

The Effect of Milk Heat Treatment on the Growth Characteristics of Lactic Acid Bacteria

I. Stulova^{1,2}, N. Kabanova^{1,2}, T. Kriščiunaite^{1,2}, T.-M. Laht^{1,2} and R. Vilu^{1,2}

¹ Tallinn University of Technology, Ehitajate tee 5, 19086, Tallinn, Estonia

² Competence Center of Food and Fermentation Technologies (CCFFT), Akadeemia tee 15B, 12618, Tallinn, Estonia; e-mails: irina.stulova@tftak.eu; natalja@tftak.eu; tiina@tftak.eu; tiiu@tftak.eu; raivo@tftak.eu

Abstract. The ability to growth in milk is an important feature for lactic acid bacteria (LAB) used as starters for fermented milk products. Several decades ago the results of the studies varied widely: some of them showed that LAB grew better in raw milk and others demonstrated improved growth of the bacteria in heat-treated milk (Foster et al., 1952). The effectiveness of heat treatment of milk as a tool for modifying the functional properties of protein components has been extensively documented in the literature (Raikos, 2010), but the information on the influence of heat treatment of milk on the growth of LAB is not exhaustive. Peculiarities of growth of *Streptococcus thermophilus* ST12 and *Lactobacillus paracasei* S1R1 were studied using isothermal batch microcalorimeter TAMIII. Bacterial growth was monitored in pasteurized and ultra-high temperature (UHT) treated milk with different fat content, and also in reconstituted skim milk (RSM) prepared from low-heat skim milk powder (LHSMP). Heat produced during different growth stages (Q_{tot} , Q_{exp}), maximal specific growth rate (μ_{max}) and lag-phase (λ) duration were determined by processing calorimetric curves, and detailed analysis of growth of the bacteria in differently pretreated milks were carried out on the basis of these data. The results of the experiments showed that primarily heat treatment and, to a minor extent, fat content of milk influenced the growth parameters of both bacterial strains, especially *Lb. paracasei*, growth of which was almost completely inhibited in UHT milk

Key words: bacterial growth, *Lactobacillus paracasei*, microcalorimetry, milk treatment, *Streptococcus thermophilus*

INTRODUCTION

Milk as a raw material has a relatively short shelf life but it can be prolonged by heat treatment, which is an essential step adopted by the dairy industry (Raikos, 2010). For a high proportion of cheese varieties, pasteurization is the sole treatment applied to the cheese-milk (Kelly et al., 2008). The temperature/time combinations for the batch heat treatments used in yoghurt manufacture are 85°C for 30 min or 90–95°C for 5 min. However, very high temperature short time (100–130 °C for 4 to 16 s) or ultra-high temperature (UHT) 140°C for 4 to 16 s treatments are also sometimes used (Lee & Lucey, 2010). Heat treatment of milk during commercial processing operations not only inactivates the microorganisms (Odriozola-Serrano et al., 2007), but also results in a number of physico-chemical changes in the milk constituents. The rheological

properties of milk gels, both chymosin and acid induced, are affected by the heat treatment applied to milk (Parnell et al., 1988), and can result in irreversible changes in milk protein structure. Some of the changes involved are whey protein denaturation and aggregation, interactions of whey proteins with casein micelles, reactions between lactose and proteins, changes in casein micelle structure, transfer of soluble calcium and phosphate to colloidal phase, changes in fat globule membranes, and decrease in pH (Singh & Waungana, 2001).

The thermophilic bacteria *St. thermophilus* are widely used in the dairy industry in the production of yoghurt and hard 'cooked' cheeses (Emmental, Gruyere, Grana). In the industrial implementations of *St. thermophilus*, fast growth of the bacteria is crucial in intense acidification of milk (Derzelle et al., 2005).

The species of *Lb. casei/paracasei* and *Lb. plantarum* are the main components of mesophilic nonstarter microflora (Laht et al., 2002) and become important as adjunct cultures for the production of fermented milk products (Dupont et al., 2000). As the starter bacteria decrease in number, a secondary microbial flora grows in the maturing cheese and it becomes dominant after 1–3 months of ripening (Laht et al., 2002).

Calorimetry is especially helpful in the studies of the growth of the bacteria in opaque media and is a useful method to obtain kinetic and thermodynamic information on microbial growth (Kabanova et al., 2009; Kriščiunaite et al., 2011). The objectives of this investigation were to study the effect of milk heat treatment on the growth parameters of thermophilic starter and non-starter lactic acid bacteria using isothermal batch microcalorimetry.

