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Unraveling the Development of A2 β-Casein Yoghurt Fortified with *Moringa oleifera* Lam. Leaf Extract from Physicochemical and Functional Aspects

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ABSTRACT

The escalating global burden of metabolic disorders and micronutrient deficiencies necessitates functional dairy innovations delivering concurrent antioxidant, bioactive component enrichments, and gastrointestinal health amelioration. This study investigated the effects of fortifying A2 β-casein yoghurt with 0%, 0.5%, and 1.5% of aqueous Moringa oleifera *Lam*. (M. oleifera) *leaf extract (MLE) on its physicochemical, rheological, and functional properties. The yoghurt formulations were evaluated for pH, titratable acidity, acetaldehyde content, proximate composition, antioxidant activity, viscosity, curd tension, and syneresis. Principal Component Analysis (PCA) was employed to identify key relationships and elucidated correlations between variables. Results showed that increasing MLE concentration significantly elevated antioxidant activity (27.12%–96.37%) and ash content (0.79%–1.17%), while maintaining stable carbohydrate levels (20.37–20.38%). Higher MLE doses reduced viscosity (1,178.26 P–897.13 P) and curd tension (26.82 g/50 mL–17.84 g/50 mL), indicating softer gel structures. Acetaldehyde content rose with MLE addition (27.76–34.80 µmol/100 g), suggesting enhanced flavor compound production. Moreover, PCA revealed strong inverse relationships between acidity-related parameters and texture, emphasizing a trade-off between functionality and sensory quality. The study demonstrates that MLE fortification at 1% (T2) optimally balances antioxidant enrichment (94.67%) with acceptable texture. These findings provide practical insights for developing functional yoghurts tailored to health-conscious consumers. This research contributes significantly to the field by providing quantitative evidence of MLE's effects on yoghurt matrix properties. The results advance the utilization of plant bioactives in dairy science, supporting the development of innovative, health-focused yoghurt products that meet evolving consumer preferences for functional foods with enhanced nutritional profiles.*

Keywords: *A2 β-casein yoghurt; Aqueous moringa leaf extract fortification; Functional dairy products; Antioxidant activity; Food quality*

INTRODUCTION

Prediabetes represents a pivotal stage for intervention, as progression to Type 2 Diabetes (T2D) can be slowed or even halted through lifestyle changes, particularly dietary adjustments (Villegas-Vazquez et al., 2025; Wajs et al., 2023). The World Health Organization (WHO), strongly advocate for the consumption of low-fat or non-fat dairy products as part of these dietary modifications (Slurink et al., 2023). This recommendation aims to reduce overall caloric intake and limit saturated fatty acids (SFAs), which are linked to improved metabolic health outcomes (Sumarmono et al., 2023). In recent years, consumer preferences have shifted toward functional foods that offer health benefits beyond basic nutrition, driven by increasing awareness of diet-related wellness (Adepoju et al., 2024; Nystrom & Winston, 2016; Shi et al., 2019). Particularly, functional dairy products have gained prominence due to their potential to deliver probiotics, prebiotics, and bioactive compounds that enhance gut health and overall immunity (Abdelshafy et al., 2022; Maftei et al., 2024; Zahid, Ranadheera, et al., 2022). Moreover, the paper by Rahmasari & Rosida (2023) present the results of research demonstrating that yogurt possesses hypocholesterolemic properties effective for

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dietary modification. It shown that yoghurt exhibits potential functionality to address hypercholesterolemia, a disorder characterized by elevated blood cholesterol levels (Rahmasari & Rosida, 2023). Yoghurt stands out as a widely consumed fermented dairy product, valued for its nutritional profile, probiotic content, and antioxidant activity (Gu et al., 2022; Okpala, 2024; Pancapalaga & Ashari, 2020; Priyashantha et al., 2025). Non-fat yoghurt, in particular, appeals to health-conscious consumers seeking low-calorie alternatives to mitigate risks associated with obesity and cardiovascular diseases.

