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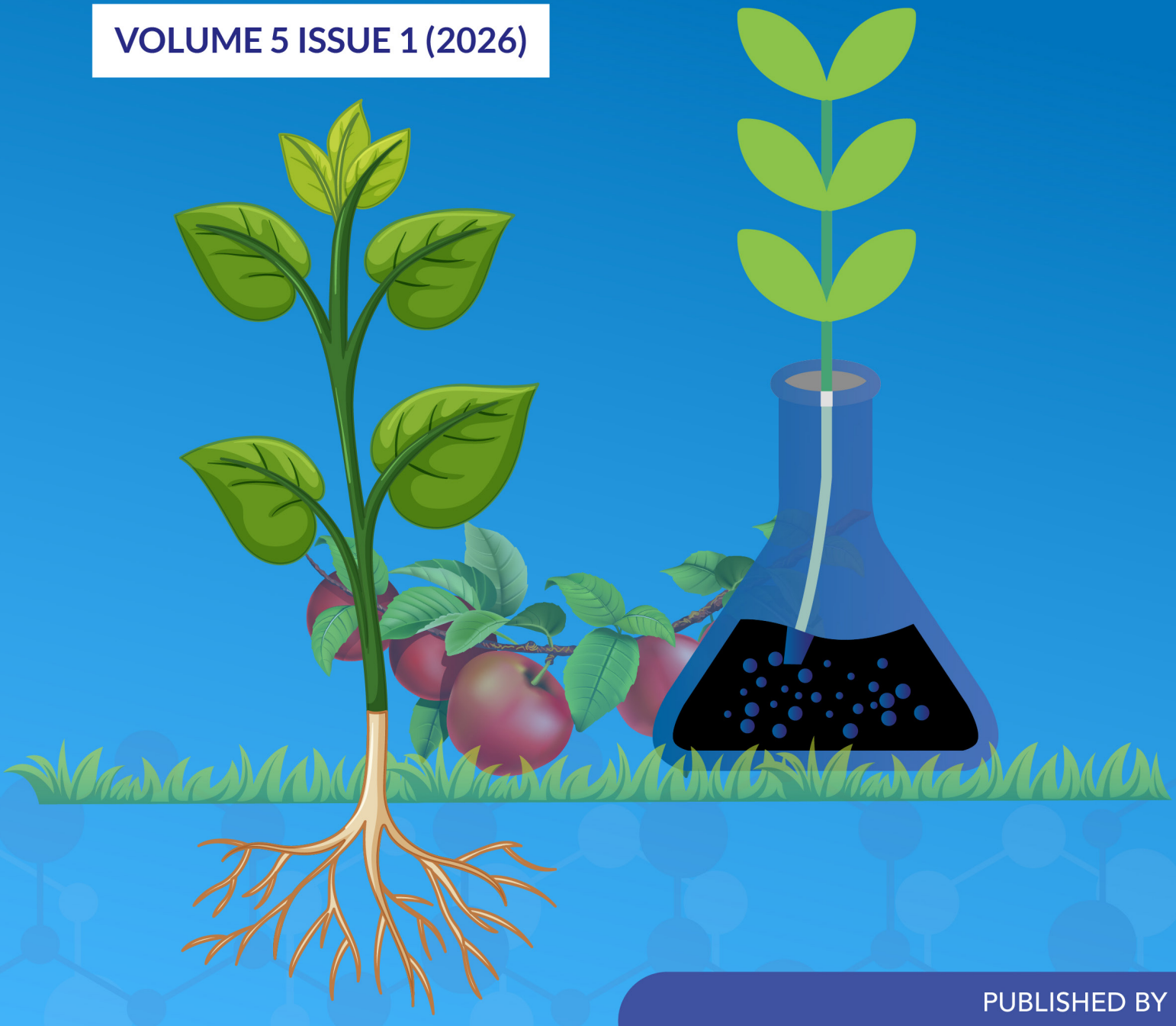

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Quality, Nutritional Composition and Effect of Various Yoghurt Blends on the Gut by-Products of Ulcerogenic Wistar Rat

Ogunka-Nnoka Cu[1*], Enyong Bu[1], Itohan E[1], Asaba E[1], Ozuru P[1], Ogonu F[1]

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ABSTRACT

The disruption of the gastrointestinal mucosal lining due to excessive secretion of gastric acid results in a gastric ulcer, which presents epigastric pain shortly after meals. The present study investigated the quality and effects of dairy-based (DBY), plant-based (PBY) and dairy/ plant-based (DPBY) yoghurts on gut metabolites in indomethacin-induced Wistar rats. The formulated yoghurt samples were subjected to physicochemical properties, proximate composition, microbiological quality, and sensory attributes, followed by an evaluation of their effect on Gut metabolites. The three yoghurt samples met Codex Alimentarius quality standards for yoghurt, including acceptable pH (3.98 ± 0.05 to 4.56 ± 0.09) and minimum total solids. The microbiological analyses showed product safety, with Total Viable Counts within International Commission on Microbiological Specifications for Foods (ICMSF) limits and high Lactic Acid Bacteria counts, suggesting probiotic capability. Results of proximate analyses showed higher fat content in DBY (6.22%) compared with PBY (2.56%), whereas protein content was greater in the PBY sample (4.02%). Colour was the most preferred attribute, while tanginess was the least favoured based on sensory evaluation. Gut microbial metabolites were significantly ($p<0.05$) influenced by the various yoghurt blends. Dairy yoghurt promoted elevated caproic (138.85504 ppm) and valeric (73.19835 ppm), plant-based yoghurt increased hexanoic (53.15172 ppm) and isovaleric acids (15.62704 ppm), and the dairy/plant-based blended yoghurt produced high caproic and propanoic acid concentrations. These findings demonstrate that all three yoghurt types are safe, nutritionally adequate, and capable of modulating SCFA production, underscoring their potential as functional dietary components for supporting gastric ulcer management.

