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Techno textural characteristics of autochthonous lactic acid bacteria isolated from naturally fermented milks

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Abstract

Naturally fermented milks are always a hub of diverse microflora. The diversity of autochthonous microflora associated with these products are linked to many factors like the type of milk, starter microflora, environment and processing practices. The lactic acid bacteria isolated from the household curd samples collected from rain fed areas of Idukki and Wayanad districts of Kerala were used in the study. IDK 120 (MT 211513), the isolate from Idukki, was capable of producing curd with good physico-technical attributes like good lactic feel with negligible syneresis at 37 °C. The culture WYD 1501 (MK 793581), the isolate from Wayanad, performed similarly at 35 °C. Both isolates succeeded in yielding coagulum with good textural attributes in their optimum growth conditions. Texture profile analysis indicated that IDK 120 curd possessed a firmness value of 175.65±11.99 g, and WYD 1501 a value of 180.46±39.71 g, both of which significantly lower than 317.16±16.92 g, the value of control curd manufactured using commercial mesophilic dahi culture NCDC 159. The cohesiveness value of IDK 120 matched with that of control dahi, while that of WYD 1501 was significantly higher than control. Unleashing the credentials of indigenous flora of naturally fermented milks will enable commercialisation of such starters. This can also contribute to development of defined multiple strain authentic starters for development of fermented foods with added attributes. The isolates IDK 120 and WYD 1501 are deposited in ICAR-Veterinary Type Culture Collection (VTCC) with accession numbers VTC DM 648B and VTC DM 592B respectively.

Keywords: Naturally fermented milks, dahi culture, texture profile analysis, firmness

Introduction

In spite of wide change in the market trend, generation gap and consumer perceptions. Dahi or curd is still a top priority in Indian diet. Dahi is considered to be the naturally fermented milks of India (Jatmiko *et al.*, (2018) [1]. The reason behind is their authenticity associated with easy digestibility, soothing aroma, proven health benefits attributed by an array of lactic acid bacteria. Nowadays, like yoghurt and thermophilic set curd, normal dahi is made available in attractive packaging like bottles, cups and stand-up pouches rather than being sold in loose or in simply tied packs mainly seen in the rural markets of India. Many small-scale entrepreneurs are venturing into dahi business seeing the increasing demand. As per the latest report, 10 percentage of total milk collected in India is being diverted for dahi manufacture, and in Kerala it comes to 15%. Above all, the curd market is showing a CAGR of more than 12% for last ten years (<https://www.imarcgroup.com/curd-market-india>) [2].

The lactic acid bacteria present in dahi has got an incredible role in making it more appealing. There are only limited studies available on the textural attributes of dahi when compared to that of yoghurt. The studies on attributes of coagulum manufactured using autochthonous organism are less. The cultures showing good textural attributes can be promoted as such or in combination with existing cultures as defined multiple strains to manufacture curd with appealing characteristics. The native 'original flavour' with improved textural attributes can reposition dahi in the current market and can be made acceptable to the newer generation who focus more on health. In this study two autochthonous cultures isolated from natural fermented milk samples collected from Kerala, as part of ongoing research were assessed for their techno textural attributes.

Materials and Methods

Maintenance of cultures

IDK 120 (*Lactiplantibacillus plantarum* (MT 211513/VTC DM 648B)) and WYD 1501

(*Lactocaseibacillus casei* (MK 793581/VTC DM 592 B). from the repository maintained at College of Dairy Science and Technology, Thiruvananthapuram, Kerala were used for the study. Both were isolated from curd samples, collected from households who have been practicing back slopping for years for curd preparation, from the interiors of Idukki and Wayanad districts of Kerala, noted for ample rainfall and low ambient temperature. The isolates were maintained in MRS broth. Freshly activated cells were used for all studies. Stock was also maintained in RSM (12% reconstituted skim milk) and glycerol (70%) at 4 °C and minus 20 °C respectively. The purity and activity of cultures were ensured by Gram staining and catalase test prior to all trials. The lyophilised culture of NCDC 159 (ICAR-NDRI, Karnal) was sub cultured three times in RSM to activate the culture.

Coagulum production potential

Technological feasibility of the cultures was evaluated by assessing optimum temperature of growth, coagulation time, pH, titratable acidity, syneresis and texture profile of the coagulum obtained when used as monocultures. Sensorial attributes were also adjudged by an expert panel.

