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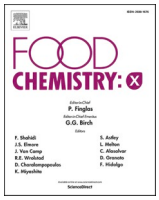

Production of low-tannin *Hibiscus sabdariffa* tea through D-optimal design optimization of the preparation conditions and the catalytic action of new tannase

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ABSTRACT

Many tannase-based industrial applications are currently being developed to successfully break down tannins in teas and juices. However, so far, no study has demonstrated the potential application of tannase to reduce tannin levels in *Hibiscus sabdariffa* tea. The D-optimal design was utilized to predict the optimal conditions for maximizing anthocyanins and decreasing tannin content in *Hibiscus* tea. Then, the effects of *Penicillium commune* tannase were evaluated by examining the physicochemical parameters and α-amylase inhibitory action of untreated and treated *Hibiscus* tea, as well as quantifying catechin content changes using HPLC. Following treatment with tannase, the esterified catechins decreased by 8.91%, while the non-esterified catechins increased by 19.76%. Additionally, tannase significantly raised the total phenolic compounds by 8.6%. In contrast, the α-amylase inhibiting activity of *Hibiscus* tea decreased by ≈28%. As a novel member of the tea family, tannase offers an excellent means of conditionally producing low-astringency *Hibiscus* tea.

Introduction

Teas are widely consumed, and *Hibiscus* spp., due to their functional health properties and chemical composition, deserves special consideration among those studied. There are over 300 cultivars of *Hibiscus*; including *H. sabdariffa* is widely consumed throughout the world, particularly in subtropical and tropical nations like India, Sudan, and Egypt (Maciel et al., 2018). Different plant parts are used to make jam, jelly, and soft drinks, while their calyces are primarily used to prepare traditional tea by infusion in cold or hot water for 5 min, followed by filtration (Monteiro et al., 2017). Extract of *H. sabdariffa* calyces is rich in organic acids (e.g., hibiscus acid, gallic acid, malic acid, and citric acid), anthocyanins (e.g., cyaniding-3-sambubioside, delphinidin 3-sambubioside, and cyanidin-3-glucoside) (Preciado-Saldaña et al., 2019), sugars, and phytochemical compounds (e.g., flavonoids including quercetin and hisbiscetrin) (Miranda et al., 2019). Gallocatechin gallate, protocatechuic acid, caffiec acid, catechin, and gallate have also been reported (Da-Costa-Rocha, Bonnlaender, Sievers, Pischel, & Heinrich, 2014). Consequently, *Hibiscus* tea plays a crucial role in reducing the risk of certain chronic health problems, including obesity (Banwo, Sanni, Sarkar, Ale, & Shetty, 2022), cancer (Laskar & Mazumder, 2020; Maciel et al., 2018), dyslipidemia, and diabetes mellitus (Shruthi & Ramachandra, 2019).

Tannins are polyphenols with a high molecular weight found naturally in plants used for food and feed. Tannins are divided into two primary categories: hydrolyzable and condensed. Hydrolyzable tannins consist of gallotannins or ellagotannins, the hydrolysis of which yields glucose and gallic acid from gallotannins and ellagic acid from ellagotannins. Condensed tannins (proanthocyanidins) are compounds composed of oligomers and polymers of the flavan-3-ol units, which are composed of different isomeric forms of catechin and gallocatechin linked by C4-C6 or C4-C8 bonds (Catelani, Bittar, Pezza, & Pezza, 2016; Wilhelmy, Pavez, Bordeu, & Brossard, 2021). Tannins may influence protein utilization by forming complexes with protein and iron when consumed as part of a human or animal's diet (Wilhelmy et al., 2021). They can bind tightly to proteins by forming hydrogen bonds between its phenolic groups and the −NH groups of peptides, which cannot be broken by digestive enzymes. In addition to their high antioxidant capacity, these compounds are notable for their astringency (Catelani et al., 2016). The astringent mouth feel is caused by the interaction of tannins with proline-rich proteins, a class of salivary proteins. In addition, the ortho-dihydroxyl groups of these compounds are an important feature for the chelation of metal ions, particularly iron. These antinutritive properties necessitate the elimination of these compounds in

