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Effect of thermization of *dahi* on post fermentation acidification during refrigerated storage#

K. Raj Silpa[1], James Ligimol[2]*, A. K. Beena[3], M. P. Divya[4], C. H. Aysha[5] and V. Sudhakaran Aparna[6]
Department of Dairy Microbiology
Verghese Kurien Institute of Dairy and Food Technology, Mannuthy, Thrissur-680651,
Kerala Veterinary and Animal Sciences University, Kerala, India

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Abstract

Post fermentation acidification is defined as the development of acidity past the desired level of fermentation or acid development. Effect of thermization at 65°C for different periods (30 sec, 60 sec, 2 min and 5 min) on post fermentation acidification of dahi samples prepared using Lacticaseibacillus rhamnosus 18 or Lacticaseibacillus casei 01 upon refrigerated storage was assessed in this study. Significant changes (p< 0.01) were observed between the two starter cultures in terms of their post acidification potential with L. rhamnosus 18 *dahi showing lower pH, higher titratable acidity and lactobacilli count than L. casei 01 during refrigerated storage. On assessing the impact of heat treatment on post acidification, significant decrease (p< 0.05) in pH, increase (p< 0.01) in titratable acidity and lactobacilli count of the heat treated and control samples were observed throughout the storage. Based on this study, it can be inferred that heat treatment at 65°C for even upto 5 min is not having any significant inhibitory effect on post fermentation acidification characteristics of the lactobacilli cultures tested.*

Keywords: *Post fermentation acidification, thermization, fermentation, dahi*

Dahi is a traditional Indian fermented dairy product well recognized as an indispensable accompaniment in Indian cuisine. From a household product prepared by backslopping, *dahi* has evolved into a commercial packaged product marketed under different brand names. The increasing

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1. *M. Tech scholar, Department of Dairy Microbiology, VKIDFT, Mannuthy, Thrissur*
2. *Assistant Professor, Department of Dairy Microbiology, VKIDFT, Mannuthy, Thrissur*
3. *Professor and Head, Department of Dairy Microbiology, VKIDFT, Mannuthy, Thrissur*
4. *Assistant Professor, Department of Dairy Chemistry, VKIDFT, Mannuthy, Thrissur*
5. *Assistant Professor, Department of Food Safety and Quality Assurance, VKIDFT, Mannuthy, Thrissur*
6. *Assistant Professor, Department of Dairy Microbiology, VKIDFT, Mannuthy, Thrissur*

**Corresponding author: lijimoljames@kvasu.ac.in, Ph: 9567086007*

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consumer preferences towards natural healthy foods and the attributed therapeutic benefits have resulted in remarkable increase in world-wide consumption of fermented milk products. According to Food Safety Standards Authority of India (2022) fermented milk is a milk product obtained by fermentation of milk, which may have been manufactured using other permitted raw material, by the action of suitable microorganisms and resulting in lowering of pH with or without coagulation. One of the main issues raised about *dahi* prepared in households and at the industrial level is the increase in acidity that occurs during its storage even after ensuring proper cold chain maintenance. This continued development of acidity after the desired fermentation, referred to as post-acidification or post-fermentation acidification is not at all desirable as it leads to wheying-off, textural defects, excessive sourness and suppression of perception of aroma compounds (Settachimongkon *et al.,* 2016). Post acidification associated defects can result in marked depreciation in product shelf-life and consumer acceptance leading to significant economic impacts. Strategies like use of additives, thermization and non-thermal processes like high hydrostatic pressure, pulse electric field are being adopted to address this issue. Thermization is defined as a mild heat treatment i.e., below pasteurization temperature which varies from 50 °C for 30 min to 70 °C for 14 seconds (Behare and Prajapati 2007). Alakali *et al.* (2009) recommended a thermization temperature of 65°C for yoghurt taking into consideration the desired shelf extension, physicochemical, sensory properties and consumer acceptability. Though Food Safety and Standards Authority of India (FSSAI) has mentioned about heat treatment of fermented milk products, studies in this regard are scanty. Taking into consideration of all these aspects, this study was conducted to understand the effect of thermization on titratable acidity, pH and lactic acid bacterial count, the parameters that are reflective of post-fermentation acidification of *dahi* during its refrigerated storage. The results obtained are reported in this manuscript.