INTRODUCTION

Yoghurt is produced by the fermentation of milk using a symbiotic culture of lactic acid bacteria, which metabolize lactose into lactic acid (El-Sayed *et al.,* 2021). Consumer interest in plant-based foods has grown substantially in recent years, driven by health concerns, lactose intolerance, dairy allergies, environmental sustainability, and ethical considerations surrounding animal-derived products. Within this expanding market, plant-based yoghurts (PBY) have emerged as a significant segment due to their ability to mimic the sensory and functional qualities of traditional dairy-based yoghurt (DBY) while offering additional nutritional and dietary benefits. The yoghurt samples are generally produced by fermenting aqueous extracts from diverse plant sources, such as oilseeds, legumes, cereals, and pseudo-cereals, each contributing distinct nutritional and functional characteristics to the final product (Makinen *et al.,* 2016).

The physicochemical properties of plant constituents play a major role in defining the texture, viscosity, and stability of plant-based yoghurts. Typically, these extracts resemble cow's milk in appearance and consistency due to processing methods such as soaking, grinding, filtration, homogenization, and thermal treatments that break down structural matrices and improve colloidal stability (Nishinari *et al.,* 2014). However, plant-derived matrices often lack optimal fermentable sugars and casein-like proteins, which are responsible for the gel structure in dairy yoghurt. To address these limitations, researchers and manufacturers often blend multiple plant substrates to leverage their complementary profiles. This blending strategy not only enhances fermentation efficiency but also improves mouthfeel, rheology, and flavour balance in the final product (Aidoo *et al.,* 2010).

Sensory characteristics remain a key determinant of consumer acceptance for both dairy and non-dairy yoghurts. Factors like taste, creaminess, scent, and consistency play a crucial role in influencing buying choices and brand loyalty. Research has shown that various formulations of plant-based yoghurt can reach sensory ratings similar to those of traditional dairy yoghurt, emphasizing the substantial advancements in enhancing the quality of non-dairy yoghurt. Such research has also identified critical formulation variables—including protein content, fat type, stabilizer choice, and fermentation culture—that profoundly impact product perception and acceptance (Grasso *et al.,* 2020). Understanding these relationships is essential for optimizing plant-based yoghurt development and ensuring market competitiveness.

Researchers have explored the potential of underutilized plant resources in yoghurt formulation. Ingredients such

[1] Department of Biochemistry, University of Port Harcourt, Choba, Rivers State Nigeria
[*] Corresponding author's e-mail: charity.ogunkannoka@uniport.edu.ng

as tiger nuts (*Cyperus esculentus*), coconuts (*Cocos nucifera*), and date fruits (*Phoenix dactylifera*) offer promising functional benefits, including natural sweetness, enhanced creaminess, dietary fibre enrichment, and improved antioxidant potential. Combining these materials has been shown to significantly enhance the sensory, nutritional, and aesthetic qualities of plant-based yoghurt, producing a product with unique flavour complexity, desirable texture, and increased consumer appeal (Baburao *et al.,* 2019). Furthermore, these ingredients contribute bioactive compounds, which are crucial in modulating the gut microbiota (Le Roy *et al.,* 2022 & Keshavarzian *et al.,* 2021).

MATERIALS AND METHODS

Sample Collection and Preparation of Raw Materials

The raw materials, including fresh pasteurized cow's milk, fresh tigernut, fresh coconut, dried date fruit, yoghurt starter culture and carboxy methyl cellulose (CMC) powder, were all purchased from Mile 3 market, Port Harcourt, Rivers State, Nigeria.

Dried and undamaged date fruits were selected, and washed with water in order to remove debris. The selected dates were soaked for about 20 minutes to soften. After the 20 minutes of soaking, the softened dates were blended with minimal water to form a smooth paste or syrup, and a muslin cloth was used to strain out the skin residues. The Coconut was also washed with water and was grated with distilled water and strained with a muslin cloth to obtain the milk. The dried tigernut was sorted and washed with clean water, then it was soaked in water for 12-24 hours. After 24 hours of soaking, it was blended with water (1:3 ratio tigernut: water).

Yoghurt Preparation

A modified method of Adejo *et al.* (2024) was used in the preparation of the various yoghurts.

