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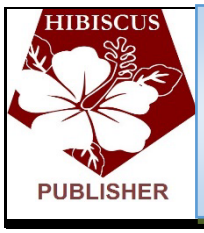

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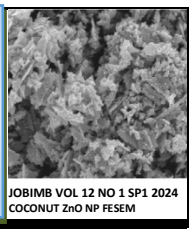

Physicochemical Properties, Nutritional Composition, and Microbial Profiles of Locally Fermented Yogurt Drink (*Lassi*) across Three Restaurants in Malaysia

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ABSTRACT

This study investigates the nutritional and microbial aspects of locally fermented lassi from three establishments in Seri Kembangan, Selangor. The microbial analysis encompasses Total Plate Count (TPC), Yeast and Mould Count (YMC), Lactic Acid Bacteria (LAB), coliform, *Listeria monocytogenes*, and *Staphylococcus aureus*. Additionally, physicochemical properties and nutritional composition, including calcium and sugar identification, viscosity, pH, titratable acidity (TA), whiteness index, Brix, and proximate analysis, were examined. The results indicate heightened levels of coliforms, YMC, and TPC, while notably lacking other pathogenic microorganisms, suggesting potential cleanliness and storage concerns. The LAB count surpasses 10^7, indicating successful fermentation, supported by low pH and TA levels, aligning with effective LAB concentrations for health benefits. These lassi variants exhibited high sugar content, potentially from the addition of table sugar. Calcium and protein levels suggest a dairy milk base, although variations among establishments hint at unique formulations.

INTRODUCTION

In recent years, fermented foods have seen a surge in popularity driven by changing consumer preferences and growing scientific interest. *Lassi*, a traditional popular South Asian beverage, is increasingly being offered in *Mamak* (Indian Muslim) restaurants in Malaysia. This smoothie-like drink comes in salted or sweet varieties and is typically made from standardized, pasteurized, and chilled milk with reduced fat content [1]. Originating in the Punjab region, *lassi* is a blend of fruits, seasonings, and yogurt, available in both sweet and salted varieties. The production process involves standardizing unprocessed milk, pasteurization, homogenization, bacterial culture addition, and stabilization with low-methoxy pectin. *Lassi*'s composition includes protein-rich yogurt, making it a good source of essential nutrients like calcium and probiotics, which aid digestion and boost immune health. Lactic acid bacteria play a key role in lassi fermentation, contributing to its probiotic properties and digestive benefits [1].

The investigation into the microbial profile and composition of local fermented *lassi* is crucial for a comprehensive understanding of its nutritional value, quality, and potential health benefits. This study aims to provide insights into the beverage's proximate, physicochemical, and microbiological characteristics, benefiting consumers in making informed choices about its consumption and addressing gaps in existing research on this culturally significant beverage.

MATERIALS AND METHOD

Sample collection

Three *lassi* samples were simultaneously obtained from eateries in Seri Kembangan, Selangor, Malaysia, over a two-week period.

(A1: Eatery 1 week 1. A2: Eatery 2 week 2, etc.). The simultaneous procurement aimed to minimize errors, ensuring that all samples were collected on the same day. The sample was stored at -20°C for 2-3 days before conducting the analysis.

Microbiological properties

Petri dishes with agar were dried in a Catalyst Class II blower. 25 mL of local fermented *lassi* was mixed with 225 mL of peptone water in a stomacher bag and stomached for 30 seconds using a BagMixer 400-P. Serial dilutions were made with sterilized peptone water. To conduct specific microbiological analyses, 0.1 mL of appropriate dilution was spread using spread plate technique on a) Plate Count Agar for total plate count (TPC); b) Potato Dextrose Agar for Yeast and Mould count (YMC); c) de Man, Rogosa, and Sharpe (MRS) agar for lactic acid bacteria (LAB) and *S. aureus* count; d) PALCAM agar for *Listeria monocytogenes* e) mannitol salt agar for *Staphylococcus aureus* and f) MacConkey broth, Brilliant Green Lactose Bile broth and Levine's Eosin-Methylene Blue for coliform count. The agar plates were incubated at 37°C for 1 to 5 days, depending on the type of agar. For MRS agar, an anaerobic condition was established; for PALCAM agar, enrichment with *Listeria* Enrichment Broth was needed. The presence of any colony, based on the morphology (e.g., grey-green with a black center and a black halo for *Listeria monocytogenes*) was counted using the colony counter and the log CFU/mL was calculated.

