

Optimization of flavonoid extraction from mulberry leaves and development of functional noodle products

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Abstract

This study aimed to optimize the extraction of flavonoids from mulberry leaves and their application in functional noodles to enhance the valorization of mulberry leaf resources. The dry-up method was selected as the optimal pretreatment based on ultraviolet spectrophotometry. Ultrasonic-assisted extraction was optimized by single-factor experiments and response surface methodology (RSM). The extract was incorporated into noodles as powder, juice, or pulp at varying concentrations to assess quality attributes and antioxidant activities. The dry-up method has proved to be most effective for drying mulberry leaves. The optimal extraction conditions identified via RSM included a solid–liquid ratio of 1:40 g/mL, 56% ethanol, a temperature of 60°C, and a time of 30 min, yielding a total flavonoid content of 26.04%. Three noodle formulations were developed: mulberry leaf powder (1.0% powder, 0.5% baking soda, and 1.0% salt), mulberry leaf juice (16.0% juice, 0.3% baking soda, and 1.0% salt), and mulberry leaf pulp (20.0% pulp, 0.4% baking soda, and 1.1% salt). The flavonoid content was 1.317 ± 0.01 mg/g for mulberry leaf powder noodles, 1.603 ± 0.01 mg/g for mulberry leaf juice noodles, and 1.413 ± 0.02 mg/g for mulberry leaf pulp noodles. All noodles exhibited significant antioxidant activity, with the mulberry leaf pulp variant demonstrating the highest radical scavenging capacity against both radical cation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS⁺) and 2,2-diphenyl-1-picrylhydrazyl (DPPH), outperforming the other two forms. The established extraction method is efficient and applicable for producing flavonoid-enriched noodles with improved antioxidant properties. This approach supports the development of functional grain foods and offers a practical route for the high-value utilization of mulberry leaves.

Keywords: antioxidant; mulberry leaf; mulberry leaf noodles; process optimization; response surface methodology; total flavonoids

Introduction

Noodles represent a fundamental traditional staple in China (Ma *et al.*, 2015). The traditional noodle dough typically comprises wheat flour, water, and salt; however, scientific analysis indicates that because of the loss of some vital nutrients during the refining process of wheat flour, traditional noodle products potentially lack essential nutrients, such as dietary fiber, vitamins, and minerals (Levent *et al.*, 2020). Moreover, regarding sensory quality, the flavor and taste of traditional wheat noodles are relatively monotonous, which may fail to satisfy consumers' demand for dietary diversity and high quality; this can be optimized through innovative processes or improvements in raw material (Shi *et al.*, 2023). Recent studies have explored the incorporation of non-traditional ingredients into noodle formulations. Partial substitution of wheat flour with horse chestnut flour has shown to improve the nutritional profile and antioxidant capacity of Ramen noodles (Ahsan *et al.*, 2023). Other innovations include the addition of banana powder, mushroom powder, quinoa, or fermented plant extracts, contributing to enhanced dietary fiber content, polyphenol levels, and functional properties, such as blood glucose regulation (Sivamaruthi *et al.*, 2018) and antioxidant activity (Jeong *et al.*, 2021). Numerous studies have investigated strategies to enhance the functional properties of noodles, such as raw material substitution and process optimization, to improve their nutritional profile and digestibility aligned with contemporary consumer health requirements.

Mulberry leaves (*Morus alba* L.), belonging to the Mulberry family, are referred to as golden mulberry leaves, winter mulberry leaves, and frost mulberry leaves. Approved by the National Health Commission of Medicinal and Food Resources, the mulberry tree is abundant in 17 essential amino acids, crude protein, crude fat, vitamins and minerals, and other nutrients, possessing high nutritional and medicinal values. Its medicinal applications were first documented in the "Fifty-Two Disease Formula" (Lu, 1995), which describes the use of mulberry juice for coating rodents. The idea of traditional Chinese medicine posits that mulberry leaves possess properties that clear the lungs, alleviate dryness, disperse wind, and enhance liver function and vision. They are frequently employed in the treatment of lung-heat dry cough, wind-heat colds, and related symptoms (National Pharmacopoeia Commission, 2020). The nutritional profile of mulberry leaves is extensive, encompassing flavonoids, polysaccharides, polyphenols, and other bioactive compounds (Li *et al.*, 2021). Contemporary medical research has identified that mulberry leaf possesses antioxidant (Varghese and Thomas, 2019), anti-inflammatory (Ma *et al.*, 2022) hypoglycemic (Chen *et al.*, 2023), blood lipid regulation (Kadam *et al.*, 2019), hepatoprotective (Feng *et al.*, 2023) properties.

Notably, flavonoids and polyphenols have garnered significant attention for their pronounced antioxidant capabilities (Chan *et al.*, 2020). The prevailing techniques for flavonoid extraction from mulberry leaves encompass water and alcohol extraction, organic solvent extraction as well as ultrasound- and microwave-assisted methods (Babamoradi *et al.*, 2018; Cao *et al.*, 2019; Chen *et al.*, 2017; Kazemi *et al.*, 2019; Zhang *et al.*, 2021). These methods are favored due to reduced extraction time, low extraction temperature, and high precision (Awad *et al.*, 2021; Thakur *et al.*, 2023). The resulting extracts exhibit notable thermal stability and compatibility with food matrices, making them suitable for fortification of products, such as noodles, cookies, and bread (Xie *et al.*, 2022). However, challenges remain regarding the retention of bioactive compounds during processing, their interactions within food matrices, and scalability to industrial production. These flavonoids exhibit significant antioxidant and anti-inflammatory properties (Lin *et al.*, 2022) and show promise for food fortification because of their thermal stability and flavor compatibility.

Response surface methodology (RSM) is widely applied in the modeling and optimization of the extraction processes of bioactive compounds (Weremfo *et al.*, 2023). It utilizes a nonlinear model that facilitates the derivation of highly accurate regression equations and enables reasonable predictions to identify optimal process conditions (Wang *et al.*, 2022a). Its primary advantage lies in minimizing the number of experiments necessary for the study. Consequently, it conserves time and diminishes the consumption of reagents and materials (Zulkifli *et al.*, 2020). Mulberry leaf noodles, a significant product derived from the utilization of mulberry leaf resources, are crafted by drying mulberry leaves, grinding them into powder, and combining them with wheat flour in a specific ratio. These noodles are favored by consumers because of their nutritional and health effects.

The current study on mulberry leaf noodles mainly focuses on industrial applications, such as mulberry leaves–wheat flour ratio. For instance, Huang *et al.* (2021) adjusted the formulation using mulberry leaves, baking soda, and salt, resulting in improved parameters for mulberry leaf noodles, which were characterized by uniform color and enhanced quality. Dai *et al.* (2023) demonstrated that incorporating mulberry leaf powder enhances dough viscoelasticity, improves cooking performance, texture, color, sensory acceptability, and antioxidant activity of fresh noodles. Fu *et al.* (2013) applied fuzzy mathematics to optimize the formulation and production process of mulberry leaf hanging noodles, establishing a methodology with substantial practical relevance. Additionally, Chen *et al.* (2024) investigated the effects of varying hot air-drying temperature (40–80°C) on the quality (total phenolics, flavonoids content,

antioxidant capacity, color, and luster) of mulberry leaf powder and the textural properties of noodles. In practical applications, hot air-drying technology is commonly employed to dehydrate fresh mulberry leaves for a prolonged preservation of raw materials; however, the drying temperature considerably influences the concentration of functionally active ingredients in raw materials, coloration, and processing characteristics. The superior performance of shade-drying is attributed to its gentle dehydration under ambient conditions, which minimizes thermal and photo-oxidative degradation of heat-sensitive flavonoids while preserving cellular integrity. Although requiring longer processing time, shade-drying offers significant industrial feasibility because of its low energy consumption, minimal operational costs, and particular suitability for resource-limited production settings.

Despite these advances, critical challenges remain. These include the lack of systematic optimization of drying methods for maximizing flavonoid retention, insufficient understanding of the stability and interaction mechanisms of flavonoids within noodle matrices during thermal processing, and a scarcity of industrial-scale validation. Moreover, comparative studies on the efficacy of different incorporated forms (powder, juice, or pulp) are limited. This study addresses the insufficient research regarding the impact of drying methods on the quality of mulberry leaf powder and its noodle processing attributes; therefore, this paper focuses on determining the total flavonoid content of mulberry leaf powder derived from various drying techniques and their influence on the antioxidant activity, flavor, and texture characteristics of mulberry leaf noodles.