Preparation of Plant-Based Yoghurt (PBY)

Exactly 500ml of tigernut milk and coconut milk were heated at 72 °C for 20 minutes and were allowed to cool to 42 - 45 °C, then 0.5g of yoghurt starter culture and 0.3g of CMC were added, and dates were also added depending on the sweetness preference. The mixture was thoroughly stirred to ensure even distribution and was incubated at 45 °C for 10 to 12 hours, and then it was allowed to cool to halt fermentation. The yoghurt was packaged into a sterile container and stored at 4 °C.

Preparation of Dairy-Based Yoghurt (DBY)

Exactly 250g of powdered milk was weighed and dissolved in 1 litre of distilled water. The milk was heated to 72 °C for 20 minutes and allowed to cool to 42 - 45 °C, then 0.5g of yoghurt starter culture and 0.3g of CMC were added, and dates were also added depending on the sweetness preference. The mixture was thoroughly stirred to ensure even distribution and was incubated at 45 °C for 6 to 8 hours, and then it was allowed to cool to halt fermentation. The yoghurt was packaged into a sterile container and stored at 4 °C.

Preparation of Dairy/ Plant-Based Yoghurt (DPBY)

Tigernut and coconut milk were measured at 300ml each and poured into a container. Exactly 100 g of powdered milk was dissolved in 300ml of water and mixed properly. The full blend was heated to 72 ℃ for 20 minutes and cooled to 42-45 ℃, followed by the addition of 0.5g of starter culture and 0.2g of CMC. Dates were added to taste and stirred to ensure even distribution, incubated at 45 ℃ for about 6-8 hours, cooled immediately to halt fermentation and packed into sterile containers and stored at 4 °C.

Determination of Physicochemical Parameters

pH was measured at 25 °C by immersing the electrode in a well-mixed sample and recording a stable reading within 60s; the electrode was rinsed between measurements and stored in an appropriate storage solution (Adejo, 2024). Titratable acidity (TA) was determined on a 10 g sample diluted with about 10–20 mL of distilled water and titrated with standardized 0.1 N NaOH to a phenolphthalein end-point (pH 8.3), according to the method described by AOAC (2012).

Determination of Proximate Composition

Determination of proximate composition was carried out using the method of AOAC (2012).

Determination of Moisture Content

Moisture was determined by oven-drying. A clean, dry Petri dish was oven-dried at 105 °C, cooled in a desiccator, and weighed (W_1). Approximately 2.00 g of homogenized yoghurt sample was added and reweighed (W_2). The dish was placed in a hot-air oven at 105 °C for 1 hour, removed, cooled in a desiccator, and reweighed (W_3). Drying and cooling cycles were repeated until a constant weight was obtained.

Moisture (%) = $W_2 – W_3 / W_2 – W_1 \times 100$

Determination of Ash Content

The total ash was determined by dry ashing in a muffle furnace. Clean, empty crucibles were first ignited at 550 °C for 1 hour, cooled in a desiccator, and weighed. Yoghurt samples (1g each) were weighed into each crucible and charred gently over a low flame to minimize spattering. The crucibles were then placed in a muffle furnace at 550 °C for 5 hours, until the residue turned greyish-white, indicating complete combustion of organic matter.

Ash (%) = Weight of ash/ Original sample weight × 100

Determination of Crude Fat

Crude fat was determined using a Soxhlet apparatus. The dried samples were wrapped in fat-free filter paper and placed in a thimble. The thimble was inserted into the Soxhlet extractor, and extraction was carried out with petroleum ether (boiling point 40–60 °C) for 6 hours,

until the solvent siphoned back clear. The difference in weight represented the extracted fat.
Fat % = Weight of fat extract/ Sample weight × 100

Determination of Crude Protein
Protein content was analyzed by the Kjeldahl method. 1.00 g of the yoghurt sample was digested with concentrated H_2SO_4 in the presence of a catalyst mixture ($CuSO_4$ + K_2SO_4) until a clear solution was obtained. After cooling, the digest was neutralized with 40% NaOH and distilled into a boric acid solution. The distillate was titrated against 0.01 N HCl until the endpoint was reached. Nitrogen content was calculated, and crude protein was obtained using the conventional factor of 6.25.
Crude Protein % = %N × 6.25

Determination of Crude Fiber
The defatted sample was boiled under reflux with dilute H_2SO_4 (1.25%) for 30 minutes, filtered through a muslin cloth, washed with hot distilled water, and then boiled with dilute NaOH (1.25%) under reflux for another 30 minutes. The residue was filtered, washed with hot water, ethanol, and acetone, dried, and weighed. The dried residue was then ashed at 550 °C for 2 hours. Crude fibre was calculated as the difference in weight before and after ashing.
Fibre % = Weight of dried residue - Ash weight/ Original weight × 100

Determination of Carbohydrate
Total carbohydrate was determined by differences between 100 and the total sum of the percentage of fat, moisture, ash, crude fiber and protein content.

Microbiological Analysis Procedures
Preparation for microbiological analysis was carried out according to the method described by ICMSF (2005). The parameters below were obtained using the method as described by Makanjuola (2012) & Matin *et al.* (2018).

