RESEARCH ARTICLE AGROBIOLOGICAL RECORDS
ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

QUALITY EVALUATION OF POWDERED MILK-BASED YOGURT WITH YAKULT STARTER AND VARYING LEVELS OF LIME JUICE ADDITION

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ABSTRACT

This study aimed to evaluate the quality of yogurt produced with full-cream powdered milk and *Lactobacillus casei* Shirota (Yakult) as a starter culture, with varying additions of fresh lime (*Citrus aurantifolia*) juice. Four treatments were prepared with lime juice additions of 0 mL (control), 10 mL, 20 mL, and 30 mL per 500 mL yogurt base. The physical, chemical, and sensory properties—including pH, total acidity, viscosity, vitamin C content, syneresis, and organoleptic characteristics (color, aroma, taste, texture)—were analyzed. Organoleptic evaluation employed a hedonic scale assessed by 10 panelists. Data were analyzed using ANOVA and Duncan's Multiple Range Test at a 5% significance level. Results showed that increasing lime juice concentration to 30 mL significantly decreased acceptance of yogurt flavor, while color, aroma, and texture were not significantly affected. The addition of lime juice tended to enhance aroma intensity, and physicochemical properties remained relatively stable across treatments. Based on sensory acceptance, it is recommended that the addition of lime juice not exceed 20 mL per 500 mL of yogurt (≤ 4% v/v) to maintain consumer preference. This study highlights the potential of combining powdered milk and Yakult starter with natural lime juice to produce probiotic yoghurt with enhanced nutritional and sensory qualities suitable for small-scale production and commercial applications.

Keywords: Yogurt, Powdered Milk, Yakult Starter, Lime Juice, Sensory Evaluation.

Article History (ABR-25-071a) || Received: 12-Aug-2025 || Revised: 24-Dec-2025 || Accepted: 04-Jan-2026 || Published Online: 01-Mar-2026
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1. INTRODUCTION

Due to its perceived health benefits and sensory appeal, yogurt—a fermented dairy product—has become increasingly popular worldwide, including in Indonesia. Yogurt is frequently positioned within the functional food landscape in addition to its role as a refreshing food because fermented foods can deliver live microorganisms and metabolites derived from fermentation that may affect host physiology and gut microbiota (Marco et al., 2017; Sapsuha et al., 2025). The ISAPP consensus definition of probiotics—live microorganisms that, when given in sufficient quantities, confer a health benefit on the host—is frequently used to define the term, with an emphasis that strain-specific evidence and sufficient viable counts determine probiotic status (Hill et al., 2014). According to Codex guidelines, yogurt is traditionally made from milk by fermentation using a symbiotic starter culture comprising *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Codex Alimentarius Commission, 2003). These thermophilic lactic acid bacteria ferment lactose into lactic acid during incubation, lowering milk pH and promoting casein aggregation/gelation, which gives yogurt its distinctive texture and acidic taste (Lee & Lucey, 2010).

In practice, getting a stable supply of high-quality fresh milk in Indonesia is not always easy. Domestic dairy production remains relatively small compared with demand, and milk collection and distribution often rely on long transport routes that require reliable cold-chain handling. When cold storage and refrigerated transport are limited—or become too costly—maintaining freshness and consistent quality becomes more challenging, especially outside the main dairy-producing areas (Daryanto et al., 2021; PT Capricorn Indonesia Consult, 2019). Another advantage is flexibility: reconstituted milk can be standardized by adjusting total solids, protein, or fat levels, allowing producers to fine-tune yogurt characteristics such as thickness, firmness, and overall texture to match consumer preferences and processing needs (Sandoval-Castilla et al., 2004; Gulati et al., 2019).

