

Harnessing the untapped potential of indigenous cow milk in producing set-type yoghurts: Case of Thamankaduwa White and Lankan cattle

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SUPPLEMENTARY FILE

Materials and methods

Experimental design

Milk sample collection

Milk samples were collected from cattle having similar physiological status (second parity, mid-lactation stage) and reared in the Dry Zone (annual rainfall < 1750 mm, average temperature 28°C) of Sri Lanka. Jersey and Friesian cattle included in this study are raised under a semi-intensive system where the farmers practice cut and fed system and no concentrates are given. LC and TW cattle are raised under an extensive management system where only free grazing is practiced. Milk from three biological replicates representing five animals from each breed/type was pooled and used to produce the set-yoghurt.

Set-yoghurt preparation

Milk was standardized to a 3% (w/v) fat level and preheated to 55-60°C followed by homogenization at the same temperature for 15 min under 10-20 MPa using a laboratory-scale homogenizer (KRH-1, Kunal International, India) and pasteurized at 90°C for 15 min. Freeze-dried starter cultures (Chr. Hansen, Horsholom, Denmark) including thermophilic yoghurt starter

culture (YF-L903) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* and probiotic culture (ABY-10) which contain *Bifidobacterium animalis* ssp. *lactis* (BB-12) were inoculated according to the manufacturer's instructions. Finally, the incubation of yoghurts was carried out at $42\pm 2^{\circ}\text{C}$ temperature for 4 hours (pH ~ 4.5) and transferred to refrigerated storage ($4\pm 1^{\circ}\text{C}$) for 21 days.

Instrumental texture analysis

Samples were allowed to reach room temperature (25°C) before the commencement of the analysis. Back extort method fitted with 10000 g load cell was undertaken to determine texture profile of set-yoghurt over the refrigerated storage period of 21 days. A cylinder probe (TA4/1000) was used to measure the texture profile of yoghurts by penetrating the samples up to 15 mm depth. The speed of the probe was fixed at 2 mm/s during the pretest compression and 1 mm/s for both tests and return speed from the samples. Texture properties including firmness, adhesiveness, cohesiveness and gumminess were calculated using a software program (Texture Pro software, Brookfield Instruments). Measurements were made in triplicates.

Apparent viscosity

The apparent viscosity of the samples was measured using a Brookfield viscometer (Brookfield RVT, USA) with a spindle no.6 and rotational speed of 2.5×10^{-4} g at $4\pm 1^{\circ}\text{C}$. Results were recorded triplicates in centipoises (cP) after 1 min of shearing.

Instrumental colour analysis

Instrumental colour analysis of set-yoghurt samples was performed by using a Minolta Chromameter (Konica Minolta Co., Ltd., Osaka, Japan). The measurement parameters consisted of a D65 light source with a measuring head hole of 8 mm. Results expressed as L*, a*, b* values according to CIE-LAB system. Each measurement was recorded by averaging triplicates.

Microstructure analysis

Samples in size of $1 \times 1 \times 0.25$ cm were excised from the middle of the set-yoghurt samples and were frozen under the -18°C . The pre-frozen set-yoghurt samples were dried in a laboratory freeze dryer (Alpha 1-4 LS basic, Christ, Germany) for 72 hours under the vacuum of 1×10^{-3} kPa. The condenser temperature of the freeze dryer was -50°C . Once the final moisture content was below 2% on a wet basis; samples were mounted onto an SEM specimen stub, coated with gold using Sputter coater (Quorum, Germany) at 1×10^{-2} kPa and examined under SEM (ZEISS EVO 18, Germany) operated at 15 kV.

Sensory property analysis

Sensory analysis was conducted with the approval from Rajarata University of Sri Lanka. The tasting panel consisted of staff members (16 male and 24 female aged 26-42 years) from the Rajarata University of Sri Lanka and prior instructions were given before the commencement of the test. Products were presented in three-digit coded white plastic containers and tested 15 minutes after removing them from refrigerated storage. As described by Mohebi and Ghodousi (2008), the panellist was requested to evaluate the set-yoghurt using 5 points hedonic scale (5- Extremely like; 1- dislike very much).

Statistical analysis

Pearson correlation coefficients were analysed among texture properties of set-yoghurts and milk composition parameters of four different cattle breeds/types as presented in previous work by Weerasingha *et al.* (2021). Also, the correlation between syneresis, pH, titratable acidity of the set-yoghurts originate from the previous study were assessed with instrumental texture properties of set-yoghurts obtained in the current study. Milk fat content was not considered in correlation analysis since the milk fat was standardized during set-yoghurt preparation.

