Original Research Paper

Flavonoids from the *Zizania latifolia* Bracts: Extraction Optimization, Antioxidant Activity and Exploration in Strawberry Preservation

[1]Tong Wang, [1]Yuzhu Zhang, [1,2]Lijie Ben, [1]Chenxin Zhai, [1]Ying Lv, [1*]Yang Zhang and [1*]Lixue Zheng

[1]*School of Biology and Food Engineering, Changshu Institute of Technology, Changshu 215500, Jiangsu, China*
[2]*School of Medical Instrument and Food Engineering,*
University of Shanghai for Science and Technology, Shanghai 200093, Shanghai, China

Corresponding Author:
Yang Zhang
School of Biology and Food
Engineering, Changshu
Institute of Technology,
Changshu 215500, Jiangsu,
China
Email: zhangyang@cslg.edu.cn

Lixue Zheng
School of Biology and Food
Engineering, Changshu
Institute of Technology,
Changshu 215500, Jiangsu,
China
Email: lixue9418@163.com

Abstract: *Zizania latifolia* bracts are *Z. latifolia*-based agricultural waste materials, distributed in various parts of the world. In the current study, the Response Surface Method (RSM) was used to optimize the cellulase-assisted extraction of Total Flavonoids from *Z. latifolia* bracts (TFZB). Then, the *in vitro* antioxidant capacity and the potential effect on strawberry preservation were assessed. The results showed that the optimal conditions for extracting TFZB was: Solid-to-liquid ratio of 1: 40 g/mL; ethanol concentration of 60% (v/v); cellulase dosage of 0.4% (w/w); incubation time of 2 h. Under these conditions, the yield of TFZB reached 0.49±0.01%. TFZB exerted certain antioxidant capacity. The free radical-scavenging rates against hydroxyl and DPPH radicals were 86±1.32% and 90±1.6% respectively at the concentration of 1.6 mg/mL and TFZB also exerted reducing power on Fe^{3+}. Finally, the potential of TFZB on strawberry preservation was investigated from three aspects: Weightlessness rate, rotting rate and Vc content. It was found that the above-mentioned profiles were all improved after the strawberries sprayed with TFZB solution during the storage period. This study suggested that the TFZB may be developed as a novel antioxidant and fruits preservative, providing an evidence for the further utilization of *Z. latifolia* bracts.

Keywords: *Zizania latifolia* Bracts, Total Flavonoids, Cellulase-Assisted Extraction, Antioxidant Activity, Strawberry Preservation

Introduction

Zizania latifolia is a perennial herb, native to China and Southeast Asia (Zhang *et al*., 2009). In China, the planting area of *Z. latifolia* is about 70,000 hm^2, which is the second largest aquatic vegetable next to the lotus root. In China, *Z. latifolia* is generally distributed along the Yangtze River and Huaihe River (Jiang *et al*., 2016). *Z. latifolia* is mainly composed of rhizomes and bracts. The rhizomes are used for daily consumption. In addition to the dietary characteristics, the rhizomes of *Z. latifolia* are rich in various physiologically active compounds, including dietary fibers, vitamins, minerals and phenolic compounds (Yan *et al*., 2018), which could produce a marked effect in reducing the risk of non-communicable diseases, such as obesity, diabetes and cardiovascular diseases (Shao *et al*., 2014). At present, some reports on *Z. latifolia* are mostly focused on

nutrients and health benefits (Surendiran *et al*., 2014). By consulting the literatures, we found that many plant bracts also contain certain physiologically active substances. However, there is little information on *Z. latifolia* bracts, most of which are discarded as agricultural wastes and their full and effective utilizations have not been constructed and realized.

Flavonoids are widely distributed in plants as secondary metabolites (Zakaryan *et al*., 2017). Because of medicinal values, scholars from all over the world have launched different degrees of researches on flavonoids, some of them focused on the extraction of flavonoids from agricultural waste materials and further explored the biological activities. For example, (Zhang *et al*., 2019) extracted Flavonoids from Corn Bracts (FCB) and found that FCB has strong antioxidant capacity and certain inhibitory effects on gram-positive bacteria. Winarsi and Yuniaty (2019) found that cardamom roots are rich in

flavonoids, which have the potential to be developed as the ingredients for antioxidant functional foods. Flavonoids from camphor leaves were extracted (Liu *et al.*, 2019a) and were found to possess strong potentials to be consumed as natural antioxidants. Cui *et al.* (2020) found that flavonoids from *Morus alba* L. leaves (MLF) has excellent antibacterial and antioxidant effects, another surprising discovery was that the shelf life of chilled meat treated with MLF was extended from 6 days to 9 days. In addition, many researchers have also carried out the studies on the application of flavonoids from plants in food preservation and the results showed that natural flavonoids usually elicit positive effects on food preservation (Liu *et al.*, 2019b).

Herein, Total Flavonoids from *Z. latifolia* Bracts (TFZB) were extracted by Cellulase-Assisted extraction (CAT) for the first time. CAT has the advantages of higher efficiency in flavonoids extraction from bracts in which cellulose can be degraded by cellulose, facilitating the release of cellular flavonoids (Huang *et al.*, 2016). In addition, CAT has good environmental compatibility, low energy consumption and simplified operation (Chen and Yen, 2007). Then, in present work, the antioxidant activities of TFZB were surveyed. Besides, the application of TFZB on the preservation of strawberry were investigated by referring the changes of weightlessness rate, rotting rate and Vitamin C (Vc) content during storage time for the first time. All the above investigations were used to estimate the potential of TFZB to be utilized in food and relating industries.

