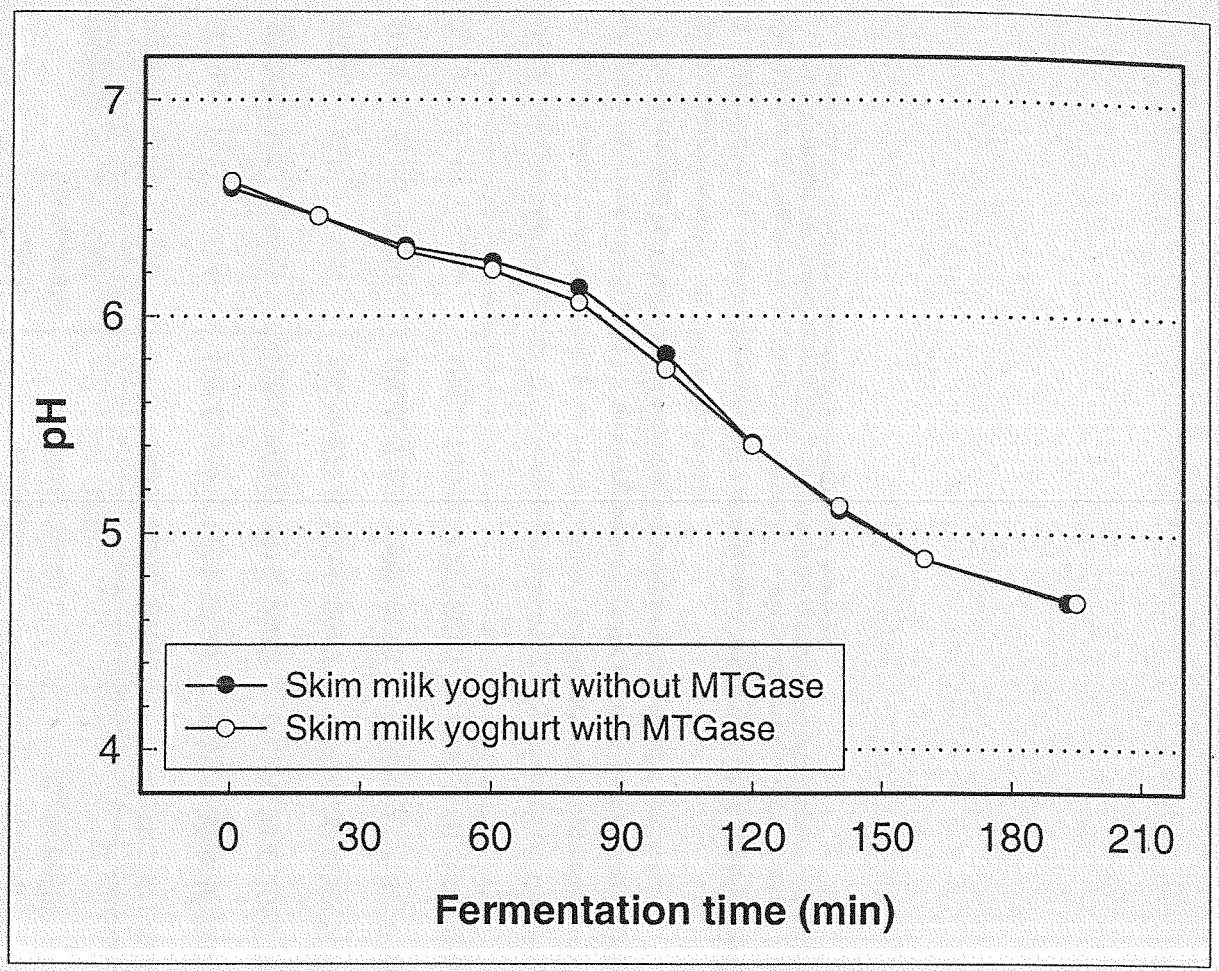

Fig. 3. Decrease of pH during fermentation of skim milk with and without non-inactivated MTGase. MTGase and yoghurt starter culture were added simultaneously.

Fig. 4 illustrates that at the end of the fermentation time, the colony counts of the two yoghurt starter strains were comparable in the untreated control samples and in the MTGase-treated products (*S. thermophilus*: 6.6×10^8 CFU/g [untreated sample] *versus* 7.8×10^8 CFU/g [enzyme-treated sample], *Lb. delbr.* subsp. *bulgaricus*: 1.3×10^8 CFU/g [untreated sample] *versus* 7.9×10^7 CFU/g [enzyme-treated sample]). The viability of the *S. thermophilus* starter strain decreased by ca. 0.5 $\log_{10}$ unit during the first 2 weeks of storage in the MTGase-treated sample, but showed no further decline during the following 4 weeks. In the untreated skim milk, no significant change in *S. thermophilus* viable counts was detected during the 6-week storage. In contrast, significant differences became apparent in the colony counts of the lactobacilli, where a marked decrease of the colony counts was documented. After a 2-week storage period, the viable count numbers in the control samples were higher than those of the MTGase-treated samples (4.8×10^7 CFU/g *versus* 1.0×10^7 CFU/g). During further storage, a rapid decrease of *Lb. delbr.* subsp. *bulgaricus* viable count number occurred in the untreated sample, while the loss of viability was less severe in the enzyme-treated sample. After 6 weeks, 3.8×10^3 CFU/g were detected in the control sample, but still 6.7×10^4 CFU/g in the MTGase-treated sample. Thus, the non-inactivated MTGase had a stabilizing effect on the viability of the lactobacilli in the yoghurt product during storage.