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order to produce more palatable foods. Subsequently, tannins were eliminated using alkaline ethanol precipitation method (Gong, Li, & Qu, 2014). Although it can remove 90 % of tannins, it has significant drawbacks, such as the need for a large amount of alkali (NaOH) and the loss of phenolic components. In contrast, tannase is the most common, eco-friendly, and cost-effective tannin removal agent (Saeed et al., 2021).

Tannase (EC 3.1.1.20) is used primarily to hydrolyze tannins by catalyzing their de-esterification to gallic acid and glucose (Saeed et al., 2021). It has been identified in numerous fungal strains, particularly those belonging to the *Penicillium* and *Aspergillus* genera, and recently purified from *Penicillium commune* HS2 with a Mw of 35 kDa (Mostafa, 2022). It exhibited high tannin tolerance and the highest optimum temperature among the fungal strains. After 2 h of incubation at 45 °C, the purified enzyme was successfully applied to remove 33.89 % of the tannins from the tea containing lemon. Tannin removal from instant teas (Aharwar & Parihar, 2021) and apple juice clarification (Andrade et al., 2021) to reduce bitterness and haze are two examples of food production applications involving tannase. However, little research has been conducted on the degradation of tannins in *Hibiscus* tea.

In terms of extraction, the yield of the target compound must be maximized. In the case of *Hibiscus*, the literature investigates factors such as time, temperature, and solvent type in an effort to maximize anthocyanins (Maciel et al., 2018). Additionally, response surface methodology (RSM) was utilized to maximize the content of phenolic compounds in functional beverages containing *Hibiscus* and green tea (Preciado-Saldaña et al., 2019). Considering that food producers are constantly looking for beverages with appealing physical and sensory characteristics, this study was initially conducted to maximize the anthocyanin content and minimize the tannin content in *Hibiscus sabdariffa* tea using the D-optimal design of RSM. This procedure was chosen because it generates optimal designs for multi-factor experiments with both quantitative and qualitative factors and offers a reasonable choice to minimize the variance of the estimated regression coefficients (Handali, Moghimipour, Rezaei, Saremy, & Dorkoosh, 2019). The effect of the newly purified tannase from *Penicillium commune* on its chemical and physical characteristics was then investigated.

Materials and methods

Standards and reagents

The following proanthocyanidin standards were purchased from Sigma-Aldrich, St. Louis, MO, USA: (−)-gallocatechin (GC), (+)-catechin (C), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), (−)-epigallocatechin gallate (EGCG), (−)-gallocatechin gallate (GCG), (−)-epicatechin gallate (ECG), and (−)-catechin gallate (CG), as well as α-amylase (EC.3.2.1.1) of the porcine pancreas Type VI-B. Additionally, DNSA (3, 5-dinitrosalysilic acid) reagent, vanillin, KIO_3, bovine serum albumin (BSA), rhodanine, gallic acid, tannic acid, and DPPH (2, 2-diphenyl-1-picrylhydrazyl) were obtained from Sigma-Aldrich, Germany. Methanol and acetonitrile (HPLC grade) were attained from Merck, Germany. All of the other chemicals and reagents were of analytical grade.