Materials and Methods

Materials

The *Z. latifolia* bracts and strawberries used in the tests were collected from Datang Ecological Agriculture Development Co., Ltd. (Jiangsu, Changshu, China). Rutin standard was purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China) and other chemical reagents were all analytical or chemical purity.

TFZB Extraction

Cellulase-Assisted Extraction (CAE)

The extraction of TFZB was referred to the previously reported method (Zhang *et al.*, 2019). The washed fresh *Z. latifolia* bracts were dried at a constant temperature of 60°C for 2 days and crushed to a particle size of 40 mesh and stored at 4°C. Powders of *Z. latifolia* bracts were immersed in Disodium Hydrogen Phosphate (DHP)-citric acid buffer solution (pH = 5, a fixed solid-to-liquid ratio of 1 g: 20 mL), then certain amounts of cellulase were added (0.2~0.6%, w/w) to be incubated at 40°C for certain time (0.5~1.5 h). After enzymatic hydrolysis, residual cellulose was inactivated at 90°C for

10 min. Lastly, certain amounts of anhydrous ethanol and deionized water were added to adjust the system to be certain concentrations of ethanol (40~90%, v/v) and certain ratios of solid-to-liicid (1: 20 g/mL ~ 1: 60 g/mL), followed by 2 h-extraction at 80°C. The particulate matter in the mixture was removed by centrifugation and the supernatant was taken to determine the content of total flavonoids.

Extraction yield of TFZB was expressed as below:

$$Y(\%) = \frac{C \times V}{M} \times 100 \tag{1}$$

Where:
C = Concentration of total flavonoids in TFZB (mg/mL)
V = Volume of filtrate (mL)
M = Weight of *Z. latifolia* bracts powders (mg)

Total Flavonoids Content in TFZB

Rutin was used as the standard to determine the flavonoids content in TFZB, the $NaNO_2$-$Al(NO_3)_3$-NaOH colorimetric method was slightly modified on the basis of the method previously mentioned (Bai *et al.*, 2015). The specific steps in this assay were as follows: 1 mL of filtrate was added into a colorimetric tube, then 0.3 mL of 5% $NaNO_2$, 0.3 mL of 10% $Al(NO_3)_3$ and 4 mL of 1mol/L NaOH solutions were added in turn, shook well and let it stand for 6 min. Finally, the mixture was attenuated to 10 mL with 60% (v/v) ethanol, after 15 min, the absorbance was determined at 510 nm, which was put into the equation of regression using rutin as the standard to calculate the total flavonoids content in TFZB. The equation of regression was expressed as below:

$$A = 2.0708C - 0.0042 \left(R^2 = 0.9995 \right) \tag{2}$$

Where:
A = The absorbance
C = Flavonoids content in TFZB (mg/mL)

Experimental Design of Response Surface Optimization

Before the response surface optimization, the single-factor experiments were used to evaluate the optimal level of the three factors of ethanol concentration (40~80%, v/v), cellulase dosage (0.2~0.6%, w/w) and solid-to-liquid ratio (1:20~1:60, g/mL) on TFZB extraction yield. Then, response surface optimization (Sheng *et al.*, 2013) was designed according to the results of single-factor experiment using Box-Behnken design via Design-Expert V8.0.6.1 software (Table 1).

Table 1: The code and actual levels of three factors used in the RSM

Independent variable	Level		
	-1	0	1
X_1: Cellulase amount (w/w, %)	0.2	0.4	0.6
X_2: Ethanol concentration (v/v, %)	40	60	80
X_3: Solid-to-liquid ratio (g/mL)	1:20	1:40	1:60

Purification of TFZB

TFZB was purified by column chromatography loaded with D101 macroporous resin (Zhang *et al.*, 2007). Before experiment, the resin was immersed in 95% (v/v) ethanol for 24 h, then washed with distilled water for several times to remove the residual ethanol. Afterwards, the treated resin was loaded on a column with volume of 300 mL and diameter to height ratio of 1: 8. The purification parameters were as follows: Loading amount of 5.0 g of dried TFZB powders; eluent of 60% ethanol (v/v); velocity of 2.0 mL/min. After purification, the content of total flavonoids in TFZB increased from 35±3.2 to 59±1.9%.

In vitro Antioxidant Activity of TFZB

DPPH Radical-Scavenging Capacity

The DPPH radical-scavenging ability was evaluated by the previously mentioned method (Chen and Yen, 2007) with slightly modifications. As much as 2 mL of TFZB solution (0.2~1.0 mg/mL) and 2 mL of 0.2 mmol/L DPPH solution (dissolved in 90% ethanol) were mixed and incubated in the dark at 37°C for 20 min, followed by measuring the absorbance at 517 nm (*As*) and each process was repeated three times. The reaction system without DPPH solution was used as normal control (*Ac*) and the system without TFZB was employed as blank solution (A_0). V_C solutions of different concentrations (0.2~1.0 mg/mL) were used as positive control. The ability to scavenge DPPH radical was calculated by the following formula:

$$DPPH\ Radical\ Scavenging\ rate\ (\%) = \frac{As - Ac}{Ao} \times 100 \quad (3)$$