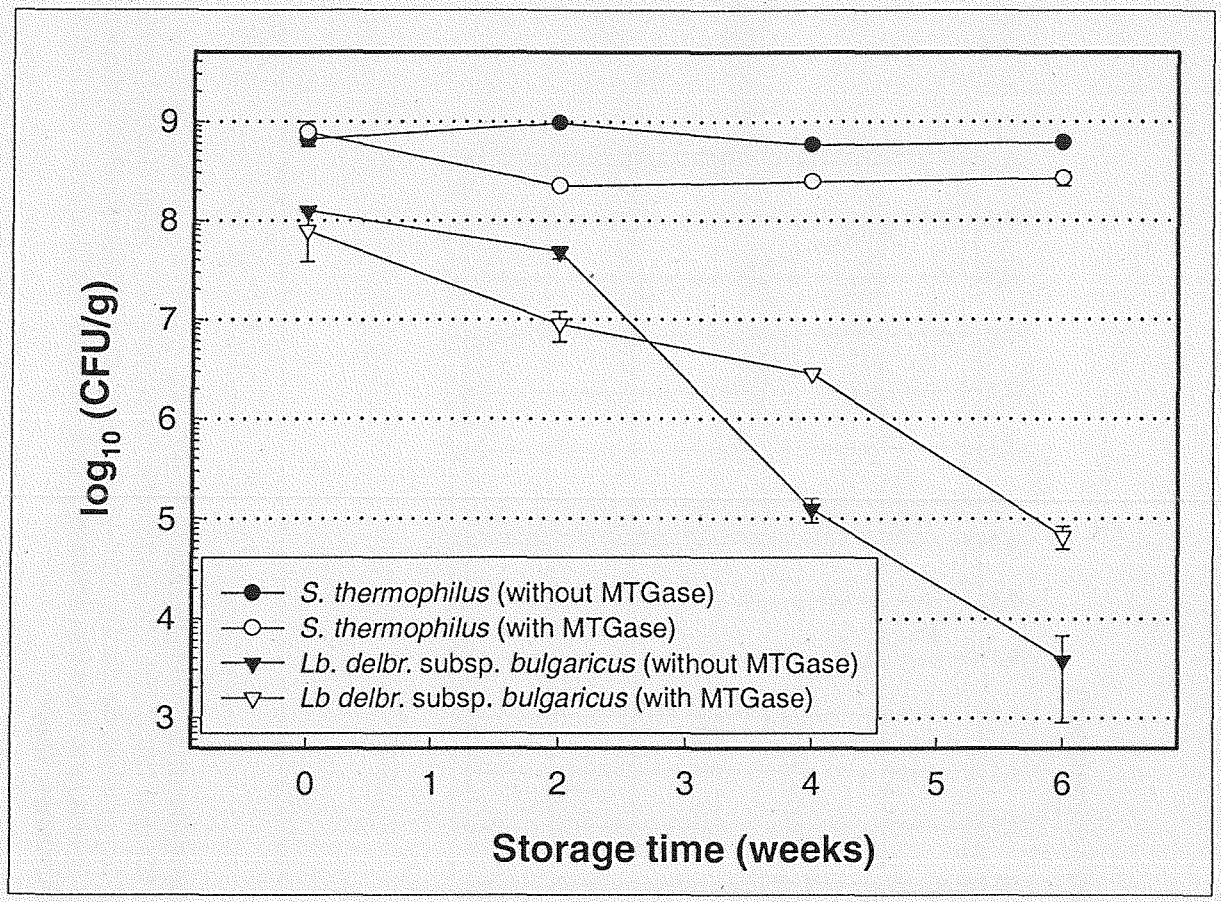

Fig. 4. Changes of viable counts of *S. thermophilus* and of *Lb. delbr.* subsp. *bulgaricus* in yoghurt made from skim milk containing non-inactivated MTGase and from untreated skim milk (control) during cold storage. The cell numbers at day 0 represent viable counts at the end of the fementation, which was terminated when a pH of 4.68 was reached. Data are mean values of two experiments (bars: standard deviation).