Total Viable Count (TVC)
For the determination of the total viable count, 10 g of the yoghurt sample was homogenized in 90 mL of sterile peptone water. The homogenate was then serially diluted, and 0.1 mL from each dilution was plated on Nutrient Agar using the spread plate technique. The plates were incubated at 37 °C for 24 h, after which visible colonies were enumerated and expressed as colony-forming units per gram (CFU/g).

Coliform Count
To determine the coliform count, MacConkey Agar was prepared at a concentration of 55 g/L and sterilized by autoclaving at 121 °C for 15 min. The medium was poured into sterile Petri dishes and allowed to solidify. Each plate was then inoculated with 0.1 mL of the diluted yoghurt suspension, which was evenly spread on the surface. The plates were incubated at 37 °C for 24 h, and colonies that appeared red or pink were counted and expressed as CFU/g.

Lactic Acid Bacteria (LAB) Count
For lactic acid bacteria enumeration, De Man, Rogosa and Sharpe (MRS) Agar was prepared at a concentration of 67.15 g/L and sterilized before use. The agar was dispensed into sterile Petri dishes, and each plate was inoculated with 0.1 mL of the yoghurt dilution. The inoculated plates were incubated anaerobically at 37 °C for 24 h. After incubation, glistening colonies characteristic of lactic acid bacteria were enumerated and further confirmed through biochemical tests.

Yeast Count
To assess the yeast count, 10 g of the yoghurt sample was homogenized in 90 mL of sterile saline solution containing 0.85% sodium chloride. The homogenate was serially diluted, and 0.1 mL aliquots were plated on Potato Dextrose Agar (PDA) acidified with 0.1% lactic acid in order to inhibit bacterial growth. The plates were incubated at ambient temperature for 24–48 h. After incubation, yeast colonies were counted, and the results were expressed as CFU/g

Mould Count
The mould count was carried out using the same procedure as yeast enumeration; however, the PDA plates were incubated at ambient temperature for 5–7 days to allow adequate fungal growth. At the end of the incubation period, visible mould colonies were enumerated and expressed as CFU/g.

Sensory Evaluation
Sensory evaluation was carried out using a 9-point hedonic scale as described by Tamime and Robinson (2007). Sensory evaluation measures consumer perception of product quality based on attributes such as taste, colour, texture, tanginess, and overall acceptability. The dairy and plant-based yoghurt was served chilled (8–10 °C) in coded, identical 30 mL cups, presented monadically in randomized order generated by a balanced design to control presentation bias. The Tanginess, Texture, Colour, Taste, Thickness, and Overall Acceptability of the yoghurt were assessed on a 9-point hedonic scale (1 = dislike extremely; 9 = like extremely), and an inter-sample interval of ≥60 s was enforced. Brief anchoring instructions clarified the attribute meanings (e.g., tanginess as perceived acidity; thickness as spoon resistance). Responses were captured on paper forms or tablets and anonymized.

Experimental Animal
Healthy Albino rats of the Wistar strain at a mean weight of 150-200g were used for this study. These rats were procured from the animal house of Basic Medical Sciences, Uniport. The rats were maintained on Growers Mash (Top feed Ltd.) and water *ad libitum* for a minimum of two weeks before the start of treatment to allow acclimatization.

Experimental Design

This study work was done using the prophylactic approach; the rats were randomly divided into six (6) groups with five (5) rats per group. The groups are designated as follows: group 1 (Normal Control) - Ulcer free and were fed with water and Normal rat chow; group 2 (Negative Control) ulcer induced, fed rat chow but remain untreated; group 3 (Positive control) treated with 20mg/kg body weight Omeprazole; group 4 (Plant-Based Yoghurt-PBY) treated with Plant-based yoghurt; group 5 (Dairy-Based Yoghurt-DBY) treated with Dairy yoghurt and group 6 treated with Dairy/Plant-based yoghurt—DPBY. All treatments were given orally for 14 days. Groups 2–6 were thereafter administered Indomethacin (30 mg/ kg), and rats were sacrificed 4 h later.

Ulcer Induction

Gastric ulceration was induced in the rats according to the procedure described by Sayanti *et al.* (2007). The rats were deprived of food for 24 hours but had free access to water before the oral administration of indomethacin, and were left for a period of 4 hours before being sacrificed. Various degrees of ulceration manifested within these 4 hours period.

Sample Collection

After the treatment of the rats with the reference drug and yoghurt for two weeks, the faeces sample collected during the period of study was stored in the refrigerator for further analysis.

Determination of Gut Byproduct

Derivatization Procedure

The procedure used was a modification of the method of Moldoveanu & David (2019). A 10 µL solution was pipetted into a 10 × 5-mm tube and dried in vacuo at 65°C. To the residue, 30 µL of acetonitrile-water-Phenylisothiocianate (2:2:1 [v/v]) was added and then removed in vacuo at 65°C. Next, 30 µL of the derivatizing reagent acetonitrile-water-Phenylisothiocianate (7:1:1:1 [v/v]) was added, and the tube was agitated and left to stand at room temperature for 20 min. Finally, the solvents were removed under a nitrogen stream, and the tube was sealed and stored at 4°C, pending analysis. Before injection, 150 µL of acetonitrile was added to each tube.