Hydroxyl Radical-Scavenging Capacity

The hydroxyl radical-scavenging ability of TFZB was carried out by method of phenanthroline-Fe^{2+} and was slightly modified on the basis of the method previously mentioned (Chen and Yen, 2007). About 2 mL of TFZB solution (0.5~2.0 mg/mL) was mixed with 1 mL of 0.75 mmol/L $FeSO_4 \cdot 7H_2O$, 1 mL of 0.01% H_2O_2 and 1 mL of 0.75 mmol/L phenanthroline (dissolved in ethanol solution) in turn. The reaction system was placed at 37°C for 60 min, followed by determining the absorbance (*As*) at 536 nm. The reaction system without TFZB was employed as blank control (A_0) and the one without H_2O_2

was defined as normal control (*Ac*). Vc solutions (the concentration ranging from 0 to 2 mg/mL) were employed as positive control. The ability to scavenge hydroxyl radical was assessed as the following formula:

$$Hydroxyl\ radical\ Scavenging\ rate(\%) = \frac{As - Ao}{Ac - Ao} \times 100 \quad (4)$$

Reducing Power Measurement

Reducing power of TFZB to ferric iron was measured based on the reports (Chen and Yen, 2007) with slightly modifications. About 1 mL of TFZB solution of different concentrations (0.5~2.0 mg/mL) was mixed with 2.5 mL of 0.2 mol/L phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) $K_3[Fe(CN)_6]$ solution subsequently, followed by being incubated at 50°C for 20 min. Then 2.5 mL of 10% (v/v) trichloroacetic acid was added and the mixture was centrifuged at 3000 rpm for 10 min. A 5 mL of supernatant was mixed with 4 mL of distilled water and 1 mL of 0.1% (w/v) $FeCl_3$ solution and then the absorbance was read at 700 nm after 10 min. V_C solution with different concentrations (0.5~2.0 mg/mL) was employed as positive control.

The Effects of TFZB on Strawberry Preservation

The Preparation of Strawberry Sample

The fresh strawberries (weigh of 15±0.5 g for each) were divided into four groups (n = 600 g), two groups of which were uniformly sprayed with sterilized distilled water, named blank control and the other two groups were uniformly sprayed with 0.5 mg/mL TFZB solution, named test sample (the volume of TFZB solution sprayed on each sample was approximately 100 mL). The blank controls and test samples were stored at 4°C and room temperature, respectively. The weightlessness rate, rotting rate and Vc content of each treatment were determined at 1, 2, 4, 6 and 9 days (Cui *et al.*, 2020).

Weightlessness Rate Measurement

The weight of strawberry before storage was weighed by the electronic balance (accuracy, 0.0001g), denoted as G_0 and the weight of strawberry after storage, denoted as G_1, then the weightlessness rate was calculated by the following formula:

$$Weightlessness\ rate\ (\%) = \frac{G_0 - G_1}{G_0} \times 100 \quad (5)$$

Rotting Rate Measurement

The rotting rate was evaluated by the previously mentioned method (Bhat and Stamminger, 2015) with some modifications. The appearance of mildew on the fruit surface was taken as the discriminant basis of rotting. According to the size of the rotting area, the extent of rotting was defined as four levels:

- One-level of rotting fruit means:

    0<the rotting area ≤1/8 area of fruit surface

- Two-level of rotting fruit means:

    1/8<the rotting area ≤1/4 area of fruit surface

- Three-level of rotting fruit means:

    1/4<the rotting area ≤3/8 area of fruit surface

- Four-level of rotting fruit means:

    3/8<the rotting area ≤1/2 area of fruit surface

Then the rotting level was denoted as *A*, the number of strawberries at corresponding level was denoted as *B*, the total number of samples examined was denoted as *C* and the total rotting rankings were defined as 4 hierarchies (One-level ~ Four-level), then the rotting rate was calculated by the following formula:

$$Rotting\ rate\ (\%) = \frac{\sum (AB)}{4C} \times 100 \qquad (6)$$

Vc Content Measurement

Vc content measurement was investigated by the previously reported method (Bhat and Stamminger, 2015) with some modifications. As much as 20 g of samples were used in the test to record the actual weight (*M*). After being mashed, the strawberry seriflux was mixed with 20 mL of 1% HCl solution (v/v, extraction agent) and shook well. Then the strawberry seriflux was centrifuged and 1 mL of supernatant (V_1) was diluted to 100 mL (V_2) with 1% HCl solution and the absorbance (A) was measured at 243 nm. Two milliliters of 1% HCl solution was employed as blank solution, while Vc solution at different concentrations ranged from 0.002 to 0.01 mg/mL was used as positive control. The regression equation using Vc as standard substance was expressed as below:

$$A = 105.75x - 0.0657 \left(R^2 = 0.9901\right) \qquad (7)$$

The *Vc* content was calculated by the following formula:

$$Vc\left(ug / g\right) = \frac{xV}{M} \times \frac{V_2}{V_1} \qquad (8)$$

Where:
x = The concentration of Vc (μg/mL)
V = The volume of the extraction agent (mL)
M = The weight of the strawberry (g)
V_1 = The volume of the supernatant of strawberry seriflux (mL)

V_2 = The constant volume diluted with 1% HCl solution (mL)

Statistical Analysis

Data were showed as means or means ± SD (Standard Deviation). SPSS19.0 Software (SPSS Inc., Chicago, USA) was applied to assess the statistical differences using *t*-test or one-way Analysis Of Variance (ANOVA).