However, the reduction of fat in milk often compromises texture and sensory properties. There are still unresolved questions related to how innovative approaches can optimally improve its functional and physicochemical quality without compromising nutritional benefits (García-Gómez et al., 2019). The reasons for limited progress in low-fat yoghurt development may be objective difficulties associated with texture preservation and the principal impossibility of completely replicating fat's functional properties (Guénard-Lampron et al., 2019). Besides, the costly part in the plan of extensive texture analysis includes evaluating several main parameters like cohesiveness, hardness, springiness, adhesiveness, and gumminess to address its physical properties and sensory perception, which makes comprehensive research impractical in industrial settings. An option to overcome these relevant difficulties can be fortification with bioactive ingredients such as *Moringa oleifera* Lam. (MLE), renowned for its exceptional nutritional and therapeutic properties that may address multiple quality parameters (Ab et al., 2024; Abdelshafy et al., 2022; Adepoju et al., 2024; Gomes et al., 2023; Pop et al., 2022). *M. oleifera* leaves are rich in essential minerals (calcium, potassium, and iron), vitamins (A and C), medicinal properties, and antioxidants like flavonoids and phenolic compounds, which contribute to anti-inflammatory, antimicrobial, and antidiabetic effects (Abdelshafy et al., 2022; Firdausy et al., 2020; Pop et al., 2022). Recent studies have demonstrated that MLE enhances the antioxidant activity and mineral content of dairy products while improving water-holding capacity and reducing syneresis (Gomes et al., 2023; Gupta et al., 2024; Okpala, 2024; Saeed et al., 2021). Nevertheless, its application in yoghurt made from A2 β-casein milk, which is a variant associated with easier digestibility and reduced gastrointestinal discomfort, remains underexplored.

The integration of synbiotics (combination of probiotics and prebiotics) with A2 β-casein milk and MLE presents a novel approach to developing functional yoghurt. While conventional yoghurt relies on A1 β-casein, A2 milk has gained attention for its potential to alleviate lactose intolerance symptoms and improve metabolic outcomes (de Gaudry et al., 2019; Fernández-Rico et al., 2022; Lv et al., 2020; Nystrom & Winston, 2016; Prabakusuma et al., 2022). Combining A2 β-casein milk with MLE could synergistically enhance the product's nutritional value, antioxidant capacity, and sensory attributes. All of this suggests that it is appropriate to conduct a study on MLE-fortified A2 yoghurt. The physicochemical interactions between these components, their impact on fermentation kinetics, and improving the quality of non-fat yoghurt require systematic investigation. This study aimed to evaluate the physicochemical quality of nonfat yoghurt made from A2 β-casein skim milk powder reconstituted in MLE. Key parameters included pH, titratable acidity, acetaldehyde content, chemical composition, antioxidant activity, curd tension, apparent viscosity, and syneresis. In this study, Principal Component Analysis (PCA) was also used to reduce data dimensionality, visualize correlations between physicochemical properties, and identify key quality drivers in MLE-fortified yoghurt (Maritha et al., 2022; Sharma & Ramanathan, 2023). It revealed the trade-

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off between antioxidant enhancement and texture deterioration for guiding optimal formulation design. The findings provide insights into optimizing functional yoghurt formulations that leverage the combined benefits of A2 β-casein milk and MLE, addressing gaps in current research while meeting consumer demand for health-focused dairy products.

METHODS

Preparation of MLE

Fresh *M. oleifera* leaves were thoroughly rinsed with distilled water to remove surface impurities. The cleaned leaves were then oven-dried at 55°C for 48 hours to ensure complete dehydration while preserving heat-sensitive bioactive compounds. After drying, the leaves were ground into a fine powder using an electric grinder to maximize surface area for extraction. The resulting powder was stored in airtight, light-protected plastic containers at ambient temperature to prevent moisture absorption and oxidative degradation. For aqueous extraction, the powdered leaves were mixed with boiled distilled water at concentrations of 0.5%, 1%, and 1.5% (*w/v*). The suspensions were continuously stirred at room temperature for 30 minutes to facilitate the diffusion of water-soluble phytochemicals. The mixtures were sequentially filtered, first through a double-layered muslin cloth to remove coarse particles, followed by a second filtration using Whatman No. 1 filter paper (Cytiva, Marlborough MA, USA) to eliminate finer residues.

Non-fat yoghurt manufacturer

The A2 β-casein skim milk powder (The a2 Milk Company™, North Sydney, NSW, Australia) was reconstituted in MLE pre-warmed to 45°C at a concentration of 14% *w/v* (140 g/L) using ultrapure water from a Milli-Q® Type 1 Ultrapure Water System (Merck Millipore, Waltham, MA, USA). The reconstituted milk was homogenized using a high-pressure homogenizer (Shanghai Donghua High Pressure Homogenizer Co., Ltd., Shanghai, China) and distributed into four sterile 1 L Schott Duran® bottles (Mainz, Germany) to create separate milk bases (Zahid, Ranadheera, et al., 2022). Three experimental treatments were formulated with different MLE concentrations: T0 (0%), T1 (0.5%), T2 (1%), and T3 (1.5%), each supplemented with 1% inulin (*w/v*), 1% psyllium husk (*w/v*), 1% milk flavor (*w/v*), 1% Stevia natural sweetener (*w/v*), and 1% food texturizer and homogenizer additives. A control treatment was prepared following the same protocol but using distilled water in place of MLE.

