RESEARCH ARTICLE

Mechanism of taro leaf extract extending the shelf life of jujube-ginger juice beverages as a dual-function stabilizing and preservative agent

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Fruit and vegetable juice beverages often face quality deterioration during storage due to physical instability such as particle aggregation and sedimentation and microbial contamination, which shorten shelf life. Natural preservatives with dual functions are needed to meet clean-label trends. This study investigated the mechanism of taro leaf extract (TLE) as a dual-effect factor for both stability enhancement and anticorrosion to extend the shelf life of jujube and ginger juice beverage. TLE was obtained by ultrasonic-assisted extraction with 70% ethanol. Its total phenol content was 156.8 mg gallic acid equivalent (GAE)/g with chlorogenic acid as the major component at the concentration of 42.3 mg/g. The extract was added to the beverage at 0.5 – 2.0 mg/mL concentrations. Physicochemical stability including turbidity, Zeta potential, particle size, browning inhibition, and antibacterial activity against *Escherichia coli*, *Saccharomyces cerevisiae*, and *Aspergillus niger* were evaluated. A shelf-life prediction model was established based on browning kinetics and microbial growth. The results showed that, at 2.0 mg/mL TLE, turbidity retention increased from 60.5% to 92.6%, absolute Zeta potential increased from 24.6 mV to 36.8 mV, and sedimentation decreased by 75.6%. The browning rate constant decreased from 0.0052/d to 0.0018/d, and the activation energy increased by 14.3 kJ/mol. The minimum inhibitory concentrations of TLE were 0.8 mg/mL against *E. coli*, 1.2 mg/mL against *S. cerevisiae*, and 1.5 mg/mL against *A. niger*. The shelf-life model indicated that 2.0 mg/mL TLE extended shelf life from 42 days to 126 days at 25°C and to 235 days at 4°C. TLE achieved a synergistic dual effect by enhancing electrostatic repulsion, scavenging free radicals, and inhibiting microbial growth. At 1.0 – 2.0 mg/mL of TLE, it met room-temperature shelf-life requirements of 3 – 4 months, providing a theoretical basis for developing natural preservatives for fruit and vegetable juices.

Keywords: taro leaf extract; jujube ginger juice beverage; shelf life; anticorrosion; dual-effect factor.

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Introduction

Jujube and ginger juice beverage has emerged as an important product category in the functional beverage market. Jujube polysaccharides exhibit immunomodulatory activity, while gingerol provides a distinctive flavor, making the beverage both nutritious and palatable. However, such beverage faces two major technical challenges during storage including poor physical stability due to protein–polysaccharide interactions leading to turbidity loss and sedimentation and high risk of microbial contamination from natural fermentation and spoilage organisms, which shorten shelf life. Industrially, sodium carboxymethyl cellulose and potassium sorbate are commonly used as stabilizer and preservative, respectively, but the clean-label

trend is driving the demand for natural alternatives.

Significant progress has been made in shelf-life prediction methodologies. Ntzimani *et al*. developed an accelerated shelf-life testing method for high-pressure processed meat products [1]. Arboretti *et al*. compared various shelf-life prediction methods, providing a statistical basis for model selection [2]. Dantas *et al*. discussed the influence of shelf life on production scheduling and vehicle routing for perishable products [3]. Rusman *et al*. explored shelf-life extension strategies through packaging modifications [4]. Ekafitri *et al*. applied the critical moisture content method for accelerated shelf-life assessment of energy banana bars [5]. Endo *et al*. analyzed factors affecting preservative efficacy attenuation in nonwovens [6]. Rauh and Xiao examined the effect of heat treatment on dairy product shelf life [7]. Nasralla *et al*. studied compositional characteristics of expired dairy products and their potential non-dairy applications [8]. Natural preservatives and antioxidants have gained widespread attention. Venkatesan and Muniyan reviewed the application of natural preservatives for extending fruit and vegetable shelf life [9]. Martínez-Carrasco *et al*. investigated Spanish consumers' preference for natural preservatives in fruits [10]. Tian *et al*. studied the residual behavior of five preservatives and their metabolites in pomegranate storage and processing [11]. Zhang *et al*. analyzed the distribution, migration and transformation of typical chemical preservatives in citrus [12]. Kumar *et al*. raised safety concerns regarding preservatives in pharmaceuticals [13]. Rajput *et al*. reviewed wood preservative development [14]. Garcia *et al*. used rapid turbidimetry to assess inhibitory effects of preservatives on bread spoilage fungi [15]. Herbon studied the economic impact of implementation costs on shelf-life extension strategies [16]. Liu *et al*. summarized antifungal mechanisms of lactic acid bacteria in baked products [17]. Taro leaves as agricultural by-products with an annual output exceeding 2 million tons and a utilization rate below 15% have attracted research interest. Dash *et al*. prepared a bio-based composite film/coating from deccan hemp seed protein, taro starch, and taro leaf extract for grape preservation [18]. Rahmawati *et al*. combined *Moringa oleifera* leaf extract with taro tuber to produce antioxidant edible films [19]. Christou *et al*. used natural deep eutectic solvents for ultrasound-assisted extraction of antioxidants from taro leaves [20]. Shehata *et al*. demonstrated that taro leaf extract synergized with probiotic lactic acid bacteria to improve antioxidant capacity in fermented milk beverages [21]. Yuan *et al*. studied the inhibitory effect of mint extract on browning of fresh-cut taro [22]. Shah *et al*. reviewed industrial applications of taro as a novel food ingredient [23]. Mitharwal *et al*. summarized the nutritional and phytochemical composition of taro leaves and their potential health benefits [24]. These studies indicate that taro leaf extract is rich in phenolic compounds and exhibits antioxidant, antibacterial, and anti-browning activities, making it a promising natural dual-effect stabilizer and preservative. However, its application in jujube and ginger juice beverages and the systematic mechanism of its influence on shelf life have not been reported.