Lacticaseibacillus rhamnosus NCDC18 (National Collection of Dairy Cultures, Karnal) and *Lacticaseibacillus casei* 01 (Chr. Hansen) were used in this study. The organisms were propagated in reconstituted skimmed milk (10% TS w/v) for use in the experiments. Purity of the bacterial cultures was ensured prior to each experiment by subjecting the cultures to Gram staining and catalase test. Homogenized, standardised (3.5% fat and 8.5% SNF), pasteurized cow milk purchased from local market was used for *dahi* preparation. For the preparation, the milk was heat treated (85°C/15 min) and cooled to incubation temperature, inoculated with dairy starter culture (7 $\log_{10}$ CFU/ml) at one per cent level and incubated at 37°C/12 h. The treatment samples of *dahi* were subjected to thermization by keeping them in a water bath set at 65 °C for 30 sec, 60 sec, 2 min and 5 min (Alakali *et al.,*2009) and subsequent immediate cooling and further storage in refrigerator at 5°C. Control sample without any heat treatment was also stored in the similar way.

The samples were analysed on days '0', '3' and '6' of refrigerated storage. Titratable acidity and pH were determined by standard methods IS: 1166 (1973) and IS: SP (Part XI) ,1981 respectively. Lactobacilli (starter culture) count was enumerated by pour plating appropriate dilutions of samples using De Man, Rogosa and Sharpe (MRS) agar (Himedia labs, Mumbai, India) and subsequent incubation at 37 ± 0.5°C for 48h (Shori and Baba, 2012).

Repeated measures ANOVA was used for comparing both the changes in the parameters between periods within each sample treatment and the changes between the sample treatments in each period. Data analyses were carried out using the Statistical Package for Social Sciences (SPSS, Version 24) and the results are presented as mean with standard error of three independent batch replications.

Activity of *L. rhamnosus 18* and *L. casei* 01 in terms of change in pH, acidity and lactobacilli count during refrigerated storage of *dahi* samples are depicted in table 1. The data clearly shows that pH values of all the *dahi* samples significantly decreased ($p< 0.01$) and the titratable acidity and lactobacilli count significantly increased ($p< 0.01$) with advancing storage irrespective of the starter culture used.

So, it can be inferred that post fermentation acidification is occurring for both the starter cultures used in this study. Significant difference ($p< 0.01$) was observed between *L. rhamnosus* 18 and *L. casei* 01 in terms of all the parameters tested throughout the study. Titratable acidity on day '0' and '6' of the *L. rhamnosus*18 fermented *dahi* were 0.69 ± 0.00 and 0.79 ± 0.006 respectively whereas for *L. casei* 01 *dahi,* the corresponding values were 0.59 ± 0.000 and 0.77 ± 0.003 respectively. Agreeing with this observation, pH of the dahi prepared using *L. rhamnosus*18 and *L. casei* 01 were 4.97± 0.015,4.74 ± 0.008 and 5.08 ± 0.019, 4.88 ± 0.007 on days'0' and '6' respectively. So, it can be inferred that *L. casei* 01 was a significantly slower ($p< 0.01$) acid producer than *L. rhamnosus*18. Such differences in technological attributes between *L. casei* and *L. rhamnosus* despite their close phylogenetic and phenotypic relationship (Toh,2013) alert us to exercise caution while extrapolating results between closely related species.

Changes in pH, titratable acidity and lactobacilli count of thermized *dahi* samples prepared using *L. rhamnosus* 18 and *L. casei* 01 are depicted in tables 2 and 3 respectively. Significant decrease ($p < 0.01$) in pH and significant increase ($p < 0.01$) in titratable acidity and lactobacilli count was observed in all samples of *dahi* prepared using *L. casei* 01 irrespective of the heat treatment employed. The same trend was observed in the case of *L. rhamnosus* 18 also except that on the third ($p < 0.01$) and sixth day ($p < 0.05$) of storage

Table 1. pH, titratable acidity and count of *Lacticaseibacillus rhamnosus 18* and *Lacticaseibacillus casei* 01fermented *dahi* samples

Parameters	Days	*Lacticaseibacillus rhamnosus 18*	*Lacticaseibacillus casei* 01	t value
pH	Day 0	$4.97± 0.015^{ax}$	$5.08 ± 0.019^{ay}$	-4.064^{*}
	Day 3	$4.87 ± 0.006^{bx}$	$4.98 ± 0.020^{by}$	-3.969^{*}
	Day 6	$4.74 ± 0.008^{cx}$	$4.88 ± 0.007^{cy}$	-10.958^{**}
Titratable Acidity (%LA)	Day 0	$0.69 ± 0.006^{ax}$	$0.59 ± 0.000^{ay}$	29^{**}
	Day 3	$0.71 ± 0.005^{bx}$	$0.68 ± 0.003^{by}$	5^{**}
	Day 6	$0.79 ± 0.006^{cx}$	$0.77 ± 0.003^{cx}$	-3.578^{**}
Lactobacilli count (Log_{10} CFU/ml)	Day 0	$8.20 ± 0.100^{ax}$	$7.70 ± 0.004^{ay}$	4.992^{**}
	Day 3	$8.65± 0.005^{bx}$	$7.98 ± 0.005^{by}$	100^{**}
	Day 6	$8.89 ± 0.005^{cx}$	$8.31 ± 0.003^{cy}$	55.340^{**}