Microbiological analyses of the non-starter microflora were done to detect: mesophilic aerobic bacteria (i.e., plate count without thermophilic starter cultures), yeasts and moulds, coliforms, aerobic and anaerobic spore producers. None of these microorganisms was neither detected in freshly prepared aqueous enzymic preparations (i.e., a 10% (wt/vol) suspension made in sterile double distilled water) nor in the freshly pasteurised milk batches (minimum detection limit: 10 CFU/ml and 0.3 MPN values/ml). No (non-starter) microbiological growth was found in the enzyme-treated yoghurt samples and untreated control samples during the 6-week storage period at 6°C (minimum detection limit: 100 CFU/g and 3 MPN values/g).

The effects of the non-inactivated MTGase on the techno-functional properties of yoghurt samples are illustrated in the Fig. 5-7 during the first $2^1/_2$ weeks of storage at 6°C. In the presence of active transglutaminase, the titratable acidity was significantly decreased in MTGase-treated skim milk yoghurt as compared with yoghurt made from untreated milk (Fig. 5). In both cases, the °SH-values increased during the first 2 weeks of storage and reached a plateau after that time. The MTGase-treatment also resulted in an increase of gel strength during the first 15 days of storage (up to 1.4 N/cm²), while the gel strength of the untreated control remained without changes at a low level of ca. 0.3 N/cm² (Fig. 6). Fig. 7 shows that serum leakage (i.e., the whey drainage ability) of the MTGase-treated yoghurt samples was also significantly reduced by 20% in comparison to the untreated yoghurt sample.

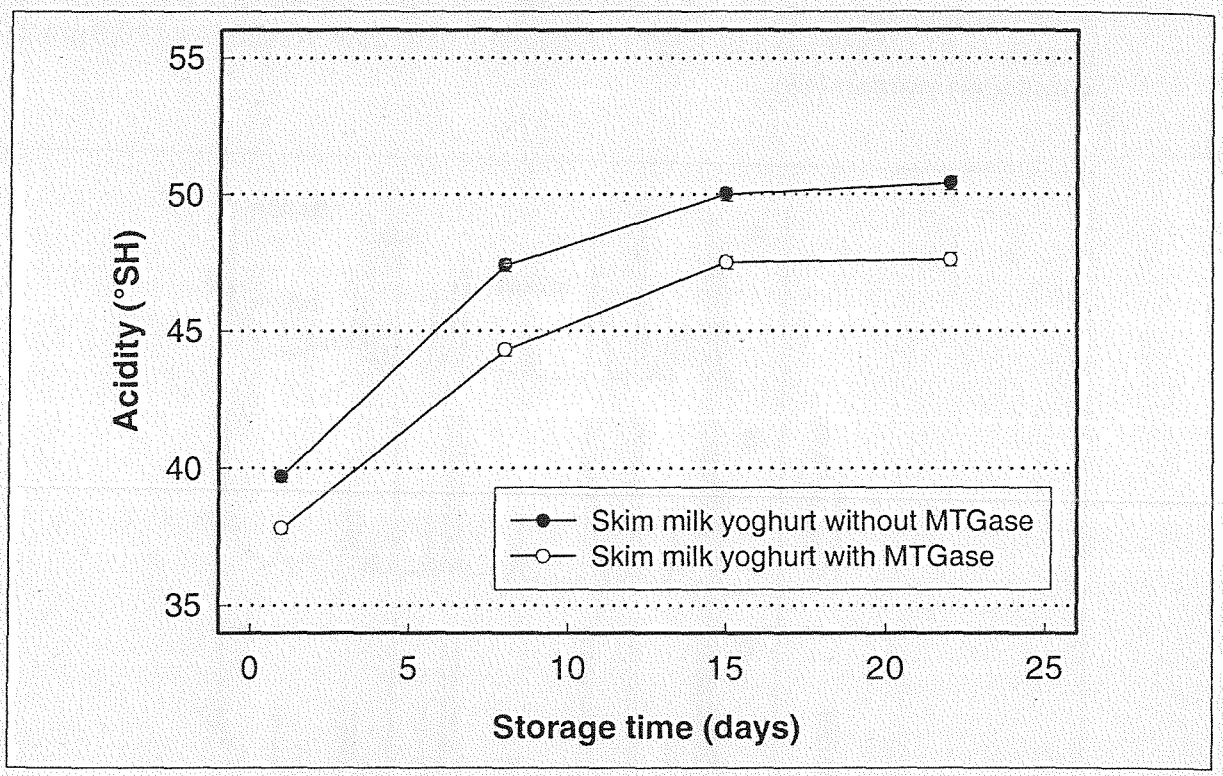

Fig. 5. Development of titratable acidity (°SH values according to the Soxhlet-Henkel method) in yoghurt made from skim milk containing non-inactivated MTGase and from untreated skim milk (control) during cold storage. Data are mean values of 3 measurements (bars: standard deviation).