Optimization of Hibiscus tea preparation conditions by RSM

Packaged dried Roselle calyces (*Hibiscus sabdariffa*) were purchased from Kenzy, Etihad Company, for packaging herbs, Giza, Egypt. The aqueous extract was prepared after analyzing the effects of three factors: two quantitative (time and calyces: water ratio) and one qualitative (preparation method). The time factor was evaluated at five distinct levels (5, 10, 15, 20, and 25 min), while the calyces: water ratio and preparation method were evaluated at three levels each (Table 1). The effectiveness of three calyces: water ratios (1:100, 2:100, and 4:100-ml water) and three preparation methods (cold water 25 ± 2 °C, hot water 90 °C, and boiling water 100 °C) was evaluated. Depending on the duration of each treatment, the extracts were stirred intermittently. The Roselle tea was subsequently filtered through a stainless-steel filter (pore size of ca. 1 mm), and the filtrate was recovered (Monteiro et al., 2017). These factors were statistically screened and optimized by the D-optimal of RSM using Design-Expert V7 (Handali et al., 2019). Based on 22 experimental runs, four responses were monitored: hydrolyzable tannins, protein-precipitable (PP) tannins, condensed tannins, and anthocyanins. Analysis of variance (ANOVA), the regression coefficient (R2), and the coefficient of variation (CV) was used to validate the model.

Degradation of tannins from Hibiscus tea

In order to degrade tannins of *Hibiscus* tea, a purified enzyme from the recently isolated *Penicillium commune* HS2 (Gene Bank accession number MT084558) was utilized. Following solid-state fermentation of the isolated fungal strain on potato peels, the extracellular enzyme was purified by ammonium sulfate precipitation, followed by gel electrophoresis of Sepharose 4-B as designated by Mostafa (2022). The *Hibiscus* tea was initially prepared by boiling 2 g of *Hibiscus* calyx powder in 100 ml of distilled water for 5 min, followed by filtration. Using a vortex mixer (LP vortex mixer from Thermo Fisher Scientific, USA), *Hibiscus* tea was mixed with purified tannase (53.1 U/ml) and incubated at 45 °C for 3 h (till no more gallic acid was produced). Tea was heated to 100 °C to inactivate tannase before being stored at −20 °C until analysis. All physicochemical properties of treated and untreated tea at the same dilution were compared.

Estimation of tannins

The hydrolyzable tannins (gallotannins and ellagitannins) were determined with modifications according to Choi and Koh (2017). One milliliter of the extract was mixed with 5 ml of 2.5 % preheated KIO_3 for 7 min at 30 °C. The tube containing the mixture was re-immersed in the same water bath for 2 min prior to its absorbance at 550 nm being measured by a UV-spectrophotometer, Unico UV-2000, USA. The standard curve was drawn using tannic acid at various concentrations (0.2–5 mg/ml). The BSA precipitation test was used to analyze the protein-precipitable (PP) tannins (de Lima et al., 2018). Two milliliters of BSA (1 mg/ml) were combined with 1 ml of *Hibiscus* tea and centrifuged at 2795×*g* (Hermle, Z300, Germany) for 15 min. In 4 ml of a mixture of 1 % sodium dodecyl sulfate and 5 % triethanolamine, the precipitate was dissolved. Then 1 ml of 0.01 M ferric chloride solution in 0.01 M HCl was added and mixed. Subsequently, using the specified

Table 1
Experimental factors and their levels for statistical screening using D-optimal design.

Factor code	Independent factor	Levels No.	Levels					Response	
			1	2	3	4	5		
A	Time	5	5	10	15	20	25	Y1	Hydrolyzable tannins
B	Calyces: water ratio	3	1:100	2:100	4:100			Y2	Protein-perceptible tannins
C	Preparation method	3	Cold	Hot	Boiled			Y3	Condensed tannins
								Y4	Anthocyanins

spectrophotometer, the absorbance at 550 nm was measured 15–30 min later. Pure tannic acid (200–1600 μg/ml) was used as a standard.

The vanillin-HCl method, as described by Mak, Chuah, Ahmad, and Bhat (2013), was used to quantify condensed tannins. Briefly, 1 ml of aqueous extract was mixed with 5 ml of a reagent mixture (1:1 ratio of 4 % vanillin in methanol and 8 % concentrated HCl in methanol). The blank contained 1 ml of extract and 5 ml of distilled water. Using the same spectrophotometer, *A500* was measured after 20 min, and a calibration curve was drawn using different concentrations of catechin.

