RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2024.55.2.284-290

Microwave treatment as a strategy to control post fermentation acidification in *dahi*#

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Citation: Raj, S.K., James, L., Devikrishna, P., Beena, A.K., Aysha, C.H. and Divya, M.P.2024. Microwave treatment as a strategy to control post fermentation acidification in dahi.
J. Vet. Anim. Sci. **55**(2):284-290
DOI: https://doi.org/10.51966/jvas.2024.55.2.284-290

Received: 04.08.2023 *Accepted: 26.03.2024* *Published: 30.06.2024*

Abstract

Post-fermentation acidification (PFA) or post-acidification is defined as the development of acidity after the desirable fermentation. PFA is not a preferred phenomenon in fermented milk products due to the detrimental effects on product quality and shelf life. So alternate methods are being attempted to control PFA in fermented milk products. The reported study aimed to assess the efficiency of microwave treatment in controlling PFA in dahi. For this, dahi samples prepared using two high post acidifying cultures Lacticaseibacillus rhamnosus NCDC 18 and Lacticaseibacillus casei 01 were subjected to microwave treatments (600 W and 720 W). Microwave treatment at 720 W resulted in product damage whereas 600 W did not elicit any detrimental effect. Samples microwaved at 600 W for 10 s, 20 s and 30 s were stored in the refrigerator (5 ± 2°C) and analysed for changes in titratable acidity, pH and starter culture count over a period of six days at three days interval. During the storage period, significant decrease ($p < 0.01$) in pH and increase ($p < 0.01$) in titratable acidity and starter culture count were observed in all the samples of dahi irrespective of the microwave treatment given and the starter culture used. However, some culture specific, microwave exposure period dependent statistically significant variations were observed. Though the microwave treatment at 600 W did not elicit any remarkable reduction in PFA, the culture specific differences observed in the impact of microwave treatment warrant further studies for starter culture specific standardisation of microwave-based heat treatments for controlling PFA.

Keywords: *Dahi, post fermentation acidification, microwave treatment, Lacticaseibacillus*

#Part of M.Tech thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

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Numerous indigenous fermented milk products are manufactured throughout the world and majority of these products rely on spontaneous or natural fermentation (Robinson and Tamime, 2006). India also has its own collection of traditional fermented milk products with regional specific techno-functional and sensory attributes. Some of them are the *dahi* of North India and Gujarat, *mishit doi* or *bhappa doi* of West Bengal, *Chilika* curd of Orissa, *Chhu* of Sikkim and *Thayir* of Kerala and Tamil Nadu. Among the various traditional fermented milk products of India, the most popular and widely prepared one is '*dahi*', the oldest Indian fermented milk product produced by fermentation of milk by food grade starter cultures of lactic acid bacteria (LAB). Due to the availability of hydrolysed carbohydrates, bioactive proteins, vitamins, and minerals, *dahi* and other fermented milk products are being associated with improved human health. Basic principle of any fermented dairy product is the conversion of lactose, the sugar in milk, to lactic acid by LAB. Though development of lactic acid during fermentation is essential for the preparation of good quality fermented milk products, development of acidity after the fermentation is undesirable as it leads to wheying-off, textural defects and excess sourness suppressing the perception of aroma compounds. Prolonged metabolic activity of acid-resistant starter cultures is considered as one of the reasons for this ongoing acidification referred to as post acidification or Post fermentation acidification (PFA). Post-acidification describes a continuous acidification that exceeds product's ideal range of acidity as a result of the continuous metabolic activity of microbes throughout the product's shelf life (Turgut, 2016). Apart from reducing the shelf life, PFA leads to a number of defects, including excessive acidity, whey syneresis, dirty flavour, and decrease in starter culture count. PFA is found to increase the hydrophobic and electrostatic interactions among proteins, cause casein particles to enlarge, colloidal calcium phosphate to dissolve, and the protein network to partially restructure itself (Guénard-Lampron *et al.,* 2020).