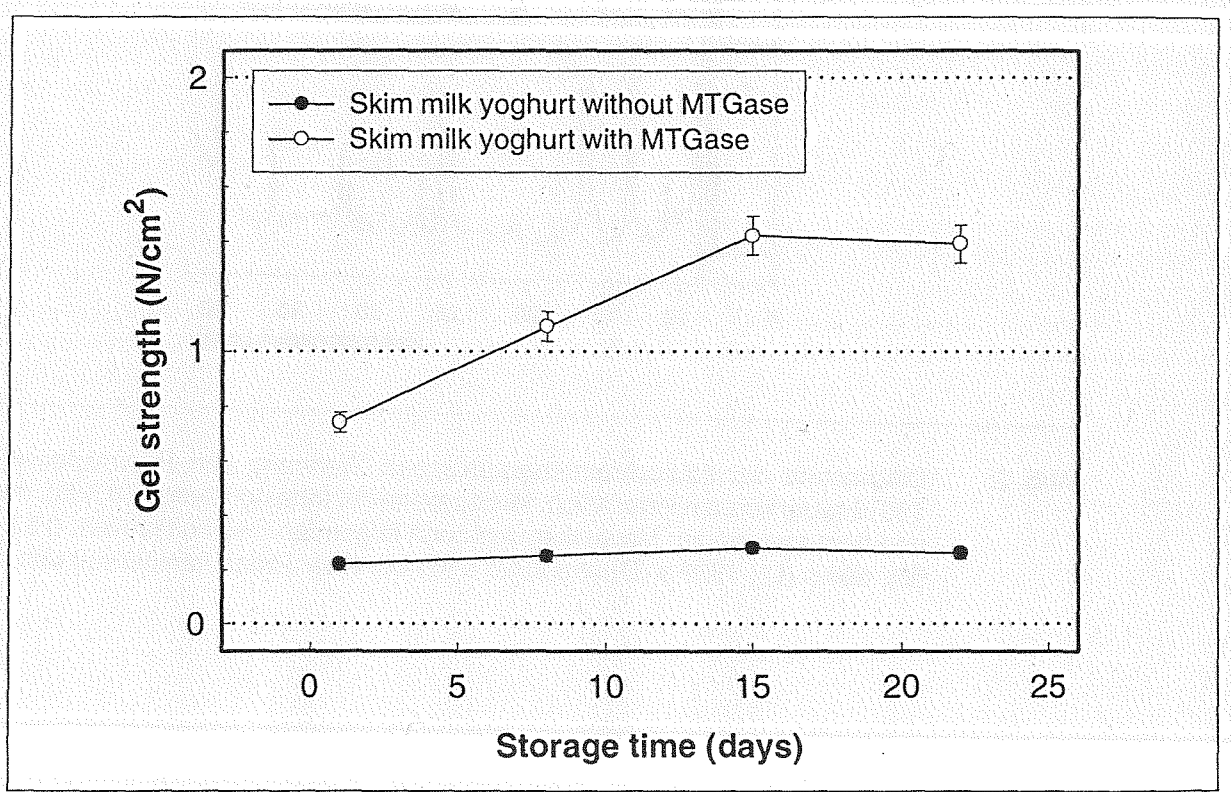

Fig. 6. Development of gel strength in yoghurt made from skim milk containing non-inactivated MTGase and from untreated skim milk (control) during cold storage. Data are mean values of 3 measurements (bars: standard deviation).