With the advancement of medicinal food industry in recent years, the comprehensive development of mulberry leaf resources has attracted more attention. Fenggang County serves as the largest sericulture county in Guizhou Province, with a cultivation scale of 52,000 Macau (mu; as of December 2024). However, the production and processing wastage in Fenggang County, alongside the waste of withered mulberry leaves after frosts, has become a prominent problem, which results in a relatively low utilization rate of resources, significantly restricting the sustainable development of the industry. Based on the above-mentioned realistic basis, the efficient utilization of mulberry leaves after frost was focused in this study, and the RSM was employed in the optimization of the extraction process of flavonoids from mulberry leaves. RSM could optimize processing conditions and reduce the cost, contributing to an increase in product yield. Besides, the research system examined key factors (such as material–liquid ratio, ethanol concentration, extraction temperature, and frequency) to determine optimal extraction parameters. Meanwhile,

preparation conditions for drying mulberry leaves and mulberry leaf surface were optimized, alongside the evaluation of the total flavonoid content and antioxidant activity, aiming to provide data support for the diversified development of mulberry leaves and the advancement of novel products. In addition, this study explored the additional benefits of mulberry leaves after frost, beside the systematic investigation of the preparation process of mulberry leaf noodles, with a view to laying a theoretical and technological foundation for the comprehensive utilization of mulberry leaf resources and the further development of novel products.

Materials and Methods

Preparation of mulberry leaf samples

The mulberry leaves used in this experiment were collected from 30 mulberry trees by Shaohuan Liu, a senior experimentalist at Guizhou Medical University, in Fenggang County, Zunyi City, Guizhou Province, China, following the first frost on 14 November 2023. Ten mature leaves, identified as those of the mulberry plant (*Morus alba* L.), were selected from the middle of the crown of each tree. The sampling area had a subtropical humid climate with an annual average temperature of 15.2°C. The soil was undisturbed sandy yellow soil. The samples were treated in a forced-air drying oven at 30°C, 60°C, and 90°C as well as natural sun-drying and shade-drying controls, and were set aside for later use.

Preparation of mulberry leaf noodles (Tao *et al.*, 2014)

Mulberry leaf powder: Fresh mulberry leaves were washed, placed them in an oven, and left them to dry at 30°C until they were somewhat twisted and brittle. The leaves were crunched into powder, followed by sifting through a 100-mesh sieve. The samples were stored in a dry atmosphere at room temperature.

Mulberry leaf juice: Fresh mulberry leaves were washed with water, dried, combined with four times the sample mass of pure water, blended with juice, and subsequently filtered.

Mulberry leaf pulp: Fresh mulberry leaves were washed, dried, and mixed with twice the sample mass of pure water to extract juice.

Precisely measured the flour, salt, and mulberry leaves (in powder, juice, and pulp), and transferred the flour and mulberry leaves in a noodles machine. Salt and baking soda were dissolved; then add them to the noodles machine. Subsequently, an appropriate quantity of pure

water was added, followed by mixing for 5–10 min and the rest of the mixture for 15 min to stretch gluten. The noodles press was employed to make noodles, and the samples were dried naturally and cut into sections for storage.

Optimization of flavonoid extraction process from mulberry leaves

Determination of total flavonoids in mulberry leaves

Precisely, 10 mg of rutin standard was weighed, and a reserve solution of 0.1 mg/mL was prepared using 75% ethanol. During the color reaction phase, 0.3 mL of 5% sodium nitrite solution (agitated for 3 min), 0.3 mL of 10% aluminum nitrate solution (agitated for 3 min), and 4 mL of 4% sodium hydroxide solution were sequentially added to 0, 1.0, 2.0, 3.0, 4.0, and 5.0 mL of rutin standard solution. Subsequently, the mixture was fixed in 75% ethanol to a final volume of 10 mL and allowed to stand for 15 min. The mixture was diluted to 10 mL with 75% ethanol and incubated in darkness for 15 min. Using 75% ethanol as a reference blank, full-wavelength scanning from 400 nm to 800 nm was performed on a UV-2700 spectrophotometer, identifying 504 nm as the optimal detection wavelength.

A mulberry leaf sample weighing exactly 0.500 g was taken, and 75% ethanol was added in accordance with the material–liquid ratio. The sample underwent extraction via ultrasonication at 100 W for 30 min at 60°C, and filtered. The filtrate was collected to yield mulberry leaf test sample solution. In all, 1 mL of each sample solution

of mulberry leaves for test was placed in a 10-mL volumetric flask, while parallel samples were prepared concurrently. The absorbance was measured at 504 nm, and the total flavonoid content was determined using a rutin reference curve:

$$\text{Total flavonoid content (mg/g)} = \frac{C \times V \times D}{V_1 \times M} \quad (1)$$

where C is the mass concentration of total flavonoids in the solution to be quantified (mg/mL); V is the volume of flavonoids to be quantified (mL); D is the dilution of the extraction solution; and V1 is the volume (3 mL) of the sample solution used for absorbance determination; and M is the mass of the sample (g).

Mulberry leaf samples by single-factor experiment

Precision weighing of 0.500 g of mulberry leaves and ultrasound-assisted extraction were carried out for each of the four variables in a single-factor experiment to examine their impact on the extraction rate of total flavonoids from mulberry leaves. The experimental design is presented in Table 1.

Response surface experimentation

To optimize the extraction parameters of total flavonoids from mulberry leaves, single-factor experiment results were analyzed using the Design-Expert V10.0 software. Employing the Box–Behnken design (BBD) principle, a response surface test was conducted, with independent variables being the (A) volume fraction of ethanol, (B) material–liquid ratio, (C) number of extractions, and (D) extraction temperature, while the content of

Table 1. Mulberry leaf samples by single-factor experiment.

Factors to be considered	Fixed factor level	Examination of the level of factors
Ethanol concentration	Material–liquid ratio 1:40, extraction temperature 60°C, extraction time 30 min, ultrasonic power set at 100 W, with a single extraction yielding	30%, 45%, 60%, 75%, and 95%
Material–liquid ratio	Ethanol concentration: 60%, extraction temperature: 60°C, extraction duration: 30 min, ultrasonic power: 100 W, extraction frequency: once	1:40, 1:60, 1:80, and 1:100 g/mL
Extraction temperature	The material–liquid ratio was 1:40, ethanol concentration was 60%, extraction duration was 30 min, ultrasonic power was 100 W, and number of extractions was one	30°C, 45°C, 60°C, and 75°C
Withdrawal time	Extraction temperature was 60°C, ultrasonic power was 100 W, and number of extractions was one. The extractions were carried out at a ratio of 1:40, with an ethanol concentration of 60% and an extraction temperature of 60°C	10 min, 30 min, 60 min, and 90 min
Extraction power	Material–liquid ratio of 1:40, ethanol concentration of 60%, extraction temperature: 60°C, extraction duration: 30 min, extraction frequency: once	100 W, 200 W, and 300 W
Number of extractions	Extraction temperature was 60°C, extraction time was 30 min, and ultrasonic power was 100 W. Material–liquid ratio was 1:40, ethanol concentration was 60%, extraction temperature was 60°C, and extraction duration was 30 min	1, 2, and 3 times

Table 2. Design of response surface factor levels for response surface testing.

Level of achievement etc.	Ethanol volume fraction (A) (%)	Liquid–material ratio (B) (g/mL)	Number of times during extraction (C) (frequency)	Extraction temperature (D) (°C)
–1	30	1:40	1	45
0	60	1:60	2	60
1	90	1:80	3	75

Table 3. Mulberry leaf noodles by single-factor experiment.

Factors to be considered	Fixed factor level	Examination of the level of factors
(Of clothes) Classifier for the number of washes	Baking soda: 0.3%, salt: 1%	36%, 38%, 40%, 42%, and 44%
Baking soda	Water: 40%, and salt: 1%	0%, 0.3%, 0.6%, 0.9%, and 1.2%
Acidifier	40% water, 0.3% baking soda	0%, 0.5%, 1%, 1.5%, and 2%
Mulberry leaf powder	40% water, 0.3% baking soda, and 1% table salt	2%, 4%, 6%, 8%, and 10%
Mulberry leaf juice	40% water, 0.3% baking soda, and 1% table salt	5%, 10%, 15%, 20%, and 25%
Mulberry leaf pulp	40% water, 0.3% baking soda, and 1% table salt	10%, 15%, 20%, 25%, and 30%

total flavonoids served as the response value. A response surface test was carried out. The detailed design of the response surface factor levels design is shown in Table 2.

Preparation and quality assessment of mulberry leaf flavonoid noodles

Utilizing the aforementioned optimization results, the extracted flavonoid from mulberry leaves was incorporated into noodle preparation to create a functional noodle characterized by high flavonoid content and anti-oxidant activity. Effects on the quality and functional properties of the noodles were examined by varying the form (mulberry leaf powder, mulberry leaf juice, mulberry leaf pulp) and ratio of the added mulberry leaf extract.

Mulberry leaf noodles by single-factor experiment

Table 3 shows the detailed single-factor experiment design to examine the influence of six single factors on the extraction rate of total flavonoids from mulberry leaf noodles.

Orthogonal design

The ideal quantities of mulberry leaf powder, mulberry leaf juice, mulberry leaf pulp, baking soda, and salt were determined through single-factor experiment, which served as a foundation for three-factor, three-level orthogonal experimental design for mulberry leaf (powder, juice and pulp) noodles, in accordance with the principle of orthogonal experimental design, as shown in Table 4. The organoleptic evaluation of the noodles was employed as an evaluation criterion.