Homogenised standardised milk was used for the coagulum preparation. The milk was subjected to a temperature of 95 °C for a period of 10 minutes. After cooling to incubation temperature, freshly activated culture was inoculated at a level of one per cent. Incubation was done at 35 °C, 37 °C and 42 °C. The incubation temperature 35 °C was selected considering the prevailing average temperature in the State. The samples were checked for coagulum formation at 4 h and then at hourly interval. The time of coagulation was calculated by assessing the time lapsing between the point of inoculation and the transition point at which a gel like consistency was attained in each case. The interval was reported with a maximum variation of 30 minutes once the coagulum was formed, the containers were transferred to refrigerator at 4 °C. Based on the syneresis, pH and coagulum characteristics after 24 h of refrigerated storage, optimum temperature of growth was ascertained. Sensory attributes of the products were also evaluated (Chaudhary *et al.*, 2018) [3]. Control dahi was prepared using freshly activated dahi culture (NCDC 159) as that of treatment samples. Incubation was done at 37 °C for 12 h. After 24h storage at 4 °C, textural properties were determined.

pH and Titratable acidity

Analysis was performed after 24h of storage. pH was determined using calibrated pH meter (Systronics). The titratable acidity of coagulum was measured as per the standard method laid down by FSSAI. (Manual of Methods,

01.035:2022) [4]. Care was taken to complete the entire titration within 20 sec and calculated using formula

$$\text{Titratable acidity as \% Lactic Acid} = \frac{9 A N}{W}$$

Where A is the ml of 0.1 N NaOH used, N; Normality of NaOH and W; the weight sample taken in g.

Syneresis

Syneresis of undisturbed curd was assessed as per Cartasev and Rudic (2017) [5]. Curd samples were weighed (W1) and kept at an angle of 45° using supports for 20 min to permit collection of whey on sides of container. The whey collected on the side was siphoned out using a syringe within 10 sec. The curd samples were weighed again (W2). Syneresis was expressed as per cent weight of the whey over the initial weight of the curd sample.

$$\% \text{ syneresis} = \frac{(W1-W2)}{W1} \times 100$$

Texture Profile Analysis

Textural properties of curd samples prepared at their optimum temperature were assessed using the Texture Analyser (TA-HD plus Texture Analyser- Stable microsystem) following the studies conducted by Veena *et al.*, (2017) [6]. The operational conditions maintained to measure the force in compression was as appended below

Mode of operation: Back extrusion

Pre-test speed: 1.00 mm/sec,

Test speed: 1.00 mm/sec

Post-test speed: 5.00 mm/sec

Trigger force: 5 g

Distance covered: 15 mm

Probe Used: A/BE-d35 diameter of 35 mm

The values were compared with that of a control mesophilic dahi prepared using commercial mesophilic dahi culture (NCDC 159). All tests were done in triplicate and statistically assessed using one way ANOVA followed by Duncan Multiple Range Test (DMRT).

Result and Discussion

Physico techno attributes of isolates

The coagulum formation potential of both the isolates were evaluated at different temperatures (Table 1 and 2)

Table 1: Coagulum characteristics of IDK 120 at different incubation temperatures

Incubation Temp (°C)	Coagulation time (h)	Acidity (% LA)	pH	Syneresis (%)
35	13.67 ^b ±0.33	0.583 ^a ±0.003	5.01 ^b ±0.01	2.56 ^b ±0.16
37	12.67 ^b ±0.33	0.637 ^a ±0.028	4.82 ^c ±0.01	1.52 ^c ±0.04
42	18.33 ^a ±0.33	0.470 ^b ±0.006	5.30 ^a ±0	5.87 ^a ±0.09
F-value (P-value)	82.33** (<0.001)	25.40** (0.001)	1450.1** (<0.001)	459.28** (<0.001)

** Significant at 0.01 level, Means having different letter as superscript differ significantly within a column

Time required for coagulum formation and acidity of coagulum obtained did not differ significantly for IDK 120 at 35 °C and 37 °C. But at 42 °C, time for coagulum formation increased suggesting its mesophilic nature. A similar increase in coagulation time at 40 °C, due to slow growth was reported

for mesophilic *Lactobacillus plantarum* Dad 13 by Wardani *et al* (2016) [7]. Though the acidity differed significantly, the pH did not. Such significant difference in acidity without concomitant significant difference in pH has been reported by Rice and Markley in 1924 [8]. Their studies clearly indicate

that the buffering capacity of milk becomes stronger at high acidity and pH will show a trend towards neutrality. Hence larger amount of acid to be produced to introduce significant change in pH when compared to milk with very low acidity or no developed acidity. Syneresis is the expulsion of whey from the curd formed by casein micelles in the form of a 3D matrix (Walstra, 1993) [9]. Syneresis differed significantly between all incubation temperatures. Highest syneresis was observed

at 42 °C. The sensory evaluation of IDK 120 curd (Table 3) at 42 °C revealed bitter taste which could be attributed to proteolysis. Low acidity of coagulum also suggests the role of proteolysis in gel formation. This also points towards the importance of incubation at optimum temperature for good lactic feel that arise from lactic acid produced by lactose utilisation of starter.