Physicochemical Properties - calcium, sugar, viscosity, pH, titratable acidity, whiteness index, brix, and proximate

A 500 mg/L calcium standard was prepared by dissolving 1.249 g of $CaCO_3$ in deionized water with 10 mL of concentrated HCl. This standard was diluted to create 1-5 mg/L standards. For the sample, 50 mL of Trichloroacetic Acid (TCA) and deionized water were added to 5 mL of *lassi*. After shaking for 30 minutes and filtering, 5 mL of the filtrate, along with 1 mL of 5% lanthanum solution, was analyzed for calcium content using an Atomic Absorption Spectrometer (AAnalyst 400).

The sugar content was analyzed using High Performance Liquid Chromatography (HPLC) Shimadzu (RID-10A). Standards of 3%, 2%, 1%, 0.5%, and 0.1% sugar were prepared. After two-fold dilution, samples were centrifuged, filtered (0.45 µm syringe filter), and 2 mL were injected into the vial for HPLC quantification by using reverse-phase column. Rheometers (Anton Paar, USA) were used to analyze the viscosity of the *lassi* which was taken directly from the refrigerator at 4ºC and placed in a cylinder cup instrument for measurement. The pH of local fermented *lassi* was determined using pH meter (Jenway 3510, Cole-Parmer Ltd., Vernon Hills, Illnois). The brix of the local fermented *lassi* was observed using an ATAGO digital portable pocket refractometer PAL-08S 1-60% (Japan) (ATAGO CO., LTD Japan).

Titratable acidity was determined by dissolving and mixing 4 mL of local fermented *lassi* samples in 16 mL of distilled water. The solution was titrated against 0.1N sodium hydroxide solution until pH 8.2 was achieved. The titratable acidity was calculated using equation [2]:

$$Titratable\ Acidity\ (\%) = \frac{Vol.NaOH\ used\ (mL) \times 0.1N\ NaOH \times milliequivalent\ factor \times 100}{mass\ of\ sample\ (g)}$$

Colour of the local fermented lassi is determined by using colorimeter (Konica Minolta, Japan) and expressed in L* (lightness), a* (redness) and b* (yellowness). 30 mL of sample is placed on the cup and the colour of the sample is measured. Whiteness Index (WI) was calculated using equation [3]:

$$WI = \sqrt{(100 - L*)^2 + a*^2 + b*^2}$$

The proximate composition of moisture, ash, protein, fat, and fibre was analyzed based on AOAC Method 934.01, AOAC Method 942.05, AOAC Method 991.20, AOAC Method 989.05 and AOAC Method 978.10, respectively. The carbohydrate content of the sample was estimated as the difference obtained after subtracting the values of protein, fat, ash, and fiber from the total dry matter [4].

Statistical Analysis

Data obtained were analysed using Minitab (v. 21) statistical package (Minitab Inc., State College, PA). One-Way analysis of variance (ANOVA) with Tukey's test was used to compare the means when a significant variation was established by ANOVA at the significance level 0.05 ($P < 0.05$).