MATERIALS AND METHODS

Bacterial culture and preparation of growth inocula

The strain of *St. thermophilus* ST12 used in this work was provided by Chr. Hansen (Denmark). Frozen cultures of *St. thermophilus* ST12 culture were thawed and pre-grown on Petri dishes with M17 Agar (LAB M, UK) for 24 h at 40°C. One colony from a pre-grown Petri dish was used as an inoculum for a 10 mL culture in sterilized RSM (Kalev Paide Tootmine AS, Paide, Estonia) at 40°C and left till coagulation. 1% of pre-grown culture was used as inoculum for the next 10 mL of RSM, left until coagulation and further used for inoculation of differently heat-treated milk samples.

A frozen *Lb. paracasei* S1R1 culture isolated from Estonian cheese (Kask et al., 2003) was thawed and pre-cultured on Petri dishes with MRS Agar (LAB M) medium for 24 h at 35°C. One colony from a pre-grown Petri dish was used as an inoculum for a 10 mL culture in liquid sterilized MRS Broth (LAB M) at 35°C. 1 mL of bacterial suspension grown overnight was used as inoculum for the next liquid 10 mL of MRS Broth and further used for inoculation of differently heat-treated milk samples.

Milk samples

Low-heat skim milk powder (Kalev Paide Tootmine AS, Paide, Estonia) was reconstituted in distilled water with thorough mixing for 1 h to yield a final concentration of 10% (w/v) milk solids. Commercial pasteurized milk with 3.5%, 2.5% and 0.05% fat content (Tere AS, Tallinn, Estonia) and commercial UHT milk with 3.5% and 0.05% fat content (Kalev Paide Tootmine AS) used in the study were obtained from retail sellers.

Calorimetric equipment and measurements

After the addition of bacteria, samples were stirred and 2 mL were transferred into the autoclaved ampoules. Isothermal batch microcalorimeter TAM III Thermal Activity Monitor (Thermometric, Järfälla, Sweden) equipped with 24 channels was used for the study of the growth of *St. thermophilus* ST12 and *Lb. paracasei* S1R1 in various milk substrates. Data analysis was accomplished using TAM Assistant program (v 0.9.1012.40, SciTech Software AB, Thermometric AB).

Statistical analysis

The experimental data were submitted to single-factor analysis of variance (ANOVA), and the differences among means were determined by Fisher's least significant difference (LSD) test.

RESULTS AND DISCUSSION

The power-time curves describing the growth of *St. thermophilus* ST12 at 40°C and *Lb. paracasei* S1R1 at 35°C in differently pretreated milk samples at the same initial inoculation rate of 10^5 cfu mL⁻¹ are presented in Fig. 1 and Fig. 2, respectively. Each curve is the average of three power-time curves, obtained with replicated samples.

The power-time curves were processed as described by Kabanova et al., 2009 and the numerical results were presented in Table 1. ANOVA of the data showed that both milk thermal processing and fat content significantly affected the growth characteristics of both bacteria ($P < 0.05$).

Table 1. Parameters describing *St. thermophilus* ST12 growth in differently pretreated milk samples: means of maximum specific growth rate (μ_{max}), heat evolved during the exponential growth phase (Q_{exp}), total heat (Q_{tot}) and time at maximum heat production rate ($t_{(dQ/dt)max}$) obtained from microcalorimetric power-time curves.

Sample	μ_{max} , h ⁻¹		Q_{exp} , J mL ⁻¹		Q_{tot} , J mL ⁻¹	$t_{(dQ/dt)max}$, h end of 2 nd exp. phase
	1 st exp. phase	2 nd exp. phase	1 st exp. phase	2 nd exp. phase		
RSM	1.72 ^a	1.38 ^a	0.44 ^a	2.43 ^a	6.73 ^a	6.69 ^a
Past 0.05%	2.03 ^b	1.80 ^b	0.07 ^b	1.89 ^b	6.51 ^a	5.88 ^b
Past 2.5%	2.03 ^b	1.55 ^c	0.08 ^c	2.05 ^c	6.51 ^a	5.88 ^b
Past 3.5%	1.94 ^c	1.47 ^c	0.07 ^b	2.01 ^{bc}	6.58 ^a	6.05 ^b
UHT 0.05%	2.03 ^b	1.22 ^d	0.10 ^d	2.39 ^a	5.98 ^b	15.87 ^c
UHT 3.5%	1.99 ^{bc}	0.98 ^e	0.11 ^e	2.41 ^a	5.88 ^b	16.02 ^c