Table 2: Coagulum characteristics of WYD 1501 at different incubation temperatures

Incubation Temp (°C)	Coagulation time (h)	Acidity (% LA)	pH	Syneresis (%)
35	15.67 ^a ±0.33	0.547 ^a ±0.009	4.85 ^c ±0	1.78 ^b ±0.04
37	13.50 ^b ±0.29	0.473 ^b ±0.007	5.20 ^a ±0.01	2.07 ^b ±0.03
42	12.33 ^c ±0.17	0.363 ^c ±0.009	5.52 ^b ±0.01	4.67 ^a ±0.23
F-value (P-value)	38.63** (<0.001)	127.72** (<0.001)	2729.54** (<0.001)	141.60** (<0.001)

** Significant at 0.01 level

In case of isolate WYD 1501, all parameters except syneresis differed significantly between the three incubation temperatures. The syneresis values did not differ significantly ($p < 0.05$) between 35 °C and 37 °C. Strains of *Lactocaseibacillus casei* exhibited a syneresis value as high as 17.8 per cent at 37 °C for 10 h (Maajid *et al.*, 2022) [10]. The less coagulation time and higher syneresis may resulted due to the high inoculation rate of three per cent. Here lower syneresis value (1.78±0.04%) with high acidity

(0.547±0.009% LA) was attained at 35 °C. Though maximum activity of organism was observed at 42 °C in terms of coagulation time (12.33±0.17 h), the sensory attributes were most ideal at 35 °C. Coagulum formation happened fast at 42 °C, but lactic feel was absent and bitterness was evident at that temperature of incubation (Table 3). Low acidity and high syneresis at 42 °C could be due to the action of proteolytic enzymes.

Table 3: Sensorial attributes of isolates at different incubation temperatures

Isolate	Incubation Temp (°C)	Sensorial observations
IDK 120	35	Smooth texture and firm body. Good lactic feel. Slight syneresis observed.
	37	Smooth texture and firm body. No syneresis observed. Good lactic feel.
	42	Grainy texture and weak body. Slightly bitter. Moderate syneresis observed.
WYD 1501	35	Soft, smooth texture, Firm body. No syneresis observed. Good lactic aroma
	37	Soft, smooth texture, Firm body. No syneresis observed. Slightly bitter
	42	Soft, smooth texture, Firm body. Slight syneresis observed. Slightly bitter

Textural characteristics of isolates

As opined by Magenis *et al.*, (2006) the textural characteristics such as hardness or firmness, cohesiveness, gumminess, chewiness, and springiness are of greater importance for fermented milk products [11]. Here the firmness, cohesiveness, gumminess and springiness were

assessed for the curd samples prepared at their optimum incubation temperature. The values obtained were compared with that of control prepared using a standard defined dahi starter.

The results are given in the fig 1a, 1b and table 4 below.

