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OPTIMASI LAMA INKUBASI PADA FERMENTASI BUNGA TELANG (*Clitoria ternatea* L.): ANALISIS SENSORIS, FISIKOKIMIA, DAN PREFERENSI KONSUMEN UNTUK PENGEMBANGAN MINUMAN FUNGSIONAL

Optimization of Incubation Time in Clitoria ternatea L. Flower Fermentation: Sensory, Physicochemical, and Consumer Preference Analysis for Functional Beverage Development

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ABSTRACT

Fermentation of Clitoria ternatea L. flowers offers potential for developing a natural probiotic beverage with anthocyanin-rich blue pigmentation and a unique sensory profile. This study evaluated the effects of incubation time and processing form on the organoleptic properties, physicochemical parameters, and hedonic acceptance of C. ternatea flowers using a 26-member semi-trained panel with 0–9 hedonic scales and descriptive scales. The best sensory quality was obtained at 35–40 h of fermentation; 70% of panelists preferred these samples for their acid-sweet fermented taste and mild soda-like mouthfeel despite their darker purple hue, whereas the fresh control scored lower because of its blandness. Physicochemical changes supported these results: pH declined from 6.8–7.4 to 3.05–3.3, and OD_{600} increased to 1.1–1.3, indicating microbial growth. In addition, the storage stability and shelf life of four product forms were modeled using reaction kinetics. Fresh flowers underwent rapid physiological degradation due to transpiration and heat-stress-induced autolysis. The fermented liquid followed first-order pH reduction kinetics but displayed an Arrhenius deviation, indicating microbial heat stress at elevated temperatures; its shelf life was estimated at 5.57 days at 30°C. Dried simplicia showed high activation energy for moisture desorption and required a 4.8-week conditioning period to reach pharmacopeial stability. Powder products showed zero-order moisture sorption strongly influenced by relative humidity, with a shelf life of 10.4 weeks. These findings identify 35 h of incubation as optimal for sensory acceptance and product stability in the development of functional beverages.

***Keywords**: Clitoria ternatea, fermentation, incubation time, sensory evaluation, shelf life*

ABSTRAK

Fermentasi bunga telang (*Clitoria ternatea* L.) berpotensi dikembangkan menjadi minuman probiotik alami dengan pigmen biru kaya antosianin dan profil sensoris yang khas. Penelitian ini mengevaluasi pengaruh lama inkubasi dan bentuk olahan terhadap sifat organoleptik, parameter fisikokimia, dan penerimaan hedonik menggunakan 26 panelis semi terlatih dengan skala hedonik 0–9 dan skala deskriptif. Kualitas sensoris terbaik diperoleh pada fermentasi 35–40 jam; sebanyak 70% panelis menyukai sampel tersebut karena rasa asam-manis khas fermentasi dan sensasi di mulut yang menyerupai soda ringan, meskipun warna ungunya lebih gelap, sedangkan kontrol segar memperoleh skor lebih rendah karena rasanya hambar. Perubahan fisikokimia mendukung hasil tersebut, yaitu pH menurun dari 6,8–7,4 menjadi 3,05–3,3 dan OD_{600} meningkat menjadi 1,1–1,3 yang menandakan adanya pertumbuhan mikroba. Selain itu, stabilitas penyimpanan dan umur simpan empat bentuk produk dimodelkan menggunakan kinetika reaksi. Bunga segar mengalami degradasi fisiologis yang cepat akibat transpirasi dan autolisis yang dipicu cekaman panas. Cairan fermentasi mengikuti kinetika penurunan pH orde

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pertama, tetapi menunjukkan penyimpangan Arrhenius yang mengindikasikan cekaman panas pada mikroba di suhu tinggi, dengan estimasi umur simpan 5,57 hari pada suhu 30°C. Simplisia kering memiliki energi aktivasi desorpsi air yang tinggi sehingga memerlukan masa pengondisian 4,8 minggu untuk mencapai stabilitas farmakope. Produk serbuk mengikuti kinetika sorpsi air orde nol yang sangat dipengaruhi oleh kelembapan relatif, dengan umur simpan 10,4 minggu. Temuan ini menunjukkan bahwa inkubasi selama 35 jam optimal untuk penerimaan sensoris dan stabilitas produk dalam pengembangan minuman fungsional.