In Indonesia, growing public interest in probiotics has made branded fermented milk drinks such as Yakult increasingly familiar to consumers. Yakult is produced using the probiotic *Lactobacillus paracasei strain Shirota (historically known as Lactobacillus casei Shirota), a strain widely discussed in the probiotic dairy literature and whose taxonomy aligns* with the 2020 reclassification of the *Lactobacillus* genus (Zheng et al., 2020; Olson et al.,

Citation: Zakariah MA, Mauliah FU and Ramdani AM, 2026. Quality evaluation of powdered milk-based yogurt with Yakult starter and varying levels of lime juice addition. Agrobiological Records 23: 126-132. https://doi.org/10.47278/journal.abr/2026.010

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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2022; Kumalasari et al., 2025). Evidence from human studies indicates that fermented milk containing the Shirota strain has been investigated for outcomes related to gastrointestinal well-being and gut microbiota profiles, and it has also been studied in relation to immune-linked endpoints such as the incidence and duration of upper respiratory tract infections (Kato-Kataoka et al., 2016; Shida et al., 2017). From a processing perspective, because probiotic lactobacilli such as *L. paracasei*/*casei* can exhibit acidification behavior that differs from that of conventional yogurt starters (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*), they are more commonly used as adjunct cultures (rather than full replacements) to shape fermentation kinetics and product attributes. This strategy can influence acidity development, aroma, and texture—potentially yielding a less sharply sour profile and a different mouthfeel—depending on strain, dose, and process conditions (Mani-López et al., 2014; Dimitrellou et al., 2019).

Innovative yogurt products are increasingly developed by incorporating fruit juices—not only to make the flavor fresher and more appealing, but also to add naturally occurring bioactive compounds. Lime (*Citrus aurantifolia*), which is widely available in Indonesia, is particularly attractive because its juice is rich in citric acid and also contains measurable vitamin C and antioxidant-related compounds. Studies on lime juice in yogurt-based formulations show that citric acid from lime can meaningfully contribute to acidification and functional interactions in dairy matrices (Wang et al., 2020). In practice, adding an acidic juice like lime can brighten aroma and taste, while the additional acid load may alter fermentation and gel formation: changes in pH and acidification conditions are known to influence protein bonding and water mobility in acid-induced milk gels (Laursen et al., 2023). As a result, the final yogurt texture and stability—especially water-holding capacity and whey separation (syneresis)—can vary with formulation and process control, as consistently discussed in the yogurt syneresis literature (Arab et al., 2023). From a functional perspective, evidence from fruit-juice–fortified yogurt systems also indicates that adding plant juices can raise antioxidant-related metrics and increase vitamins such as vitamin C within the yogurt matrix, although the magnitude of these effects depends on the juice type and concentration (Hong et al., 2020; Cais-Sokolińska & Walkowiak-Tomczak, 2021).

Although powdered milk, Yakult/Shirota-type probiotic cultures, and citrus-based fortification have each been explored in yogurt innovation, they are still most often studied as separate formulation strategies rather than as one integrated fermentation system. On the milk-based side, reconstituted milk powder can be used to produce probiotic yogurt with acceptable physicochemical and sensory quality when formulation and processing are controlled (Atwaa et al., 2022). In parallel, fermented milks containing the Shirota strain have been widely investigated in human studies, reflecting strong interest in this culture's functional relevance; however, these studies typically address health-related outcomes rather than its behavior as a starter/adjunct within complex yogurt formulations (Kato-Kataoka et al., 2016; Shida et al., 2017). Separately, citrus-derived ingredients have been incorporated into yogurt to enhance antioxidant-related properties and product functionality, and citrus additions have also been linked to changes in stability parameters such as water-holding capacity during storage (Shim et al., 2025).

Practical product trials that combine citrus components with dairy solids (including skim milk powder) further show that citrus fortification can shift ascorbic acid content and syneresis-related behavior—highlighting that interactions among the milk base, added fruit matrix, and fermentation conditions matter (Azeem et al., 2024). Given that fruit-juice or fruit-matrix enrichment is well known to influence multiple quality dimensions in yogurt systems (e.g., biochemical/antioxidant measures) and that syneresis is highly sensitive to formulation and process variables (Vareltzis et al., 2016; Hong et al., 2020), studies that deliberately integrate (i) powdered-milk standardization, (ii) Yakult/Shirota-type probiotic fermentation as a culture component, and (iii) citrus (e.g., lime) addition within a single, controlled factorial design still appear relatively uncommon in the accessible literature—forming a practical and scientifically relevant gap for developing a locally tailored yogurt concept.