This study investigated the effect mechanism of taro leaf extract on the shelf life of jujube and ginger juice beverage. The contents of total phenols, total flavonoids, and major monomeric phenols in taro leaf extract were determined to identify the active substance basis. The effects of adding taro leaf extract at different concentrations on the physicochemical stability of the beverage were evaluated, and key stability indicators were measured to analyze the stabilization mechanism. The inhibitory effects of taro leaf extract against the dominant spoilage microorganisms in the beverage and the minimum inhibitory concentrations were determined. A modified Gompertz kinetic model based on microbial growth was established to fit the change in total colony counts under different temperature conditions. A first-order reaction kinetic model based on browning degree change was constructed, and the activation energy was

calculated using the Arrhenius equation. The shelf-life endpoint was determined by integrating physicochemical and microbiological indices, and the predicted shelf lives of products with different amounts of taro leaf extract were compared under different storage conditions. The synergistic mechanism of taro leaf extract was elucidated, and the quantitative relationship between the amount of taro leaf extract added and shelf-life extension was established to provide data support for the application of natural dual-effect stabilizer and preservative factors in fruit and vegetable juice beverages.

Materials and methods

Plant materials and sampling

Fresh taro leaves (*Colocasia esculenta* L. Schott var. Lipu) were harvested from Shuangqiao Town, Wuming District, Nanning City, Guangxi Zhuang Autonomous Region, China (22°85′N, 108°32′E) on August 15, 2024, between 8:00 am and 10:00 a.m. A total of 200 healthy mature leaves with a total fresh weight of 2.5 kg without visible disease or pest damage were collected. Immediately after harvest, the leaves were placed in polyethylene bags and transported to the laboratory in an insulated container at 4°C within 4 h. Upon arrival, the leaves were washed with tap water followed by distilled water, blotted dry with paper towels, and stored at −80°C for no longer than 7 days before further processing.

Preparation of taro leaf extract (TLE)

The frozen taro leaves were cut into 2 cm × 2 cm pieces and freeze-dried at −50°C for 48 h using a LGJ-10N freeze dryer (Beijing Songyuan Huaxing, Beijing, China). Dried leaves were ground into fine powder and passed through a 60-mesh (aperture 250 μm) sieve. For each extraction, 10.0 g powder was mixed with 200 mL 70% (v/v) ethanol in a 500 mL conical flask. Ultrasonic-assisted extraction was performed using KQ-300DE ultrasonic cleaner (Kunshan Shumei, Kunshan, Jiangsu, China) with 300 W power and 40 kHz frequency. The extraction temperature was maintained at 50 ± 1°C using a circulating water bath, and the extraction duration was 40 min. The mixture was then vacuumed through Whatman No. 1 filter paper (Whatman, Maidstone, Kent, UK). The filtrate was concentrated under reduced pressure using RE-52AA rotary evaporator (Shanghai Yarong, Shanghai, China) at 45°C, 0.09 MPa, until a viscous concentrate was obtained. The concentrate was freeze-dried again to obtain the TLE dry powder with the extraction yield of 8.7 ± 0.3%. The TLE powder was stored in airtight dark glass bottles at −20°C until use.