All yoghurt bases were refrigerated overnight at 6±1°C to ensure complete hydration of milk solids before being pasteurized at 80±5°C for 30 minutes. Following pasteurization, the bases were cooled to 40 ± 2°C and inoculated with 0.05% (*w/v*) of a commercial direct vat set (DVS) YO-MIX® 300 LYO 10 DCU yoghurt starter culture (Danisco, Copenhagen, Denmark), which contained defined strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* with a minimum viability of 10^{11} CFU/g (Adepoju & Selezneva, 2024). Fermentation was conducted at 40±2°C until reaching a target pH of 4.6±0.2, typically achieved within 4-5 hours, with pH monitored periodically using a calibrated digital pH meter (Adepoju et al., 2024). Upon reaching the desired acidity, the coagulated yoghurt was immediately cooled in an ice water bath to 20°C, gently stirred to ensure uniform texture, portioned into sterile 100 mL polypropylene containers, and stored at 5±1°C for 24 hours to allow proper cooling and texture development before subsequent analysis (Adepoju & Selezneva, 2024; Zahid, Ali, et al., 2022).

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Analysis of non-fat A2 β-casein yoghurt samples

Measurement of pH, titratable acidity, and acetaldehyde content

The non-fat A2 β-casein yoghurt's stability was assessed by monitoring pH and titratable acidity (TA) during 4°C storage. pH was measured using a calibrated pH meter with E-201F electrode (China), following AOAC 947.05 after daily calibration with pH 4.01/7.01 buffers (Hanna, Australia). TA was determined by titrating 10 g yoghurt (homogenized with 20 mL water) with 0.1 M NaOH using a Dosimat burette (Switzerland) to pH 8.2 endpoint (Zahid, Ranadheera, et al., 2022). Results were calculated as lactic acid percentage (Eq. 1):

$$\%TA = V\ x\ N\ \frac{90.08}{W\ x\ 10} \quad (1)$$

where, V: NaOH volume, N: normality, and W: sample weight. All measurements were triplicated at 25±0.5°C following ISO 2963:2010. Acetaldehyde was quantified via colorimetric method. The reaction with sodium nitroprusside in alkaline medium produced a 572 nm-measurable purple complex. After triplicate measurements, results were expressed as µmol/100 g yoghurt, which is providing reliable quantification while minimizing interference from other carbonyls (Li et al., 2024; Tian et al., 2020).

Proximate composition analysis

The A2 β-casein yoghurt's composition was analyzed using standard methods. Protein content was determined via Kjeldahl analysis (AOAC 991.20) using a K9840 analyzer (Hanon, China), applying a 6.38 conversion factor. Fat content was measured by Mojonnier extraction (AOAC 996.06) with a SOX406 system (Lanyi, China). Carbohydrates were calculated by difference. Moisture (AOAC 934.01) was assessed by drying at 105°C (DHG-9070A oven, Yiheng, China), while ash content (AOAC 925.45) was determined by combustion at 550°C (SX2-4-10 furnace, Jing Hong, China). All analyses were performed in triplicate to ensure accuracy (Zahid, Ranadheera, et al., 2022).

Apparent viscosity, curd tension, and syneresis measurements

The rheological properties of the yoghurt samples were characterized through comprehensive texture and viscosity analyses. Apparent viscosity measurements were conducted at 4 ± 0.5°C using a controlled shear rate ramp from 0.1 to 100 s^{-1} over 120 seconds. Gel strength was evaluated via penetration testing with a TAXT Plus texture analyzer (Stable Micro Systems Ltd., Godalming, UK). Parallel viscosity measurements were performed using a Fungilab Premium series viscometer (Fungilab China, Shanghai, China) with TR2 spindle rotating at 100 rpm (equivalent to 128 s^{-1} shear rate) for 30 seconds, with several modification (Guénard-Lampron et al., 2019). All determinations were replicated three times, with viscosity reported in Pascal-seconds (Pa·s) and gel hardness in Newtons (N). Precise temperature control (±0.1°C) was achieved using a DC-0506 refrigerated circulator (Shanghai Hengping Instrument Factory, Shanghai, China). Curd tension (g/50 ml) measured gel resistance, while syneresis (g/50 ml) assessed whey separation. Both tests were also conducted in triplicate under controlled conditions.