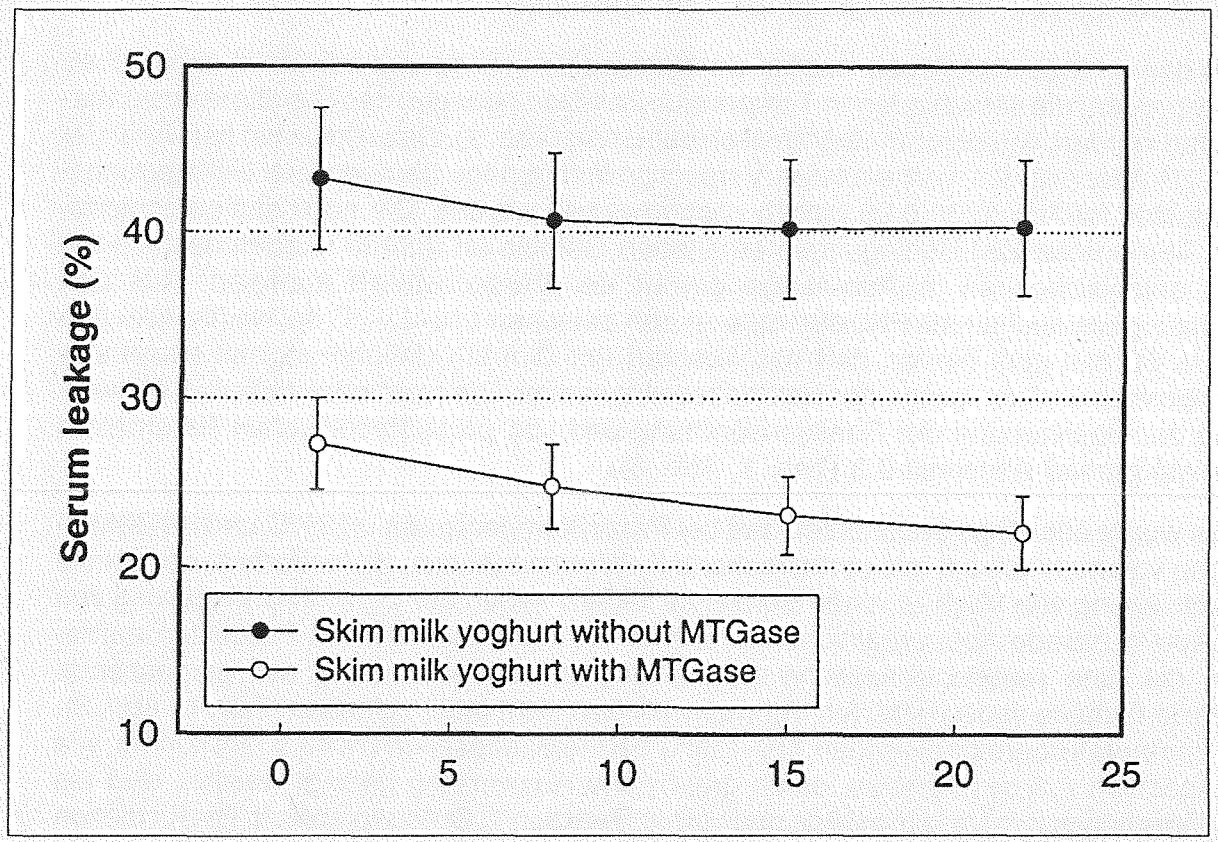

Fig. 7. Development of serum leakage ability (i.e., whey syneresis) of yoghurt made from skim milk containing non-inactivated MTGase and from untreated skim milk (control) during cold storage. Data are mean values of 3 measurements (bars: standard deviation).

4. Discussion

We have shown that pre-treatment of skim milk with MTGase for 2 h causes a minor imbalance of the growth association between *S. thermophilus* and *Lb. delbr.* subsp. *bulgaricus*. In the first phase of fermentation, the starter bacteria revealed good growth in the enzyme-treated milk comparable to the control trial, while the lactobacilli grew slower during the second fermentation phase in MTGase-treated skim milk. The observed minor decline of the lactobacilli cell counts is probably causing the observed minor delay in the fermentation time in the MTGase-treated skim milk sample. The effect, that yoghurt starter cultures require a longer fermentation time in milk treated with MTGase, is already known from other studies (11, 21). It is well established that *S. thermophilus* and *Lb. delbr.* subsp. *bulgaricus* benefit from each other during milk fermentation by providing compounds promoting the growth of the partner strain. *Lb. delbr.* subsp. *bulgaricus* is known to possess active proteolytic systems, while the majority of *S. thermophilus* strains reveal either weak or no proteolytic activities (12, 13, 15, 17, 19, 31, 32). Hence, amino acids liberated from proteolytic lactobacilli are the main growth-promoting factors for *S. thermophilus* starter strains. In return, *S. thermophilus* cells provide other growth-stimulating factors (i.e., formic acid and CO_2) for the lactobacilli (34, 36). The majority of *S. thermophilus* strains does not express or express a very low level of cell wall proteinase. Small peptides (1000 to 2500 Dalton) containing lysine or arginine promote growth of *S. thermophilus*, while larger peptides have an inhibitory effect (7). An efficient complex oligopeptide transport system comprising of three different functional oligopeptide-binding proteins with overlapping substrate specificities has been described recently for

S. thermophilus (14). This unusually broad transport system allows uptake of small oligopeptides composed of 3 to 23 amino acids into the starter cells. MTGase-treatment of milk results in a finer–meshed protein network and prevents whey leakage from the milk gel (10, 11, 21, 28). Hence the cross-linking prevents the occurrence of excessive local phase separation. The development of a cross-linked protein network during MTGase-treatment of skim milk may bring the starter bacterial cells into closer physical contact with these growth-stimulating soluble small peptides. Lauber *et al.* (2000) have shown recently that only a very limited number of lysine and glutamine residues are prone to modification by transglutaminase resulting in intermolecular bonds between casein molecules. Although tested with a guinea pig liver TGase, Christensen *et al.* (1996) have shown before that only 5 of the 21 glutamine residues of ß-casein are in fact involved in TG-mediated cross-linking. Hence a sufficiently high number of oligopeptides is expected to result from the proteolytic activity of the lactobacilli to become available for stimulating growth of *S. thermophilus*.