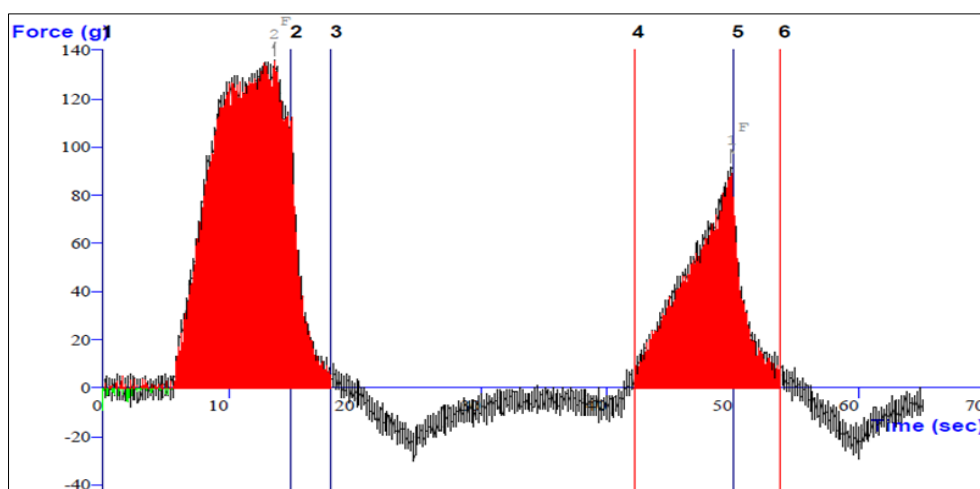


Fig 1: Texture profile analysis of IDK 120

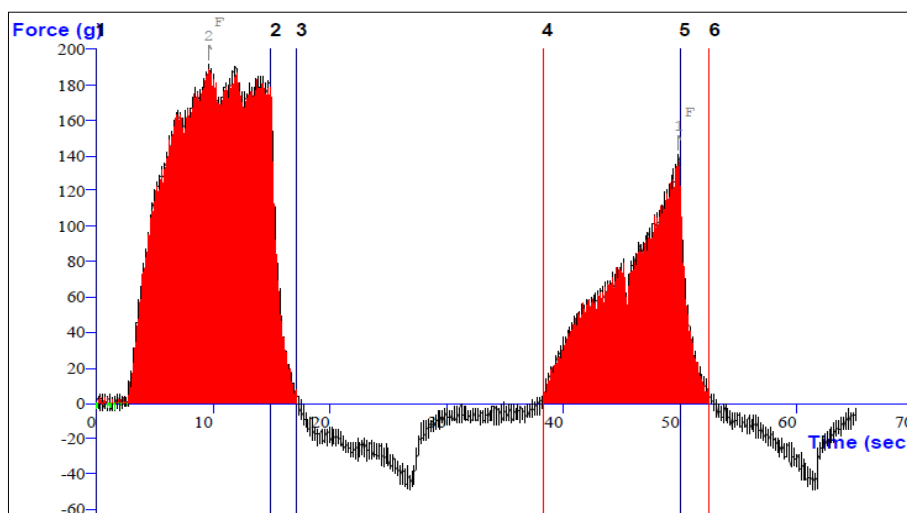


Fig 2: Texture profile analysis of WYD 1501

Table 4: Comparison of different textural parameters between samples

Isolate (Dahi)	Firmness (g)	Cohesiveness	Gumminess	Springiness
IDK 120	175.65 ^b ±11.99	0.397 ^{ab} ±0.047	70.89±12.54	0.772±0.035
WYD 1501	180.46 ^b ±39.71	0.463 ^a ±0.022	83.1±17.13	0.679±0.088
Control (NCDC 159)	317.16 ^a ±16.92	0.283 ^b ±0.029	89.35±8.23	0.556±0.064
F-value (P-value)	9.650* (0.013)	6.989* (0.027)	0.510 ^{ns} (0.624)	2.697 ^{ns} (0.146)

* Significant at 0.05 level; ns non-significant ($p > 0.05$), Means having different letter as superscript differ significantly within a column

The table shows that there is no significance difference was observed for firmness and cohesiveness for both the isolates at 95% confidence level. But the firmness was significantly lower when compared to control curd. In case of cohesiveness, control and IDK 120 dahi possessed almost similar values. Firmness value obtained for control curd is in agreement with the reported value of 308.37 ± 2.14 g by Joon *et al.*, (2017) [12]. Rejeesh *et al.*, (2013) reported that mesophilic curd samples showed lower firmness when compared to thermophilic curd samples. The scanning electron microgram of curd samples revealed that firmness is very much related to the arrangement of casein micelles. When casein micelles are seen in the form of long chains or big clusters, with their identities merged and separated by interspaces, the curd will be less firm. When caseins micelles were compact with less interstitial spaces, the coagulum was firmer [13]. Mesophilic nature of IDK 120 and WYD 1501 could be the reason for low values for firmness observed in this study.

Conclusion

Naturally fermented milks are reservoirs of diverse microflora. The strains assessed in this study performed well in mesophilic temperature range. They have produced good coagulum with appealing lactic feel and low syneresis. They also exhibited moderate firmness on textural studies. More extensive molecular studies and evaluation of probiotic characteristics may position these cultures on the top of the list. Ample scope is there for the use of these strains along with primary starters as defined multiple starters.

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Conflict of Interest: The authors declare that they have no

conflict of interest.

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