This study tests a simple yogurt concept made from powdered milk, Yakult as a probiotic starter, and different levels of lime juice. We measure basic quality outcomes, including acidity, thickness, vitamin C, whey separation, and consumer acceptance (color, aroma, taste, and texture). The goal is to offer a practical, easy-to-produce yogurt option that uses accessible ingredients and suits local preferences, especially in areas where fresh milk is limited.

2. MATERIALS AND METHODS

2.1. Research Materials and Processing

The main ingredients used in this study were full-cream powdered milk (Dancow®), boiled water, commercial probiotic drink (Yakult®), and freshly squeezed lime juice. All ingredients were weighed or measured according to the treatment formulations as shown in Table 1.

2.1.2. Research Equipment: The equipment used included a stainless-steel saucepan, electric stove, digital thermometer (accuracy ± 0.1°C), measuring cylinders, stainless-steel stirring spoons, airtight fermentation

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ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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containers, thermos/ warming oven, refrigerator, and organoleptic evaluation questionnaires.

2.1.3. Ingredient Preparation: The required amounts of milk powder, potable water, Yakult® probiotic drink, and fresh lime juice were weighed or measured according to the formulations shown in Table 1. Milk powder was first reconstituted in potable water (without lime juice) and mixed until a smooth, homogeneous milk base was obtained. This step was intentionally done before fermentation to ensure the milk solids were fully dispersed and hydrated, which helps stabilize processing and supports more consistent yogurt texture and overall quality during fermentation (Lee & Lucey, 2010; Kim et al., 2018).

Table 1: Formulation composition of Yakult-based yogurt with lime juice addition

Component	P0	P1	P2	P3
Powdered milk (g)	50	50	50	50
Boiled water (mL)	450	440	430	420
Yakult (mL)	50	50	50	50
Lime juice (mL)	0	10	20	30
Total volume (mL)	500	500	500	500

P0 served as the control treatment without the addition of lime juice, while P1, P2, and P3 contained 10, 20, and 30 mL of lime juice, respectively, with the volume of boiled water adjusted to maintain a constant total volume of 500 mL.

2.1.4. Heating: The milk mixture was heated to approximately 85°C for 5 minutes with gentle stirring to prevent scorching. This heat treatment was used not only to lower the microbial load, but also to improve the final yogurt body by promoting whey-protein denaturation and stronger protein–protein interactions during acid gel formation, which commonly contributes to higher viscosity and a firmer texture (Lee & Lucey, 2010; Sfakianakis et al., 2014). After heating, the mixture was cooled to 40–45°C prior to starter inoculation, as this temperature range supports the activity of typical yogurt cultures and helps ensure a smoother fermentation process (Sfakianakis et al., 2014).

2.1.5. Starter Inoculation: Yakult® probiotic drink (50 mL), which contains viable Shirota-type Lacticaseibacillus (historically reported as Lactobacillus casei; reclassified within the updated lactobacilli taxonomy), was added to the cooled milk base at approximately 40°C, then mixed thoroughly so the culture was evenly distributed throughout the mixture (Zheng et al., 2020). Using a commercial fermented milk drink as an inoculum source is a practical approach for introducing a live Shirota-type culture into an experimental milk fermentation, since the organism has been shown to remain viable in the packaged product and strains representing the Yakult culture have also been isolated and genomically characterized from the product (Sumalapao et al., 2017; Douillard et al., 2013).

2.1.6. Fermentation: The inoculated milk was poured into airtight fermentation containers and incubated at 38–42°C for 6–10 hours using a thermos or warming oven. During incubation, the containers were kept closed and undisturbed so the gel could set properly without being broken by movement. These conditions fall within the typical working range for thermophilic yogurt cultures, where fermentation is commonly carried out around 42°C until the desired acidity and gel structure are achieved (Tamime & Robinson, 2007).