Determination of optimal cooking duration

In all, 50 times the mass of water sample was placed into a pot and brought to a gentle boil on an adjustable induction cooker. Forty whole mulberry leaf noodles were randomly placed into the boiling water and the timer was initiated. One mulberry leaf noodle was taken out every 30 s and compressed between two glass plates to examine dark-colored selenocentric lines inside. Upon the disappearance of selenocentric lines, the corresponding duration was recorded as an optimal cooking time.

Determination of ripening breakage rate

In all, 50 times the mass of water sample was poured into a pot and heated on an adjustable induction cooker to a gentle boil. Forty whole mulberry leaf noodles were randomly placed and timed. Upon achieving optimal cooking duration, mulberry leaf noodles were extracted using chopsticks and the number of intact roots (X) was counted, from which the cooked breakage rate was derived:

$$Y = \frac{40 - X}{40} \times 100 \quad (2)$$

where Y represents the rate of cooked broken strips (%) of mulberry leaf noodles, and X is the number of intact roots on mulberry leaf noodles.

Measurement of cooking loss rate

An appropriate quantity of mulberry leaf noodles was processed into powder, and 3 g of the powder was precisely weighed using a moisture meter to measure the moisture content. Subsequently, an appropriate amount of mulberry leaf noodles was boiled in pure water for an optimal cooking time, followed by removing the noodles

Table 4. Orthogonal experimental design of noodles incorporating mulberry leaf powder, juice, and pulp.

Type of test	Level of achievement etc.	Mulberry leaf additives (%)	Amount of baking soda added (%)	Salt addition (%)
Mulberry leaf powder noodles	1	1	0.3	0.9
	2	2	0.4	1.0
	3	3	0.5	1.1
Mulberry leaf juice noodles	1	14	0.3	0.9
	2	15	0.4	1.0
	3	16	0.5	1.1
Mulberry leaf pulp noodles	1	19	0.3	0.9
	2	20	0.4	1.0
	3	21	0.5	1.1

and rinsing them in warm water. The rinse water was combined with the cooking water, and the mixture was transferred into a 500-mL volumetric flask for volume measurement. Then, 100 mL of the mixed solution was taken for drying. To ascertain the cooking loss rate, this study referred to the national standard GB/T 40636-2021 for ‘Hanging Noodles’ and the cooking loss rate was calculated according to the following formula:

$$Y = \frac{5M_2}{M_1(1 - W)} \times 100 \tag{3}$$

where Y is the cooking loss rate (%); M1 represents the mass of the sample (g); M2 is the mass of 100 mL of noodle broth after drying (g); and W indicates the water content of mulberry leaf noodles (%).

Sensory assessment

In accordance with the methodology of Liu *et al.* (2023) for improvement, a sensory evaluation panel consisting of 15 students from traditional Chinese medicine, pharmacy and food disciplines assessed the prepared mulberry leaf noodles. The sensory evaluation form was improved in accordance with the national standard GB/T 40636-2021 for ‘Hanging Noodles,’ with a total score of 100 points, and the scoring criteria are shown in Table 5.

Determination of total flavonoid content in mulberry leaf noodles

Precisely, 0.025 g of rutin control was weighed, and 0.5-mg/mL standard solution was prepared using 75% ethanol to a final volume of 50 mL. Mulberry leaf sample powder was weighed and extracted with 75% ethanol at a material–liquid ratio of 1:40 for 30 min using

ultrasonic assistance. Then the mixture was pumped, filtered, and adjusted to a final volume of 25 mL to yield the test solution. During chromogenic reaction phase, 0, 0.5, 1.0, 1.5, 2.0, 2.5 mL of rutin standard solution was taken and diluted in a gradient. Subsequently, 0.3 mL of 5% sodium nitrite (allowed to stand for 3 min), 0.3 mL of 10% aluminum nitrate (standing for 3 min), and 4.00 mL of 4% sodium hydroxide were added in succession. The solution was then adjusted to a final volume of 10 mL with 75% ethanol and allowed to stand for 15 min, and extracted by a full band scan (200–600 nm). The optimal detection wavelength of 358 nm was established using full band scanning (200–600 nm), and the standard curve was constructed.

Appropriate quantities of mulberry leaf flour and common noodle powder were weighed to prepare test solution, which was added with color developer and stabilized with 75% ethanol. The absorbance (A) was measured at 358 nm using 75% ethanol as a reference blank, and the total flavonoid content was calculated using the following formula:

$$Y = \frac{(A - 0.0699)W}{0.0258G \times 1000} \tag{4}$$

where Y represents the total flavonoid content (mg/g); A is the absorbance of the sample; W indicates the sample dilution; and G is the sample mass (g).

Determination of antioxidant activity of mulberry leaf noodles extract

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity assessment method was modified based on the approach of Li *et al.* (2024). A sufficient quantity of DPPH was measured to make 0.05-mmol/L solution. In all, 2 mL of total flavonoid extract

Table 5. Sensory evaluation of mulberry leaf noodles.

Sports event	Value of a score	Evaluation criteria
Color and luster	10	Green (mulberry leaf powder and pulp noodles) or light brown (mulberry leaf juice surface), bright surface (8–10 points), brighter surface (6–8 points), darker, grayish color, poor brightness (0–6 points)
Odor	10	With mulberry leaf clear flavor (6–10 points), basically no odor (3–6 points), with other odor (0–3 points)
Apparent state	16	Smooth and fine surface (10–16 points), fair (6–10 points), severely deformed and rough surface (1–6 points)
Tackiness	10	Chewing is crisp and non-sticky (6–10 points), more crisp and slightly sticky (3–6 points), not crisp and sticky (0–3 points)
Palatability (soft and hard)	16	Taste noodles with moderate force (10–16 points), slightly firm or soft (5–10 points), too firm or too soft (0–5 points)
Hardness	16	Chewy and elastic (10–16 points), fair (5–10 points), poor bite and lack of elasticity (0–5 points)
Smoothness	10	Tastes noodles with a lubricious texture (6–10 points), a more lubricious texture (3–6 points), and a rough texture (0–3 points)
Food and drink	12	When tasting noodles, they are clear and full of flavor, with a sweet aftertaste (8–12), no obvious flavor, no bitterness, astringency, or odor (4–8 points), acidic, with a bitter aftertaste, and with a heavy alkaline taste (0–4 points)

Note: Score values exhibit greater variability to highlight the significance of specific sensory attributes of noodles.

solution (concentrations of 400, 800, 1,200, 1,600, and 2,000 µg/mL) from different mulberry leaf noodle samples was extracted under the conditions described in the previous section, mixed thoroughly, and reacted in the dark for 1 h. Subsequently, 2 mL of anhydrous ethanol as a reference blank was utilized and the absorbance was measured at 517 nm. The rate of free radical scavenging was determined using the following formula:

$$Y = \frac{A_0 - A_1}{A_0} \times 100\% \quad (5)$$

where Y denotes the DPPH radical scavenging rate; A₀ is the absorbance of blank solution; A₁ signifies the absorbance of sample solution.

The ability of mulberry leaf noodle extracts to scavenge 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radicals was assessed according to the method described by Wen *et al.* (2022). For assessment, 7-mmol/L ABTS solution and 2-mmol/L potassium persulfate solution were prepared and combined in a 1:1 ratio to react for 24 h in the absence of light to produce ABTS working solution. The ABTS working solution was diluted 10 times prior to use. Then, 2 mL of the total flavonoid extract solution (concentrations of 400, 800, 1200, 1600, and 2000 µg/mL) was added from different mulberry leaf noodle samples extracted under the conditions described in the previous section, mixed thoroughly, and allowed to react for 1 h in the dark. Finally, 2 mL of anhydrous ethanol was used as a reference blank, and the absorbance was measured at 734 nm. The free radical scavenging rate was calculated using Equation (5).

Correlation between mulberry leaf extract and antioxidant activity

By conducting a statistical analysis of Pearson's coefficients between mulberry leaf noodle extracts with varying concentrations of total flavonoids and the free radical scavenging rates of DPPH and ABTS, the degree of closeness between two variables was assessed, with the range of the parameter quantifying linear correlation from -1 to 1. A higher absolute value indicated a stronger correlation, while values approaching 0 signified a weaker correlation.

Statistical analysis

Sample determination was performed using three replicates. Data processing was conducted using Excel for data organization and significance analysis. GraphPad Prism 9.5.0 was employed to generate graphs, perform one-way analysis of variance (ANOVA), and present the results as mean ± standard deviation (SD). Additionally, Design Expert 13 was utilized for performing RSM analysis.

Results and Analysis

Optimization results of mulberry leaf flavonoid extraction process

Determination of total flavonoid content of Morus alba by methodological investigation

The standard curve was constructed with absorbance on the vertical axis and concentration on the horizontal

axis. The regression equation, $y = 9.1057 \times +0.0145$ was derived with coefficient of determination (R^2) = 0.9997. The data indicated a strong linear relationship within the concentration range of 0.00–0.05 mg/mL.

Control solution, 1 mL, was extracted and fixed after the color development reaction, and the absorbance of rutin at 504 nm was measured successively for six times, followed by the calculation of the RSD value. The mean absorbance value of rutin was 0.627 ($n = 3$) with an RSD of 0.19%, signifying the efficacy of the experimental apparatus.