Quantification by GC-FID

The analysis of the metabolites was performed on an Agilent 6890 Gas chromatography equipped with a flame ionization detector. A RESTEK 15-meter MXT-1 column (15m x 250um x 0.15um) was used. The injector temperature was 280 °C with splitless injection of 2 µL of sample and a linear velocity of 30cms-1, Helium 5. 0pa.s was the carrier gas with a flow rate of 40 mlmin-1. The oven operated initially at 200°C, it was heated to 330°C at a rate of 3°C min-1 and was kept at this temperature for 5min. The detector operated at a temperature of 320°C.

Statistical Analysis

All data were expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using SPSS version 25.0. Differences between treatment groups were assessed using one-way analysis of variance (ANOVA) followed by both Tukey's post hoc test and Duncan's multiple range tests for multiple comparisons. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Physicochemical Parameters

The pH (Table 1) of the dairy-based yoghurt (DBY) was found to be 4.00 ± 0.04 in contrast to that of plant-based yoghurt (PBY), which was 3.98 ± 0.05, and that of the blend yoghurt (DPBY), found to be 4.56 ± 0.09, which falls within the Codex Alimentarius Standard recommended range of 4.0–4.6 for fermented milk products. The titratable acidity (TTA) was recorded at 12.26 ± 0.15 mL for diary-based yoghurt, 6.57 ± 0.12mL for plant-based, and 7.92 ± 0.28mL for blend yoghurt, which corresponds to moderate lactic acid development compared to the Codex permissible range of 0.6–1.5% lactic acid for yoghurt. The salinity of the dairy-based yoghurt was 0.41 ± 0.00%, that of plant-based 0.17 ± 0.10 %, and the blend yoghurt 0.36 ± 0.02%, reflecting the natural mineral content of the milk base and additives. The total solids content was 21.54 ± 1.80% for diary-based yoghurt,13.70 ± 1.16% for plant-based, and 22.42 ± 1.10 %for blend yoghurt, which is considerably higher than the minimum FAO/WHO requirement of 8.2% for yoghurt. The sugar content was 13.00 ± 0.00% for diary-based yoghurt,8.00 ± 1.00% for plant-based yogurt and 13.00 ± 1.00% for blend yoghurt (DPBY), well above the 4–6% typically found in plain yoghurt.

Table 1: Physicochemical Properties of Formulated Yoghurts

Parameter	DBY	PBY	DPBY
pH	4.00 ± 0.04[a]	3.98 ± 0.05 [a]	4.56 ± 0.09 [a]
Titratable Acidity (mL)	12.26 ± 0.15[a]	6.57 ± 0.12 [b]	7.92 ± 0.28 [b]
Salinity (%)	0.41 ± 0.00[a]	0.17 ± 0.10 [b]	0.36 ± 0.02 [a]
Total Solids (%)	21.54 ± 1.8 [a]	13.70 ± 1.16 [b]	22.42 ± 1.10 [a]
Sugar Content (%)	13.00 ± 0.00 [a]	8.00 ± 1.00 [b]	13.00 ± 1.00 [a]

Values were expressed as the mean of triplicate determination ± standard error of the mean (SEM). Means followed by different superscript letters in the same row indicate a significant difference at p<0.05

The quality of yoghurt is largely determined by its physicochemical properties. In this study, the physicochemical properties of the three yoghurts (DBY, PBY, and DPBY) were compared to internationally accepted standards and findings from previous studies. The pH, which reveals the level of acidity, is vital for ensuring microbiological safety, as it creates an environment unfavourable for the growth of most pathogenic and spoilage microorganisms. Additionally, it contributes to the development of the characteristic tart and refreshing taste of yoghurt. The value obtained is similar to that reported by Adejuyitan *et al.* (2014), who observed a pH of 4.12 in fortified yoghurt products, although slightly lower than the 4.42 reported by El-Kholy *et al.* (2019) in plain cow's milk yoghurt, possibly due to variations in fermentation conditions and compositional differences. The titratable acidity level indicates effective fermentation by lactic acid bacteria, though the relatively lower value compared to previous studies (Ozer, 2009), who reported 17.8 mL in fruit-sweetened yoghurt, may be attributed to the sugar content of date syrup, which could mask acid perception while balancing flavour. This balance between acidity and sweetness is essential for consumer acceptability. The salinity of yoghurt reflects the natural mineral content of the milk base and additives. Although salinity is not always a major focus in yoghurt characterization, it influences osmotic balance and taste perception. Similar salt levels have been reported in yoghurt formulations, where they contribute to overall flavour without being perceptible as "salty" (Massomian *et al.,* 2025). High total solids improve the viscosity, mouthfeel, and retention of flavour compounds, resulting in enhanced textural and sensory quality. The value is slightly higher than the 21.1% reported in other sweetened yoghurt formulations, indicating that the addition of date syrup significantly contributed to the solid content. The sugar level in the various yoghurts was elevated (Hussein *et al.,* 2017). This elevated sugar level is largely due to the natural glucose, fructose, and sucrose in date syrup, in addition to residual lactose from milk. Similar findings were reported by Hasnae *et al.* (2017), who noted sugar values around 12.8% in date syrup-sweetened yoghurt. The high sugar content enhances palatability, masks acidity, and makes the yoghurt more appealing to consumers.