Antioxidant activity measurement

The antioxidant capacity of A2 β-casein yoghurt was evaluated following an adapted protocol from Adepoju and Selezneva (2024). For sample preparation, 10 g aliquots were

centrifuged at 6,000 × g for 20 minutes at 4°C using a CR22G III refrigerated centrifuge (Hitachi Koki, Tokyo, Japan). The resulting supernatant was filtered through 0.45 µm pore size Whatman™ cellulose membranes (GE Healthcare Life Sciences, Maidstone, UK) and immediately stored at -80°C in a Sanyo ULT-1386 ultra-low temperature freezer (Panasonic Healthcare, Osaka, Japan) to preserve antioxidant integrity prior to analysis. The DPPH radical scavenging assay was performed by reacting 0.5 mL of yoghurt extract with 0.1 mM methanolic DPPH solution (100 µM final concentration) (Ul et al., 2025). After 30 minutes of dark incubation at 25±1°C, absorbance measurements at 517 nm were conducted using a SpectraMax® M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA) with Corning® Costar 96-well plates (Corning Incorporated, NY, USA) (Adepoju et al., 2024; Adepoju & Selezneva, 2024). The assay included appropriate controls consisting of methanol blanks and DPPH solution controls. Quantification was achieved through an ascorbic acid standard curve (5-50 µg/mL concentration range). Radical scavenging activity was technically calculated according to the equation 2:

$$DPPH\ scavenging\ activity\ (\%)\ =\ [1-\left(\frac{Abs_{\ sample}}{Abs_{\ control}}\right)\times 100\%] \quad (2)$$

where, Abs sample: absorbance of the test solution; and Abs control: absorbance of the DPPH control solution. This standardized methodology provides reliable quantification of the antioxidant potential in dairy matrices while accounting for potential interference from sample matrices. All measurements were performed in triplicate to ensure analytical precision.

Statistical Analysis

The study employed a completely randomized design to evaluate the effects of MLE fortification on non-fat s A2 β-casein yoghurt. Data were analyzed using IBM SPSS Statistics 27 (IBM Corp., Chicago, USA) for one-way analysis of variance (ANOVA) with Tukey's post-hoc test (α = 0.05). Triplicate results are expressed as mean ± SD, with normality (Shapiro-Wilk, p>0.05) and homogeneity (Levene's test) confirmed (Adepoju & Selezneva, 2024). Principal component analysis was conducted in OriginPro 2024 (OriginLab, Northampton, MA, USA) to assess variable correlations (OriginLab, 2024). Data were mean-centered and unit-variance scaled before singular value decomposition with Eigenvalues >1. Biplots visualized loadings (>|0.5|) and scores, with model validity confirmed by R^2 and Q^2 > 0.5. The first two components explained >70% variance, which identifying key physicochemical drivers. PCA complemented ANOVA by revealing multivariate patterns in yoghurt quality parameters and enhancing interpretation of treatment effects.

RESULTS AND DISCUSSION

pH Value, Titratable Acidity, And Acetaldehyde Content

The physicochemical and functional properties of A2 β-casein yoghurt fortified with aqueous MLE demonstrated significant variations across treatments, revealing critical insights into the interactions between MLE concentration and yoghurt matrix components. The comprehensive analysis of pH, titratable acidity, acetaldehyde content, gross composition, rheological properties, and antioxidant activity, supported by multivariate PCA, provides a systematic understanding of how MLE fortification influences product quality. Table 1 reveals that increasing MLE concentration (0–1.5%) progressively lowered pH values (4.55 to 4.49) and elevated titratable acidity (0.89% to 0.94% lactic acid). This trend aligns with the findings of

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Zhang et al. (2019), who reported enhanced acidification in MLE-fortified yoghurt due to bioactive compounds in MLE stimulating lactic acid bacteria (LAB) activity (Zhang et al., 2019). The acetaldehyde content, a key flavor compound, increased significantly (27.76 to 34.80 µmol/100g) with MLE concentration. This suggests that MLE polyphenols may act as electron acceptors, promoting pyruvate metabolism in LAB toward acetaldehyde production rather than lactate (Li et al., 2024; Tian et al., 2020). However, the pH reduction remained within the optimal range for yoghurt stability (pH 4.2–4.6), indicating that MLE fortification does not compromise microbial safety.