Results and Discussion

Optimization of CAE Parameters for TFZB Extraction

Effects of Cellulase Dosage, Solid-to-Liquid Ratio and Ethanol Concentration on the Extraction Yield of TFZB

The effects of cellulase dosage (0.2~0.6%, w/w) on TFZB extractions were tested and the results are showed in Fig. 1a, as shown in it, TFZB extraction rate increased dramatically with the increase of cellulase dosage in the range of 0.2 to 0.4% and reached a peak of 0.45±0.007% at 0.4% dosage, but obviously decreased within the range of 0.4 to 0.6%. These results suggested that cellulase could promote the release of TFZB by the way of destroying cellulose in the cell wall, however, the decrease of TFZB yield with further increasing amount of cellulase could be due to the fact that all the binding sites on the cell walls of test samples were dominated by cellulase and excessive cellulase could make the solution viscous, which is not beneficial for the enzymatic hydrolysis (Huang *et al.*, 2016).

The ethanol concentration was also conducted and the results were showed in Fig. 1, TFZB extraction rate increased slowly with the increase of ethanol concentration in the range of 40 to 50%, but, followed by decreasing dramatically with the further increase of ethanol concentration. Too high or too low ethanol concentrations are both adverse to the ideal extraction of flavonoids, which depends on the types of flavonoids in the extracts and the polarity of ethanol solution (Wei *et al.*, 2012).

The effects of solid-to-liquid ratio (1: 20 g/mL ~ 1: 60, g/mL) on TFZB extractions were examined and the results are provided in Fig. 1c, as shown in it, TFZB extraction rate increased dramatically with the increase of solid-to-liquid ratio in the range of 1: 20 g/mL ~ 1: 40 g/mL and then declined gradually. The increase of TFZB yield may be due to the fact that the over-increasing contact areas between the liquid and the material may result in the decrease of mass transfer resistance (Najafian, 2013). The decrease of TFZB yield perhaps could be attributed to the changes of extraction polarity with the increasing amount of solvent, which would reduce the solubility of some flavonoids in TFZB (Wei *et al.*, 2012). Thus, the optimum solid-to-liquid ratio for TFZB extraction was deemed to be 1: 40 g/mL.
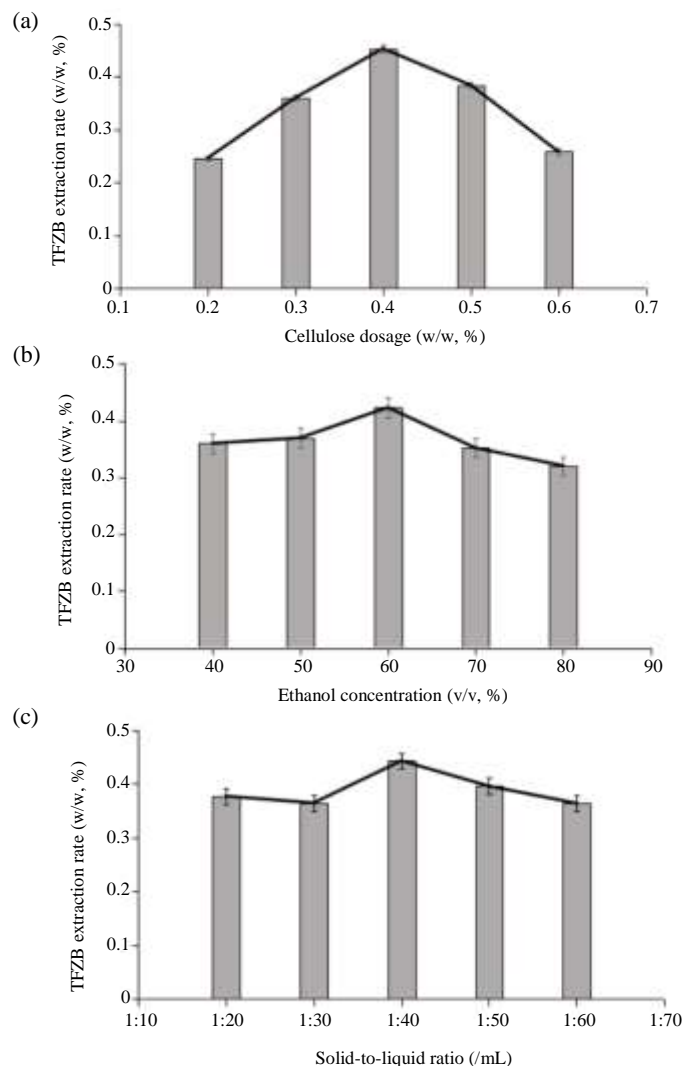
Fig. 1: The effect of single factors on the extraction of TFZB. (a) Cellulase dosage (b) Ethanol concentration (c) Solid-to-liquid ratio. Data was shown as means ± SD (n = 3)

RSM Analysis

The extraction of TFZB was optimized by RSM. Table 2 listed the code and actual levels of three factors including cellulase dosage (X_1), ethanol concentration (X_2) and solid-to-liquid ratio (X_3) and used in the process of this assay. A total of 17 experimental combinations including 5 zero-point experiments and 12 factorial tests and response values (Y) were reported in Table 2.