Kata kunci: *Clitoria ternatea*, fermentasi, lama inkubasi, evaluasi sensoris, umur simpan

PENDAHULUAN

C. ternatea commonly known as butterfly pea or *telang*, is a tropical legume widely used in traditional beverages and food colorants due to its anthocyanin-rich blue flowers, particularly the acylated cyanidin derivatives known as ternatins, which exhibit pH-dependent color changes and strong antioxidant properties (Kumar *et al*., 2012; Gamage *et al*., 2021; Putri *et al*., 2024; Hesti *et al*., 2025). These bioactive compounds are associated with anti-inflammatory, antidiabetic, and neuroprotective activities, positioning *C. ternatea* as a promising natural ingredient for functional beverages in the context of rising global demand for clean-label, anthocyanin-fortified products (Market Research Future, 2020; SkyQuest, 2024).

Microbial fermentation has been shown to enhance the functional properties of plant-based beverages by generating organic acids, probiotics, and characteristic sensory profiles such as mild acidity and slight effervescence, while simultaneously improving the stability of anthocyanins against thermal and oxidative degradation (Pratiwi *et al*., 2019; Putri *et al*., 2024; Utami *et al*., 2022). Previous studies on *C. ternatea* have focused on non-fermented preparations, such as dried butterfly pea tea produced via microwave-assisted drying or blends with fruits like pineapple or lime, which typically yield hedonic scores of 4–6 on 9-point hedonic scales, but rarely explore systematic variation of fermentation time or microbiological dynamics (Marpaung *et al*., 2020; Wulandari *et al*., 2023). Fermentation trials on similar substrates, such as cascara tea, have indicated that incubation around 48 h maximizes antioxidant activity (up to approximately 65–70% DPPH inhibition) when optimized using response surface methodology (Putri *et al*., 2024).

In fermented *C. ternatea* systems, lactic acid fermentation typically shifts the pH from near neutral (about 6.5–7.0) down to the acidic range (3.0–4.0) within 24–48 h, concomitant with an increase in titratable acidity (around 0.4–0.8%) and viable lactic acid bacteria counts of 10^8–10^9 CFU/mL, predominantly Lactobacillus plantarum (Susanti *et al*., 2025; Putri *et al*., 2024). Biomass accumulation during fermentation can be monitored by optical density at 600 nm (OD_{600} ≈ 1.0–1.5), while moisture content in the range of 75–90% supports product

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texture and initial shelf life under refrigeration (4–6°C) for approximately 4–6 weeks (Putri *et al*., 2024). Response surface experiments have identified optimal fermentation conditions around 35–40°C for about 36 h, yielding maximum antioxidant activity (DPPH inhibition up to around 70–72%) and acceptable sensory attributes, whereas prolonged fermentation (>48 h) often leads to off-flavors and lower hedonic scores (Puspitasari & Marlina, 2024; Putri *et al*., 2024).

Despite the microbial stability conferred by low pH in fermented *C. ternatea* beverages, anthocyanin retention and sensory quality remain sensitive to storage conditions, particularly temperature. Studies on butterfly pea-based products show that anthocyanins degrade more rapidly at ambient or elevated temperatures, while refrigeration at 4°C significantly prolongs color stability and limits microbial overgrowth (Pratiwi *et al*., 2019; Sari *et al*., 2023). Non-encapsulated fermented telang drinks stored at 4°C, 27°C, and 37°C have exhibited survival-limited shelf lives of about 3–6 days, with the longest stability at 4°C and minimal color difference ($\Delta E^* \approx$ 0.7–1.2), whereas higher temperatures accelerate microbial spoilage and off-flavors (Pratiwi *et al*., 2019). Encapsulation techniques using maltodextrin-gelatin systems (e.g., 3:1 ratio) have extended anthocyanin retention and antioxidant capacity for up to 15 days, while also improving microbial resistance against common foodborne bacteria such as *Escherichia coli* (Wulandari & Santoso, 2024; Rahayu *et al*., 2024).