An appropriate amount of test solution was taken and placed at 0, 20, 40, 60, 80 and 100 min, and the absorbance was quantified at the detection wavelength, A. The average absorbance value was calculated as 0.629 ($n = 3$), with an RSD of 1.64%, indicating satisfactory instrumental precision for this experiment.

An appropriate volume of test solution was taken and 75% ethanol was used as a reference blank. Absorbance value, A, of six samples was measured at 504 nm and RSD value was calculated. The calculated mean absorbance value was 0.626 ($n = 6$) with an RSD of 0.99%, indicating that the precision of this experimental apparatus was satisfactory.

Appropriate amount of test solution was taken, and three parallel samples were established by adding rutin control in the ratios of 0.5:1, 1:1, and 1.5:1, resulting in nine samples. Absorbance values, A, of these nine samples were determined at 504 nm and RSD values were calculated using 75% ethanol as a reference blank. The results indicated a good recovery performance with an average recovery of 100.14% ($n = 3$) and a relative RSD of 0.6%.

Extraction procedure for total flavonoids from Morus alba leaves by single-factor experiment

Figure 1A illustrates that the total flavonoid content of *Morus alba* increased initially and then decreased with increase in ethanol concentration. This phenomenon could be ascribed to the increased solubility of flavonoids, and ethanol, as an organic solvent, can engage in interactions, such as hydrogen bonding, with flavonoids, thereby augmenting the extraction rate, with optimal solubility observed at 60% ethanol. However, when the concentration of ethanol exceeded 75%, its polarity decreased, resulting in the release of fat- and alcohol-soluble compounds in the sample, thus resulting in reduced extraction rate of total flavonoids. Therefore, excessively high and excessively low ethanol concentrations were detrimental to the extraction of total flavonoids from mulberry leaves.

Figure 1B indicates that the total flavonoid extraction rate may decline with the gradual increase of liquid–material

ratio. Increase in extraction solvent could result in excess extractant dissolving numerous impurities, which obstructs the dissolution of total flavonoids in samples, thus leading to a decrease in the extraction rate of total flavonoids.

Figure 1C illustrates that the extraction rate of total flavonoids from mulberry leaves was optimized at an extraction temperature of 60°C. With the increase of extraction temperature, solubilization of total flavonoids by ethanol increased. Nonetheless, an additional rise in temperature may result in a reduction of the extraction rate of total flavonoids, as elevated temperatures compromise the structural integrity of flavonoids and diminish extraction efficiency (Li et al., 2022).

Figure 2D illustrates that the total flavonoid extraction rate gradually increased with the extension of extraction time in the range of 10–60 min. However, when the time was increased to 60 min, the extraction yield declined. Prolonging the extraction time may increase flavonoid yield; however, once an ideal period of 30 min was attained, further extension could compromise flavonoid structure, resulting in a diminished extraction rate.

Figure 1E illustrates that the extraction rate of total flavonoids from mulberry leaves attained its optimum level at an extraction power of 100 W. The extraction rate of total flavonoids from mulberry leaves attained an optimum level at an extraction power of 100 W. However, when power increased, the extraction rate decreased correspondingly. Excessive microwave power elevated the temperature of extraction solvent, which destroyed the structure of flavonoids, consequently affecting the extraction rate. High temperature denatures and vaporizes flavonoids, compromising their integrity in the solvent and thus influencing the extraction rate (Deng et al., 2024).

Figure 1F shows that as the number of extractions increases, the total flavonoid content in the extracted herb exhibits a progressive decrease. This phenomenon could be attributed to the increased extraction cycles disrupting the structure of certain flavonoid compounds, thereby reducing their overall content (Cao et al., 2025). Maximum total flavonoid content was observed after a single extraction; thus, the extraction cycle was determined to be one.

Response surface methodology to optimize experimental design and analysis of results

On the basis of one-way experiment, four main factors affecting the extraction amount of total flavonoids from mulberry leaves were selected: ethanol concentration (A), material–liquid ratio (B), extraction frequency (C), and extraction temperature (D). Additionally, based on the Response Surface Central Combination BBD test, total flavonoids extracted from mulberry leaves were used as a

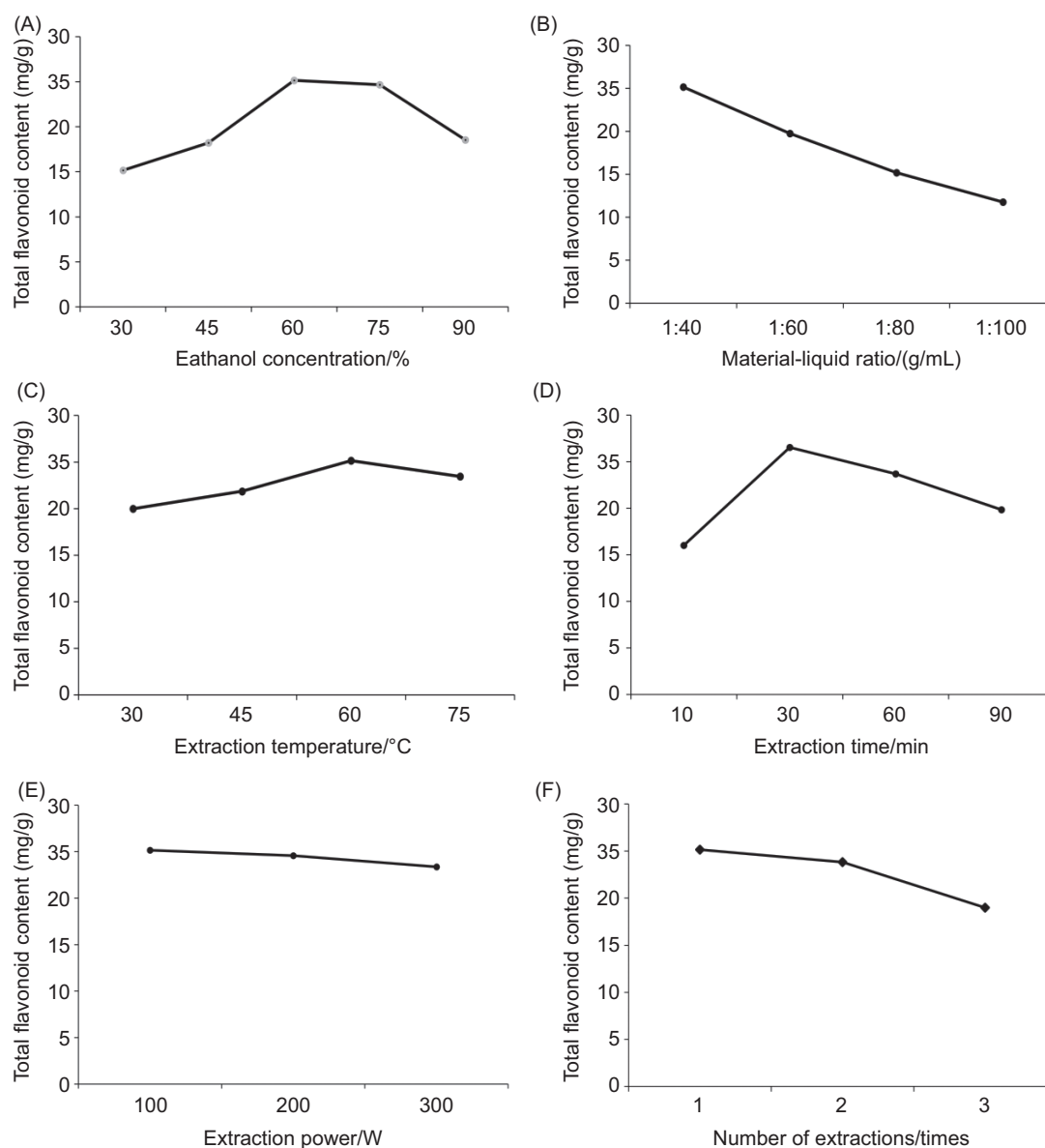


Figure 1. Results of single-factor experiment on mulberry leaf samples.

response value to determine the optimal extraction process of total flavonoids from mulberry leaves. Results of the response surface tests are shown in Table 6.

Numerical regression model using the Design-Expert 10.0 software produced a quadratic multiple regression equation:

$$\begin{aligned} \text{Total flavonoid extraction rate} = & 23.32 - 1.31 \times A - 2.31 \times B - 1.22 \times C + 0.2744 \\ & \times D - 0.4153 \times AB + 0.4262 \times AC + 2.35 \times AD + 1.68 \\ & \times BC + 0.4515 \times BD + 1.23 \times CD - 4.82 \times A^2 - 1.90 \\ & \times B^2 - 0.5122 \times C^2 - 2.22 \times D^2. \end{aligned}$$

The analysis of variance is shown in Table 7. According to statistical analysis shown in Table 7, the significance level

of quadratic regression model, $P < 0.0001$, demonstrates that the model is extremely significant. Meanwhile, P value of misfit term (0.5453), which exceeded 0.05, indicated that the method was reliable for estimating the extraction rate of total flavonoids from mulberry leaves. The model's coefficient of determination, $R^2 = 0.9637$, and the calibration coefficient, $R^2 \text{ adj} = 0.9274$, demonstrated a strong correlation with the test data, indicating a good fit (Sun *et al.*, 2022). Consequently, the model and the regression equation were applicable for analyzing and predicting test results. The factors affecting the extraction rate of total flavonoids from mulberry leaves were ranked as follows: material-liquid ratio (B) > ethanol concentration (A) > extraction frequency (C) > extraction temperature (D). The impact of AD, BC, A^2 , B^2 and D^2 on flavonoid extraction rate was extremely

Table 6. Response surface test results.