Therefore, post-acidification must be prevented utilising appropriate processing or treatment procedures during fermentation and/or preservation stages. The currently accepted commercial strategy for preventing post-acidification is the transportation and storage of fermented products at low temperature (Deshwal *et al*., 2021). It is sought to prevent metabolic processes while still keeping live microorganisms alive. However, even chilled-chain logistics is found ineffective at preventing post acidification of fermented dairy products over the course of their shelf-life. This issue is even worse in tropical and resource-poor developing countries. Production of acidic compounds by the residual metabolic processes of the starter cultures or their enzymes even at low temperature (4-10°C) is identified as the reason for development of PFA during cold-chain storage and transportation. Various approaches like thermization (Routray and Mishra, 2011, Silpa *et al.*, 2023), use of additives (Rajapaksha *et al*., 2013), high hydrostatic pressure (Jankowska *et al.,* 2005), pulsed electric fields (Chanos *et al.*, 2020) and genetic engineering (Chuah and Mao, 2020) were attempted to reduce or avoid post-acidification. Though, thermal treatment after fermentation is reported to resolve post-acidification issues to some extent by inactivating bacterial activity, certain disadvantages like loss of considerable cell viability and textural changes has limited the scope of its widespread use (Demir *et al*., 2021). Another approach which could be adopted even at household levels is the microwave treatment. Due to the convenience it provides and the advantages it offers over conventional methods, like reduction in processing time and costs, while retaining the sensory quality and nutritional value of food (Ekezie *et al.,* 2017) microwave treatment could be a highly attractive option for controlling PFA. If found effective in controlling PFA this process will be a highly suitable and adoptable method, especially at household levels. During microwave treatment heat energy is generated from the electromagnetic energy formed through the process of selective absorption and dissipation (Guo *et al.,* 2017). Though microwave treatment is suggested as an option for PFA control, not many studies have been conducted in this direction. So, the current study aimed to assess the effect of microwave treatment on post acidification fermentation during refrigerated storage of *dahi*

prepared using two high post acidifying lactic acid bacterial cultures in terms of changes in pH, acidity and starter culture count.

Materials and methods

Two lactic acid bacterial cultures namely *Lacticaseibacillus rhamnosus* NCDC 18 (National Collection of Dairy Cultures, Karnal) and *Lacticaseibacillus casei* 01 (Chr. Hansen, Denmark) identified as high post acidifying (Silpa, 2022) were used as starter cultures in this study.

Microwave treatment of Dahi samples

For *dahi* preparation milk was heated to 85°C for 15 min and subsequently cooled to 37°C. This milk was inoculated with the starter cultures at 1% level (7 log cells/ mL), mixed well and incubated at 37°C till coagulation. These samples were subjected to microwave treatment by placing them in a preheated microwave oven (Frequency 2450 MHz) maintained at 720 W and 600 W for 10 s, 20 s and 30 s (Turgut, 2016). Microwaved samples were then cooled and stored (5 ± 2°C) in the refrigerator. *Dahi* samples without any further treatment served as the control. All the samples (both control and treatment) were analysed for changes in titratable acidity, pH and starter culture count at three days intervals during the six days of refrigerated storage.

Determination of pH, acidity, starter culture count of Dahi samples

pH of the *dahi* samples was determined by the method described in IS: SP (Part XI) (1981) using digital laboratory pH meter (Eutech instruments PC 510) after proper calibration of the meter at pH 4.0 and pH 7.0 before each trial. Titratable acidity of the *dahi* samples as per cent lactic acid was measured by standard method described in IS: 1166 (1986). Pour plating appropriate dilutions on De Mann, Rogosa and Sharpe (MRS) agar and subsequent aerobic incubation at 37°C for 48 h was used for determining starter culture count (Shori and Baba, 2012). Counts obtained were presented in log values as the mean ± standard error values of three replications.