Proximate Composition

The moisture content of the yoghurts ranged from 78.46 ± 1.10% 87.46 ± 1.16%, with the PBY having the highest value (Table 2). The ash content of 0.636% for DBY,0.988% for PBY and 0.755% for DPBY. DBY had the highest fat content of 6.22%, followed by 4.86% and 2.56% for DPBY and PBY, respectively. There was no significant ($p>0.05$) difference in the fibre content of the three formulated yoghurts. The fiber ranged from 0.14% for DBY to 0.18% for PBY. Protein content was 3.50% for DBY, 4.02% for PBY, slightly above the Codex minimum requirement of 2.7% for yoghurt. While carbohydrate content ranged from 1.87% (DBY) to 12.21% (DPBY).

Table 2: Proximate Composition of Formulated Yoghurts (%)

Parameter	DBY	PBY	DPBY
Moisture Content (%)	78.46 ± 1.80	87.46 ± 1.16	78.46 ± 1.10
Ash Content (%)	0.64± 0.00 [a]	0.99± 0.00 [b]	0.76± 0.01 [a]
Fat Content (%)	6.22± 0.02 [a]	2.56± 0.00 [b]	4.86± 0.04 [c]
Fibre Content (%)	0.14± 0.02[a]	0.18± 0.03 [a]	0.14± 0.00 a
Protein Content (%)	3.50± 0.04 [a]	4.02± 0.02 [a]	1.05± 0.00 [b]
Carbohydrate Content (%)	1.87± 0.01 [a]	4.05± 0.80 [b]	12.21± 1.03 [c]

Values were expressed as the mean of triplicate determination ± standard error of the mean (SEM). Means followed by different superscript letters in the same row indicate a significant difference at $p<0.05$

The moisture content of the various yoghurts agrees with the moisture content of most commercial yoghurts (75–88%). Adequate moisture content ensures a semi-solid consistency, which is desirable for yoghurt texture and consumer perception. This value also complements the high total solids, indicating a balanced composition that supports both texture and overall acceptability. The fat content of 6.22% in the dairy-based yoghurt (DBY) places it in the category of whole-milk yoghurt according to Codex standards, which specify >3.0% fat for whole-milk varieties. This is higher than values for low-fat yoghurts (0.5–2%), like the plant-based yoghurt (PBY) that has a fat content of 2.56%, but comparable to the 6.4% reported by Leeward *et al.* (2023) in full-cream milk yoghurt fortified with fruit puree. High fat levels contribute positively to creaminess, viscosity, and flavour perception, making the yoghurt more appealing to consumers who prefer richer textures. The protein content ranged from 1.05% to 4.02%. This protein level is comparable to the 3.4% reported by El-Kholy *et al.,* (2019), indicating that the use of powdered milk preserved protein concentration despite dilution from the syrup, and the DPBY (1.05%), which is very low in protein. However, it is lower than the 4.0–4.5% reported in some concentrated Greek-style yoghurts. The ash content of (0.64% - 0.99%) reflects the mineral richness of the product, likely enhanced by date syrup, which is known to contain potassium, magnesium, calcium, and trace elements. This aligns with the 0.68% ash content reported by Amadou *et al.* (2017) in yoghurt fortified with date paste. The fibre content (0.14% - 0.18%) originates from the natural soluble and insoluble fibre in date syrup,

which can also act as a prebiotic to support beneficial gut microbiota (Holscher, 2017). The carbohydrate content in the proximate analysis was 1.87% for dairy, which seems low compared to the sugar content measured in the physicochemical analysis. This discrepancy is because lactose conversion to lactic acid during fermentation reduces the carbohydrate fraction in proximate calculations, while total sugar analysis measures both lactose and added sugars.

The microbiological quality of the yoghurts met international safety standards with a value of 2.8 × 10^3 CFU/g for DBY, 1.9 ×103 for PBY and 2.3 ×103 DPBY blend (Table 3). Lactic acid bacteria (LAB) were recorded at 2.0 × 10^2 CFU/g for DBY, 2.2 ×102 CFU/g for PBY and 1.6 ×107 CFU/g for DPBY blend. Yeast counts ranged from 2.2 × 10^2 CFU/g (DBY) to 9.0 × 101 CFU/g (DPBY). Coliform and Moulds were basically absent. However, the plant-based recorded Coliform content as 8.0 × 102 CFU/g.

The International Commission on Microbiological Specifications for Foods (ICMSF) recommends that yoghurt should contain <10^6 CFU/g of total viable bacteria for safety, and the concentrations obtained in this study (1.9 ×103 - 2.8 × 10^3 CFU/g) are within the acceptable limit. Lactic acid bacteria (LAB) level recorded confirms active fermentation and probiotic potential (Sidhu *et al.,* 2020). The value for DBY and PBY is slightly lower than the 10^6–10^8 CFU/g recommended for probiotic-labelled products, suggesting that while the product has fermentative activity, higher starter culture inoculation or shorter storage before analysis might improve probiotic viability (Olson *et al.,* 2022). Yeast counts were low, indicating minimal spoilage activity. The absence of mould suggests good packaging and storage practices, as mould contamination is a common cause of yoghurt spoilage in tropical environments.