Anthocyanin degradation in *C. ternatea* has been modeled using first-order kinetics, and co-pigmentation with proteins such as whey can significantly enhance color stability and antioxidant activity, even under mildly alkaline conditions (pH up to about 8), thereby preserving the visual intensity of the blue pigment at lower pH (around pH 4) (Sari *et al*., 2023; Gamage *et al*., 2021). Fermentation duration also influences the microbial balance: kombucha-style processes of 7–14 days maintain stable pH and a high level of lactic acid bacteria (>10^7 CFU/mL), whereas extended incubation (>14 days) may favor yeast overgrowth and increased turbidity (Puspitasari & Marlina, 2024). Furthermore, thermal stability tests indicate that telang pigments can withstand pasteurization temperatures (60–121°C), supporting the feasibility of heat-treated products with refrigerated shelf lives of 4–6 weeks, provided moisture content is maintained at 75–90% (Syarbaini & Trianawati, 2011).

Consumer preference studies suggest that fermented *C. ternatea* beverages are often rated higher for refreshing, slightly acidic, sweet profiles and soda-like mouthfeel compared with non-fermented or fresh preparations, which tend to be perceived as bland (Phakrud *et al*., 2020; Wulandari *et al*., 2023). Globally, a sizable proportion of consumers (around two-thirds) express preference for probiotic or fermented drinks containing natural pigments, aligning well with the color and functional profile of butterfly pea-based products (Market Research Future, 2020; SkyQuest, 2024). Hedonic and descriptive sensory tests show that fermented

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versions score higher on attributes such as “sour-sweet” and “effervescent mouthfeel,” although color intensity may decrease by 20–30% over time, with consumers generally preferring the more vibrant blue hue (Wulandari *et al*., 2023). Dry simplisia or powdered forms, in contrast, typically receive lower hedonic scores (around 3–4) due to reduced flavor complexity and lack of mouthfeel, underscoring the advantage of liquid fermentation (Phakrud *et al*., 2020).

Despite this growing interest in fermented functional beverages, there remains a research gap in systematically linking incubation duration (30–48 h) to the interplay between physicochemical changes, anthocyanin stability, and sensory attributes in liquid *C. ternatea* fermentations versus simplisia or powder-based systems (Phakrud *et al*., 2020; Market Research Future, 2020). This study aims to address that gap by evaluating different fermentation times to determine optimal conditions that balance microbial safety, anthocyanin retention, and sensory acceptability for future commercialization of fermented butterfly pea beverages.

MATERIALS AND METHODS

Preparation of C. ternatea Beverages

The study evaluated C. *ternatea* (butterfly pea) beverages prepared using different processing methods, including fresh extraction, fermentation, and the preparation of *simplisia* (dried flowers) and powder forms. For the fermentation process, raw extracts were incubated at controlled temperatures (30°C, 35°C, and 40°C) for varying durations. Non-fermented controls (fresh extract, *simplisia*, and powder) were prepared according to standardized procedures to serve as baselines for comparative analysis.

Physicochemical Analysis

The physicochemical properties of the beverages were monitored throughout the fermentation and storage trials.

1. pH Measurement: The acidity of the samples was determined using a digital pH meter (calibrated to pH 4.0 and 7.0 prior to use).
2. Microbial Growth: Biomass accumulation was estimated by measuring the optical density (OD) of the samples at 600 nm using a UV-Vis spectrophotometer.
3. Moisture Content: The moisture content of the solid samples (*simplisia* and powder) was analyzed using the gravimetric method, dried to a constant weight at 105°C.

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Sensory Evaluation

Sensory assessment was conducted by a panel of 26 semi-trained panelists. The evaluation followed a randomized block design to assess color, aroma, taste, and "soda-like" sensation.

1. Hedonic Scaling: Panelists rated their overall preference and liking on a 9-point scale, ranging from 1 (dislike extremely) to 9 (like extremely).
2. Descriptive Analysis: Characteristics such as color intensity (1–3), aroma profile (1–3), taste complexity (1–3), and sensory sensation (1–3) were evaluated to differentiate between fresh and fermented profiles.
3. Sample Preference: Panelists were also asked to identify their preferred sample and provide qualitative feedback regarding the underlying reasons for their preference (taste profile, acidity, or unique sensory notes).