Serial No.	Ethanol concentration (%)	Material–liquid ratio (g/mL)	Number of extractions (frequency)	Extraction temperature (°C)	Total flavonoid extraction rate (mg/g)
1.	90	60	2	75	17.42
2.	30	60	2	75	15.54
3.	60	60	3	45	18.83
4.	30	80	2	60	15.16
5.	60	60	2	60	23.65
6.	90	40	2	60	18.52
7.	90	60	2	45	12.18
8.	60	60	2	60	23.65
9.	60	60	2	60	23.93
10.	60	80	2	75	18.15
11.	30	60	2	45	19.71
12.	30	60	1	60	22.15
13.	60	80	2	45	15.64
14.	90	60	3	60	15.14
15.	60	60	3	75	20.80
16.	60	60	2	60	21.69
17.	60	40	2	75	22.31
18.	60	60	2	60	23.65
19.	30	40	2	60	19.14
20.	60	60	1	75	19.53
21.	60	40	3	60	19.82
22.	60	40	2	45	21.60
23.	90	60	1	60	17.74
24.	60	80	1	60	18.50
25.	60	60	1	45	22.50
26.	30	60	3	60	17.85
27.	60	80	3	60	19.21
28.	90	80	2	60	12.88
29.	60	40	1	60	25.83

significant ($P < 0.01$); however, the effect of CD on flavonoid extraction rate was substantial ($P < 0.05$).

The F-value in the response surface ANOVA table represents the ratio of the model's mean square to the error's mean square. It serves as the core metric for determining whether the constructed regression model can significantly explain the variation in response values (i.e., whether the model is valid). A larger F-value coupled with a corresponding p-value below the significance level (e.g., 0.05) indicates stronger model significance.

Effect of factor interactions on the extraction rate of total flavonoids

The gradient of the response surface and the elliptical shape indicate a strong interaction between the two factors (Wang *et al.*, 2022b). Figure 2 illustrates that the relationships between ethanol concentration and

extraction temperature, feed–liquid ratio, and number of extractions, as well as the number of extractions and extraction temperature, significantly influenced the extraction rate of total flavonoids from mulberry leaves. The interactions between ethanol concentration and feed–liquid ratio, ethanol concentration and number of extractions, and feed–liquid ratio and extraction temperature exhibited negligible effects on extraction rate, aligning with the findings from ANOVA of the quadratic regression equation.

The optimal process parameters evaluated using the Design Expert 10.0 software, with extraction power set at 100 W, were as follows: ethanol concentration 55.582%, material–liquid ratio 1:41.189 g/mL, frequency of extractions 1.132 times, and extraction temperature 59.579°C. Under these conditions, the predicted extraction rate of total flavonoids of *Morus alba* leaf was 25.969

Table 7. ANOVA results.

Source	Sum of squares	Degree of freedom	Mean square	F value	P value	
Model	314.66	14	22.48	26.53	−0.0001	Significant
A	20.44	1	20.44	24.14	0.0002	
B	63.82	1	63.82	75.34	−0.0001	
C	17.74	1	17.74	20.94	0.0004	
D	0.90	1	0.90	1.07	0.3193	
AB	0.69	1	0.69	0.81	0.3820	
AC	0.73	1	0.73	0.86	0.3700	
AD	22.17	1	22.17	26.17	0.0002	
BC	11.26	1	11.26	13.29	0.0026	
BD	0.82	1	0.82	0.96	0.3432	
CD	6.07	1	6.07	7.17	0.0180	
A ²	150.68	1	150.68	177.89	−0.0001	
B ²	23.49	1	23.49	27.73	0.0001	
C ²	1.70	1	1.70	2.01	0.1782	
D ²	31.97	1	31.97	37.75	−0.0001	
Residual	11.86	14	0.85	–	–	Not significant
Lack-of-fit	8.49	10	0.85	1.01	0.5453	
Pure error	3.37	4	0.84	–	–	
Cor total	326.52	28	–	–	–	

Note: $P < 0.05$ indicates a significant effect, and $P < 0.01$ indicates a highly significant effect.

mg/g. For convenience of actual operation, the process parameters were adjusted to 1:40 g/mL, ethanol volume fraction to 56%, extraction temperature to 60°C, and a single extraction. Three parallel tests were conducted under these conditions, yielding real flavonoid extraction rates of 26.15 mg/g, 26.12 mg/g, and 25.84 mg/g. The average extraction rate of total flavonoids from mulberry leaves was 26.04%, aligning well with the anticipated value.

Total flavonoid content of Morus alba subjected to different drying methods

The optimized results from the response surface method indicated that the total flavonoid content in samples subjected to different drying methods was quantified by ultraviolet (UV) determination of the sample solution of mulberry leaves via precise aspiration, revealing a statistically significant difference ($P < 0.05$). The results are shown in Table 8 and Figure 3. The findings of the drying methods indicated the following efficacy: shade-drying > sun-drying > 30°C > 60°C > 90°C, with shade-drying being the most optimal drying method. This suggested that shade-drying conditions were more favorable for the metabolic transformation and accumulation of effective medicinal components of mulberry leaves. The

findings indicated that flavonoid content in mulberry leaves did not increase with the increase of drying temperature over many gradients, and excessively high temperatures would facilitate its transformation into other compounds.

Mulberry leaf noodle products and quality evaluation

Mulberry leaf noodle products

According to Figure 4, the surface of mulberry leaf powder exhibited a dark green hue, and dark green dots appeared on the surface due to the particles present in the powder, resulting in uneven integration with flour; nonetheless, this had a minimal impact on smoothness. The mulberry leaf juice exhibited a light brown hue, and due to its preparation from mulberry leaf filtrate, the surface was smoother with no obvious impurities. Mulberry leaf pulp noodles exhibited a dark green hue, deeper than that of mulberry leaf flour noodles, with a large number of dark green dots on the surface. The texture was rough when moist but turned smooth upon drying. The three varieties of noodles had the fragrance of mulberry leaves.