The response variable Y (TFZB extraction rate) could be related by the following quadratic polynomial regression equation:

$$Y = 0.09 + 1.89375X_1 + 0.00025X_2 + 0.0076X_3 \\ + 0.0019X_1X_2 - 0.005X_1X_3 - 0.000019X_2X_3 \\ - 2.06250X_1^2 - 0.0000125X_2^2 - 0.00004375X_3^2$$

Where:
Y = TFZB extraction rate (w/w, %)
X_1 = Cellulase dosage (w/w, %)
X_2 = Ethanol concentration (v/v, %)
X_3 = Solid-to-liquid ratio (g/mL)

The one-way Analysis Of Variance (ANOVA) for the regression equation was exhibited in Table 3, the P value of the model was <0.0001 and the lack of fit was 0.3258 (>0.05), indicating that the mathematical model obtained was highly significant. The determination coefficient value (Adj-R^2) of the model was 0.9598, suggesting that 95.98% of the response variable could be clarified by the model. In this model, the linear coefficients (X_1, X_2 and X_3), the interaction terms (X_1X_3) and the quadratic coefficients (X_1^2) were highly significant ($P<0.01$). The quadratic

terms (X_3^2) was significant effects ($P<0.05$). The interaction terms (X_1X_2, X_2X_3) and the quadratic coefficients (X_2^2) were not significant ($P>0.05$). The F value of the model is directly proportional to the response variable (Sheng *et al.*, 2013). Thus, the influence of various factors on the extraction rate of TFZB was: $X_1>X_2>X_3$. In addition, the interaction effects of X_1X_2, X_1X_3 and X_2X_3 were analyzed (Fig. 2). The steepness of the response surface is directly proportional to the significance of the interaction and the ellipticity of the contour map can also directly

reflect the strength of the interaction. The larger the ellipticity of the contour, the more significant the interaction is (Sheng *et al.*, 2013). As shown in Fig. 2b and Fig. 2d, the response surfaces were smooth and the contour line Fig. 2a and 2c tended to be round, which indicated that the interaction parameters (X_1X_2 and X_2X_3) had little effect on TFZB extraction rate, however, Fig. 2f showed that the response surface was steep and the contour line Fig. 2e tended to be oval, which indicated that the interaction parameter X_1X_3 had a greater effect.
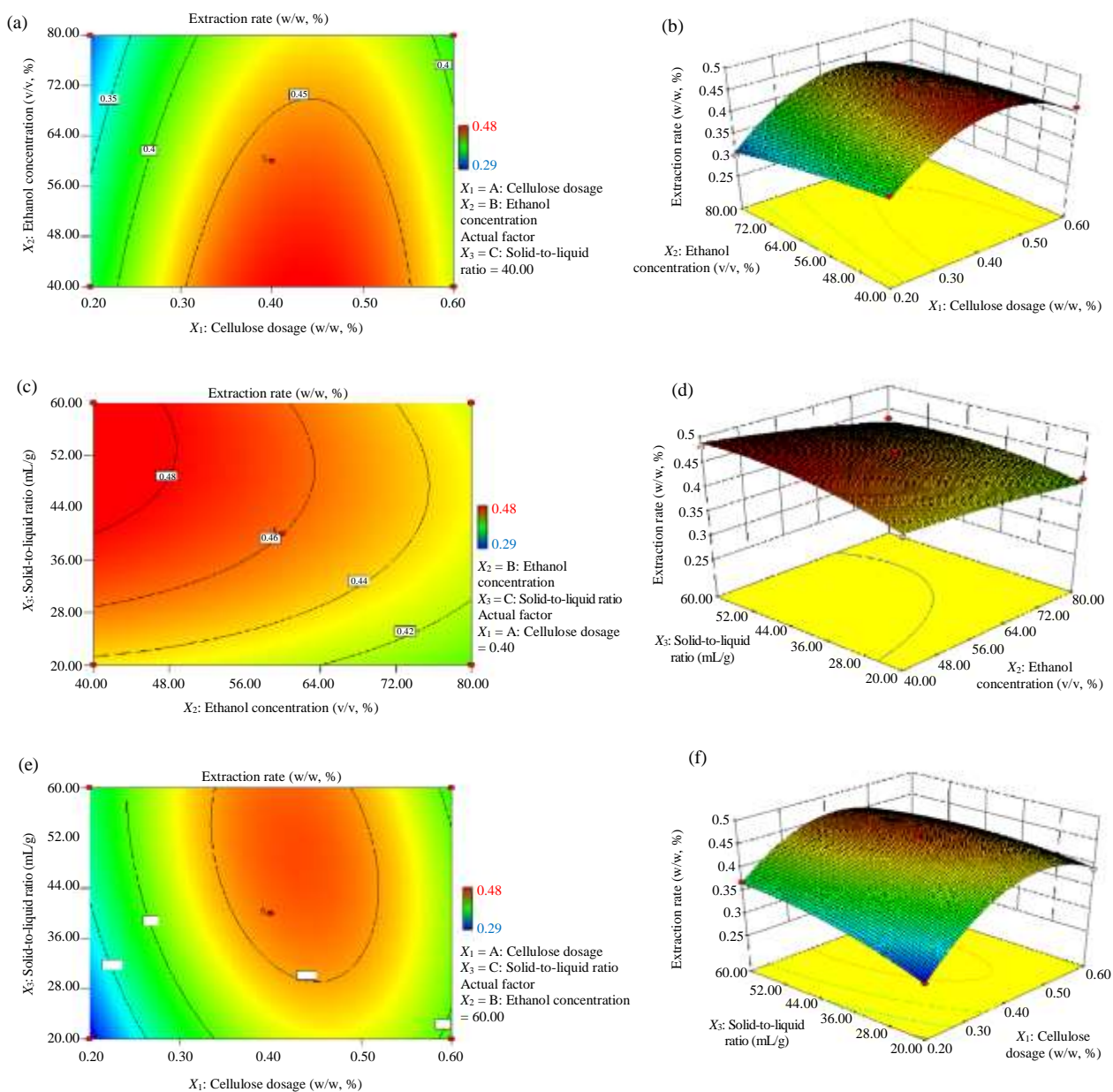