Preparation of jujube and ginger juice beverage

Jujube fruits (*Ziziphus jujuba* Mill. cv. Ruoqiang) (Yipin Jujube Co., Ltd., Cangzhou, Hebei, China) were washed, pitted, and extracted with 8 volumes (v/w) of deionized water at 90°C for 60 min in a water bath. The extract was filtered through four layers of cheesecloth to obtain jujube juice. Fresh ginger (*Zingiber officinale* Roscoe cv. Laiwu) obtained from a local farmers' market (Laiwu, Shandong, China) was peeled, diced, and blended with 5 volumes (v/w) of deionized water for 2 min using a household blender. The ginger slurry was sieved through a 0.125 mm mesh sieve to obtain ginger juice. Jujube juice and ginger juice were mixed at a volume ratio of 7:3. Food grade sucrose (COFCO, Beijing, China) was added to reach a soluble solid content of 10° Brix, that meant 10 g soluble solids per 100 g solution, measured by ATAGO hand-held refractometer (ATAGO, Tokyo, Japan) at 20°C. The pH was adjusted to 3.8 ± 0.1 with food grade citric acid (Weifang Ensign, Weifang, Shandong, China). TLE was added at four concentration levels of 0 (control), 0.5, 1.0, and 2.0 mg/mL, respectively. The mixture was stirred at 500 rpm for 15 min to dissolve TLE completely, then homogenized twice at 20 MPa using ATS AH-BASIC laboratory homogenizer (ATS Engineering, Brampton, ON, Canada). The beverage was filled into 50 mL sterile polypropylene centrifuge tubes with minimal headspace, sealed, and stored under defined conditions.

Determination of active components in TLE

Total phenolic content (TPC) was determined by dissolving TLE in 70% ethanol to prepare a 1 mg/mL solution followed by Folin–Ciocalteu method. Briefly, 0.5 mL of sample solution was mixed with 2.5 mL of 10-fold diluted Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) and allowed to stand for 5 min. 2.0 mL of 7.5% Na_2CO_3 solution was then added. After 60 min incubation at room temperature in the dark, the absorbance was measured at 765 nm using Shimadzu UV-1800 UV-vis spectrophotometer (Shimadzu, Kyoto, Japan). Gallic acid (Sigma-Aldrich, St. Louis, MO, USA) was used as standard, and results were expressed as mg gallic acid equivalent (GAE) per g of dry extract. Total flavonoid content (TFC) was determined by the aluminum nitrate colorimetric method. A 1 mL aliquot of 1 mg/mL sample solution was mixed with 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$. After 5 min, 0.3 mL of 10% $Al(NO_3)_3$ was added for another 5 min followed by 2 mL of 1 M NaOH. The mixture was diluted to 10 mL with distilled water, and the absorbance was measured at 510 nm. Rutin (Sigma-Aldrich, St. Louis, MO, USA) was used as standard, and results were expressed as mg rutin equivalent (RE) per g of dry extract. The composition of phenolic compounds was analyzed by using Agilent 1260 Infinity II high-performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector (DAD) and a Zorbax SB-C18 column (4.6 mm × 250 mm, 5 µm). The mobile phase consisted of 0.1% formic acid in water and methanol. Gradient elution was performed at 1.0 mL/min and 30°C as 0 – 10 min using 10% methanol, 10 – 25 min using 10 – 30% methanol, 25 – 40 min using 30 – 50% methanol followed by re-equilibration. Injection volume was 20 µL, and detection wavelengths were 280 nm and 320 nm. Compounds were identified by comparing retention times and UV spectra with authentic standards of chlorogenic acid, caffeic acid, ferulic acid, rutin, and quercetin. Quantification was based on calibration curves of each standard.

Physicochemical stability assessment

Beverage samples with different TLE concentrations of 0, 0.5, 1.0, 2.0 mg/mL were stored at 4°C, 25°C, and 37°C in the dark. At predetermined time points of 0, 7, 14, 21, 30, 45, 60, 90 days, three independent tubes per condition were taken for analysis. Turbidity was measured using WGZ-2000 turbidimeter (Shanghai Xinrui, Shanghai, China). Samples were gently inverted three times to homogenize, then 15 mL was transferred to the turbidity cell. Readings were taken after equilibration for 5 min at room temperature. The turbidity retention rate was calculated as (Turbidity at time t / Initial turbidity) × 100%. Zeta potential and particle size distribution were determined using a Zetasizer Nano ZS90 (Malvern Panalytical, Malvern, Worcestershire, UK). The beverage samples were diluted 10-fold with deionized water to avoid multiple scattering. For Zeta potential, the diluted sample was injected into a DTS1070 disposable capillary cell, equilibrated at 25°C for 120 s, and measured in triplicate. For particle size, the same dilution was measured in a DTS0012 disposable cuvette using laser diffraction. The volume-weighted mean diameter D[4, 3] was automatically calculated using Zetasizer Software v7.12 as follows.

$$D[4,3] = \frac{\sum(d_i^4 \cdot n_i)}{\sum(d_i^3 \cdot n_i)} \quad (1)$$

where d_i was the diameter of particle i. n_i was the number of particles of that diameter. Three measurements per sample were averaged. Sedimentation was measured by centrifugation of 10 mL beverage at 4,000 rpm for 20 min at 20°C using H2100R high-speed refrigerated centrifuge (Hunan Xiangyi, Changsha, Hunan, China). The supernatant was discarded, and the wet weight of the precipitate was recorded.

Browning degree measurement

Browning degree was evaluated by measuring the absorbance at 420 nm. Beverage samples were filtered through a 0.45 µm hydrophilic syringe filter (Millipore, Burlington, MA, USA). The filtrate was transferred to a quartz cuvette, and the absorbance was measured against

deionized water as blank. The browning inhibition index (BII) was calculated as (A_{420} at time t / A_{420} at time 0) × 100%.