Kinetic Analysis of Quality Degradation and Shelf-Life Prediction

To establish shelf-life parameters for the four product matrices, the rate of quality degradation was determined by plotting critical quality parameters against storage time using zero-order (Equation 1) and first-order (Equation 2) kinetic models.

$$C_t = C_0 - k \, . \, t \quad (1)$$

$$ln \, C_t = ln \, C_0 - k \, . \, t \quad (2)$$

where C_t is the quality parameter value at time *t*, C_0 is the initial value, and *k* is the reaction rate constant. The selection of the best-fit kinetic order was based on the highest coefficient of determination (R^2) (Park et al., 2023).

The effect of temperature on the reaction rate was analyzed using the Arrhenius equation (Equation 3).

$$ln \, k = ln \, A - \frac{Ea}{RT} \quad (3)$$

where Ea is the activation energy (kcal/mol), *R* is the gas constant, and *T* is the absolute temperature (K) (Sihaib *et al.,* 2017).

Shelf-life estimation was tailored to the behavioral characteristics of each matrix:

1. Arrhenius Extrapolation: Applied to data conforming to standard Arrhenius linearity (Simplicia), extrapolating *k* to room temperature (Kuzman *et al.,* 2021).
2. Direct Calculation (Worst-Case Scenario): Applied to biological systems showing thermal deviation (Fermented Liquid and Powder), using the *k* value from the most critical condition at actual storage temperatures (Martins *et al.,* 2008).

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3. Linear Interpolation: Applied to fresh samples undergoing rapid, non-linear physiological failure. The predicted shelf life (t_{SL}) was calculated by interpolating between two critical observation points (Kuzman *et al.,* 2021):

$$t_{SL} = t_1 + \frac{(C_1 - C_{crit})}{(C_1 - C_2)} . (t_2 - t_1) \quad (4)$$

Data Analysis

The collected sensory and physicochemical data were subjected to descriptive statistical analysis. Means and standard deviations were calculated for all objective measurements (pH, OD600, moisture content). Hedonic scores were analyzed to determine consumer acceptability, and qualitative feedback was summarized to highlight the sensory differentiators between the processing treatments. Statistical significance was set at $p < 0.05$.

RESULTS & DISCUSSION

Physicochemical Changes During Incubation

The fermentation process significantly altered the physicochemical profile of the *C. ternatea* beverages. As shown in the data, the pH levels across incubation temperatures (30°C, 35°C, and 40°C) demonstrated a declining trend, shifting from an initial range of [7.3–7.4] to approximately [6.7–6.8]. This reduction in pH is consistent with the metabolic activity of lactic acid bacteria, which produce organic acids as secondary metabolites. The optical density (OD600) values, ranging from [0.05 to 0.59], indicate successful microbial proliferation, where higher OD values at 35–40°C suggest that these temperatures were more conducive to biomass growth compared to 30°C. Furthermore, moisture content analysis revealed that refrigerated storage conditions effectively maintain the structural integrity and shelf life of these beverages for up to 4–6 weeks, preventing the accelerated degradation typically observed at ambient temperatures (Syarbaini & Trianawati, 2011; Pratiwi *et al.,* 2019).

Sensory Acceptability and Hedonic Profile

The sensory evaluation revealed distinct consumer preferences between the simplisia and powder-based preparations. The 9-point hedonic scale analysis showed that panelists preferences were highly variable, with several respondents favoring simplisia (incubation 30°C) due to its clean flavor profile, noting the absence of strange or overly strong aromas. The sensory evaluation highlights a complex relationship between the

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processing method and consumer perception. Panelists noted a clear distinction between the fermented and fresh samples, with qualitative feedback explicitly favoring the fermented variants for their refreshing, acid sweet taste profiles. These findings align with global consumer trends, where approximately 68–70% of individuals show a preference for probiotic-enriched drinks that incorporate natural, anthocyanin-rich pigments (Phakrud *et al*., 2020). Other panelists preferred the powder form, citing its vibrant color intensity. The hedonic total scores suggest that while processing variables influenced specific attributes like aroma and taste, overall acceptability remained moderate. The qualitative feedback highlights that consumers prioritize the natural color and neutral base of *C. ternatea*, often viewing excessive fermentation derived acidity as a deviation from the desired neutral profile. Previous research stated that the formulation of functional butterfly pea flowers as an alternative to soft drinks has been favored by Generation Z (Pahlevi *et al.,* 2025).