Fig. 2: Contour and surface plots showing the effects of the ratio of two factors. (a, b) Cellulase dosage versus Ethanol concentration; (c, d) Ethanol concentration versus Solid-to-liquid ratio; (e, f) Cellulase dosage versus Solid-to-liquid ratio

Table 2: Box-Behnken design and experimental data

| Run | Independent variable | | | Y |
	X_1	X_2	X_3	
1	-1	0	-1	0.37
2	0	0	0	0.42
3	0	0	0	0.31
4	0	0	0	0.39
5	-1	-1	0	0.29
6	0	0	0	0.39
7	1	1	0	0.37
8	1	0	1	0.39
9	-1	0	1	0.44
10	0	1	-1	0.40
11	0	-1	1	0.49
12	0	1	1	0.42
13	-1	1	0	0.46
14	1	-1	0	0.46
15	0	-1	-1	0.46
16	0	0	0	0.46
17	1	0	-1	0.36

Table 3: Variance analysis of extracted equation of TFZB

Source	Sum of squares	Mean square	F-value	P-value	Significance[a]
Model	0.049	5.424×10^{-3}	43.39	<0.0001	**
X_1	7.813×10^{-3}	7.813×10^{-3}	62.50	<0.0001	**
X_2	5.000×10^{-3}	5.000×10^{-3}	40.00	0.0004	**
X_3	2.813×10^{-3}	2.813×10^{-3}	22.50	0.0021	**
$X_1 X_2$	2.250×10^{-4}	2.250×10^{-4}	1.80	0.2216	n.s.
$X_1 X_3$	1.600×10^{-3}	1.600×10^{-3}	12.80	0.0090	**
$X_2 X_3$	2.250×10^{-4}	2.250×10^{-4}	1.80	0.2216	n.s.
X_1^2	0.029	0.029	229.26	<0.0001	**
X_2^2	1.053×10^{-4}	1.053×10^{-4}	0.84	0.3893	n.s.
X_3^2	1.289×10^{-3}	1.289×10^{-3}	10.32	0.0148	*
Residual	8.750×10^{-4}	1.250×10^{-4}			
Lack of fit	4.750×10^{-4}	1.583×10^{-4}	1.58	0.3258	n.s.
Pure error	4.000×10^{-4}	1.000×10^{-4}			
Cortotal	0.050				
Adj-R^2 = 0.9598					

a**P<0.01 highly significant; *P<0.05 significant; P>0.05 not significant (n.s.)

Verification of Predictive Model

According to the quadratic multinomial regression equation of the extraction rate of TFZB, the optimal extraction conditions were obtained as follows: Cellulase dosage of 0.40% (w/w), the liquid-to-solid ratio of 41.15: 1 mL/g, the ethanol concentration of 41.05% (v/v), the predicted extraction rate of TFZB was 0.48% (w/w). Considering the operability of process, the optimal extraction conditions were modified as below: Cellulase dosage of 0.40% (w/w), liquid-to-solid ratio of 41: 1 mL/g, ethanol concentration of 41% (v/v). The actual extraction rate of TFZB was 0.49±0.01% (w/w) (n = 3), which was a 2.1% deviation compared with the model predicted. These results showed that the model has a good fitting degree and the reliability of the predictive model was also verified.

In Vitro Antioxidant Activity of TFZB

The DPPH radical-scavenging capacities of TFZB were investigated and the results was provided in Fig. 3a, as shown in it, the scavenging capacities of TFZB and Vc on DPPH radicals had a significant dose-effect relationship with the concentration ranging from 0.2 to 1.0 mg/mL. The half inhibitory concentration (IC_{50}) values of TFZB and Vc against DPPH were 0.39 and 0.09 mg/mL, respectively. Therefore, the scavenging capacities of TFZB against DPPH was close to that of Vc when the concentration was in the range of 0.6~1.0 mg/mL, especially when the concentration was 0.8 mg/mL, the scavenging capacities against DPPH were similar, both of which were 88%.

The hydroxyl radical-scavenging capacities of TFZB were also investigated and the results were provided in Fig. 3b, as shown in it, the scavenging capacities of TFZB

and Vc on hydroxyl radicals also elicited significant dose-effect relationship within the concentrations spanning from 0.4 to 2.0 mg/mL. The IC_{50} values of TFZB and Vc against hydroxyl radicals were 0.70 and 0.12 mg/mL, respectively, indicating that the hydroxyl radicals-scavenging capacity of TFZB is superior to that of Vc.