Antimicrobial activity assay

Three spoilage microorganisms commonly found in fruit and vegetable juices were tested including *Escherichia coli* ATCC 25922 (Gram-negative bacterium), *Saccharomyces cerevisiae* CICC 1374 (yeast), and *Aspergillus niger* CICC 2475 (mold). All strains were obtained from China Center of Industrial Culture Collection (CICC) (Beijing, China). *E. coli* was cultured in Luria-Bertani (LB) broth at 37°C for 24 h, while *S. cerevisiae* was cultured in Yeast Extract-Peptone-Dextrose (YPD) broth at 28°C for 48 h, and *A. niger* was cultured on potato dextrose agar (PDA) at 28°C for 5 days, and spores were harvested with sterile saline containing 0.05% Tween 80. The minimum inhibitory concentration (MIC) of TLE was determined by the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. TLE was dissolved in sterile deionized water and serially diluted twofold in 96-well plates to final concentrations ranging from 0.1 to 3.0 mg/mL. Each well contained 100 µL of TLE solution, 80 µL of appropriate broth, and 20 µL of microbial suspension with the final inoculum of 10^6 CFU/mL for bacteria/yeast and 10^5 spores/mL for mold. Positive controls (without TLE) and negative controls (without inoculum) were included. Plates were incubated under optimal conditions with 37°C for 24 h for *E. coli*, 28°C for 48 h for yeast, 28°C for 72 h for mold. MIC was defined as the lowest TLE concentration with no visible microbial growth (turbidity or pellet). All tests were performed in triplicate.

Microbiological analysis during storage

At each sampling time point of 0, 7, 14, 21, 30, 45, 60, 90 days, three independent tubes per condition including TLE concentrations and storage temperatures were analyzed. Under a clean bench, 1 mL of beverage sample was serially diluted 10-fold with sterile 0.85% saline. For total viable count (TVC), 0.1 mL of appropriate dilutions were spread onto plate count agar (PCA) (Beijing Land Bridge, Beijing, China) and incubated at 36 ± 1°C for 48 ± 2 h. For yeast and mold enumeration, 0.1 mL of dilutions were spread onto Rose Bengal Chloramphenicol Agar (Beijing Land Bridge, Beijing, China) and incubated at 28 ± 1°C for 120 ± 2 h. Colonies were counted and expressed as colony-forming units per mL (CFU/mL). Counts below the detection limit (10 CFU/mL) were recorded as < 10. All dilutions were plated in duplicate.

Shelf-life prediction modeling

Browning kinetics was determined by fitting the changes in browning degree (A_{420}) over time to a first-order reaction model below.

$$\ln(A_t) = \ln(A_0) - k \cdot t \quad (2)$$

where A_t and A_0 were the absorbances at time t and time 0, respectively. k was the rate constant (d^{-1}). The temperature dependence of k was modeled using the Arrhenius equation as follows.

$$k = A_f \cdot \exp\left(-\frac{E_a}{R \cdot T}\right) \quad (3)$$

where A_f was the frequency factor. E_a was the activation energy (J/mol). R was the gas constant (8.314 J/mol·K). T was the absolute temperature (K). Linear regression of ln(k) versus 1/T yielded E_a and A_f. Microbial growth kinetics were determined by fitting total viable counts (logCFU/mL) to the modified Gompertz model as follows.

$$N(t) = N_0 + (N_{max} - N_0) \cdot \exp\left\{-\exp\left[\frac{\mu \cdot e}{N_{max} - N_0} \cdot (\lambda - t) + 1\right]\right\} \quad (4)$$

where N(t) was the log count at time t (logCFU/mL). N_0 and N_{max} were the initial and maximum log counts. µ was the maximum specific growth rate (logCFU/d). λ was the lag time (d). e was Euler's number (≈ 2.71828). Nonlinear regression was performed using OriginPro 2021 (OriginLab, Northampton, MA, USA). The shelf life was defined as the time when either the browning degree (A_{420}) reached 0.3 determined by preliminary sensory evaluation or the total viable count exceeded 10^4 CFU/mL

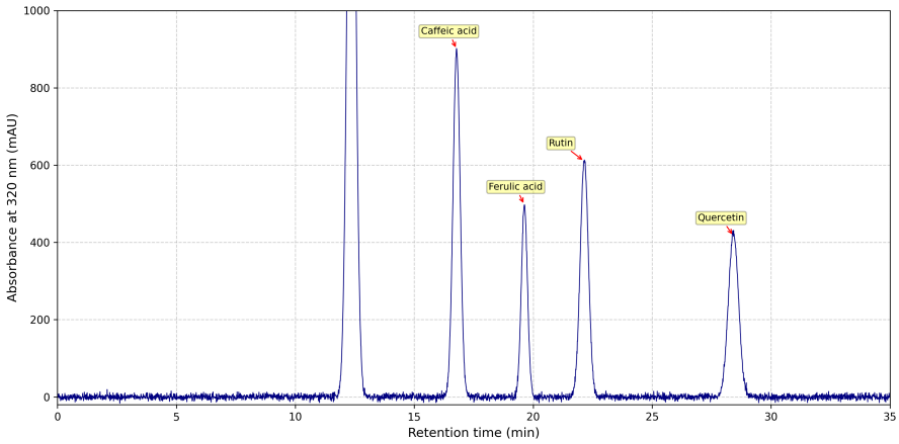

Figure 1. HPLC chromatogram of TLE.