Principal component analysis (PCA) was used in multivariate analyses of milk composition parameters, syneresis, pH, titratable acidity of set-yoghurts which was obtained in a previous study by the authors and instrumental texture parameters of set-yoghurts using the software simca 16.0 (Sartorius Stedim Data Analytics AB, Sweden). The variables were preprocessed with UV centering. PCA score scatter plots were generated for evaluating similarities and groupings of studied parameters, while PCA loading scatter plots were used to interpret the score to scatter plots to display similarities or differences among all variables.

Supplemental Tables

Supplementary Table S1: Milk compositional analysis of four different cattle breeds/types

Breed/Cattle type	Fat (%)	Protein (%)	Lactose (%)	Solid-non-fat (%)
Thamankaduwa white	4.56±0.50 ^A	3.20±0.32 ^A	4.28±0.18 ^A	7.89±0.15 ^A
Lankan	4.35±0.32 ^A	3.14±0.26 ^A	4.29±0.25 ^A	7.80±0.46 ^A
Jersey	4.13±0.75 ^B	3.18±0.28 ^A	4.11±0.79 ^A	7.37±1.47 ^B
Friesian	3.15±1.21 ^C	3.06±0.18 ^A	4.35±0.39 ^A	7.29±0.25 ^B

Supplementary Table S2. Correlation between physicochemical properties of milk/set-yoghurt and textural properties of set-yoghurt

	Apparent Viscosity	Hardness	Adhesiveness	Cohesiveness	Gumminess
Milk Protein	0.251	0.195	0.134	0.319*	0.257
Milk Lactose	0.078	0.125	0.075	0.103	0.089
Milk Solid Non Fat (SNF)	0.503**	0.037	0.037	0.326*	0.348*
Syneresis of set- yoghurt	-0.602**	0.426**	0.221	0.427**	0.455**
Set-yoghurt pH	0.034	-0.022	0.013	0.359*	0.142
Titratable Acidity of set-yoghurt	0.060	0.075	0.299	0.201	0.130

*indicate correlation coefficient is significant where $p < 0.05$

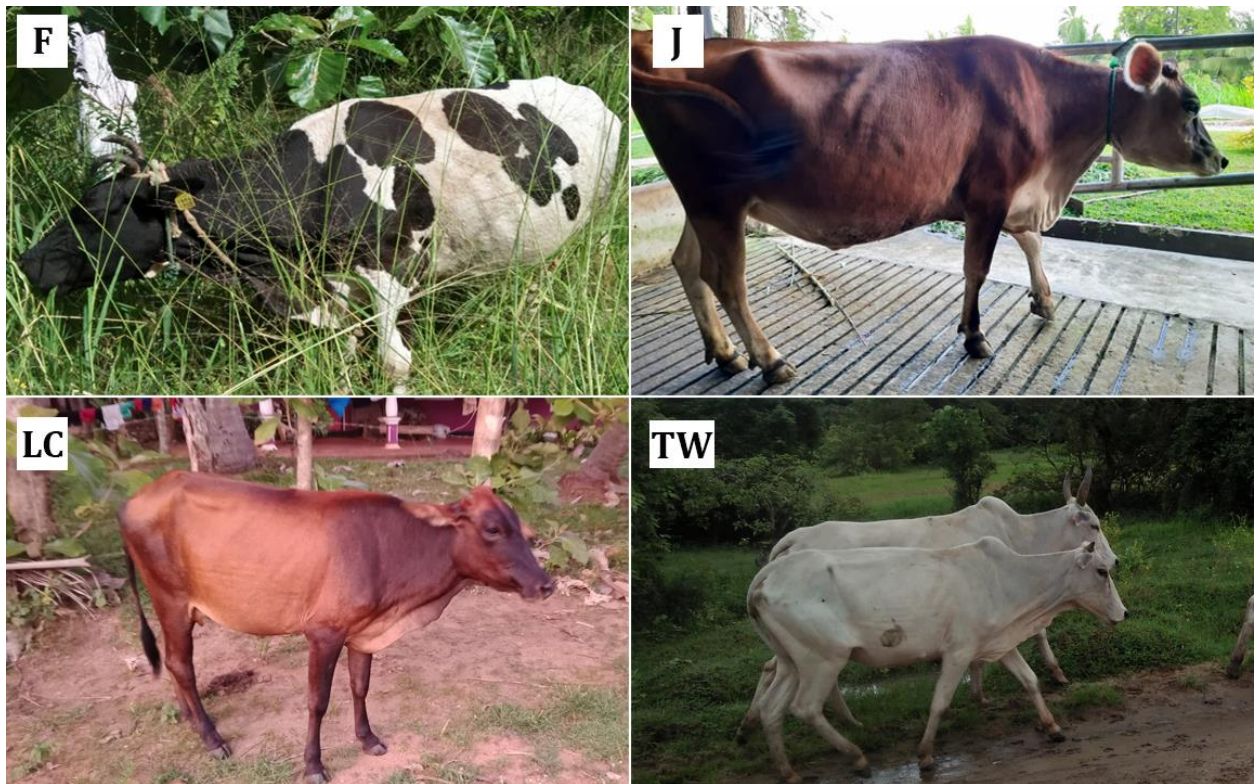
**indicate correlation coefficient is significant where $p < 0.01$