Gross chemical composition, apparent viscosity, curd tension, and syneresis

Table 2 shows no significant changes in moisture (91.27–91.32%) or carbohydrate content (20.37–20.38%) across treatments, confirming that MLE primarily contributes minerals and bioactive compounds rather than altering macronutrient distribution. The ash content increased linearly (0.79% to 1.17%), reflecting MLE's high mineral density (Peñalver et al., 2022). Notably, antioxidant activity surged from 27.12% (control) to 96.37% (T3), consistent with Gomes et al. (2023), who attributed this to MLE's flavonoid content (quercetin, kaempferol, epicatechin, and catechin) (Gomes et al., 2023). The stability of protein content (3.58–3.44%) despite MLE addition contradicts Gomes et al. (2023), possibly due to differences in MLE preparation methods affecting protein-polyphenol interactions.

Table 1. pH value, titratable acidity, and acetaldehyde content.

Formulation	pH value	Titratable acidity (%)	Acetaldehyde content (µmol/100 g)
T0 (0%)	4.55±0.02^{a}	0.89±0.02^{a}	27.76±0.75^{a}
T1 (0.5%)	4.54±0.02^{ab}	0.91±0.02^{b}	29.22±0.27^{a}
T2 (1%)	4.52±0.02^{ab}	0.92±0.01^{c}	31.20±1.24^{b}
T3 (1.5%)	4.49±0.02^{b}	0.94±0.01^{d}	34.80±0.11^{c}

T0: Nonfat non-fat s A2 β-casein yoghurt samples manufactured from reconstituted skim milk powder in distilled water. T1, T2, and T3: Nonfat non-fat s A2 β-casein yoghurt samples manufactured from reconstituted A2 β-casein skim milk powder in aqueous MLE 0.5, 1, and 1.5%, respectively. Means ± standard deviations with different small letters represent significantly different among formulations (p<0.05).

Table 2. Gross chemical composition and antioxidant activity.

Formulation	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Antioxidant (%)
T0 (0%)	91.27±1.56^{a}	0.79±0.03^{a}	2.35±0.06^{a}	3.58±0.04^{a}	20.37±0.04^{a}	27.12±0.26^{a}
T1 (0.5%)	91.28±0.03^{a}	0.95±0.01^{a}	2.58±0.04^{ab}	3.54±0.03^{ab}	20.38±0.06^{a}	92.80±0.94^{b}
T2 (1%)	91.29±0.79^{a}	0.98±0.11^{a}	2.72±0.04^{bc}	3.47±0.04^{bc}	20.38±0.06^{a}	94.67±0.39^{c}
T3 (1.5%)	91.32±0.20^{a}	1.17±0.03^{a}	2.91±0.02^{c}	3.44±0.04^{c}	20.38±0.05^{a}	96.37±0.65^{d}

T0: Non-fat A2 β-casein yoghurt samples manufactured from reconstituted skim milk powder in distilled water. T1, T2, and T3: Non-fat A2 β-casein yoghurt samples manufactured from reconstituted A2 β-casein skim milk powder in aqueous MLE 0.5, 1, and 1.5%, respectively. Means ± standard deviations with different small letters represent significantly different among formulations (p<0.05).

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Table 3. Apparent viscosity, curd tension, and syneresis.

Formulation	Apparent viscosity (P)	Curd tension (g/50 ml)	Syneresis (g/50 ml)
T0 (0%)	1,178.26±8.44^{d}	26.82±0.34^{d}	2.21±0.12^{c}
T1 (0.5%)	1,075.10±7.14^{c}	22.82±0.13^{b}	1.52±0.17^{c}
T2 (1%)	992.97±6.48^{b}	20.56±0.22^{c}	0.78±0.01^{b}
T3 (1.5%)	897.13±3.27^{a}	17.84±0.36^{a}	0.60±0.04^{a}

T0: Non-fat A2 β-casein yoghurt samples manufactured from reconstituted skim milk powder in distilled water. T1, T2, and T3: Non-fat A2 β-casein yoghurt samples manufactured from reconstituted A2 β-casein skim milk powder in aqueous MLE 0.5, 1, and 1.5%, respectively. Means ± standard deviations with different small letters represent significantly different among formulations (p<0.05).