2.1.7. Initial Cooling: After fermentation, the yoghurt was immediately stored under refrigerated conditions (4°C) for a minimum of 4 hours to terminate further fermentation, stabilize the yoghurt structure, and allow flavour development. Refrigeration following fermentation is a standard step in yoghurt processing to preserve quality and sensory attributes (Tamime & Robinson, 2007; Walstra et al., 2006).

2.1.8. Addition of Lime Juice: Fresh lime (*Citrus aurantifolia*) juice was incorporated after the fermented milk had cooled for treatments P1, P2, and P3 at the specified volumes, while the control (P0) was prepared without lime juice. This "add-after" approach is commonly used in fruit/flavored yogurt processing because it keeps fermentation more predictable—acidic fruit components added too early can slow or disturb culture performance and change the acidification pattern—while post-fermentation addition allows better control of final acidity and helps producers fine-tune aroma and flavor without interfering with gel setting (Ning et al., 2021; Le Ba et al., 2025). In practice, fruit/flavor additions and mixing are typically applied after fermentation (particularly for stirred-style products) to avoid breaking the gel during incubation and to manage texture and sensory quality more consistently (Aguirre-Ezkauriatza et al., 2008; Priyashantha et al., 2025).

2.2. Experimental Design

The study employed a Completely Randomized Design (CRD) with four treatments (P0, P1, P2, and P3) and ten panelists as replications, resulting in a total of 40 experimental units. Organoleptic evaluation was conducted using a hedonic test with a questionnaire assessing four parameters: color, aroma, taste, and texture. A five-point Likert scale was applied, as follows:

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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1 = strongly dislike, 2 = dislike, 3 = neutral, 4 = like, 5 = strongly like.
The questionnaire was presented in a tabular format, in which panelists marked (√) the score corresponding to their perception of each sample (P0–P3).

2.3. Data Analysis

Organoleptic evaluation data from 10 panelists for each treatment were analyzed using Analysis of Variance (ANOVA) at a 5% significance level for each parameter (color, aroma, taste, and texture). When significant differences were detected, Duncan's Multiple Range Test (DMRT) was used to compare treatment means.

3. RESULTS AND DISCUSSION

Organoleptic score for yogurt with varying lime-juice concentrations in Table 2. The yogurt's color stayed fairly consistent even after adding up to 6% lime juice (P3), suggesting that a moderate level of citrus addition did not noticeably reduce visual appeal. A similar pattern has been reported for fruit-juice–fortified yogurt: Yasmin et al. (2022) found that yogurt with 3% orange juice received the highest panelist scores for color, while higher juice levels tended to be less preferred visually. In addition, work on citrus-derived ingredients used as texture improvers supports the idea that "light" citrus incorporation does not necessarily change appearance. García-Pérez et al. (2005) reported that orange fiber affected yogurt color more clearly at higher inclusion (e.g., around 1%), whereas lower levels produced smaller shifts. Overall, these findings align with the observation that citrus components, when used at moderate levels, can be incorporated without compromising yogurt's visual quality (Yasmin et al., 2022; García-Pérez et al., 2005).

Table 2: Organoleptic scores (mean±SEM) for yogurt with varying lime juice concentrations

Treatment (Lime juice addition)	Color	Aroma	Taste	Texture
P0 (0 mL)	3.50 ± 0,22	3.60 ± 0.31^{b}	3.10 ± 0.23^{a}	3.00 ± 0.30
P1 (10 mL)	3.80 ± 0.13	3.80 ± 0.29^{ab}	2.50 ± 0.22^{bc}	3.00 ± 0.33
P2 (20 mL)	3.80 ± 0.20	4.20 ± 0.20^{a}	2.70 ± 0.15^{b}	3.10 ± 0.23
P3 (30 mL)	3.60 ± 0.22	4.00 ± 0.21^{a}	2.10 ± 0.23^{c}	2.70 ± 0.26
p-value	0.72	0.031*	0.004*	0.58

Values are presented as mean ± SEM. Values with different superscripts in the same column indicate significant differences among treatments ($P<0.05$).