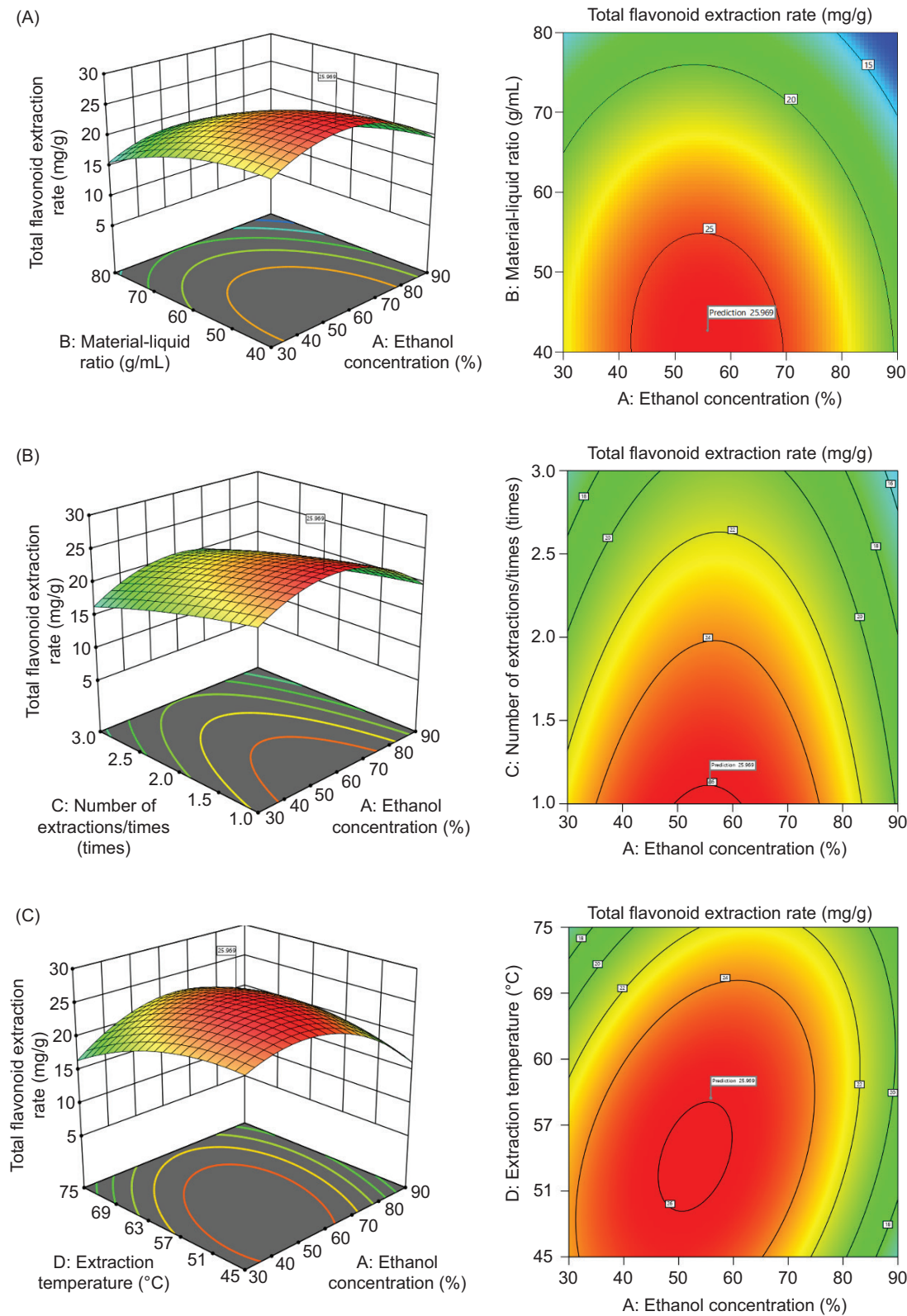


Figure 2. Interaction of different variables in response surface. (A) Ethanol concentration and feed–liquid ratio; (B) ethanol concentration and number of extractions; (C) ethanol concentration and extraction temperature; (D) material–liquid ratio and extraction frequency; (E) material–liquid ratio and extraction temperature; and (F) extraction frequency and extraction temperature.

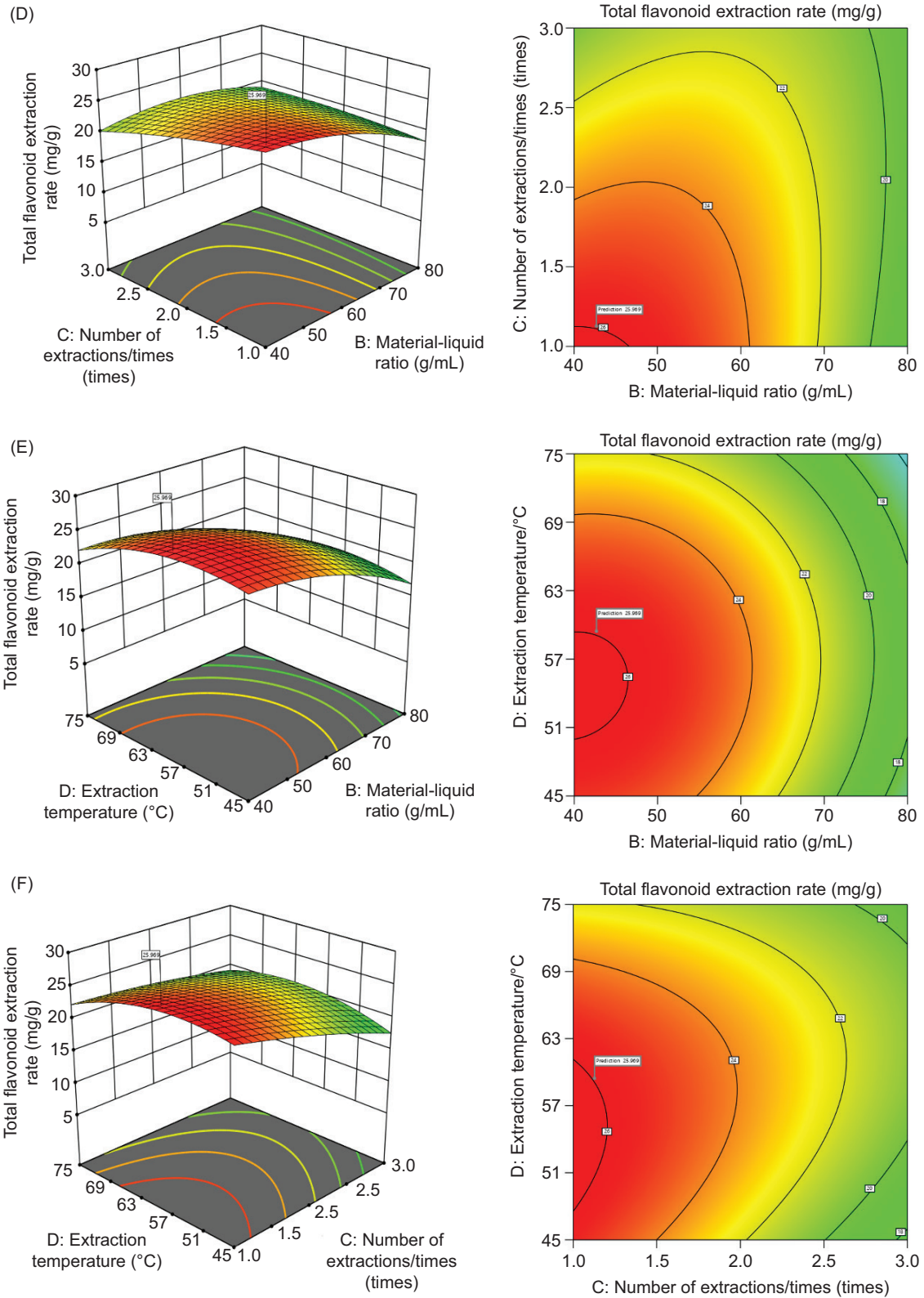


Figure 2. Continued.

Analysis of results of single-factor experiment on mulberry leaf noodles

During the dough mixing and resting process, the starch and protein present in wheat flour absorbed water and expanded to create a net-like gluten structure, which determined the ductility and elasticity of noodles. Therefore, the amount of water added directly influenced dough's structure and the subsequent pressing and cutting processes, which in turn affected the quality and texture of noodles. Experiments were conducted to analyze cooked breakage rate, cooking loss rate, and sensory evaluation of noodles with varying quantities of water, baking soda, salt, mulberry leaf powder, mulberry leaf juice, and mulberry leaf pulp (refer to Table 9).

Orthogonal design results

Through a comprehensive analysis of sensory orthogonal design results (see Table 10), the optimal process formulation for mulberry leaf powder noodles was established. The results revealed that the priority sequence for addition of mulberry leaf powder was KA1 > KA3 > KA2, while for addition of baking soda, it was KB3 > KB2 > KB1, and for addition of salt, it was KC2 > KC3 > KC1. Therefore, when all indices were comprehensively evaluated, the optimal process formulation for mulberry leaf flour noodles was determined to be A1+B3+C2, wherein mulberry leaf powder, baking soda, and salt were incorporated at concentrations of 1.0%, 0.5%, and 1.0% respectively. In addition, examination of R-value magnitudes demonstrated that the addition of mulberry leaf powder

exerted the most significant influence on sensory quality, followed by the addition of salt, while addition of baking soda was found to have the least impact.

Through a comprehensive analysis of sensory orthogonal design results (see Table 11), the optimal process formulation for mulberry leaf juice noodles was established. The analysis revealed that the priority sequence of mulberry leaf juice addition was KA3 > KA1 > KA2, while for baking soda addition, it was determined to be KB1 > KB3 > KB2, and for salt addition, KC2 > KC3 > KC1. Consequently, the optimal formulation of mulberry leaf juice noodles was identified as A3+B1+C2, wherein mulberry leaf juice, baking soda, and salt were incorporated at concentrations of 16.0%, 0.3%, and 1.0%, respectively. In addition, R-value analysis demonstrated that salt addition exerted the most pronounced effect on sensory quality, followed by mulberry leaf juice addition, with baking soda addition exhibiting the least significant impact.

The optimal process formulation for mulberry leaf pulp noodles was established through a systematic analysis

Table 8. Effect of different drying methods on the total flavonoid content of mulberry leaves.

Type of test	Drying method	Total flavonoid extraction rate (mg/g)
1	Dry-up	23.932±0.12
2	Sun-drying	23.151±1.53
3	30°C	22.246±0.39
4	60°C	21.878±0.09
5	90°C	9.775±0.46

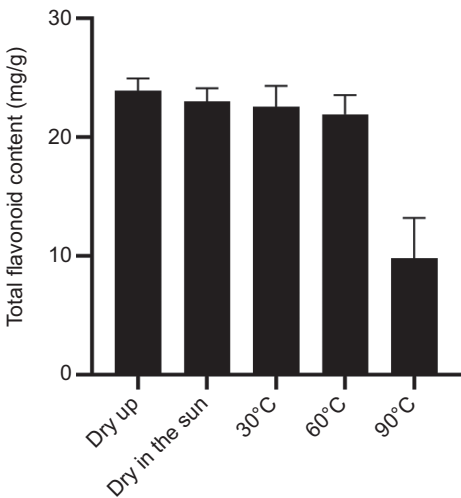


Figure 3. Content of total flavonoids in mulberry leaves subjected to different drying methods.



Figure 4. Mulberry leaf noodle samples. (A) Mulberry leaf powder noodles; (B) mulberry leaf juice noodles; and (C) mulberry leaf pulp noodles.

Table 9. Results of single-factor experiment on mulberry leaf noodles.