Table 3 demonstrates concentration-dependent effects of MLE on yoghurt texture. Apparent viscosity decreased from 1,178.26 P (T0) to 897.13 P (T3), while curd tension declined (26.82 to 17.84 g/50ml). These changes align with Al-Ahwal et al. (2017), who proposed that MLE polyphenols interfere with casein cross-linking and weakening the gel network. Syneresis reduction (2.21 to 0.60 g/50ml) indicates improved water retention, likely due to MLE polysaccharides forming hydrophilic complexes with milk proteins (Yang et al., 2023). The inverse correlation between viscosity and antioxidant activity (r = -0.81934, Fig. 2) suggests a trade-off between textural and functional properties that requires optimization.

Principal component analysis (PCA)

The PCA biplot (Fig. 1) and eigenvalue tables (Supplementary Tables S1–S3) provide a holistic view of parameter interactions. PC1 (86.85% variance) strongly associates with acidity-related parameters (negative loading, including viscosity and curd tension; positive loading, including titratable acidity and ash), while PC2 (11.36%) separates carbohydrate and antioxidant activity from other variables (Fig. 1). The near-perfect negative correlation between titratable acidity and curd tension (r = -0.99916, Fig. 2) on PC1 confirms that acidification drives structural weakening. The clustering of T3 with high antioxidant activity and low viscosity in the biplot illustrates the functional-textural dichotomy.

The observed trends can be explained through three biochemical mechanisms, including polyphenol-casein interactions disrupting hydrophobic bonds in the gel network (Shahidi & Dissanayaka, 2023; Yuan et al., 2024), mineral cations (Ca^{2+} and Mg^{2+}) from MLE modulating casein micelle charge (Bauland et al., 2020); and MLE flavonoids scavenging free radicals during fermentation and altering LAB metabolism (Zhang et al., 2019). The PCA loadings (Supplementary Tables S2 and S3) highlight that viscosity and protein content oppose antioxidant activity on PC2, which suggesting that maximizing functionality may require compromising traditional textural benchmarks. However, the absence of carbohydrate variation contrasts possibly due to differences in MLE carbohydrate content based on extraction protocols. The PCA-derived correlations provide novel insights beyond previous univariate approaches, particularly in quantifying the acidity-texture-antioxidant interplay. This present study demonstrates that MLE concentrations up to 1% (T2) balance functionality (94.67% of antioxidant activity) with acceptable texture (992.97 P of viscosity) (Tables 2 and 3). For industry applications, this suggests that MLE fortification should be tailored based on whether antioxidant potency or textural attributes are prioritized.

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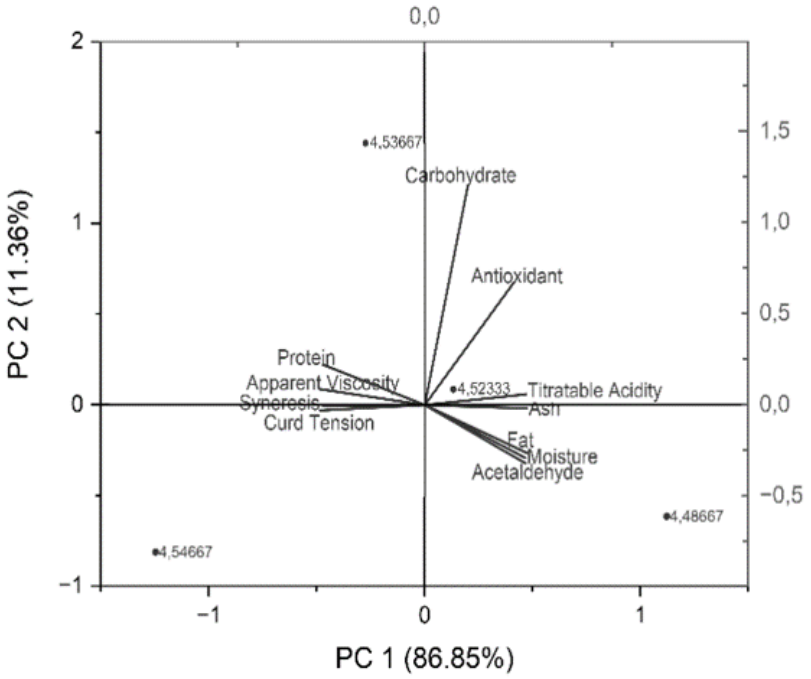

Figure 1. Biplot for four formulations of non-fat s A2 β-casein yoghurt.