The reducing power of TFZB to ferric iron was also studied and the results were provided in Fig. 3c, as shown in it, the reducing powers of TFZB and Vc to ferric iron also showed a dose-effect relationship within the concentrations of 0.4~2.0 mg/mL and the median effect concentration (EC_{50}) values of TFZB and Vc to ferric iron were 0.39 and 0.12 mg/mL, respectively, indicating that the reducing power of TFZB to ferric iron is slightly inferior to that of Vc.
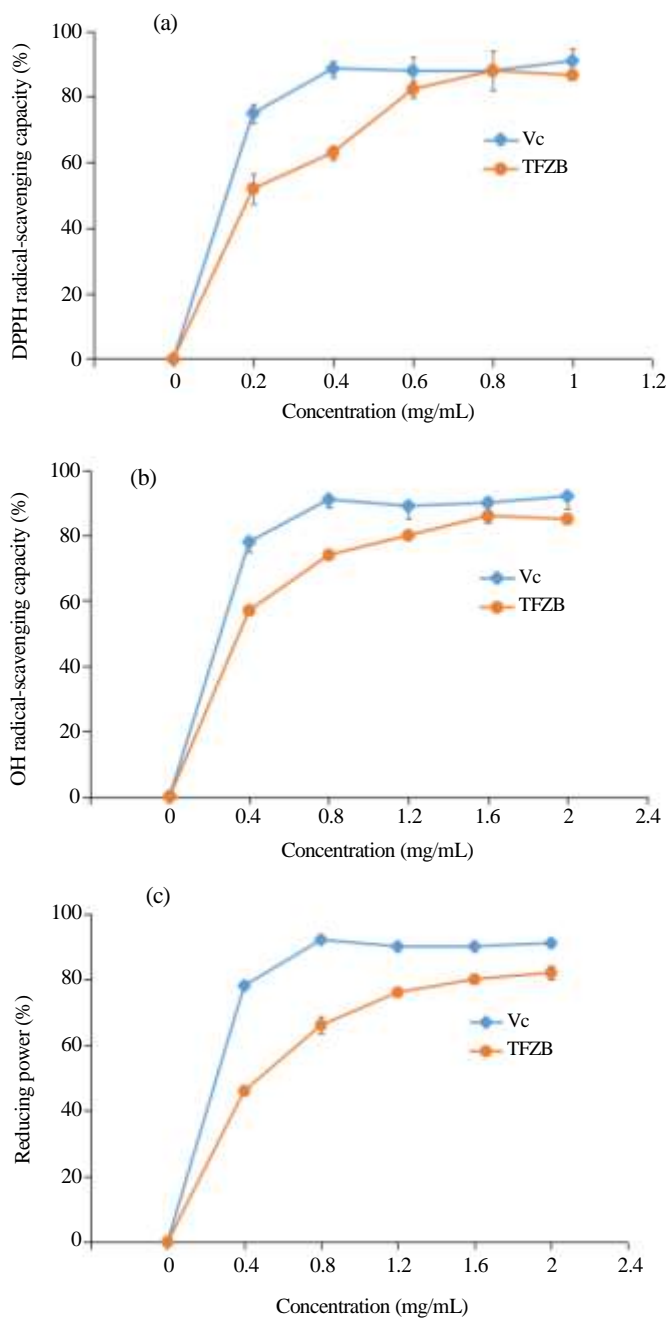

Fig. 3: The *in vitro* antioxidant evaluation of TFZB using Vc as positive control. (a) DPPH radical-scavenging capacities; (b) Hydroxyl radical-scavenging capacities; (c) Reducing power. Data was shown as means ± SD (n = 3)
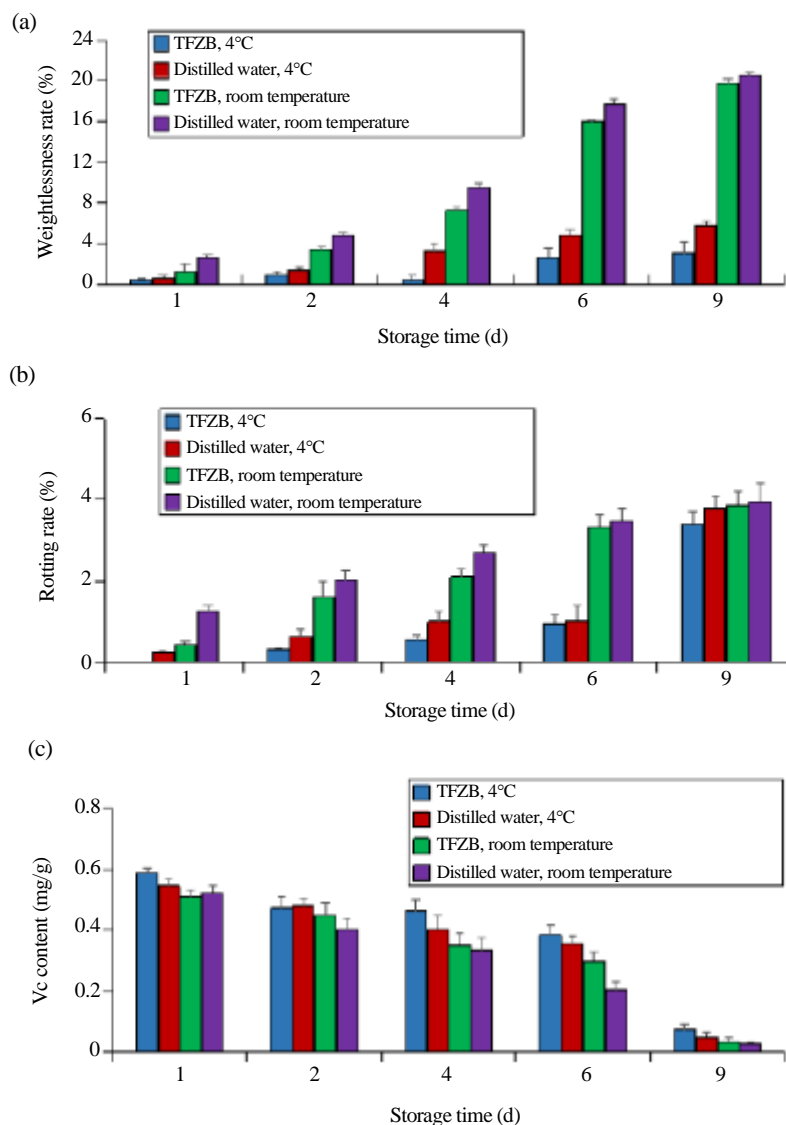
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Fig. 4: The effects of TFZB on strawberry preservation (a) Weightlessness rate; (b) Rotting rate; (c) Vc content. Data was expressed as means ± SD (n = 3)