Detection of catechins by HPLC

A modified version of Saito, Welzel, Suyenaga, and Bueno (2006) method for catechin detection in tea was utilized. 100 mg of freeze-dried sample was dissolved in 60 ml of methanol, sonicated for 10 min (SB-50 Ultrasonicator, 50 W, Scientz, China), and then diluted to 100 ml with the mobile phase. Finally, it was filtered through a 0.22 μm PVDF membrane Millex filter. The HPLC system (Agilent 1200) was used, along with a Merck (Germany) LiChrosorb RP-18 column (12.5 cm long × 4 mm i.d. and 5 μm particle diameter) equipped with a guard column (1.0-cm long × 4.6 mm i.d.). The mobile phase was Solution A: 0.01 % orthophosphoric acid in HPLC-grade water that was degassed for 3 min in a sonicator, and Solution B: acetonitrile. Gradient elution was applied as follows: 11 % B was eluted initially, followed by 25 % B after 30 min, 100 % B from 35 to 39 min, and 11 % B from 40 to 50 min. The injection volume was 10 μl, the flow rate was 1.0 ml/min, and the temperature was maintained at 30 °C. At 280 nm, the eluents were detected and analyzed with a UV–Visible detector. This system was validated by calculating the peak tailing factor = 1.146 (< 2), the relative standard deviation percentage of the peak area = 0.25 (< 2 %), and the theoretical plates = 6832 (> 2000) of 6 injections of the standard solution. The catechin standards were prepared by mixing 150 ml of methanol with 3 mg of GC, 3.7 mg of C, 3.7 mg of EGC, 5.2 mg of EC, 5.2 mg of EGCG, 5.5 mg of GCG, 5.5 mg of ECG, 7 mg of CG, and 8.5 mg of CG. After 10 min of sonication, 10 ml was diluted to 60 ml with methanol and thoroughly mixed. The obtained mixture (5.0 ml) was mixed with 10 ml of mobile phase and injected as the sample. Catechin compounds were quantified in terms of mg/g DW by comparing the peak area of the UV response at the specified wavelength to that observed in the presence of pure standards.

Determination of reducing sugar, anthocyanin, total polyphenol, and gallic acid content

The reducing sugars were analyzed by the DNSA method (Miller, 1959), followed by an absorbance measurement at 540 nm by the UV-spectrophotometer of Unico, UV-2000, USA. Anthocyanin content as cyanidin-3-glucoside (C-3-G) was determined as described by Amaya-Cruz, Peréz-Ramírez, Pérez-Jiménez, Nava, and Reynoso-Camacho (2019), with modifications. Briefly, 0.5 ml of the extract was combined with 1.75 ml of either KCl buffer (pH 1, 0.025 M) or potassium acetate buffer (pH 5, 0.01 M), and the absorbances *A*510 and *A*700 of each test tube were recorded using the specified spectrophotometer. Next, the C-3-G (mg/100 ml extract) was calculated using the following equation:

$$Anthocyanin\ content\ as\ C-3-G\ =\ [(A510-A700)\mathrm{pH1}-(A510-A700)\mathrm{pH5}]\ x\ \frac{MW\ x\ DF}{\varepsilon\ x\ D} \quad (1)$$

where MW is the molecular weight of C-3-G (484.8 g/mol), DF is the dilution factor, Ɛ is the molar absorptivity coefficient (26,900/M.cm), and D is the width of the cuvette (1 cm).