Results and discussion

Lcb.rhamnosus 18 and *Lcb. casei* 01 *dahi* samples were subjected to microwave treatment at 720 W and 600 W. But considering the product damage in terms of wheying off and textural changes occurred on employing 720 W (Fig.1) further experiments were conducted at 600 W only. It was observed that heating of coagulum leads to its contraction and subsequent expulsion of whey from it (Tamime and Robinson, 1988). So, the wheying off or whey separation occurred on microwaving at 720 W could be attributed to the generation of heat during the treatment. On subjecting the *dahi* samples to 600 W for 10, 20 and 30 s their temperatures increased to 35ºC, 44ºC and 60ºC, respectively. So, the higher temperature attained at 720 W could be the reason for the ill effects observed in the samples subjected to that treatment.

Fig. 1: *Lcb. rhamnosus*18 *dahi* sample microwaved at 720W

During the storage period, significant decrease ($p < 0.01$) in pH and increase ($p < 0.01$) in titratable acidity and lactobacilli count were observed in all the samples of *dahi* irrespective of the microwave treatment given and the starter culture used (Tables 1 and 2). After 6 days of storage, pH values of the *Lcb. casei* 01 *dahi* control samples decreased from 5.09 to 4.88. In a similar way pH of the *dahi* samples treated at 600 W for 10, 20, and 30 s also decreased from the initial value of 5.09 to 4.88, 4.90, and 4.91, respectively. Similarly, in the case of *Lcb. rhamnosus* 18 *dahi* samples, the pH of the control and samples treated for

Table 1: pH, titratable acidity and starter culture count of *Lcb. casei* 01 fermented *dahi* samples subjected to different microwave treatments

Parameter	Days	Control (C0)	Treatment samples – MW treatments given			F value
			600W/10 s (C1)	600W/20 s (C2)	600W/30 s (C3)	
pH	Day 0	5.09 ± 0.005^{ax}	5.09 ± 0.007^{ax}	5.09 ± 0.003^{ax}	5.09 ± 0.003^{ax}	0.333^{ns}
	Day 3	4.98 ± 0.007^{bx}	4.98 ± 0.005^{bx}	4.98 ± 0.005^{bx}	5.00 ± 0.005^{bx}	2.562^{ns}
	Day 6	4.88 ± 0.000^{cx}	4.88 ± 0.000^{cx}	4.90 ± 0.005^{cy}	4.91 ± 0.003^{cy}	17^{*}
Acidity (%TA)	Day 0	0.59 ± 0.003^{ax}	0.59 ± 0.005^{ax}	0.59 ± 0.005^{ax}	0.59 ± 0.005^{ax}	0.1^{ns}
	Day 3	0.68 ± 0.007^{bx}	0.68 ± 0.007^{bx}	0.68 ± 0.005^{bx}	0.67 ± 0.003^{bx}	0.667^{ns}
	Day 6	0.78 ± 0.003^{cx}	0.78 ± 0.007^{cx}	0.76 ± 0.005^{cy}	0.75 ± 0.003^{cy}	6.394^{*}
Starter culture count (Log_{10} CFU/ml)	Day 0	7.70 ± 0.003^{ax}	7.70 ± 0.000^{ax}	7.70 ± 0.000^{ax}	7.70 ± 0.003^{ax}	0.667^{ns}
	Day 3	7.99 ± 0.005^{bx}	7.99 ± 0.007^{bx}	8.00 ± 0.007^{bx}	7.90 ± 0.005^{bx}	2.529^{ns}
	Day 6	8.32 ± 0.003^{cx}	8.32 ± 0.000^{cx}	8.3 ± 0.000^{cy}	8.29 ± 0.005^{cy}	18^{*}

Figures are mean ± standard error of 3 replications, **- Significant at one percent level (p<0.01) *- Significant at five percent level (≤0.05), 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

10, 20, and 30 s at 600 W on day zero were 4.93, 4.94, 4.95, 4.95, respectively. After 6 days of storage these values were decreased to 4.75, 4.77, 4.77, 4.75, respectively. In general, the adopted microwave treatments did not elicit any significant (p ≤0.01) reduction in the development of PFA in the *dahi* samples prepared using the tested LAB cultures. Kosikowski (1982) observed that microwave treatment reduced excessive acid formation in yoghurt by destroying the viable bacteria. Hence it can be inferred that destruction of starter culture organisms by microwave treatment could be one the mechanisms by which it can affect the PFA in fermented milk products. As there was no significant reduction but rather an increase in the starter culture count of microwave treated samples compared to the control samples it can be concluded that the adopted microwave treatment could not inhibit/kill the starter cultures under the study. In general, the adopted microwave treatments could not exhibit any significant effect on PFA and could not be considered as a suitable method for controlling PFA of the tested starter cultures.