Table 3: Microbiological Quality of the Formulated Yoghurts

Samples	TVC (CFU/g)	Coliform (CFU/g)	LAB (CFU/g)	Yeast (CFU/g)	Mould (CFU/g)
DBY	2.8 × 10^{3a}	Nil	2.0 × $10^{2\ a}$	2.2 × $10^{2\ a}$	Nil
PBY	1.9 × $10^{3\ a}$	8.0 × $10^{2\ b}$	2.2 × $10^{2\ a}$	2.4 × $10^{2\ a}$	NIL
DPBY	2.3 × $10^{2\ a}$	NIL	1.6 × $10^{7\ a}$	9.0 × $10^{1\ b}$	NIL

Values were expressed as the mean of triplicate determination ± standard error of the mean (SEM). Means followed by different superscript letters in the same column indicate a significant difference at p<0.05

The sensory evaluation revealed that Colour received the highest mean score (6.50), indicating strong visual appeal (Table 4). Thickness and texture were moderately rated, consistent with the influence of high total solids on mouthfeel. Tanginess was the lowest-rated attribute. Taste and overall acceptability were moderate.

Table 4: Sensory Scores of the Formulated Yoghurts

Attribute	DBY	PBY	DPBY
Tanginess	4.73 ± 0.64^a	4.30 ± 0.07 ^{a}	6.50 ± 0.06 ^{a}
Texture	5.50 ± 0.99 ^{a}	4.90± 0.10 ^{a}	7.90 ± 0.10 ^{ab}
Colour	7.41 ± 0.53 ^{a}	4.41 ± 0.03 ^{b}	7.73 ± 0.20 ^{a}
Taste	5.23 ± 0.05 ^{a}	4.62 ± 0.80 ^{a}	7.33 ± 0.07 ^{ab}
Thickness	5.86 ± 0.73 ^{a}	5.11 ± 0.60 ^{a}	7.24 ± 0.50 ^{a}
Overall Acceptability	5.45 ± 0.92 ^{a}	4.84 ± 0.82 ^{a}	5.30 ± 0.02 ^{a}

Values were expressed as the mean of triplicate determination ± standard error of the mean (SEM). Means followed by different superscript letters in the same row indicate a significant difference at p<0.05

The sensory evaluation revealed interesting patterns in consumer perception. Colour received the highest mean score, indicating strong visual appeal. This is likely due to the golden-brown hue imparted by date syrup, which previous studies (Al-Farsi *et al.,* 2005) have found to be associated with natural and wholesome food products in consumer minds. Thickness and texture were moderately rated, consistent with the influence of high total solids on mouthfeel. However, the scores suggest that further adjustment of milk solids or stabiliser content could improve consistency. Tanginess was the lowest-rated attribute, reflecting reduced acidity perception due to the masking effect of high sugar content from date syrup. This finding corroborates the findings of Ozer *et al.* (2009), who reported that sweet syrups can suppress sourness, leading to more neutral-tasting yoghurt. Taste and overall acceptability were moderate, showing that while the product was generally liked, opinions were diverse, possibly due to individual differences in sweetness–acidity preference balance.

The results obtained from the study of Gut microbiota metabolites revealed that DBY, PBY and the DPBY play a distinct role in modulating the gut microbiota, leading to the production of certain byproducts or metabolites

such as Short Chain Fatty Acids (SCFA) and MCFA. The results, as seen in Table 5, revealed that omeprazole (positive control group) and plant-based yoghurt (PBY) significantly reduced the concentrations of most Short Chain Fatty Acids, including acetic acid and butyric acid, in contrast with the Negative control group (indomethacin untreated group), which had a significant concentration of acetic acid and butyric acid. High concentration of valeric acid and Hexanoic acid/isovaleric acid concentration were observed in the positive control group and plant-based group, respectively. Group treated with Dairy yoghurt (DBY) had a high concentration of caproic acid (138.85504 ppm), followed by valeric acid (73.19835) and PBY had high concentration of hexanoic acid (53.15172 ppm), while the dairy/plant-based (DPBY) group recorded high capronic acid (92.83699 ppm).