Two different cell wall proteinases are known to be present in *Lb. delbr.* subsp. *bulgaricus* strains, reflecting the high proteolytic activity of these strains. One of them is a serine proteinase, the other a zinc-dependent proteinase (15, 32). The first enzyme is probably only active during the first phase of milk fermentation, since its activity is decreasing during acidification. The latter enzyme is supposed to be active during the late phase of milk fermentation. Stefanitsi & Garel (1997) reported that below a pH value of 6.5, the zinc-dependent proteinase was responsible for more than half of the caseinolytic activity of *Lb. delbr.* subsp. *bulgaricus*. Since we have shown that this starter strain grows slower in MTGase-treated skim milk in the late phase of fermentation, it is possible that the activity of the metalloproteinase is in particular affected by enzymatic cross-linking of the milk proteins.

Growth studies performed with *Lb. delbr.* subsp. *bulgaricus* strains in a chemically defined medium have shown that a number of amino acids (in particular proline) are required in high concentration for efficient growth of this yoghurt bacterium (2). During the optimisation of the composition of the defined growth medium, the authors had to raise the concentration of lysine from initially 0.27 mM to a final concentration of 2.4 mM. This amino acid is thus - among various others - required for bacterial growth. If some of the lysine residues are covalently linked in isopeptides, they are taken away from the pool required by the starter organisms for optimal growth.

In addition to cell wall proteases and oligopeptide transport systems, intracellular peptidases are further key components of the proteolysis system of lactic acid bacteria. Several aminopeptidases, di- and tripeptidases, endopeptidases and peptidases required for proline-containing peptides are known today to be present in the thermophilic yoghurt starters (4). Deutsch *et al.* (2000) recently performed an analysis of amino acids released from ß-casein (prehydrolysed by a mixture of trypsin and chymotrypsin to mimick an extracellular protease activity) by cell extracts of *Lb. delbr.* subsp. *bulgaricus* and *S. thermophilus*. Although tested at a rather low suboptimal temperature of 24°C, incubation either with an *S. thermophilus* or a *Lb. delbr.* subsp. *bulgaricus* cell extract led to high amounts of free amino acids released from the ß-casein peptides with the predominating amino acids glutamine, proline, leucine, valin and lysine. Since the first and the last amino acid are involved in MTGase-induced cross-linking, it cannot be excluded, that peptidase activities are also affected by the enzymic reaction.

We have further shown, that the concomitant incubation of skim milk with MTGase and with the yoghurt starter culture and omission of the enzyme heat inactivation step did not result in a longer fermentation profile. Hence it is concluded that the yoghurt starter

bacteria are able to break down sufficient amounts of milk casein before efficient cross-linking is occuring. During the first 2 weeks of storage, a decline of viability (ca. 0.5 $\log_{10}$ cycle) was observed both for *S. thermophilus* and *Lb. delbr.* subsp. *bulgaricus* in MTGase-treated yoghurt. Since the gel strength of the enzyme-treated yoghurt is increasing during the first 2 weeks of storage, we assume that the MTGase is still active during this period. Due to the significant changes of the yoghurt micro-structure as a result of MTGase action and pH-variation during fermentation and storage, further lysine- and glutamine-residues may become available for the enzyme to induce further cross-linking. Apparently, the yoghurt starters are still metabolically active during the first 2 weeks of storage, since the amount of acidity is still accumulating during these 2 weeks. It should be noted that the enzyme-treated samples cannot be addressed any more as typically "yoghurt-like", since the gel strength and whey leakage ability differ significantly from those values obtained in earlier yoghurt production trials, where MTGase activity was restricted to a 2-h incubation prior to fermentation (21). The consistency of yoghurt containing active transglutaminase looks like a heat-induced whey protein gel rather than like an acid-induced casein gel.

The most striking outcome of our study is the considerable stabilising effect of a (long-term) MTGase treatment of yoghurt on the viability of *Lb. delbr.* subsp. *bulgaricus* after 2 weeks of storage, where the acidity of the yoghurt had reached a maximum level. Since the acidity of the MTGase-treated yoghurt is reduced, the yoghurt starters will face a weaker pH stress situation during yoghurt storage. At low pH values, adaptation mechanisms are induced in the bacterial starters (26). Lactobacilli are known to be more acid-tolerant than the other lactic acid bacteria. Siegumfeldt *et al.* (2000) have recently decribed that the intracellular pH in *Lb. delbr.* subsp. *bulgaricus* yoghurt starter cells decreases rapidly in response to a rapid drop in extracellular pH while different rates of intracellular pH decrease were observed for *S. thermophilus* strains. Lim *et al.* (2000) have further shown recently that *Lb. delbr.* subsp. *bulgaricus* is producing a series of stress response proteins during growth under acidic conditions.