The antioxidant capacity of compounds mainly depends on the protons that are supplied to neutralize free radicals at different reaction stages. Meanwhile, the main reason why flavonoids have antioxidant activity is that phenolic hydroxyl groups in flavonoids can react with free radicals to terminate the chain reaction. Besides, the methoxyl groups at the ortho-positions of phenolic hydroxyl in aromatic ring have a synergistic effect on the antioxidant activity and the most important active site of flavonoids are catechol hydroxyl at the position of C-3',4' (Omosa *et al.*, 2014). According to the antioxidant analysis in this assay, the comprehensive antioxidant capacity of TFZB seems to be strong, deserving of deep investigation. However, present paper focused on the extraction optimization of TFZB, the components of TFZB are still lacking and whether there are any of the above-mentioned structures or other chemical structures that can enhance their antioxidant capacity is still unclear. In the future work, we will analyze the components of TFZB to further establish the structure-activity relationship between compound structure and antioxidant activity.

Effects of TFZB on Strawberry Preservation

The changes in weight loss of strawberries after being treated with TFZB and distilled water at room temperature and 4 h were determined and the results were provided in Fig. 4a, as indicated in it, the

weightlessness rate of strawberry increased continuously with prolonging the storage time, at the same time, compared with the blank group, the weightlessness rate of strawberries slowed down when treated with TFZB at a concentration of 0.5 mg/mL. The weightlessness rate at room temperature was significantly higher than that of the samples at 4℃, which could be induced by the enhanced transpiration at higher temperature and the fast respiration rate of strawberry itself (Bhat and Stamminger, 2015). From the cellular level, Li found that the water loss during strawberry storage is caused by the increasing permeability of the cell membrane (Bhat and Stamminger, 2015). According to this hypothesis, the decrease in weightlessness rate during storage of strawberries after TFZB treatment may be due to the fact that TFZB could reduce cell damage and degeneration and maintain cell stability.

The changes in rotting rate of strawberries after being treated with TFZB and distilled water at room temperature and at 4℃ were measured and the results were provided in Fig. 4b, as indicated in Fig. 4b, the rotting rate of strawberry also increased continuously with prolonging the storage time, meanwhile, compared with the blank group, the rotting rate of strawberries slowed down when treated with TFZB at a concentration of 0.5 mg/mL. Besides, the rotting rate at room temperature was significantly higher than that of the samples at 4℃, which could be associated with the inhibition of the growth and reproduction of microorganisms at low temperatures. Strawberry spoilage is mainly caused by various pollutions induced by molds (Han-Bing *et al.*, 2020). Mounting evidences have pointed out that flavonoids have an inhibitory effect on microorganisms (Cui *et al.*, 2020). Molds belong to the class of fungi. Li *et al* emphasized that the antifungal compounds tend to be more lipophilic. Therefore, the inhibitory effects of TFZB on the decay of strawberries observed in this assay may be contributed by the lipophilic compounds in TFZB, which could aggregate into membranes on the surface of the strawberry, thus playing a role in water retention, which was another reasonable explanation for the changes in the weightlessness rate in the assay. Hence, the antifungal potential and activity-contributed compounds, particularly lipophilic ingredients in TFZB are needed to be further explored.

The changes in Vc content of strawberries during storage was shown in Fig. 4c. As indicated in it, the Vc content in strawberries decreased gradually with prolonging the storage time. Surprisingly, under the same conditions, the Vc content in strawberries treated with TFZB solution was higher than that in blank control, which complies with the antioxidant capacity of TFZB. As antioxidant, TFZB could quench the free radicals on the surface of the strawberries to protect Vc from being destroyed.

In summary, TFZB plays a central role in strawberry preservation. However, in this assay, the concentration of TFZB solution was only selected a lower level of 0.5 mg/mL. According to the observed results that the antioxidant capacity of TFZB increased with the increase of concentration, therefore, it is speculated that increasing the amount of TFZB in strawberry preservation may have better preservation effect, which will be another focus in our future research.

Conclusion

Z. Latifolia is the second largest aquatic vegetable in China, how to reasonably utilize the discarded bracts is an urgent concern regarding the sustainable and comprehensive utilization of *Z. Latifolia* species. In present investigation, the bract of *Z. Latifolia* was taken as the research object, Total Flavonoids from *Z. latifolia* Bracts (TFZB) were extracted and optimized and the yield of TFZB reached 0.49±0.01% after the RSM optimization. Besides, TFZB exerted certain antioxidant capacity and reducing power on Fe^{3+}. The application of TFZB in strawberry preservation was explored for the first time and the positive effects on strawberry preservation were verified by several tests. Further studies are still needed to clarify the flowing issues: (1) TFZB composition analysis and antifungal experiments are needed to be carried out to verify the association between compound structure, antioxidant and antifungal activity; (2) The preservation effect curves under different concentrations of TFZB should be constructed to determine the best preservation scheme. Nevertheless, it is hoped that this work will provide ideas for the comprehensive development and utilization of discarded *Z. latifolia* bracts.

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Author's Contributions

Tong Wang and Yuzhu Zhang: Perform the extraction process optimization and draft the manuscript.

Lijie Ben, Chenxin Zhai and Ying Lv: Carry out the evaluation of antioxidant capacity and strawberry preservation.

Yang Zhang and Lixue Zheng: Design the experiments and polish the manuscript.

Ethics

There is no ethical issue that may arise after the publication of this manuscript.

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