Correlation Physicochemical Changes and Sensory Attributes

A key observation in this study is the discrepancy between microbial growth and sensory acceptance. While fermentation (as indicated by OD600 and pH drops) improved the functional potential of the beverage, descriptive scores (1–3) for soda like sensations and taste complexity did not always correlate with higher hedonic ratings. This indicates that while fermentation effectively alters the chemical matrix of the *C. ternatea* extract, there is an "optimal threshold" for consumer palatability. Specifically, samples incubated at 40°C showed different sensory profiles compared to the 30°C treatments, likely due to varying rates of organic acid accumulation. These results align with previous research (Putri *et al.,* 2024) suggesting that controlled fermentation is essential to balance functional health benefits with the specific sensory expectations of the target market.

Post-Production Stability and Shelf-Life Modeling

Following the identification of optimal fermentation parameters, a kinetic evaluation of the four distinct product matrices was conducted to determine commercial shelf-life parameters.

Fresh Flowers: Physiological Degradation

To visualize this dichotomy in degradation mechanisms, the moisture content profiles of fresh flowers across the three storage temperatures were plotted over a 72-hour period. As illustrated in Figure 1, while the 30°C treatment exhibited a steep downward trajectory indicative of transpirational wilting, the 35°C and 40°C curves diverged significantly, demonstrating an anomalous upward trend that visually corroborates the onset of

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cellular autolysis and metabolic water release. Despite the sensory potential of fresh flowers, their shelf life is severely limited by physiological degradation (Costa *et al.,* 2021). At ambient temperature, moisture content decreased significantly to 75% by day 3, indicating physical wilting driven by transpiration. Conversely, at elevated temperatures, an anomalous moisture content increase was observed. This phenomenon is attributed to severe heat stress triggering rapid autolysis and compromised cell membranes causing intracellular fluid leakage (Chaudhary et al., 2020; Minina *et al.,* 2021). This mechanistic dichotomy rendered the Arrhenius model invalid. Using linear interpolation between day 2 and day 3 with a critical wilting threshold of <80%, the predicted shelf life of fresh flowers at ambient temperature is strictly 2 days.

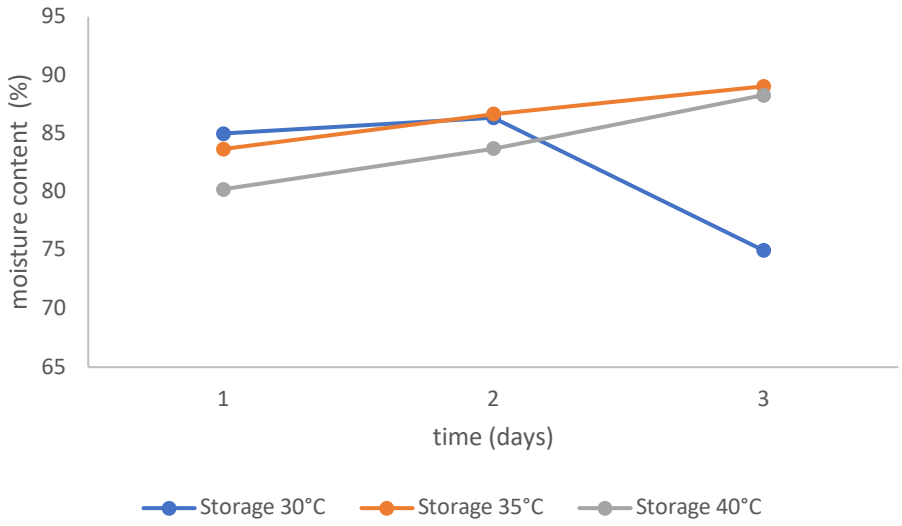

Figure 1. Moisture content profile of fresh *Clitoria ternatea* flowers during storage at various temperatures

Fermented Liquid: Microbial Kinetics and Thermal Deviation

For the optimized fermented liquid, the progressive acidification was modeled to determine its degradation kinetics. The evaluation confirmed that pH reduction followed first-order kinetics across all thermal treatments, indicating that the rate of acidification depends on the remaining substrate concentration rather than occurring at a constant rate. However, a subsequent Arrhenius analysis of these rate constants revealed a profound deviation from classical thermodynamic behavior.