The total phenolic content (TPC) was determined by mixing 1000 μl of the extract or methanol (blank) with 2.5 ml of a 10 % Folin-Ciocalteu reagent and 2.0 ml of sodium carbonate (7.5 %) solution. Before measuring the absorbance at 760 nm with the UV-spectrophotometer Unico UV-2000, USA; the mixture was incubated in the dark for 30 min. TPC was calculated as mg gallic acid equivalents/100 ml sample (Singh, Thrimawithana, Shukla, & Adhikari, 2021). As described by Sharma, Bhat, and Dawra (2000) the gallic acid content of the *Hibiscus* tea extract was determined before and after tannase treatment using the rhodanine reagent. One milliliter of the tea was mixed with 1500 μl of methanolic rhodanine (0.667 %) and left for 5 min before adding 500 μl KOH (0.5 M). After 5 min, *A*520 was measured. A freshly prepared gallic acid solution (1–35 ng/ml) was utilized to generate the standard curve.

Determination of pH, titratable acidity, and color density

Using a pH meter (301, Orion Research, Boston, USA), the pH value of the extract was determined, while the titratable acidity was determined by titration with NaOH solution (0.1 N) until pH 8.2 was reached and then calculated as hibiscus acid (Ifie, Ifie, Ibitoye, Marshall, & Williamson, 2018). The changes in the color density were estimated by measuring *A*420, *A*520, and *A*700 (Monteiro et al., 2017), and then calculated using the following equation:

$$\text{Color density} = (A420 - A700) + (A520 - A700) \quad (2)$$

Antioxidant potential activity determination

The DPPH free radicals assay (Zhang et al., 2023) was used to determine the antioxidant effect of *Hibiscus* tea prior to and following tannase treatment. Briefly, the sample was diluted 1:3 with methanol before being combined with 1 ml of methanolic DPPH solution (0.2 mM). After 30 min of incubation in the dark, the absorbance (*A*517) was measured with a UV-spectrophotometer, Unico UV-2000 (USA), and methanol was used as a control. The DPPH scavenging activity was calculated as follows:

$$\%\ \text{DPPH scavenging} = \frac{Acontrol - Asample}{Acontrol} \times 100 \quad (3)$$

α-Amylase inhibiting activity determination

The anti-diabetic activity of *Hibiscus* tea before and after tannase treatment was determined using the Siegień et al. (2021) method for measuring α-amylase inhibitory activity. 200 μl of *Hibiscus* tea and 100 μl of α-amylase solution (12.5 U/ml in sodium phosphate buffer, 50 mM, pH 6.9) were incubated for 10 min. Consequently, 700 μl of starch solution (1 %) was added to each tube and incubated for another 10 min at 37 °C. In order to determine the formed reducing sugars, 1 ml of DNSA reagent was appended to each tube and placed in a water bath at boiling temperature for 5 min. After cooling, *A540* was measured, and glucose was used to plot the standard curve. Next, the degree of inhibition of α-amylase activity was calculated using this equation:

$$\text{Inhibition activity}\ (\%) = \frac{A\ \text{blank} - A\ \text{sample}}{A\ \text{blank}} \times 100 \quad (4)$$

Sensory evaluation

An untrained panel of 40 students and staff members from Cairo University's Food Science Department, Faculty of Agriculture, evaluated the untreated and tannase-treated *Hibiscus* tea samples. It was served at 25 °C in glass cups (50-ml) to evaluate various sensory attributes such as taste, color, bitterness, acidity, and overall acceptance (using a 9-point hedonic scale). Samples were evaluated for these attributes, with 9 representing liked extremely and 1 representing disliked extremely (Monteiro et al., 2017).

Statistical analysis

CoStat software (Berkeley, CA, USA) was used to compare samples using one-way Analysis of Variance (ANOVA) (Gauderman, 1988). Duncan's test was applied to the means of three replicates at a significant level of $p < 0.05$. Using the software Design-Expert V7 (Stat-Ease, Minneapolis, MN, USA), the investigational design to maximize anthocyanin and reduce tannin content in *Hibiscus* tea was statistically analyzed.