The yoghurt starter culture used in our investigation has also been included by us in a previous study as a reference culture (24). In this study, yoghurt was not produced in plastic cups but instead in screw capped glass bottles. Under these conditions, the *Lb. delbr.* subsp. *bulgaricus* strain exhibited high cell counts even after 4 week storage at 8°C, indicating that this species with a preference for anaerobic conditions is better surviving in glass containers than in plastic cups. Dave & Shah (1997) noted better survival of *Lb. delbr.* subsp. *bulgaricus* in yoghurt produced in glass bottles probably due to the lower oxygen content compared to yoghurt made in plastic containers. In contrast, survival of *S. thermophilus* was almost 50% higher in the latter containment (5).

In conclusion we have shown that pretreatment of skim milk with MTGase is inducing a minor imbalance of the yoghurt starter bacteria during fermentation. Furthermore - if the enzyme is supplied simultaneously with the yoghurt starter bacteria without subsequent inactivation - an increased viability of the *Lb. delbr.* subsp. *bulgaricus* culture during cold storage of the yoghurt was observed. A long-term application of MTGase in yoghurt does not result in the emerging of non-starter microorganisms during yoghurt storage, reflecting the high microbiological quality of the enzyme preparation used. Since many "mildly acidified" yoghurt products contain cultures of *Lb. acidophilus* and bifidobacteria (34), it may be interesting now to study the effect of an MTGase-treatment on the growth behaviour and viability of these alternative bacterial "yoghurt" cultures. We have further elucidated the effect of an MTGase-treatment on the micro-structure of fermented milk products by scanning electron microscopy. The results of this investigation will be reported elsewhere.

Acknowledgement

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5. Summary

H. Neve, P. Chr. Lorenzen, A. Mautner, E. Schlimme, K. J. Heller: **Effects of transglutaminase treatment on the production of set skim milk yoghurt: microbiological aspects.** Kieler Milchwirtschaftliche Forschungsberichte **53** (4) 347-361 (2001)

26 Microbiology (yoghurt, transglutaminase)

Crosslinked set-style yoghurt was produced from skim milk pretreated with microbial transglutaminase for 2 h. The enzyme was inactivated before inoculation of the skim milk with a traditional yoghurt starter culture (composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*). Compared to the untreated control trial, the

enzymic cross-linking step led to a minor imbalance of the associative growth (i.e., the protosymbiosis) of the yoghurt starter culture. In the second phase of the milk fermentation, a minor decrease in growth of the lactobacilli was observed. This reduction in cell counts correlated well with the concomitant occurrence of a delay in the fermentation profile (i.e., a delayed drop of pH in the enzyme-treated yoghurt sample). In a second set of experiments, transglutaminase and yoghurt starter culture were added simultaneously to the skim milk, in order to ensure enzyme activity during fermentation and subsequent cold storage. Under this condition, no delay was observed in the fermentation profile of the enzyme-treated sample in comparison to the untreated control. During the first 2 weeks of cold storage of the enzyme-treated yoghurt, a minor decline of viability was found for both starter culture strains (approx. 0.5 $\log_{10}$ unit reduction). However, during further storage (from week 2 to 6), the enzyme treatment resulted in a stabilising effect on the viability of *Lb. delbr.* subsp. *bulgaricus*, as this strain demonstrated a better surviving ability than in the control yoghurt. Analysis of chemical-technological factors confirmed prolonged activity of the cross-linking enzyme during the first 2 weeks of cold storage as seen by an increase in gel strength. Contaminating non-starter microorganisms (i.e., mesophilic aerobic bacteria, yeasts and mould, coliforms, aerobic and anaerobic spore formers) were not found in the transglutaminase preparation and were absent in the cross-linked and the control yoghurt batches during the 6-week storage period at 6°C.

Zusammenfassung

H. Neve, P. Chr. Lorenzen, A. Mautner, E. Schlimme und K. J. Heller: **Einfluß einer Transglutaminasebehandlung auf die Herstellung von stichfestem Magermilchjoghurt: Mikrobiologische Aspekte.** Kieler Milchwirtschaftliche Forschungsberichte **53** (4) 347-361 (2001)