Table 1. Contents of main phenolic compounds in TLE.

Compound	Retention time (min)	Content (mg/g)	Peak area percentage (%)
Quinic acid	12.36 ± 0.05	42.3 ± 1.8	38.2
Caffeic acid	16.78 ± 0.04	18.6 ± 0.9	16.8
Ferulic acid	19.63 ± 0.06	9.4 ± 0.5	8.5
Rutin	22.15 ± 0.07	12.5 ± 0.7	11.3
Quercetin	28.42 ± 0.08	8.2 ± 0.4	7.4
Other unidentified peaks	-	65.8 ± 2.1	17.8
Total phenol	-	**156.8 ± 3.2**	100
General flavone	-	**89.4 ± 2.1**	-

Note: The content was calculated on a dry basis. The total phenols were expressed in gallic acid equivalent (GAE), and the total flavonoids were expressed in rutin equivalent (RE). The data were expressed as mean ± standard deviation (n=3).

according to National food safety standard – Drinks (GB 7101-2022) (https://www.chinesestandard.net/PDF.aspx/GB7101-2022) for fruit and vegetable juice beverages, whichever occurred first.

Statistical analysis

SPSS 26.0 (IBM, Armonk, NY, USA) was employed for statistical analysis. All experimental data were presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was performed. Nonlinear curve fitting for kinetic models was carried out using OriginPro 2021 with the Levenberg–Marquardt algorithm with max iterations 400 and tolerance 10^{-9}. The goodness of fit was evaluated by the coefficient of determination (R^2), root mean square error (RMSE), and Akaike information criterion (AIC). P value less than 0.05 was defined as statistically significant difference.

Results

Analysis of active components of taro leaf extract

HPLC analysis of TLE identified five main phenolic compounds (Figure 1). Chlorogenic acid was predominant with 42.3 mg/g and 27.0% of total phenols followed by caffeic acid, rutin, ferulic acid, and quercetin. Total phenol content was 156.8 mg gallic acid equivalent (GAE)/g, and total flavonoid content was 89.4 mg rutin equivalent (RE)/g. These phenolic acids constituted the major antioxidant and antibacterial components of TLE (Table 1).

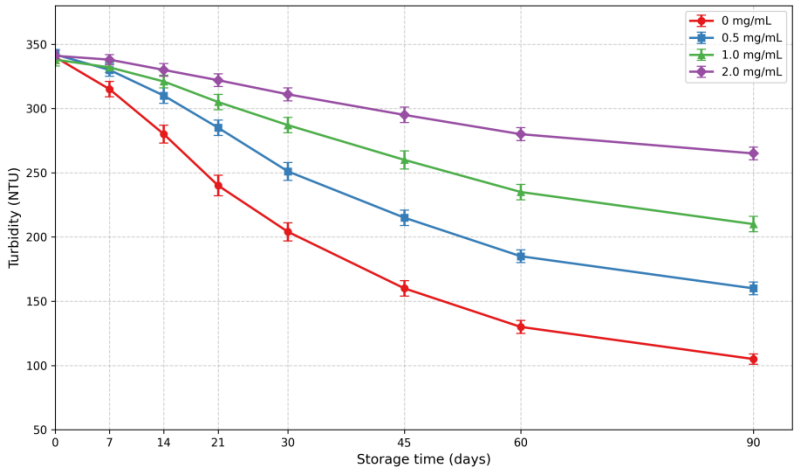

Figure 2. Turbidity of beverage with different TLE addition amount.

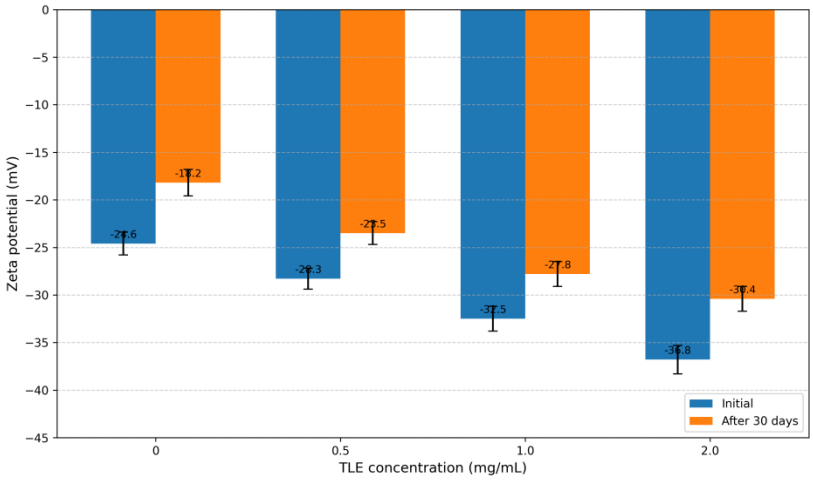

Figure 3. Zeta potential changes of beverages with different TLE addition.