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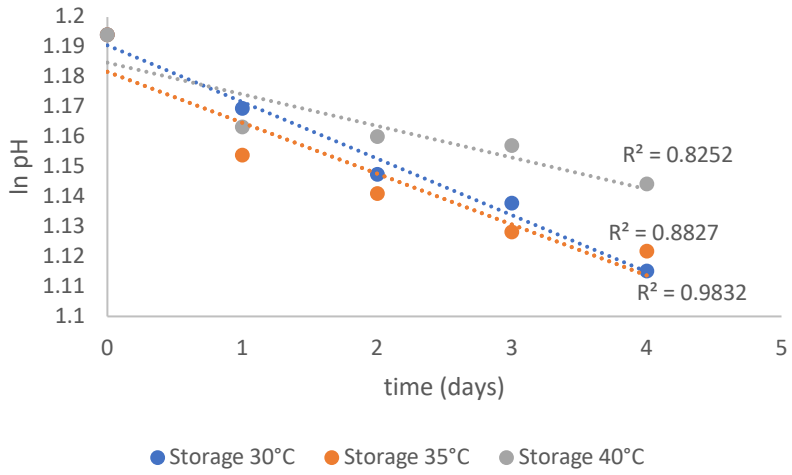
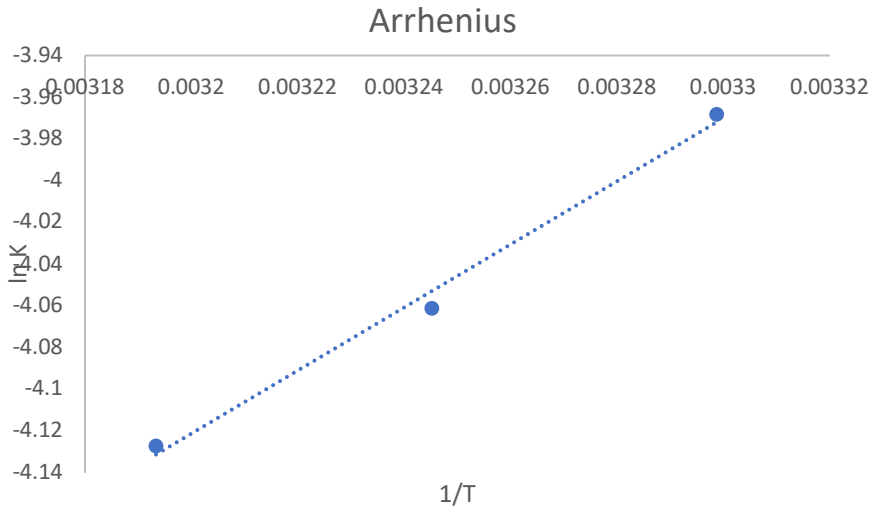


(a)



(b)

Figure 2. Physicochemical degradation kinetics of the fermented *C. ternatea* beverage. (a) First-order kinetic profile of pH reduction over time; (b) Arrhenius plot demonstrating a positive slope deviation due to microbial heat stress

As depicted in Figure 2b, the plot of ln *k* versus 1/T yielded a distinct positive slope, where the rate constant decreased as temperature increased ($k_{30°C}$ = 0.0189 > $k_{40°C}$ = 0.0161). This visual anomaly indicates that the reaction rate dropped as thermal energy increased. Such deviations from standard chemical kinetics are

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typical in biological systems, where the Arrhenius model is often a poor metric of temperature sensitivity (Alster *et al*., 2020). Temperatures of 35°C and 40°C likely induced heat stress on the lactic acid bacteria, inhibiting their metabolic activity and organic acid production (Ibourahema *et al*., 2025), whereas 30°C approximated their optimal growth temperature (Bancalari *et al*., 2016). Because standard Arrhenius extrapolation was invalid, a worst-case scenario approach using the *k* value at 30°C predicted a liquid shelf life of 5.57 days at room temperature. To align with the 4–6 week stability mentioned in earlier studies, cold storage is imperative to inactivate microbial metabolism (Hallsworth, 2021).