Supplemental Figures

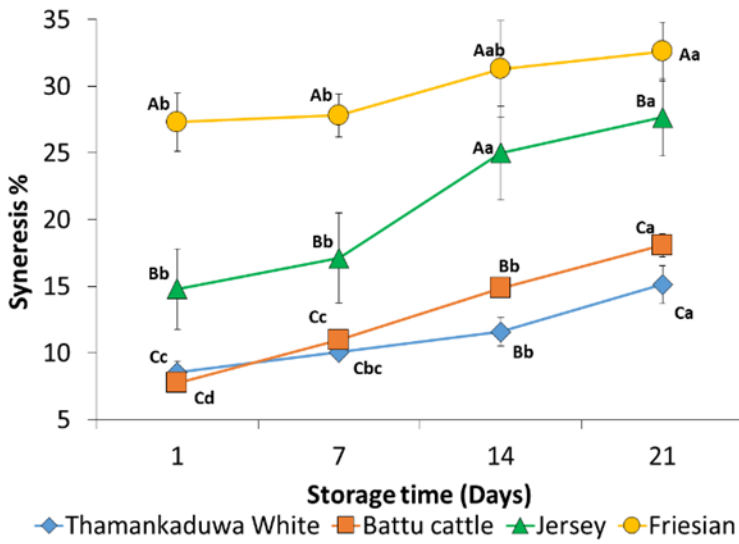
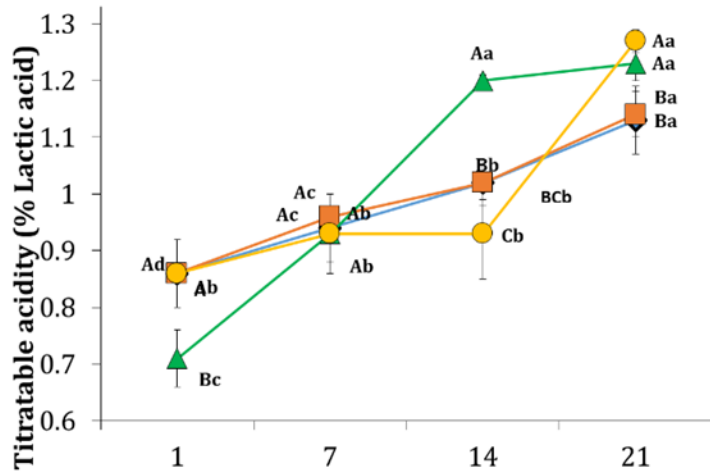
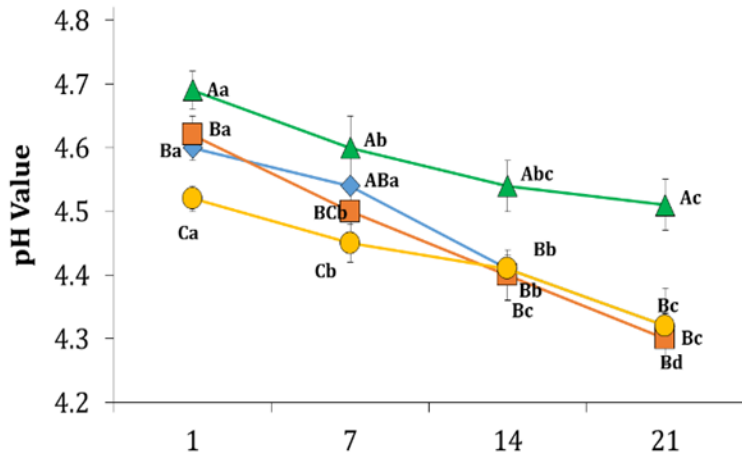
Supplementary Figure S1. Phenotypic view of the cattle breeds/types used in the current study; J- Jersey, F- Friesian, LC-Lankan cattle and TW-Thamankaduwa White

Supplementary Figure S2. Variation of pH, titratable acidity and syneresis of set-yoghurt made from TW, Lankan, Jersey and Friesian milk during storage of 21 days at $4 \pm 1^\circ\text{C}$.