RESULTS AND DISCUSSION

Microbiological parameters

In **Fig. 1**, the Total Plate Count (TPC), yeast and mold (YMC), and lactic acid bacteria (LAB) counts among various restaurants at different timeframes exhibited no significant differences demonstrating a high level of uniformity during in *lassi* preparation. Notably, YMC demonstrated the highest growth, suggesting potential contamination with fungi species in the restaurant serving fermented milk. This aligns with findings by Yazid *et al.* [5], indicating fungi as a notable contributor to food poisoning cases in Malaysia [5]. The TPC count ranged from 6.78 to 7.25 log CFU/mL, exceeding the 5 log CFU/mL limit set by the Malaysia Food Act 1983 [6]. However, it's important to note that this standard is designed for non-fermented milk, which logically tends to have higher TPC due to the growth of LABs on general agar media. All *lassi* samples exhibited robust LAB growth at around 7 log CFU/mL, meeting the minimum level associated with conferring beneficial effects on human health.

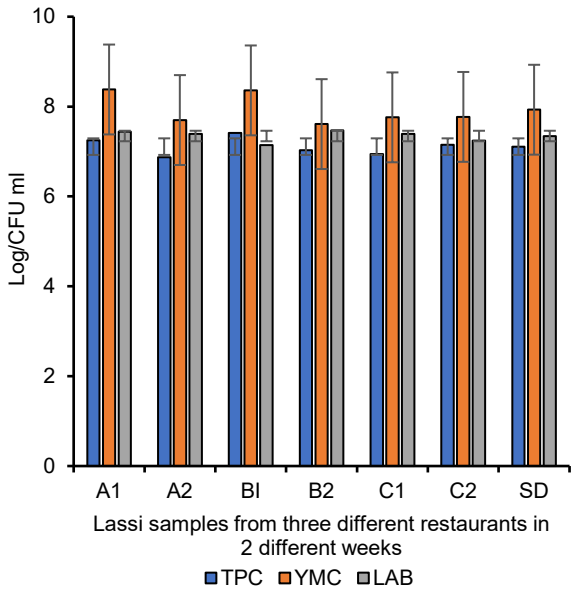

Fig. 1. Total Plate Count Total Plate Count (TPC), Yeast and Mould Count (YMC), and Lactic Acid Bacteria Count (LAB) of Local Fermented *lassi* from 3 different restaurants collected on week 1 and week 2. No significance was detected across the three restaurants.

Table 1 indicates that all local fermented *lassi* samples from three restaurants tested positive for coliform, except for the second-week sample from restaurant A. The positive samples were confirmed through both presumptive and confirmatory tests using MacConkey and BGLB broths, respectively. EMB agar was used if both tests were positive. The presence of coliform suggests possible contamination, which could be due to hygiene issues or the use of contaminated or unpasteurized milk. Coliform count serves as a definitive indicator of potential faecal contamination and the presence of enteric pathogens, posing health risks [7]. Safe levels of coliform in cultured products are limited to <10 CFU/mL [8].

Table 1. The presence (+) or absence (-) of common foodborne pathogens.

Sample	Presence of coliform	Presence of *Listeria monocytogenes*	Presence of *Staphylococcus aureus*
A1	+	-	-
A2	-	-	-
B1	+	-	-
B2	+	-	-
C1	+	-	-
C2	+	-	+

In contrast, no *Listeria monocytogenes* was detected in any of the samples. Apart from the absence of *Listeria monocytogenes* the method used might have limitations, as traditional assays can struggle to identify L. mo*nocytogenes* when overshadowed by other *Listeria* spp., like *L. innocua*. The absence of *L. monocytogenes* in *lassi* is positive, given its favorable growth conditions [9].

Staphylococcus aureus was detected in sample C2 from the second week of collection in restaurant C. This presence could signal poor hygiene practices, especially from food handlers, as *Staphylococcus aureus* commonly resides in human skin, nose, and gastrointestinal systems [10]. Asymptomatic workers might contaminate food through manual contact or respiratory secretions, leading to staphylococcal food poisoning.