The effect of TLE on the physical and chemical stability of beverages

Turbidity measured in Nephelometric Turbidity Units (NTU) reflected beverage stability. At 25°C after 30 days, the control group showed a turbidity retention rate of 60.5%, while 2.0 mg/mL TLE increased retention to 92.6%. Intermediate TLE concentrations gave intermediate values (Figure 2). The results indicated that TLE inhibited particle aggregation and sedimentation in a concentration dependent manner. Zeta potential's absolute value indicated colloidal stability. Initially, the control group showed −24.6 mV. After adding 2.0 mg/mL TLE, it increased to −36.8 mV. After 30 days at 25°C, the control group decreased to −18.2 mV, while 2.0 mg/mL TLE group remained at −30.4 mV. The results showed that negatively charged phenolics in TLE adsorbed onto particles, enhancing electrostatic repulsion and stability (Figure 3). Particle size distribution reflected particle aggregation degree. The results demonstrated that the particle size distribution curve of beverages with different TLE additions after 30 days storage showed broad unimodal distribution in the control group with a volume average diameter D[4,3] of 1,245 nm, indicating particle aggregation. As TLE addition increased, the curve shifted left, and the peak became narrow. The D[4,3] of the 0.5 mg/mL group was

Table 2. Turbidity retention rate and precipitation amount of beverages with different TLE addition (storage for 30 days at 25℃).

TLE addition (mg/mL)	Initial turbidity (NTU)	30 days turbidity (NTU)	Turbidity retention rate (%)	Precipitation amount (g/10mL)
0	340 ± 5	204 ± 7	60.5 ± 2.1	0.86 ± 0.05
0.5	342 ± 4	251 ± 7	74.0 ± 2.3	0.58 ± 0.04
1.0	338 ± 5	287 ± 6	84.9 ± 2.0	0.35 ± 0.03
2.0	341 ± 4	311 ± 5	92.6 ± 1.8	0.21 ± 0.02

Note: Data were expressed as mean ± standard deviation (n = 3). The precipitation amount was 10 mL, and the wet weight of the sample was precipitated after centrifugation.

876 nm, while the 1.0 mg/mL group was 652 nm, and the 2.0 mg/mL group was only 423 nm (Figure 4). The 2.0 mg/mL group's particle size distribution was close to the initial state of 380 nm, showing that TLE effectively inhibited particle aggregation and maintained the system's fine dispersion, which might be due to the steric - hindrance layer formed by polysaccharides and flavonoids in TLE on the particle surface combined with electrostatic repulsion to synergistically stabilize the system.

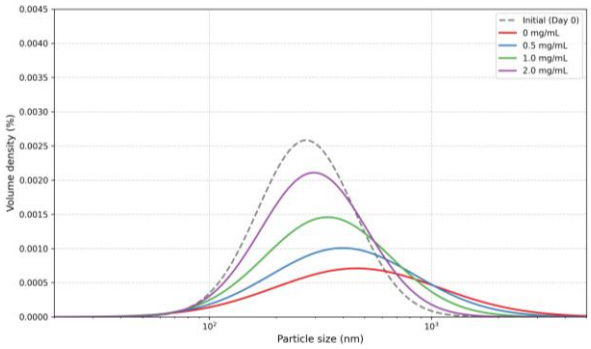

Figure 4. Particle size distribution of beverage after 30 days storage.

After 30 days of storage, the control group had a precipitation of 0.86 g/10 mL. Adding 2.0 mg/mL TLE reduced precipitation to 0.21 g/10 mL, which was close to the initial 0.18 g/10 mL (Table 2). Thus, TLE reduced precipitation and maintained beverage homogeneity in a concentration dependent manner.

Inhibitory effect of TLE on browning degree of beverage

The first-order reaction kinetic model provided an excellent fit to the browning data at 25°C with $R^2 > 0.98$ for all TLE concentrations. The browning rate constant (k) decreased progressively with increasing TLE concentration. The control group showed the highest rate constant of 0.0052/d, while the 2.0 mg/mL TLE group showed the lowest rate constant of 0.0018/d, indicating that TLE effectively slowed the browning reaction (Figure 5). The intermediate TLE concentrations of 0.5 and 1.0 mg/mL yielded intermediate rate constants (data not shown).