Figures are mean ± standard error of 3 replications, *- Significant at five percent level ($p<0.05$), **- Significant at one percent level ($p<0.01$), ns- non-Significant ($p>0.05$). a-c: means with different superscripts vary significantly ($p < 0.01$) within a column, x-y: means with different superscripts vary significantly within a row.

Table 2. pH, titratable acidity and count of *Lacticaseibacillus rhamnosus 18* fermented *dahi*samples subjected to thermization

Parameters	Days	Control	Treatment Samples - Heat Treatment Given				F value
			65⁰C/30sec	65⁰C/60sec	65⁰C/2min	65⁰C/5min	
pH	**Day 0**	$4.97± 0.015^{ax}$	$4.96 ± 0.008^{ax}$	$4.94 ± 0.012^{ax}$	$4.96 ± 0.009^{ax}$	$4.98 ± 0.000^{ax}$	1.385^{ns}
	Day 3	$4.87 ± 0.006^{bx}$	$4.86± 0.012^{bx}$	$4.87 ± 0.006^{bx}$	$4.87 ± 0.006^{bx}$	$4.86 ± 0.017^{bx}$	0.441^{ns}
	Day 6	$4.74 ± 0.008^{cx}$	$4.75 ± 0.020^{cx}$	$4.75 ± 0.006^{cx}$	$4.74 ± 0.011^{cx}$	$4.76 ± 0.012^{cx}$	0.604^{ns}
Acidity (%LA)	**Day 0**	$0.69 ± 0.006^{ax}$	$0.69 ± 0.003^{ax}$	$0.69 ± 0.003^{ax}$	$0.68 ± 0.003^{ax}$	$0.68 ± 0.003^{ax}$	0.300^{ns}
	Day 3	$0.71 ± 0.005^{bx}$	$0.70 ± 0.005^{bx}$	$0.71 ± 0.003^{bx}$	$0.71 ± 0.008^{bx}$	$0.72 ± 0.006^{bx}$	1.861^{ns}
	Day 6	$0.77 ± 0.011^{cx}$	$0.76 ± 0.012^{cx}$	$0.75 ± 0.005^{cx}$	$0.75 ± 0.006^{cx}$	$0.76 ± 0.003^{cx}$	0.879^{ns}
Lactobacilli count Log_{10} CFU/ml	**Day 0**	$8.20 ± 0.100^{ax}$	$8.30 ±0.000^{ax}$	$8.20 ± 0.101^{ax}$	$8.30 ± 0.010^{ax}$	$8.10 ± 0.100^{ax}$	1.148^{ns}
	Day 3	$8.65± 0.005^{bx}$	$8.63 ± 0.017^{bx}$	$8.62 ± 0.042^{bx}$	$8.61 ± 0.005^{bx}$	$8.48 ± 0.010^{by}$	10.279^{**}
	Day 6	$8.89 ± 0.005^{cx}$	$8.87 ± 0.014^{cx}$	$8.89 ± 0.010^{cx}$	$8.87 ± 0.014^{cx}$	$8.78 ± 0.035^{cy}$	4.326^{*}

Figures are mean ± standard error of 3 replications, *- Significant at five percent level ($p<0.05$), **- Significant at one percent level ($p<0.01$), ns- non-Significant ($p>0.05$). a-c: means with different superscripts vary significantly ($p< 0.01$) within a column, x-y: means with different superscripts vary significantly within a row.