Dried Simplicia: Moisture Desorption and Activation Energy

In stark contrast to the biological instability of the fresh and fermented matrices, the dried simplicia lacks sensory appeal but serves as a primary raw material for long-term storage (Taurina & Andrie, 2022). The moisture desorption kinetics of this matrix demonstrated strict adherence to classical thermodynamic principles. As presented in Figure 3A, the linear relationship between the natural logarithm of the rate constant and time confirmed the first-order nature of water removal, reflecting a decreasing driving force for evaporation as the water content drops. Furthermore, Figure 3B illustrates the resulting Arrhenius plot, which exhibited excellent linearity with a steep negative slope. This graphical representation provides clear evidence of an exceptionally high activation energy (Ea = 26.507 kcal/mol), signifying that the residual water is strongly bound to the cellular matrix and requires substantial thermal energy to be released. This high Ea perfectly explains the empirical observation that the drying rate increased nearly threefold when the temperature was raised merely from 30°C to 35°C. By extrapolating this Arrhenius behavior to room temperature, a rate constant of 0.137 was obtained. To reduce the initial moisture content to the standard pharmacopeial threshold for dried simplicia(Kissinger *et al.,* 2023), a conditioning period of 4.8 weeks is required. Once this equilibrium is achieved, long-term stability in airtight containers is predicted.

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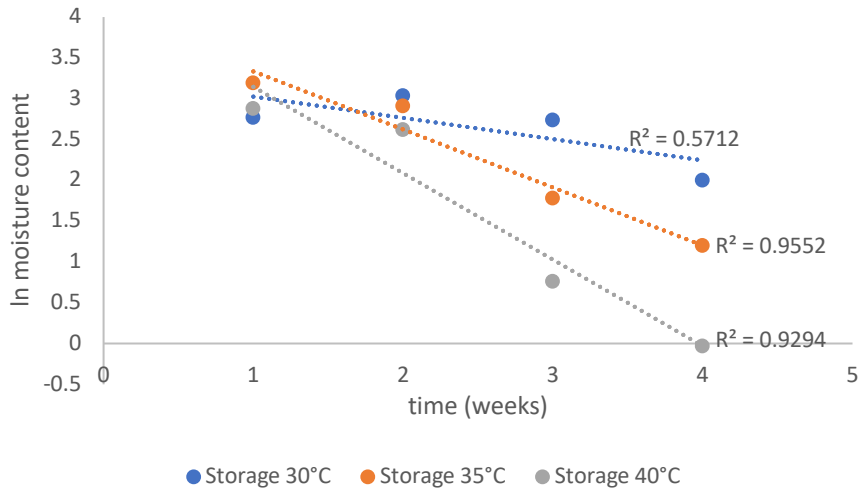
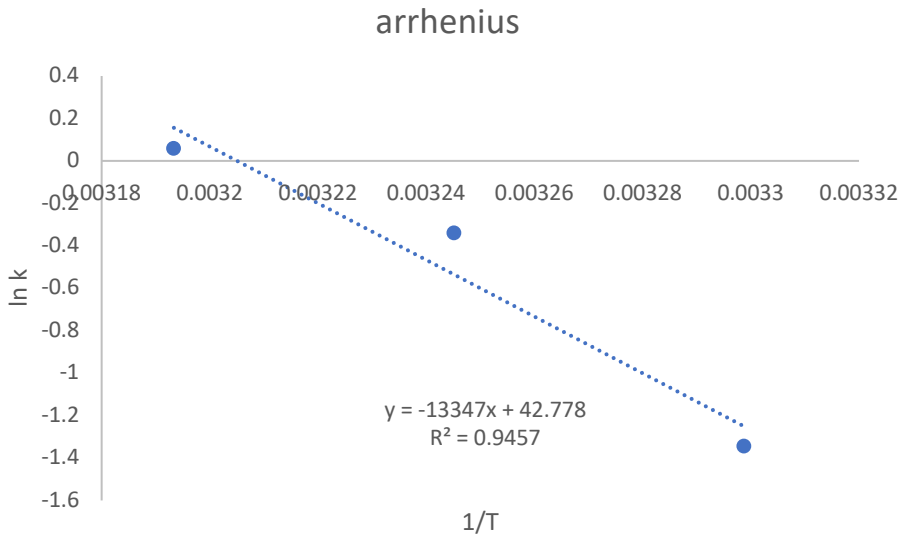


(a)



(b)

Figure 3. Thermodynamic parameters of moisture desorption in dried *C. ternatea* simplicia. (a) First-order kinetic plots for the moisture desorption of dried *C. ternatea* simplicia; (b) Arrhenius plot depicting the linear relationship between the natural logarithm of the rate constant and the reciprocal of absolute temperature.