Evaluation of antibacterial activity of tale

The results showed that the MIC of TLE against *Escherichia coli* ATCC 25922 was determined to be 0.8 mg/mL, while the MICs were 1.2 mg/mL and 1.5 mg/mL against *Saccharomyces cerevisiae* CICC 1374 and *Aspergillus niger* CICC 2475, respectively. The results indicated that TLE exhibited the strongest inhibitory effect against the Gram-negative bacterium followed by the yeast with relatively weaker activity against the mold. The relative growth inhibition rates of three spoilage bacteria at different TLE concentrations showed that, at 2.0 mg/mL TLE, inhibition exceeded 90% for *Escherichia coli*, yeast, and mold with the inhibition rate being calculated as (1 − colony forming units (CFU) of treatment / CFU of control) × 100% (Figure 6). TLE exhibited broad spectrum, concentration dependent antimicrobial activity, attributed to phenolic compounds such as chlorogenic acid disrupting cell membranes and enzyme function.

Shelf-life prediction model and its verification

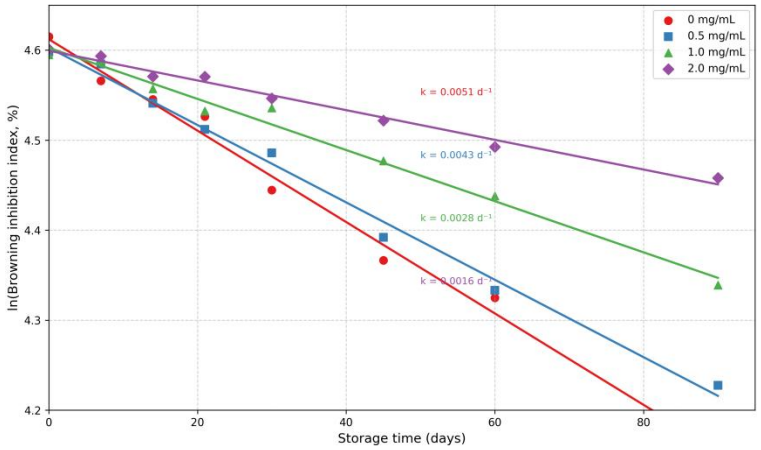

Figure 5. Browning degree fitting straight line based on first-order kinetic model.

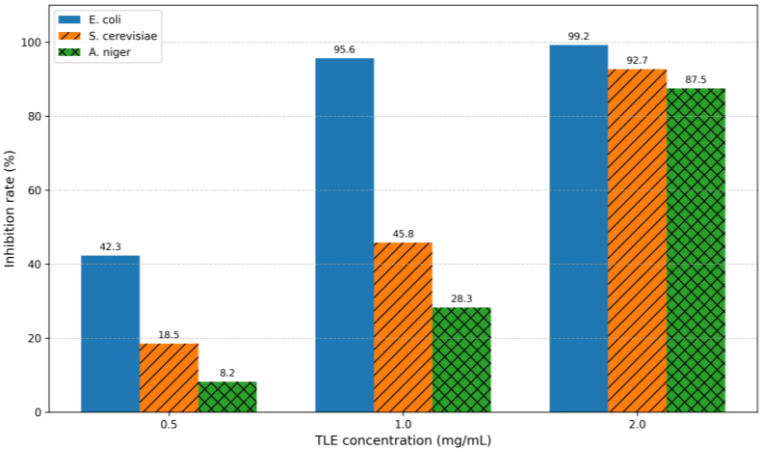

Figure 6. Inhibitory effect of TLE on dominant spoilage bacteria (yeast, mold, *Escherichia coli*) in beverages.

Under 25°C storage condition, modified Gompertz model fitted total bacterial count changes in beverages with different TLE additions. The results demonstrated that the control group exceeded 10^4 cfu/mL on day 14, while 0.5 mg/mL group reached limit on day 30 and 1.0 mg/mL group on day 45. 2.0 mg/mL group remained below threshold for 90 days. Gompertz parameters showed that TLE extended lag phase and reduced growth rate with $r^2 > 0.96$ for all fits. Based on browning and colony count models with Arrhenius extrapolation, shelf life at 4°C, 25°C and 37°C was determined. The control group shelf-life ranged from 18 - 98 days. TLE prolonged shelf-life dose-dependently as 2.0 mg/mL group achieving 235, 126, and 68 days at the different temperatures tested, which were 2.4 - 3.8 times that of control group, showing significant preservation effects, especially at room temperature (Table 3). The relationship between shelf-life and TLE addition showed that, at three storage temperatures, the shelf-life was linearly prolonged following the increase of the concentration of TLE with the linear correlation coefficients r as 0.996 at 4°C, 0.999 at 25°C, and 0.997 at 37°C, respectively. The slope reflected the synergistic degree of TLE, which was the largest at 25°C that prolonged shelf-life by about 41 days for every increase of 1 mg/mL of TLE, the smaller at 4°C that prolonged by about 68 days for every increase of 1 mg/mL, and the smallest at 37°C that prolonged by about 25 days for every

Table 3. Predicted shelf-life (days) of beverages at 4℃, 25℃, and 37℃ with different TLE additions.