26 Mikrobiologie (Joghurt, Transglutaminase)

Zur Herstellung eines stichfesten Joghurts wurde Magermilch für 2 Stunden mit mikrobieller Transglutaminase vorbehandelt. Vor dem Beimpfen der Magermilch mit einer traditionellen Joghurtstarterkultur (bestehend aus *Streptococcus thermophilus* und *Lactobacillus delbrueckii* subsp. *bulgaricus*) wurde das Enzym inakiviert. Im Vergleich zu dem unbehandelten Kontrollansatz führte die enzymatische Quervernetzung zu einer geringfügigen Unausgeglichenheit des assoziierten Wachstums (auch Protosymbiose genannt) der Joghurtstarterkultur. In der zweiten Phase der Milchfermentation wurde eine geringe Abnahme des Wachstums der Laktobacillen beobachtet. Die Abnahme der Zellzahl korrelierte gut mit dem gleichzeitigen Auftreten einer Verzögerung im Fermentationsprofil (d.h. eine verzögerte pH-Absenkung in der enzymbehandelten Joghurtprobe). In einem zweiten Versuchsansatz wurden die Transglutaminase und Joghurtstarterkultur gleichzeitig der Magermilch zugesetzt, um Enzymaktivität während der Fermentation und der folgenden Kaltlagerung zu gewährleisten. Unter diesen Bedingungen wurde keine Verzögerung im Fermentationsprofil der enzymbehandelten Probe im Vergleich zur unbehandelten Kontrolle beobachtet. Während der ersten 2 Wochen der Kaltlagerung wurde eine geringe Abnahme der Lebensfähigkeit beider Starterkulturenstämme festgestellt (Abnahme um ca. eine halbe Zehnerpotenz). Während der weiteren Lagerung von der 2. bis zur 6. Woche führte die Enzymbehandlung zu einem stabilisierendem Effekt auf die Lebensfähigkeit von *Lb. delbr.* subsp. *bulgaricus*, da dieser Stamm eine höhere Überlebensrate zeigte als in dem unbehandeltem Kontrolljoghurt. Die Untersuchung der chemisch-technologischen Parameter bestätigte die fortlaufende Aktivität des quervernetzenden Enzyms während der ersten beiden

Wochen der Kaltlagerung, erkennbar an der Zunahme der Gelfestigkeit. Mikrobielle Kontaminanten (mesophile aerobe Bakterien, Hefen und Schimmelpilze, coliforme Bakterien, aerobe und anaerobe Sporenbildner) wurden in dem Transglutaminase-präparat nicht vorgefunden und ließen sich auch nicht in den quervernetzten und in den Kontroll-Joghurtansätzen während der 6-wöchigen Lagerung bei 6°C nachweisen.

Résumé

H. Neve, P. Chr. Lorenzen, A. Mautner, E. Schlimme, K. J. Heller: **Effets du traitement avec de la transglutaminase sur la production de yaourt caillé à base de lait écrémé: aspects microbiologiques.** Kieler Milchwirtschaftliche Forschungsberichte **53** (4) 347-361 (2001)

26 Microbiologie (yaourt, transglutaminase)

Pour la production de yaourt caillé, du lait écrémé fut prétraité avec de la transglutaminase microbienne pour 2 heures. Avant d'inoculer le lait écrémé avec une culture traditionnelle de starter de levain de yaourt (composée de *Streptococcus thermophilus* et de *Lactobacillus delbrueckii* subsp. *bulgaricus*) l'enzyme a été inactivé. Comparée à la mixture de contrôle non-traîtée, la réticulation enzymatique a mené à un léger déséquilibre de la croissance associative (connue également sous le nom de protosymbiose) de la culture de yaourt. Lors de la seconde phase de fermentation du lait, une croissance légèrement réduite des lactobacilles a été observée. La réduction du dénombrement cellulaire est en bonne corrélation avec l'apparition simultanée d'un retardement dans le profile de fermentation (c'est-à-dire, une diminution retardée du ph dans l'échantillon de yaourt traité à l'enzyme). Lors d'une seconde mixture d'essai, la transglutaminase et la culture de starter de yaourt furent simultanément ajoutés au lait écrémé, afin de garantir une activité enzymatique pendant la fermentation et le stockage au frais. Sous ces conditions, on n'a pas constaté de retardement dans le profile de fermentation de l'échantillon traité à l'enzyme comparé à l'échantillon de contrôle non-traité. Pendant les deux premières semaines de stockage au frais du yaourt traité à l'enzyme, une légère diminution de la viabilité a été constatée pour les deux souches de culture de starter (diminution d'environ 0,5 $\log_{10}$). Cependant, pendant un stockage consécutif (semaine 2 à 6), le traitement enzymatique a eu un effet stabilisateur sur la viabilité de *Lb. delbr.* subsp. *bulgaricus*, comme cette souche a démontré une meilleure capacité de survie que celle constatée dans le yaourt de contrôle. L'analyse des paramètres chimiques-technologiques a confimé une activité prolongée de l'enzyme réticulé pendant les deux premières semaines de stockage au frais, devenue apparente par une solidité de gel accrue. Des contaminants microbiens (bactéries mésophiles aérobes, levains et moisissures, coliformes, bactéries sporulées aérobes et anaérobes) n'ont pas été détectés dans la préparation à la transglutaminase, ni dans les lots de yaourt réticulés et de contrôle pendant le stockage de 6 semaines à 6°C.