Type of test	Level of achievement etc.	Ripening breakage rate (%)	Cooking loss rate (%)	Sensory evaluation score
Water addition (%)	36	14.17	4.3	67.08
	38	10.83	4.46	64.92
	40	5.83	5.37	74.38
	42	8.33	4.26	70.62
	44	13.33	3.97	66.77
Amount of baking soda added (%)	0	4.17	3.89	66.15
	0.2	4.17	3.29	66.31
	0.4	3.33	3.25	71.85
	0.6	8.33	3.82	70.08
	0.8	5	4.46	65.62
Salt addition (%)	0	5.83	4.61	69.38
	0.5	4.17	4.62	72.31
	1	2.5	4.29	73.77
	1.5	4.17	4.73	72.38
	2	6.67	5.24	72.46
Mulberry leaf powder additive (%)	2	2.5	4.5	67.31
	4	3.33	4.56	64.54
	6	2.5	4.92	62.23
	8	6.67	6.48	61.38
	10	5	6.3	60.08
Mulberry leaf juice addition (%)	5	5.83	6.26	68.69
	10	5.83	9.44	67.38
	15	4.17	5.53	71.23
	20	4.17	5.74	67.92
	25	6.67	6.89	66.69
Mulberry leaf pulp addition (%)	10	3.33	3.23	64.08
	15	4.17	3.19	65.45
	20	3.33	4.33	66.08
	25	9.17	4.94	64.69
	30	8.33	4.69	59.69

of sensory orthogonal design results (see Table 12). The analysis revealed that the priority sequence for mulberry leaf pulp addition was KA2 > KA1 > KA3, while for baking soda addition, it was determined as KB1 > KB2 > KB3, and for salt addition, KC3 > KC2 > KC1. Consequently, the optimal formulation for mulberry leaf pulp noodles was identified as A2+B1+C3, wherein mulberry leaf pulp, baking soda, and salt were incorporated at concentrations of 20.0%, 0.4%, and 1.1%, respectively. In addition, the R-value analysis showed that the addition of baking soda exerted the most significant influence on sensory quality, followed by addition of salt, while addition of mulberry leaf pulp exhibited a comparatively modest effect. The most pronounced impact of baking soda on sensory attributes was due to its integrated mechanism of action: as a weak base, it triggered conformational rearrangements of

gluten proteins by increasing the system's pH while reacting with acidic components to release carbon dioxide, forming a network of fine pores. Additionally, it stabilized flavor-active components, thereby comprehensively improving textural properties and palatability.

Methodological investigation of mulberry leaf noodles

The regression equation of the standard curve was determined as $Y = 0.0258 \times X + 0.0699$, where Y represents the absorbance of the solution; X denotes the concentration of the solution, and $R^2 = 0.9997$, which proved that this method exhibited a good linear relationship in the concentration range of 5–25 µg/mL.

The test solution was subjected to color development and fixed with 75% ethanol. Following a 15-min equilibration

Table 10. Results and analysis of sensory orthogonal design of mulberry leaf flour noodles.

Test number	(A) Mulberry leaf powders added (%)	(B) Baking soda added (%)	(C) Salt added (%)	Sensory evaluation score/points
1	3.0	0.5	0.9	74.54
2	1.0	0.4	1.1	76.62
3	3.0	0.3	1.1	73.98
4	1.0	0.5	1.0	76.52
5	2.0	0.5	1.1	74.58
6	3.0	0.4	1.0	75.18
7	2.0	0.4	0.9	73.76
8	2.0	0.3	1.0	74.18
9	1.0	0.3	0.9	74.23
K1	227.37	222.39	222.53	–
K2	222.52	225.56	225.88	–
K3	223.7	225.64	225.18	–
Excellent level	A1	B3	C2	–
k1	75.79	74.13	74.18	–
k2	74.17	75.19	75.29	–
k3	74.57	75.21	75.06	–
R-value	1.62	1.08	1.12	–

Note: R-value (regression coefficient) represent the range (max. & min.) of mean indices across different factor levels, indicating the significance of each factor. Larger R values denote more pronounced effects on the experimental outcome.

Table 11. Results and analysis of mulberry leaf juice noodles sensory orthogonal design.

Test number	(A) Mulberry leaf juice added (%)	(B) Baking soda added (%)	(C) Salt added (%)	Sensory evaluation score/points
1	16.0	0.3	1.0	79.75
2	15.0	0.5	1.0	75.13
3	14.0	0.5	1.1	72.65
4	14.0	0.4	1.0	75.33
5	16.0	0.5	0.9	73.87
6	14.0	0.3	0.9	70.11
7	16.0	0.4	1.1	73.25
8	15.0	0.3	1.1	72.12
9	15.0	0.4	0.9	68.48
K1	218.09	221.98	212.46	–
K2	215.73	217.06	230.21	–
K3	226.87	221.65	218.02	–
Excellent level	A3	B1	C2	–
k1	72.70	73.99	70.82	–
k2	71.91	72.35	76.74	–
k3	75.62	73.88	72.67	–
R	3.71	1.64	5.92	–

Note: R values represent the range (max. & min.) of mean indices across different factor levels, indicating the significance of each factor. Larger R values denote more pronounced effects on the experimental outcome.

period, the absorbance was measured at 358 nm. The results indicated that the absorbance values ranged from 0.3230 to 0.3253 with RSD = 0.13%, confirming the excellent precision of the instrument.

The test solution was prepared by adding color developer and 75% ethanol to the scale line. After a 15-min standing period, triplicate measurements were performed at 358 nm and averaged at 10, 20, 30, 40, 50, and 60 min. The absorbance values were observed to range from 0.3277 to 0.3350 with an RSD of 0.78%, thereby demonstrating the stability of the method over a 60-min duration. The test solution was prepared, color developer was added, and the volume was adjusted with 75% ethanol. After standing for 15 min, the absorbance was measured at 358 nm, and the total flavonoid content and RSD value were calculated using the regression equation. The results showed no significant variability among the values, with an RSD of 1.18%, confirming the reproducibility of analytical method.

Nine portions of mulberry leaf flour powder of known content were precisely weighed, and rutin standard was added in ratios of 1:1, 2:1, and 3:1. The test solutions were prepared, color developer was added, and volumes were adjusted. Following a 15-min equilibration period, the absorbance was measured at 358 nm and the total flavonoid content, recovery, and RSD value were calculated

using the regression equation. As shown in Table 13, the recoveries of samples ranged from 98.12% to 102.36%, with RSD values were 1.07%, 1.01%, and 0.94%, confirming the excellent accuracy of analytical method.

Determination and analysis of total flavonoid content and antioxidant active components in mulberry leaf noodles

The results of flavonoids content are presented in Table 14. It shows that total flavonoids of four types of noodles were determined, and it was observed that all mulberry leaf (powder, juice, and pulp) noodles contained flavonoids, while the content of total flavonoids of conventional noodles was 0.162 mg/g, which was much lower than that of total flavonoids in mulberry leaf noodles.

The antioxidant activity assessments of mulberry leaf powder noodles, mulberry leaf juice noodles, and mulberry leaf pulp noodles were conducted using both DPPH and ABTS methods, which showed (Figures 5 and 6, and Tables 13 and 14) that free radical scavenging rates of the three varieties of mulberry leaf noodles exhibited concentration-dependent enhancement in the concentration range of 400–2,000 µg/mL. Specifically, results of the DPPH method indicated that scavenging ability was ranked as follows: mulberry leaf pulp noodles > mulberry leaf juice noodles > mulberry leaf powder noodles, with mulberry leaf powder noodles displaying a more stable trend

Table 12. Sensory orthogonal design results and analysis of mulberry leaf pulp noodles.

Test number	(A) Mulberry leaf pulp added (%)	(B) Baking soda added (%)	(C) Salt added (%)	Sensory evaluation score/points
1	20.0	0.3	1.0	70.83
2	21.0	0.3	1.1	74.10
3	19.0	0.5	1.0	71.57
4	20.0	0.5	1.1	71.45
5	19.0	0.3	0.9	71.55
6	20.0	0.4	0.9	71.68
7	19.0	0.4	1.1	69.85
8	21.0	0.5	0.9	68.21
9	21.0	0.4	1.0	70.18
K1	212.97	216.48	211.44	–
K2	213.96	211.71	212.58	–
K3	212.49	211.23	215.4	–
Excellent level	A2	B1	C3	–
k1	70.99	72.16	70.48	–
k2	71.32	70.57	70.86	–
k3	70.83	70.41	71.80	–
R	0.49	1.75	1.32	–

Note: R values represent the range (max & min) of mean indices across different factor levels, indicating the significance of each factor. Larger R values denote more pronounced effects on the experimental outcome.

line. Conversely, experimental data of the ABTS method showed that scavenging capacity was ranked as follows: mulberry leaf pulp noodles > mulberry leaf powder noodles > mulberry leaf juice noodles. This discrepancy may be attributed to the differential response properties of antioxidant components (e.g., flavonoids) within various mulberry leaf noodles toward the fat- and water-soluble radicals. Both methods confirmed the optimal antioxidant activity of mulberry leaf pulp noodles, a phenomenon potentially associated with processing-induced variations in the dissolution rate of active ingredients.