Physicochemical properties

Physicochemical analyses (**Table 2**) exclusively utilized the first-week sampling (A, B, and C). All *lassi* samples exhibited calcium concentrations ranging from 609 mg/L to 984 mg/L. A full cream milk sample served as a positive control, yielding a value of 1302 ± 10.616b (data not shown in the Table). Sample A displayed the lowest calcium (609 mg/L) and lactose (0.6 g/mL) content, suggesting a source other than real milk was also added, especially considering its elevated glucose content (0.35 g/L) compared to other samples. However, all samples exhibited high sucrose content (>8.94 g/L), with Sample B showing significantly higher sucrose, Brix content, and viscosity, indicating the potential addition of table sugar typical for sweet *lassi*.

Sample B also displayed a notably higher whiteness index, suggesting a possibly shorter fermentation period. Longer fermentation tends to reduce whiteness due to Maillard and browning reactions, protein breakdown by lactic acid bacteria (LAB), and the development of metabolites that may impact the whiteness index of the *lassi* [11]. The higher pH and lower titratable acidity in Sample B support this observation.

Table 2. The physicochemical properties of lassi.

	A	B	C
Calcium (mg/L)	609 ± 3.105^{b}	984 ± 1.554^{a}	981 ± 5.029^{a}
Glucose amount (g/l)	0.35 ± 0.004	Undetected	Undetected
Sucrose amount (g/l)	8.94 ± 0.069^{c}	13.14 ± 0.241^{a}	10.37 ± 0.176^{b}
Lactose amount (g/l)	0.60 ± 0.012^{c}	3.29 ± 0.015^{a}	2.14 ± 0.005^{b}
Viscosity (mPas)	6.37 ± 0.058^{c}	10.83 ± 0.058^{a}	9.433 ± 0.306^{b}
pH	3.89 ± 0.061^{c}	4.78 ± 0.010^{a}	4.57 ± 0.006^{b}
Titratable acidity (%)	0.50 ± 0.0002^{a}	0.41 ± 0.0004^{c}	0.45 ± 0.0001^{b}
Whiteness Index	19.16 ± 0.632^{b}	24.84 ± 0.546^{a}	18.27 ± 0.145^{b}
Brix	13.07 ± 0.058^{b}	18.63 ± 0.153^{a}	11.43 ± 0.116^{c}

[a-c] Means with different superscripts within the same row are significantly different ($p < 0.05$).

High carbohydrate content in sample B than that of samples A and C, is potentially contributed by the higher content of sucrose in the formulation for sample B (Table 3). Correspondingly, as observed in Table 2 where Sample B exhibited the highest calcium levels, it also returns the highest ash content. Interestingly, Sample C, contrary to expectations, contains the highest fat content, suggesting that other samples might employ a base of low-fat milk or yogurt cultures with lower fat production. Another surprising finding is that Sample A has the highest protein content, even though **Table 3** suggests the use of a mixture of real milk as its base. Additionally, Sample A showed the presence of glucose, while other samples did not exhibit this sugar component.

Table 3. The proximate composition of different lassi samples.

Sample	A	B	C
moisture content (%)	86.60 ± 0.564^{b}	80.50 ± 0.155^{c}	88.29 ± 0.155^{a}
protein content (%)	5.44 ± 0.239^{a}	$4.72 \pm 0.170^{a, b}$	3.89 ± 0.558^{b}
fat content (%)	0.37 ± 0.058^{c}	0.73 ± 0.058^{b}	3.97 ± 0.116^{a}
ash content (%)	0.35 ± 0.040^{b}	0.90 ± 0.035^{a}	0.29 ± 0.138^{b}
fibre content (%)	0	0	0
carbohydrate content (%)	7.24 ± 0.836^{b}	13.14 ± 0.143^{a}	3.56 ± 0.707^{c}

[a-c] Means with different superscripts within the same row are significantly different ($p < 0.05$).

CONCLUSION

Lassi exhibits rich nutrients and functional benefits, exemplified by its high protein, calcium, and Lactic Acid Bacteria content. However, attention should be directed towards reducing its sugar content for improved health. Enhanced hygiene practices are crucial, considering the presence of specific foodborne pathogens. In the future, regular checks on food establishments are recommended to enhance overall food safety.

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