The correlation matrix demonstrates strong negative relationships between acidity-related parameters and textural properties. Titratable acidity shows near-perfect inverse correlation with curd tension (r = -0.99916) and strong negative association with apparent viscosity (r = -0.99276) (Fig. 2). These findings align with the gel weakening theory proposed by Lee and Lucey (2019), where increased acidification disrupts casein micelle interactions (Lee & Lucey, 2010). The positive correlation between ash content and titratable acidity (r = 0.96423) suggests MLE-derived minerals may accelerate LAB activity, consistent with the Zhang et al. (2019). Antioxidant activity exhibits moderate positive correlation with ash (r = 0.80122) and fat content (r = 0.74019), but negative correlation with protein (r = -0.76692). This pattern indicates that MLE's bioactive compounds interact differently with milk components and polyphenols may bind preferentially to fat globules rather than caseins. The stability of carbohydrate content across treatments (r < 0.45 with almost all variables, exclude antioxidant) confirms its minimal role in the observed quality changes. The PCA results (Supplementary Table S1) explain 98.21% of variance in the first two components, providing exceptional dimensionality reduction. PC1 (86.85% variance) (Fig. 1) clearly separates samples by MLE concentration, with positive loading for functional properties (antioxidant activity: 0.27636; ash: 0.31607) and negative loading for texture parameters (viscosity: -0.32285; curd tension: -0.32343) (Supplementary Table S3). This axis represents the fundamental trade-off between nutritional enhancement and structural integrity. PC2 (11.36% variance) reveals secondary differentiation, with strong positive loading for carbohydrate (0.80794) and antioxidant activity (0.45201). The biplot visualizes how T3 (1.5% MLE) clusters in high-antioxidant space, while T0 (control) occupies the high-texture quality quadrant (Fig. 1). The intermediate position of T1–T2 (0.5–1% MLE) in PCA space indicates an optimal balance by avoiding extreme texture degradation (seen in T3) while maintaining significant antioxidant benefits (92.8–94.7% activity). Their central location reflects moderate scores on both PC1 (functionality-texture

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trade-off) and PC2 (nutrient interactions), supported by weaker negative protein-antioxidant correlations than T3 (Fig. 1).