Correlation analysis between mulberry leaf noodles extract and antioxidant activity

Pearson’s correlation coefficient was employed to examine the degree of association between the two variables (Wang *et al.*, 2022b). As presented in Table 15, *P* values derived from the correlation analysis ranged from 0.0035 to 0.0038, which were less than 0.01, indicating a significant positive correlation between the flavonoid components of mulberry leaf noodles and their corresponding antioxidant activity.

Discussion

This study successfully optimized the ultrasonic-assisted extraction (UAE) process for mulberry leaf flavonoids using RSM, identifying 56% ethanol, a solid–liquid ratio of 1:40 g/mL, and extraction at 60°C for 30 min as optimal parameters, achieving a yield of 26.04%. Crucially, pretreatment by shade-drying, which minimizes photo-thermal oxidation and enzymatic degradation within a low-temperature, light-protected environment while preserving cellular integrity, proved significantly more effective than sun-drying for bioactive component retention. Applying the optimized extract, three functional noodle variants were developed: mulberry leaf powder noodles (1.317 ± 0.01 mg/g), mulberry leaf juice noodles (1.603 ± 0.01 mg/g), and mulberry leaf pulp noodles (1.413 ± 0.02 mg/g). This study confirmed the compliance of these mulberry leaf noodles with key industry standards (e.g., LS/T 3212-2014), demonstrating their strong potential for pilot-scale production. All exhibited potent antioxidant capacity, with IC₅₀ values for both ABTS and DPPH radical scavenging assays below 0.60 mg/mL. The observed variations in IC₅₀ values

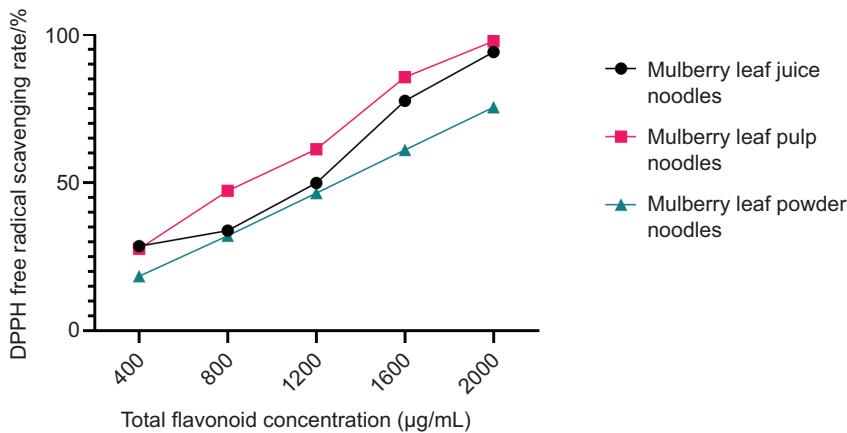


Figure 5. DPPH scavenging rates of mulberry leaf powder, juice, and pulp noodle extracts.

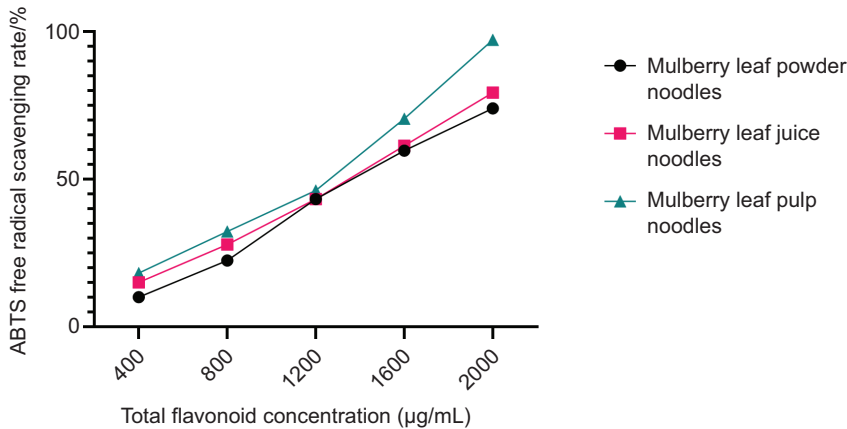


Figure 6. ABTS scavenging rates of mulberry leaf powder, juice, and pulp noodle extracts.

Table 13. Antioxidant activity measurements for mulberry leaf noodles.

Assay	Concentration (ug/mL)	Mulberry leaf powder	Mulberry leaf juice	Mulberry leaf pulp
DPPH	400	18.40 ± 0.15	28.53 ± 0.15	27.64 ± 0.15
	800	32.00 ± 0.27	33.78 ± 0.15	47.20 ± 0.41
	1,200	46.48 ± 0.15	49.87 ± 0.31	61.33 ± 0.31
	1,600	61.07 ± 0.56	77.60 ± 0.41	85.60 ± 0.31
	2,000	75.47 ± 0.31	94.13 ± 0.56	97.78 ± 0.15
ABTS	400	10.06 ± 0.46	15.04 ± 0.63	18.17 ± 0.15
	800	22.42 ± 0.71	27.86 ± 0.52	32.28 ± 0.46
	1,200	43.30 ± 0.59	43.31 ± 0.34	46.23 ± 0.15
	1,600	59.73 ± 0.38	61.39 ± 0.92	70.56 ± 0.41
	2,000	74.01 ± 0.36	79.36 ± 0.38	97.32 ± 0.27

Note: DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid).

Table 14. Determination of total flavonoids in mulberry leaf noodles.

Sample name	Mean total flavonoid content (mg/g)	DPPH IC ₅₀ (mg/mL)	ABTS IC ₅₀ (mg/mL)
Mulberry leaf powder	1.317 ± 0.01	0.4640	0.5054
Mulberry leaf juice	1.603 ± 0.01	0.4102	0.4799
Mulberry leaf pulp	1.413 ± 0.02	0.3327	0.4299
Plain noodles	0.162 ± 0.05	–	–

Note: DPPH IC₅₀: scavenging 50% of 2,2-diphenyl-1-picrylhydrazyl; ABTS IC₅₀: scavenging 50% of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid).

between ABTS and DPPH assays are attributed to their distinct underlying mechanisms—single-electron transfer for DPPH versus electron/hydrogen atom transfer for ABTS—combined with the influence of specific flavonoid structural characteristics and matrix effects within different noodle formulations. Notably, the superior performance of mulberry leaf pulp noodles is attributed to two mechanistic factors: (1) preservation of native flavonoid structures through minimal processing of whole mulberry leaf pulp, and (2) synergistic protection provided by the coexisting dietary fiber and polyphenols within the mulberry leaf pulp matrix, which may reduce oxidative degradation during processing. From a broader perspective, our findings provided both scientific and practical advances: (1) We established a scientifically validated UAE extraction method that maximizes flavonoid yield while preserving bioactivity; and (2) we demonstrated the commercial potential of mulberry leaf utilization through development of functional noodle products with enhanced bioactive properties and industry compliance.

Conclusion

Mulberry leaves provide significant concentrations of dietary fiber and essential micronutrients. Incorporation into noodle formulations modulates the food matrix architecture while conferring gastrointestinal benefits attributable to the physicochemical properties of dietary fiber. This study addresses two critical gaps in functional food development: (1) a scalable approach for valorizing agricultural byproducts, and (2) a nutrient-fortified staple food that combines traditional acceptability with enhanced bioactivity. Despite these promising results, the following translational challenges persist: (i) batch-to-batch variability in raw material composition affecting extract standardization; (ii) thermal degradation of flavonoids during industrial-scale drying processes; and (iii) consumer preference for conventional noodle textures. Therefore, the future investigations should prioritize *in vivo* validation of metabolic benefits, microencapsulation technologies for the stabilization of bioactive compounds, and sensory optimization through hybrid formulations with the incorporation of buckwheat or yam flour. Notably, our methodology aligned with China's 'East Mulberry-West Relocation' initiative, which demonstrates how regional specialty crops can be transformed into high-value functional ingredients. In conclusion, this dual-focused strategy not only enhances nutraceutical efficacy and agricultural sustainability but also provides a replicable model for global functional food innovation, effectively bridging the gap between scientific discovery and commercial application.

Data Availability Statement

No datasets were generated or analyzed in the current study.

Table 15. Results of correlation coefficient assay of flavonoid components with antioxidant activity.

Serial No.	Samples	DPPH		ABTS	
		<i>R</i> ²	<i>P</i>	<i>R</i> ²	<i>P</i>
1.	Mulberry leaf powder noodles	0.9998	0.0035	0.9978	0.0034
2.	Mulberry leaf juice noodles	0.9563	0.0037	0.9943	0.0035
3.	Mulberry leaf pulp noodles	0.9918	0.0038	0.9817	0.0036

Note: DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid; *R*²: coefficient of determination).

Author Contributions

Xirui Rao: conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing—original draft, visualization, supervision, and project administration; Di Deng and Jiang Lu: methodology (noodle formulation development) and investigation (antioxidant activity assays); Jing Miao, Churui Chang, and Xiangchun Shen: writing—review & editing, and interpretation of results; Shaohuan Liu: supervision, funding acquisition, writing—review & editing. All authors had read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declared no conflict of interest.

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