Addition amount of TLE (mg/mL)	4 ℃	25℃	37℃
0	98 ± 5	42 ± 3	18 ± 2
0.5	135 ± 7	63 ± 4	28 ± 3
1.0	182 ± 9	89 ± 5	45 ± 4
2.0	235 ± 12	126 ± 7	68 ± 5

Note: Shelf-life was calculated by the time when browning degree or total number of colonies whichever reached the threshold first. The data were expressed as mean ± standard deviation (n = 3).

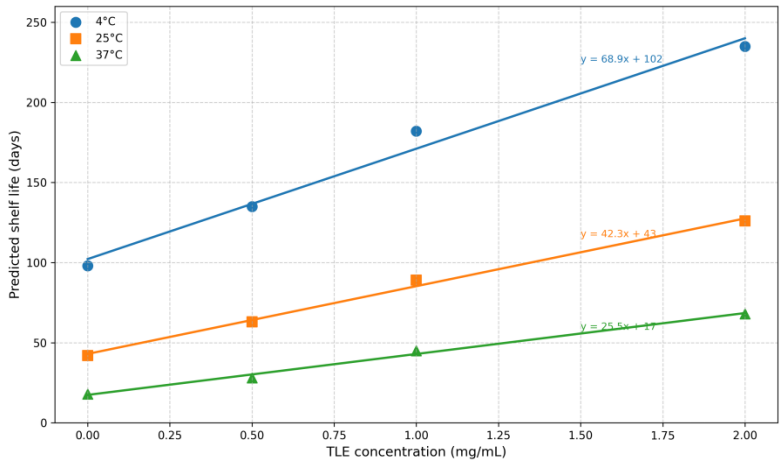

Figure 7. Relationship curve between predicted shelf-life and TLE addition.

increase of 1 mg/ml (Figure 7). TLE had the highest shelf-life improvement efficiency at room temperature, which was suitable for actual circulation scenarios. The linear characteristics of the relationship curve provided a convenient quantitative basis for determining the amount of TLE added according to the target shelf-life in production practice.

The application scenario

According to the experimental results, TLE served as a dual-effect stabilizer and preservative. Recommended addition was 1.0 – 2.0 mg/mL, achieving 89 – 126 days shelf-life at room temperature, while combining 1.0 mg/mL TLE with 4°C storage could extend shelf-life to 6 months. The shelf-life prediction model supported formulation optimization for other juice systems.

Discussion

Under laboratory conditions, TLE demonstrated good stability and antiseptic effects, but industrial application faces challenges including seasonal raw material variation with more than 30% total phenol fluctuation, scale-up reduces extraction yield from 8.7% to less than 6%, solubility limits in high-protein/fat systems, cost about¥0.80/L for 1.0 mg/mL, not yet listed in GB 2760 because safety evaluation takes 18 – 24 months, powder caking necessitates liquid premixes, and real-world shelf-life as predicted 126 days at 25°C might drop to 80 – 90 days due to temperature fluctuations, light, and vibration.

Based on existing research, four future directions were proposed. The first was the isolation and identification of core active monomers. 42.3 mg/g chlorogenic acid with 27% of total phenols might synergize with unidentified components, and the activities could be verified *via* preparative HPLC. The second was to clarify interaction mechanisms between TLE and

beverage proteins and/or polysaccharides, distinguishing electrostatic from steric contributions to Zeta potential increase. The third was to incorporate sensory evaluation including color, aroma, taste into a multi-factor shelf-life model beyond browning and microbial counts. The fourth was to extend microbiology to naturally contaminated samples to identify dominant spoilage organisms and optimize TLE dosage. Extraction process could be improved by replacing ethanol with deep eutectic solvents to raise yield and reduce solvent residues. Stability tests should be conducted across varying pH levels and sugar contents to establish a response surface model. Eventually, the human safety evaluation including acute oral toxicity, genotoxicity, and 90-day feeding should be completed to support food additive registration.

TLE contained abundant phenolic compounds with chlorogenic acid as the dominant component. TLE functioned as a natural dual-effect stabilizer and preservative in jujube and ginger juice beverage through three synergistic mechanisms including enhancing electrostatic repulsion and steric hindrance to prevent particle aggregation, scavenging free radicals to delay browning, and inhibiting microbial growth *via* membrane disruption. The shelf-life prediction model confirmed that TLE addition at 1.0 – 2.0 mg/mL extended the room-temperature shelf-life to 3 – 4 months, meeting commercial requirements. This study provided a theoretical foundation for utilizing agricultural by-products (taro leaves) as a source of natural food additives, offering an alternative to synthetic stabilizers and preservatives in fruit and vegetable juice beverages.

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