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Powder Product: Vapor Sorption and RH Anomalies

For the powder format, chemical parameters remained stable, establishing moisture content as the Critical Quality Attribute (CQA), as minimal moisture uptake induces sugar crystallization and caking (Juarez-Enriquez *et al*., 2022; Lad *et al.,* 2022). Despite data variability, a zero-order model was selected based on mass transfer theory in a closed system; the initial vapor pressure differential remains relatively constant, justifying a linear sorption model (Koga *et al*., 2022). Figure 4 maps the empirical increase in moisture content over time under these varying conditions. The graphical distribution of the data points explicitly reveals the fluctuating nature of the sorption process; notably, the curve for 35°C visually diverges from the expected thermal progression, yielding a lower reaction rate (k = 0.291) compared to the 30°C treatment (k = 0.349). This anomaly is explained by sorption thermodynamics in a closed incubator; a temperature increase without humidity control inherently lowers Relative Humidity (RH). The reduction in environmental water vapor pressure decreases the driving force for mass transfer, overpowering the thermal kinetic energy effect (Duong *et al.,* 2017; Edar *et al.,* 2021). Utilizing the Direct Method at 30°C (representing tropical storage conditions), with an initial moisture content of 1.37% and a critical caking limit of 5%, the predicted shelf life of the powder is 10.4 weeks (~2.5 months).

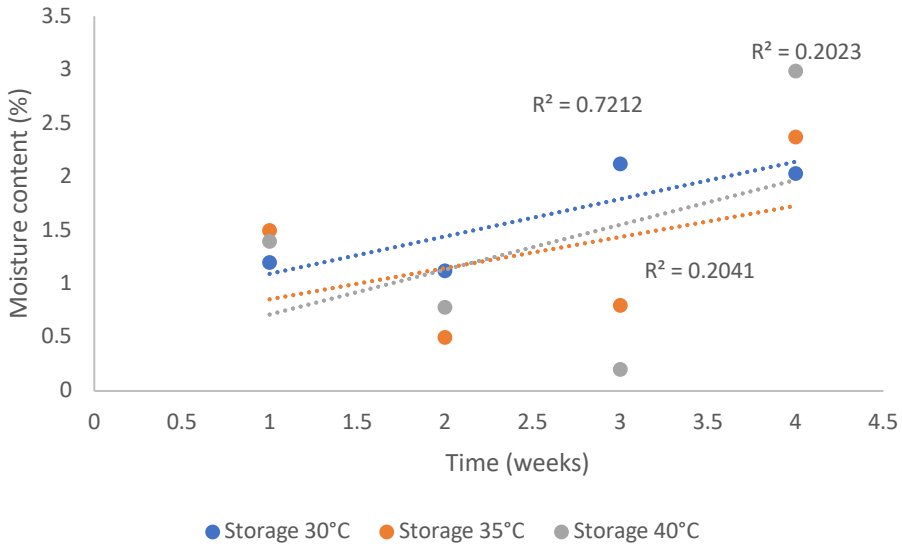

Figure 4. Zero-order kinetics of moisture sorption in *C. ternatea* powder products during storage

CONCLUSION

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This study successfully identified 35 h as the optimal incubation time for *C. ternatea* fermentation, striking a critical balance between microbial proliferation, acid-sweet sensory appeal, and soda-like mouthfeel, outperforming fresh, dried, and powdered formats in hedonic testing. However, the subsequent kinetic analysis reveals that commercialization requires highly matrix-specific storage strategies. The inherent physiological instability of fresh flowers limits their shelf life to merely 2 days at ambient temperatures. The optimized fermented liquid, while sensory-superior, exhibits a shelf life of only 5.57 days at room temperature due to continuous microbial acidification, necessitating cold chain distribution. Conversely, solid formats offer extended but condition-dependent stability: dried simplicia require a 4.8-week conditioning period to overcome high activation energy barriers for moisture desorption, while powdered products are highly susceptible to caking via zero-order moisture sorption, yielding a 10.4-week shelf life that can only be secured via high-barrier packaging and desiccants. Together, these findings provide a comprehensive roadmap from product optimization to shelf-life engineering for butterfly pea-based functional beverages.

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