Table 5: Concentrations of Gut byproduct (ppm) extracted from the faecal matter of Wistar rats fed with the formulated yoghurts

Byproduct	Normal Control	Negative Control	Omeprazole	DBY	PBY	DPBY
Acetic acid	11.77339	34.07148	4.61232	5.31692	6.08923	19.69051
Propanoic acid	60.81805	25.58375	1.01833	64.02499	0.848676	25.49035
Butyric acid	24.88348	51.86744	2.01720	61.79941	0.782021	11.78075
Isobutyric acid	2.37919	12.68210	7.23539	12.78516	2.17143	2.72455
Isovaleric acid	3.32804	15.18337	8.44093	15.15977	15.62704	0.988662
Valeric acid	1.72306	12.32459	101.06866	73.19835	4.40466	7.73700
Hexanoic acid	2.16389	12.07867	4.30008	1.39930	53.15172	0.980037
Caproic acid	3.79619	19.83602	2.48948	138.85504	10.58862	92.83699

The results, as seen in Table 5, revealed that omeprazole significantly reduced the concentrations of most Short Chain Fatty Acids, including acetic acid and butyric acid. Omeprazole intake reduces the production of short-chain fatty acids (SCFAs) in the gut of ulcerogenic rats primarily due to the alteration of the gut microbiota composition and the reduction in the abundance of beneficial SCFA-producing bacteria, such as Lactobacillus (Imahan *et al.,* 2016). Omeprazole, a proton pump inhibitor (PPI), works by suppressing gastric acid secretion, which significantly increases the stomach's pH. The less acidic environment compromises the stomach's natural barrier against ingested bacteria, allowing oral and other non-commensal bacteria to survive and reach the lower gastrointestinal tract in higher numbers (Koo *et al.,* 2019). This leads to an alteration or dysbiosis of the gut microbiota. The intake of certain plant-based yoghurts may reduce the production of short-chain fatty acids (SCFAs) in ulcerogenic rats primarily due to differences in fatty acid composition and the nature of the plant additives used. Some plant additives cause a significant decrease in saturated fatty acids (SFAs) and SCFAs, while increasing polyunsaturated fatty acids (PUFAs). Not all plant-based yoghurts contain the specific types or quantities of prebiotics (like certain fibers) or live probiotic strains (such as Lactobacillus gasseri) that are efficiently fermented into beneficial SCFAs in the gut of the specific rat model. The specific ingredients in the plant-based yoghurt may not support the growth of the key SCFA-producing bacteria in the gut microbiome of the ulcerogenic rats as effectively as other diets. Differences in how the plant material is processed into yoghurt (e.g., heat treatment may affect probiotic viability) can influence the final product's ability to support SCFA production. The increase in acetic acid and butyric acid concentration in the gut of ulcerogenic rats is related to gut microbial dysbiosis and the body's response to inflammation and injury. Ulcers disrupt the normal gut environment, leading to a shift in the bacterial populations and their metabolic activities (Jackson *et al.,* 2016; Imhann *et al.,* 2016).

However, the high concentration of valeric acid in the Positive control group suggests that omeprazole allowed the growth of other gut microbiota, which favours the fermentation pathway of this byproduct. A significant increase in Hexanoic acid and isovaleric acid concentration suggests that plant-based yoghurt, known for its rich fibers and phytochemical content, can selectively modulate microbial populations, promoting pathways for the fermentation of different gut byproducts distinct from those in dairy substrates (Marco *et al.,* 2017). In contrast, group treated with Dairy yoghurt had a high concentration of caproic (138.85504 ppm) and valeric (73.19835 ppm), and a mild concentration of isobutyric acids (12.78516 ppm) and Isovaleric acid (15.15977 ppm), supporting the fact that Dairy yoghurt is a traditional probiotic carrier which enhances the production of gut byproducts with gastroprotective properties (Lorea *et al.,* 2007). The group treated with Plant-based yoghurt produced a high concentration of hexanoic acid, indicating an enhanced lipid metabolism. Hexanoic acid is a medium-chain fatty acid (MCFA) which plays a role in the gastrointestinal tract. Hexanoic acid maintains the gut microbial balance through its antimicrobial properties. Studies have shown that medium-chain fatty acids, including hexanoic acid, can disrupt bacterial cell membranes, thereby reducing the growth of pathogenic microbes (Zhou *et al.,* 2025). Hexanoic acid has been reported by Liu *et al.* (2021) to play a crucial role in modulating inflammation and gut

barrier integrity, thereby enhancing mucosal defence and supporting a healthier gut environment. In contrast, the DPBY group had a high concentration of caproic acid (92.83699 ppm), which is also a medium-chain fatty acid similar to hexanoic acid. This medium-chain fatty acid has antimicrobial properties, modulating inflammation, thus enhancing mucosal defence.

CONCLUSION

This study formulated and evaluated DBY, PBY, and DPBY, confirming they are feasible, safe for consumption, and also align with established international quality standards. The physicochemical, proximate, and microbiological analyses demonstrated that all three yoghurt samples met recommended ranges for pH, acidity, and milk solids, thereby ensuring microbial stability and nutritional benefit. Specifically, the microbiological quality for all samples was within safety limits, with viable Lactic Acid Bacteria (LAB) counts confirming active fermentation and probiotic potential. Furthermore, the study suggests that DBY, PBY and DPBY play a crucial role in modulating the gut microbiota by enhancing the production of beneficial gut byproducts, particularly Short Chain Fatty Acids (SCFAs). In general, these yoghurts offer a safe, consumer-acceptable, and functional food approach for the potential management of gastric ulcer conditions through the positive modulation of the gut microbiota.

Conflict of Interest

Authors have declared that no competing interests exist.

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