Table 3. pH, titratable acidity and count of *Lacticaseibacillus casei 01 dahi* samples subjected to thermization

Parameters	Days	Control	Treatment Samples- Heat Treatment Given				F value
			65^0C/30sec	65^0C/60sec	65^0C/2min	65^0C/5min	
pH	Day 0	5.08 ± 0.019^{ax}	5.08 ± 0.013^{ax}	5.08 ± 0.009^{ax}	5.07 ± 0.012^{ax}	5.08 ± 0.000^{ax}	0.126^{ns}
	Day 3	4.98 ± 0.020^{bx}	4.95 ± 0.016^{bx}	4.98 ± 0.033^{bx}	4.93 ± 0.027^{bx}	4.99 ± 0.009^{bx}	0.857^{ns}
	Day 6	4.88 ± 0.007^{cx}	4.85 ± 0.023^{cx}	4.86 ± 0.005^{cx}	4.87 ± 0.004^{cx}	4.81 ± 0.019^{cx}	0.525^{ns}
Acidity (%LA)	Day 0	0.59 ± 0.000^{ax}	0.58 ± 0.003^{ax}	0.58 ± 0.005^{ax}	0.58 ± 0.005^{ax}	0.58 ± 0.000^{ax}	1.643^{ns}
	Day 3	0.68 ± 0.003^{bx}	0.68 ± 0.000^{bx}	0.67 ± 0.002^{bx}	0.67 ± 0.003^{bx}	0.66 ± 0.010^{bx}	1.344^{ns}
	Day 6	0.79 ± 0.006^{cx}	0.78 ± 0.003^{cx}	0.78 ± 0.010^{cx}	0.78 ± 0.010^{cx}	0.80 ± 0.005^{cx}	1.132^{ns}
Lactobacilli count Log_{10} CFU/ml	Day 0	7.70 ± 0.004^{ax}	7.69 ± 0.002^{ax}	7.67 ± 0.015^{ax}	7.67 ± 0.010^{ax}	7.66 ± 0.024^{ax}	1.074^{ns}
	Day 3	7.98 ± 0.005^{bx}	7.98 ± 0.003^{bx}	7.98 ± 0.003^{bx}	7.97 ± 0.003^{bx}	7.97 ± 0.003^{bx}	2.643^{ns}
	Day 6	8.31 ± 0.003^{cx}	8.27 ± 0.027^{cx}	8.20 ± 0.082^{cx}	8.20 ± 0.082^{cx}	8.20 ± 0.082^{cx}	0.394^{ns}

Figures are mean ± standard error of 3 replications, *- Significant at five percent level (p<0.05), **- Significant at one percent level (p<0.01), ns- non-Significant (p>0.05). a-c: means with different superscripts vary significantly (P < 0.01) within a column, x-y: means with different superscripts vary significantly within a row.

the lactobacilli count of samples thermized at 65°C/5min was significantly lower than those of other thermized samples. Observations of this study are different from most of the earlier reports. No significant increase in the acidity of lassi subjected to heat treatment of more than 65 °C during storage was reported by Kumar *et al*. (2003a, b) and Behare and Prajapati (2007). Mohammed *et al.* (1986) reported improvement in the quality and shelf life of yoghurt heat treated at 70, 75 and 80°C for 5 minutes upon 21 days of cold storage. On assessing the impact of thermization at 65 °C, 75 °C and 85 °C on the shelf life of yoghurt, Alakali *et al*. (2009) reported significant increase in titratable acidity and significant reduction in pH during storage at room temperature. They also reported that the samples thermized at 85°C had least increase in titratable acidity and least decline in pH during storage. A different trend observed in the current study could be due to the lower intensity of the heat treatment adopted in the study. Difference in the starter cultures used could also have contributed to the differential response observed in the study. Decrease in rates of increase in acidity and decrease in LAB count in thermized (65 °C for 5 min) sorghum based fermented milk beverage samples compared to the non-thermized samples was reported by Hussain *et al*. (2014). Observations of the current study are in agreement with that of Hussain *et al.* (2014), in that, there was increase in acidity during refrigerated storage even in thermized products. However, decrease in the rate of increase in acidity of thermized products as reported by Hussain *et al.* (2014) was not observed in the current study. Observations of the current study can be effectively made use to plan further research to develop post fermentation acidification control methods with minimum adverse quality on product quality.

Summary

In this study, possibility of adopting heat treatment at 65°C for different periods to address the post fermentation acidification in *dahi* was investigated. Contrary to the previous reports of efficiency of heat treatment in increasing the shelf life of fermented milk products this study could not establish any inhibitory effect of thermization at 65°C for 30 sec, 60 sec, 2 min and 5 min on post fermentation acidification. However, a different trend observed on the third and sixth day of storage on the lactobacilli count of *Lacticaseibacillus rhamnosus* 18 fermented samples thermized at 65°C/5 min is a positive note to consider heat treatment-based starter culture specific method to control post fermentation acidification.

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Conflict of interest

The authors declare that they have no conflict of interest.

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