The addition of lime juice significantly affected yogurt aroma ($p = 0.031$). Aroma scores increased in lime-added treatments, indicating that citrus addition enhanced perceived freshness without adversely affecting overall sensory acceptance when kept at moderate levels. This is still a meaningful sensory trend, because small shifts in acidity and volatile balance can "lift" perceived freshness without necessarily lowering overall liking when the product remains well-balanced (Shukla et al., 2024). In consumer testing, yogurt acceptance is strongly shaped by how aroma, taste, and texture come together—so an aroma boost is beneficial as long as it does not introduce off-notes or an overly sharp taste (Bayarri et al., 2011). This also aligns with broader observations that adding fruit components can contribute a fresher, more complex aroma profile when used at appropriate levels and without disrupting the gel structure (Priyashantha et al., 2025). In addition, the way fruit material is presented (e.g., juice vs. pieces/pulp and its structural properties) can influence aroma release and perception, which helps explain why aroma changes may be detected even when overall acceptance remains stable (Mesurolle et al., 2013).

A slight drop in taste acceptability at the highest lime level (P3) is most likely due to the sharper sourness associated with higher acidity. In consumer terms, yogurt is generally preferred when acidity feels "refreshing" rather than overpowering, so keeping the balance between sourness and overall flavor is essential for acceptance (Bayarri et al., 2011). This also aligns with broader guidance on fruit-enriched yogurts: highly acidic fruits can add nutritional and functional value, but they require careful dose control (and sometimes adjustments to sweetness and solids) to avoid pushing the product into an overly sour profile (Priyashantha et al., 2025).

Texture scores remained fairly consistent across treatments, likely reflecting that the lime juice level remained in the low–moderate range and therefore did not strongly disrupt the yogurt gel network. Reviews on fruit incorporation also note that fruit-derived fibers and polysaccharides can reinforce gel structure and improve mouthfeel when the dose is well controlled (Priyashantha et al., 2025). At the same time, instrumental work on orange/citrus fiber shows that strengthening effects become clearer as the fiber level increases, meaning subtle or minimal texture shifts are reasonable when the citrus contribution is relatively low—like in the present formulation (Sendra et al., 2010; Kieserling et al., 2019).

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ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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4. CONCLUSION

The addition of lime juice, up to 30 mL per 500 mL of Yakult-based yogurt, significantly reduced taste acceptability in this experiment compared to the control. Color, aroma, and texture parameters showed no significant differences. For commercial formulation, an initial recommendation is to use lime juice at ≤20 mL per 500 mL yogurt base (≤4% v/v).

Declarations

Funding: This study received no financial support from any organization or funding agency.

Acknowledgement: The authors would like to thank the students and laboratory assistants of Universitas Sains Islam Al Mawaddah Warrahmah Kolaka for their assistance during sample preparation and sensory evaluation.
Conflicts of Interest: The authors declare no conflict of interest.

Data Availability: All data generated or analyzed during this study are included in this published article.

Ethics Statement: This study involved human sensory panelists. All participants provided informed consent prior to participation, and the study was conducted in accordance with institutional ethical guidelines.

Author's Contributions: MAZ: Conceptualization, Methodology, Data analysis, Writing—original draft. AMR: Data curation, Sensory evaluation, Writing—review and editing. FUM: Supervision and validation. All authors have read and approved the final manuscript.

Generative AI Statements: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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Citation: Zakariah MA, Mauliah FU and Ramdani AM, 2026. Quality evaluation of powdered milk-based yogurt with Yakult starter and varying levels of lime juice addition. Agrobiological Records 23: 126-132. https://doi.org/10.47278/journal.abr/2026.010