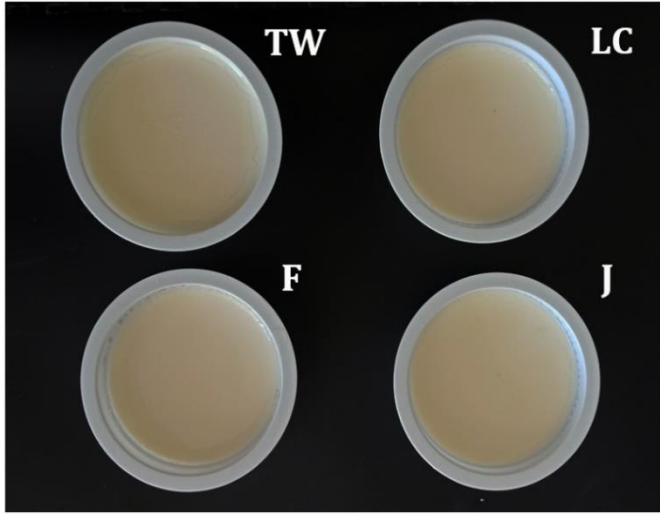
Supplementary Figure S3. Visual appearance of yoghurt colour (A) and disturbed gel (B and C) of the set-yoghurt gels made from Jersey (J), Friesian (F), Lankan cattle (LC) and Thamankaduwa White (TW) milk



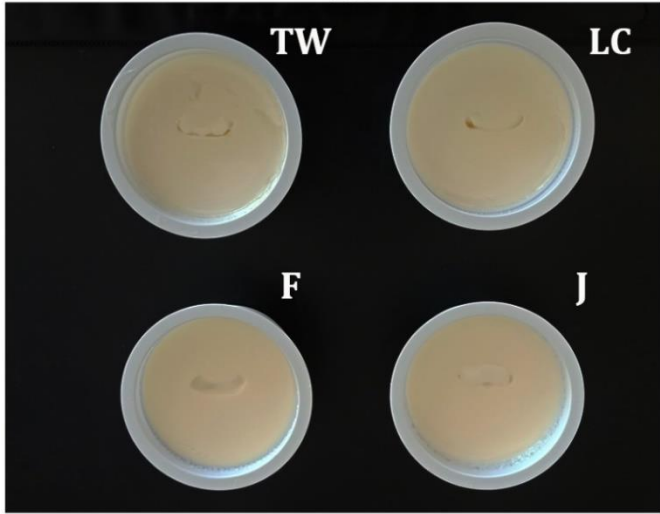
Supplementary Figure 1.



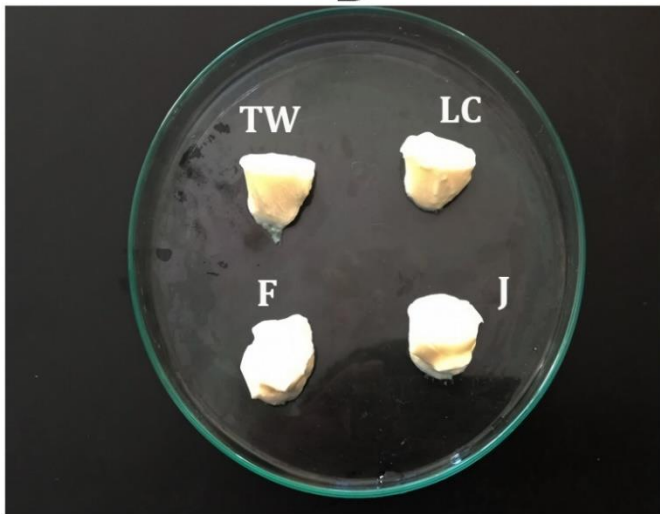
Supplementary Figure 2



A



B



C

Supplementary Figure 3.

References

Mohebi M and Ghodousi HB (2008) Rheological and sensory evaluation of set-yoghurts containing probiotic cultures. *Journal of Agricultural Science & Technology* **10**, 145-155.

Weerasingha WVVR, Ranadheera CS, Prasanna PHP, Silva GLLP and Vidanarachchi JK (2021) Probiotic Viability and Physicochemical Properties of set-yoghurt made of indigenous and exotic cow milk. *Tropical Agricultural Research* **32**, 39-48.