The dual-peak power-time curve of diauxic growth of *St. thermophilus* ST12 was registered in reconstituted milk prepared from LHSMP, also in pasteurized and UHT milk. Two peaks observed correspond to two growth phases: the first exponential growth phase (a shoulder of the curve), and the second (major) exponential growth phase. However, power-time curves in the milk subjected to various heat treatments were completely different. In RSM the heat evolved during the first exponential phase was 6.5% of the total, whereas the contribution of this phase was 1.1–1.9% in other

milks. There were no significant differences in Q_{tot} (6.5–6.7 J mL⁻¹) between pasteurized milk with 0.05%, 2.5%, 3.5% fat content and RSM ($P > 0.05$), but the total evolved heat Q_{tot} was the smallest in UHT milk (5.9 J mL⁻¹).

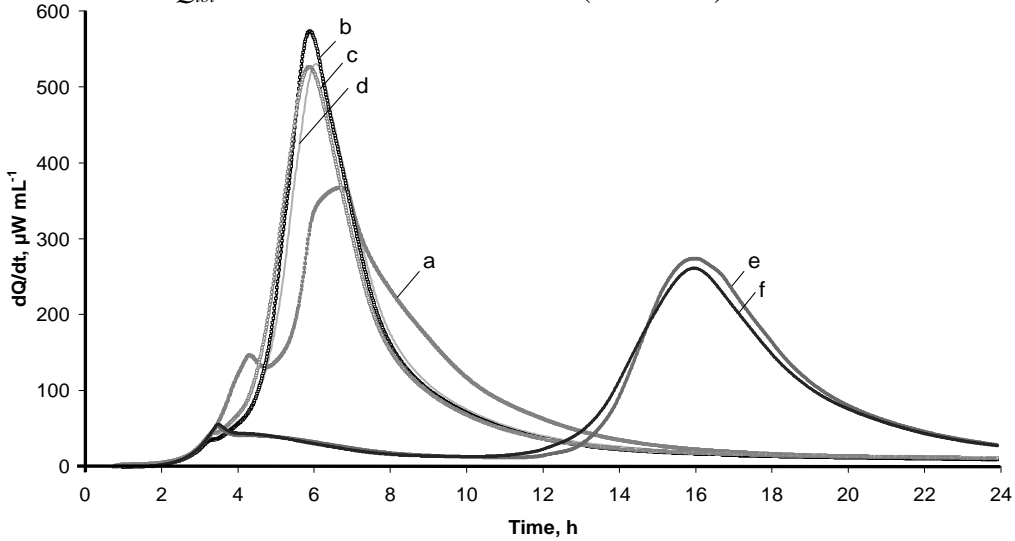


Figure 1. Thermal profiles of differently pretreated milk inoculated with thermophilic lactic starter bacteria *St.thermophilus* ST12. Mean power-time curves ($n = 3$) of milk prepared from low-heat skim milk powder (a), pasteurized milk with 0.05% (b), 2.5% (c), 3.5% (d) fat content, and UHT milk with 0.05% (e) and 3.5% (f) fat content.