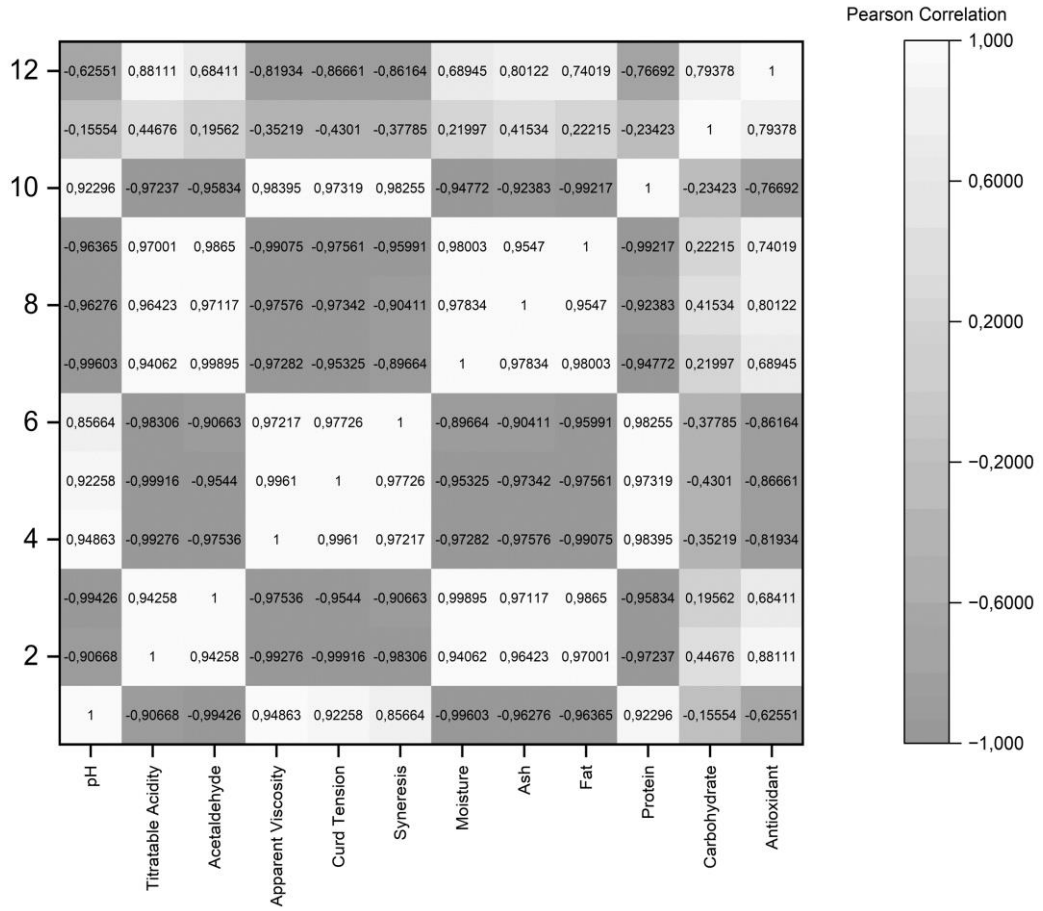

Figure 2. Correlation coefficient of physicochemical and biochemical variables in four formulations of non-fat s A2 β-casein yoghurt.

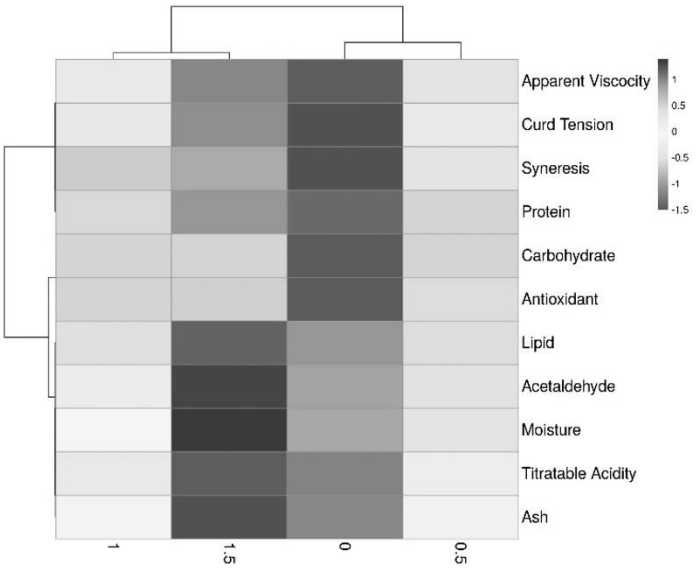

Figure 3. Heatmap of score plot for physicochemical and biochemical variables in four formulations of non-fat s A2 β-casein yoghurt.

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The hierarchical clustering heatmap in Fig. 3, separates variables into three distinct clusters, including texture-related (viscosity, curd tension, and syneresis); fermentation markers (acidity, moisture, acetaldehyde, and lipid), and nutritional components (protein, carbohydrate, antioxidants, lipid, and syneresis). This clustering validates the hypothesis that MLE affects yoghurt quality through multiple independent mechanisms of mineral contribution, polyphenol-protein interactions, and microbial modulation. Notably, protein shows stronger association with texture cluster than nutritional cluster, which is emphasizing its structural role (An & Zheng, 2025). The heatmap's dose-dependent color gradients confirm that MLE effects are concentration-linear, with 1.5% treatment causing most significant changes (Fig. 3).

CONCLUSION

This study systematically evaluated the impact of aqueous MLE (0–1.5%) on non-fat A2 β-casein yoghurt, establishing clear formulation guidelines for functional dairy development. Results demonstrated that MLE significantly enhanced antioxidant capacity (up to 96.37%) and mineral content (1.17% ash), while maintaining stable carbohydrate levels, confirming its role as a nutrient-dense fortificant without altering macronutrient balance. Multivariate analysis revealed a critical trade-off: increased acidity from MLE weakened gel strength, reducing viscosity (24%) and curd tension (33%) at higher concentrations. The 1% MLE formulation (T2) emerged as optimal, delivering 94.67% antioxidant activity while preserving acceptable texture, outperforming previous approaches reported by Zhang et al. (2019) for plant extract fortification. These findings advance functional dairy science by quantifying the precise threshold where bioactive benefits outweigh structural compromises. Future work should test sensory appeal and explore encapsulation to preserve texture. This research paves the way for innovative plant-based fortification in dairy product formulation, which meeting demand for nutritious and functional foods.

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