Agreeing with the decrease in pH, significant (p ≤ 0.01) increase in titratable acidity was observed for the control and all the treatment samples during the study period. The significant (p ≤0.01) increase observed in the starter count very well explains the increase in titratable acidity as well as the decrease in pH. In the case of *Lcb. casei* 01 *dahi* samples, on the zeroth day and third day there were no significant differences in between any of the treatment samples and control samples in any of the parameters tested. However, the samples microwave treated at 600W for 20 s and 30 s exhibited significantly (p < 0.05) higher pH, lower titratable acidity and starter culture count than the control and the sample subjected to microwaving for 10 seconds on the sixth day of refrigerated storage. This observation clearly demonstrates that the inhibitory effect of microwave treatment became evident only after extended period of storage and is affected by the period of microwave treatment. Different from the observations obtained for *Lcb. casei* 01 *dahi* samples, microwave treatment affected only the starter culture count in the case of *Lcb. rhamnosus* 18 *dahi* samples. No significant differences were observed in between the pH values and titratable acidities of the control and all the three treatment samples throughout the storage period. While there were no significant (p >0.05) differences between the starter culture count of control and treatment samples on the zeroth day, *Lcb. rhamnosus* 18 *dahi* sample microwaved at 600 W for 30 s had significantly lower starter culture count than that of the other treatment and control samples on the third (p < 0.01), sixth day (p < 0.05) of storage. This is in concurrence with the report of significantly lower starter culture count of *Lcb. rhamnosus* 18 *dahi* samples thermized at 65°C/5min on the third (p < 0.01) and sixth day (p < 0.05) of storage than

Table 2: pH, titratable acidity and count of *Lcb. rhamnosus* 18 fermented *dahi* samples subjected to different microwave treatments.

Parameter	Days	Control	Treatment samples – MW treatments given			F value
			600W/10 s	600W/20 s	600W/ 30 s	
pH	Day 0	4.93 ± 0.012^{ax}	4.94 ± 0.019^{ax}	4.95 ± 0.010^{ax}	4.95 ± 0.027^{ax}	0.126^{ns}
	Day 3	4.85 ± 0.022^{bx}	4.85 ± 0.000^{bx}	4.85 ± 0.022^{bx}	4.83 ± 0.014^{bx}	0.346^{ns}
	Day 6	4.75 ± 0.004^{cx}	4.77 ± 0.010^{cx}	4.77 ± 0.010^{cx}	4.75 ± 0.005^{cx}	1.096^{ns}
Acidity (%TA)	Day 0	0.69 ± 0.003^{ax}	0.69 ± 0.003^{ax}	0.69 ± 0.003^{ax}	0.68 ± 0.007^{ax}	0.633^{ns}
	Day 3	0.72 ± 0.007^{bx}	0.72 ± 0.006^{bx}	0.73 ± 0.009^{bx}	0.72 ± 0.008^{bx}	0.360^{ns}
	Day 6	0.78 ± 0.005^{cx}	0.78 ± 0.005^{cx}	0.77 ± 0.005^{cx}	0.77 ± 0.005^{cx}	1.000^{ns}
Starter culture count (Log_{10} CFU/ml)	Day 0	8.2 ± 0.005^{ax}	8.2 ± 0.005^{ax}	8.2 ± 0.005^{ax}	8.1 ± 0.005^{ax}	0.750^{ns}
	Day 3	8.67 ± 0.007^{bx}	8.67 ± 0.003^{bx}	8.66 ± 0.005^{bx}	8.60 ± 0.000^{by}	34.30^{**}
	Day 6	8.89 ± 0.000^{cx}	8.88 ± 0.000^{cx}	8.86 ± 0.005^{cx}	8.78 ± 0.003^{cy}	7.418^{*}