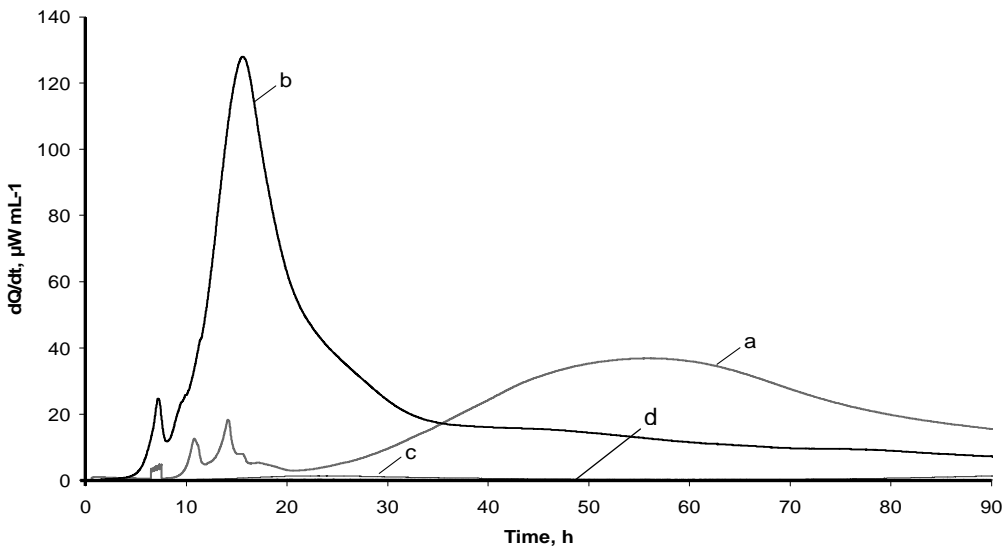


Figure 2. Thermal profiles of differently pretreated milk inoculated with non-starter lactic acid bacteria *Lb.paracasei* S1R1. Mean power-time curves ($n = 2$) of milk prepared from low-heat skim milk powder (a), pasteurized milk with 2.5% fat content (b), UHT milk with 3.5% fat content (c) and UHT milk with 0.05% fat content (d) – no heat production detected.

The differences in time scales and heat evolution scales on Fig. 1 and Fig. 2 reflect the differences in growth of the bacteria.

The mean fermentation times needed to reach the maximum heat production rate ($t_{(dQ/dt)_{max}}$) on the power-time curves were equal to approximately 6 h for pasteurized milk with 0.05%, 2.5% and 3.5% fat content, and they were different from the UHT milk with 0.05% and 3.5% fat content, where $t_{(dQ/dt)_{max}}$ was reached in 16 h.

It was shown for *St. thermophilus* ST12 that the calculated μ_{max} in the first exponential growth phase was higher than in the second exponential phase irrespective of milk substrate. These results are in agreement with the results obtained earlier (Letort et al., 2002; Kriščiūnaite et al., 2011). After inoculation, the cells start growing exponentially presumably using the amino acids, dipeptides, tripeptides, and oligopeptides that are freely available in milk. Subsequently, amino acids become limiting, and the culture enters a post-exponential growth phase in which the synthesis of extracellular protease is needed and initiated for the production of free amino acids. Finally, in a second exponential phase, the proteolytic system is able to supply sufficient peptides for exponential growth, but here the growth rate is lower than in the first exponential phase, probably due to the limited capacity of the peptide uptake systems (Letort et al., 2002; Sieuwerts et al., 2008).

It has been reported that the growth of several strains of LAB was identical in whole milk and in the same milk with fat removed, but the organisms grew much better in milk heated at 115°C for 15 min compared to milk heated at 80°C for 10 min (Foster, 1952). As seen from our data, *St. thermophilus* ST12 had higher values of μ_{max} in the first exponential growth phase growing in milk with 0.05% fat content than in milk with 3.5% fat content. A remarkable decrease of the value of μ_{max} in the second exponential growth phase was also observed in both pasteurized and UHT milks with 3.5% fat content, compared with low-fat samples – from 1.94 ± 0.02 to 1.47 ± 0.01 in the case of 3.5% pasteurized milk, and from 1.99 ± 0.05 to 0.98 ± 0.05 in the case of 3.5% UHT milk.

The duration of the lag-phase was the same in all milk substrates, but the start of the second exponential growth phase in UHT milk was markedly delayed. Poor growth of *St. thermophilus* ST12 in UHT milk could be explained by specific amino acid requirements which cannot be met by the proteolytic action of the bacteria on casein, taking into account that casein micelles in heated milk are coated with denatured whey proteins (Vasbinder et al., 2003).

It was shown for *Lb. paracasei* S1R1 that the power-time curves corresponded to the two-stage growth pattern in pasteurized milk and multiphase growth in RSM. *Lb. paracasei* S1R1 was characterized by low μ_{max} in the second exponential growth phase in pasteurized milk with 2.5% fat content and three times lower in reconstituted milk (data not shown). No heat production was recorded during 90 h in UHT milk with 0.05% fat content and very negligible growth occurred in UHT milk with 3.5% fat content.

CONCLUSIONS

It has been shown that milk thermal processing significantly affected the growth characteristics of starter culture and NSLAB during fermentation, whereas fat content had minor effect on bacterial growth. It was demonstrated in the present study that UHT-treatment of milk led to a decrease of growth rate of *St. thermophilus* ST12 and almost completely inhibited the growth of *Lb. paracasei* S1R1. The values of

maximum growth rates depended notably on the milk fat content, especially in the second exponential phase. The results obtained showed also that microcalorimetry is a very powerful instrument in studying quantitative detailed peculiarities of fermentation processes in milk.

ACKNOWLEDGEMENTS: This investigation was financially supported by the grant No.7636 of Estonian Science Foundation, by the European Regional Development Fund project EU29994, and Ministry of Education, Estonia, through the grant SF0140090s08.

REFERENCES

- Derzelle, S., Bolotin, A., Mistou, M-Y. & Rul, F. 2005. Proteome analysis of *Streptococcus thermophilus* grown in milk reveals pyruvate formate-lyase as the major upregulated protein. *App. and Environ. Microbiol.* **71**(12), 8597–8605.
- Dupont, I., Roy, D. & Lapointe, G. 2000. Comparison of exopolysaccharide production by strains of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* grown in chemically defined medium and milk. *J Ind Microbiol Biotechnol.* **24**, 251–255.
- Foster, E. M. 1952 The effect of heat on milk as a culture medium for lactic acid bacteria. *J. Dairy Sci.* **35**(12), 988–997.
- Kabanova, N., Kazarjan, A., Stulova I. & Vilu R. 2009. Microcalorimetric study of growth of *Lactococcus lactis* IL1403 at different glucose concentration in broth. *Thermochim Acta.* **496**, 87–92.
- Kask, S., Adamberg, K., Orlowski, A., Vogensen, F. K., Møller, P. L., Ardö, Y. & Paalme, T. 2003. Physiological properties of *L. paracasei*, *L. danicus* and *L. curvatus* strains isolated from Estonian semi-hard cheese. *Food Res Int.* **36**, 1037–1046.
- Kelly, A. L., Huppertz, T. & Sheehan, J. J. 2008. Pre-treatment of cheese milk: principles and developments. *Dairy Sci. Technol.* **88**, 549–572.
- Kriščiunaite, T., Stulova, I., Kabanova, N., Laht, T-M. & Vilu, R. 2011. The effect of hydrogen peroxide on the growth of thermophilic lactic starter and acid gelation of UHT milk. *Int. Dairy J.* **21**, 239–246.
- Laht, T-M., Kask, S., Elias, P., Adamberg, K. & Paalme, T. 2002. Role of arginine in the development of secondary microflora in Swiss-type cheese. *Int. Dairy J.* **12**, 831–840.
- Lee, W. J. & Lucey, J. A. 2010. Formation and physical properties of yogurt. *Asian-Aust. J. Anim. Sci.* **23**(9), 1127–1136.
- Letort, C., Nardi, M., Garault, P., Monnet & V., Juillardi, V. 2002 Casein utilization by *Streptococcus thermophilus* results in a diauxic growth in milk. *App. and Environ. Microbiol.* **68**(6), 3162–3165.
- Odrizola-Serrano, I., Bendicho-Porta, S. & Martín-Belloso, O. 2006. Comparative study on shelf life of whole milk processed by high-intensity pulsed electric field or heat treatment. *J. Dairy Sci.* **89**, 905–911.
- Parnell-Clunies, E., Kakuda, Y., deMan, J.M. & Cazzola, F. 1988. Gelation profiles of yogurt as affected by heat treatment of milk. *J. Dairy Sci.* **71**, 582–588.
- Raikos, V. 2010. Effect of heat treatment on milk protein functionality at emulsion interfaces. A review. *Food Hydrocoll.* **24**, 259–265.
- Sieuwerts, S., de Bok, F. A. M., Hugenholtz, J. & van Hylckama Vlieg, J. E. T. 2008 Unraveling microbial interactions in food fermentations: from classical to genomics approaches. *App. and Environ. Microbiol.* **74**(16), 4997–5007.
- Singh, H. & Waungana, A. 2011. Influence of heat treatment of milk on cheese-making properties. *Int. Dairy J.* **11**, 543–551.
- Vasbinder, A. J., Rollema, H. S. & de Kruif, C. G. 2003. Impaired rennetability of heated milk; study of enzymatic hydrolysis and gelation kinetics. *J. Dairy Sci.* **86**, 1548–1555.