Figures are mean ± standard error of 3 replications, *- Significant at five percent level (p≤0.05), 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 control and sample thermized at 65ºC for 2 minutes (Silpa, 2023). It is interesting to note that the decrease in starter culture count of *Lcb. rhamnosus* 18 *dahi* samples microwaved at 600 W for 30 s was not reflected in the other two tested parameters, as both the pH value and acidity, continued to decrease and increase respectively on the sixth day also. This observation is suggestive of the residual or extended activity of the enzymes involved in acid production. Based on the obtained data it can be inferred that the effects of microwave treatment are highly dependent on the starter culture used, intensity of microwave treatment and the period of storage. So, further extensive studies using more starter cultures, microwave dosages and extended periods of storage are warranted to derive a conclusion on recommending microwave treatment as a PFA control method.

Microbiological decontamination effect similar to that of boiling was reported by Termonte *et al. (*2014) on subjecting raw milk to microwave treatment at 900 W for 75 s. The fact that the same study also reported the ineffectiveness of microwave treatment to effect microbial decontamination at 750 W for 75 s underlines the need of food matrix specific optimisation of dosage of microwave treatments during its usage for antimicrobial effects. Ahmed (1998) reported that microwave heating to a temperature of 70ºC for 1 min caused marked reduction in the starter culture count and titratable acidity of yoghurt during storage. Effectiveness of microwave treatment (720 W/10 s, 20 s and 30 s) to delay the increase in starter culture count, titratable acidity and reduce the rate of pH reduction during refrigerated storage of yoghurt sample was reported by Turgut (2016). Current study is in agreement with the study of Turgut (2016) in that, there was increase in acidity, starter culture count and decrease in pH during refrigerated storage even in microwaved *dahi* samples. However, the reported study could not establish any notable inhibitory effect of microwave treatment on the tested high post acidifying cultures or their acid production potential. As the increase in temperature caused by microwave exposure plays a key role in microbial inactivation (Rougier *et al*., 2014; Shaw *et al*., 2021) the low extent of temperature increase attained on using microwaving at 600W might be one of the reasons for the non-inhibitory effect observed in the current study. Compositional differences in between the products tested could be another reason as the rate of conversion of microwaves into heat is highly dependent on the dielectric properties of the food matrices (Verma *et al.,* 2020). Studies on impact of microwave treatment on PFA are scanty and those reported are on yoghurt. To the best of our knowledge the current study is the first one on use of microwave treatment for controlling PFA in *dahi*. The unique observation of differential responses of starter cultures to microwave treatments emphasizes the need of culture wise standardisation of microwave treatment for harnessing the beneficial effects of

this process. Further the differential responses also open up the possibilities of recommending microwave treatment for control of PFA in microwave sensitive starter cultures.

Conclusion

This study assessed the possibility of using microwave treatment as a method for controlling post-fermentation acidification in *dahi* samples prepared using two high post acidifying cultures namely, *Lcb. casei* 01 and *Lcb. rhamnosus* 18. Starter culture wise differences were observed in the effectiveness of microwave treatment in controlling post acidification in the tested *dahi* samples. The observed culture specific differential responses to microwave treatment are suggestive of the need of in-depth studies, incorporating more starter cultures and different microwave dosages to derive proper conclusions on the suitability of microwave treatment for post fermentation control. As microwaves have become very common in Indian households, if proven efficient in controlling post-fermentation acidification there will be many takers for this microwave-based technology of post-fermentation acidification control.

Acknowledgement

The authors acknowledge the financial support by Kerala Veterinary and Animal Sciences University to carry out this post graduate research work.

Conflict of interest

The authors declare that they have no conflict of interest.

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