Results and discussion

RSM-based optimization of Hibiscus tea extraction conditions

Anthocyanins play a crucial role in the sensory quality of this plant-based beverage and are important functional components. In order to optimize the extraction conditions (time, calyces-to-water ratio, and preparation method) for maximum anthocyanin and minimum tannin (hydrolyzable, PP, and condensed) content in *Hibiscus* tea, response surface methodology was utilized in the current study. The cumulative effect of these factors on the anthocyanin and tannin content was investigated using a D-optimal design consisting of 22 experiments. The conditions of each experiment and the experimental values of the four responses are presented in Table 2. RSM is less time-consuming than other approaches because it requires fewer runs to monitor the interaction between independent and dependent variables. The D-optimal design was chosen as it was suitable for investigating the least number of experiments to aid in the selection of the optimal composition or conditions (Handali et al., 2019).

Model validation

ANOVA and regression coefficient were used to assess whether the model fits the experimental data well and can be used to predict the optimal conditions for *Hibiscus* tea preparation for the experimental design employed (Table 3).

The ANOVA for hydrolyzable and protein-perceptible (PP) tannins, with respective F-values of 807.92 and 1157.25, was highly significant, with a very low probability value (p-model > F = 0.001) that is significant at the 95 % confidence interval. Simultaneously, the lower values of the coefficient of variation (CV = 3.44 % and 3.55 %, respectively) indicated that the conducted experiments were more precise and reliable. The determination coefficient (R^2) was used to verify the model's validity. The results of R^2 = 0.9989 and 0.9992 in these responses indicate that independent factors account for 99.8 % and 99.9 %, respectively, of the total variation in hydrolyzable and PP tannin concentrations. The predicted R^2 values of 0.9893 and 0.9951 corresponded reasonably well to the adjusted R^2 values of 0.9976 and 0.9984, respectively. A sufficient level of accuracy measures the signal-to-noise ratio. A value greater than four is desirable and a ratio of 97.278 for hydrolyzable tannins and 105.228 for PP tannins indicated an acceptable signal. The model was found to be significant for hydrolyzable tannins, with model terms A, B, C, AB, BC, and A^2 being significant. In contrast, the model was found to be significant for PP tannins with A, B, C, AC, BC, and B^2. Based on these results, the subsequent quadratic models were fitted.

$$\text{Hydrolyzable tannins} = +107.97 + 29.11^{*}\,A + 60.25^{*}\,B - 38.62^{*}\,C[1] - 19.14^{*}\,C[2] + 14.59^{*}\,AB + 1.17^{*}\,AC[1] + 0.78^{*}\,AC[2] - 17.74^{*}\,BC[1] - 10.72^{*}\,BC[2] - 7.51^{*}\,A^2 - 0.90^{*}\,B^2$$

$$\text{Protein-perceptible (PP) tannins} = +59.70 + 8.83^{*}\,A + 32.88^{*}\,B - 44.37^{*}\,C[1] - 2.84^{*}\,C[2] + 1.62^{*}\,AB + 2.34^{*}\,AC[1] - 3.61^{*}\,AC[2] - 26.51^{*}\,BC[1] + 0.80^{*}\,BC[2] + 2.27^{*}\,A^2 - 2.93^{*}\,B^2$$

where A, B, and C are the time, calyces: water ratio, and preparation method, respectively.

Concerning condensed tannins and anthocyanins, ANOVA (Table 3) for the 2-factorial model was significant with an F-value of 168.86 and 357.63, respectively, with a very low probability value of < 0.0001. Lower values of the coefficient of variation (CV = 5.87 %) indicate greater precision and reliability of the studies conducted. The R^2 value was also used to examine the goodness of fit of both responses. R^2 = 0.9922 for condensed tannins indicates that 99.2 % of the total variation is attributable to independent factors. In contrast, R^2 = 0.9963 was observed for anthocyanins, indicating that 99.6 % of the total variation is attributed to the independent factors. The predicted R^2 of 0.9515 was reasonably in agreement with the adjusted R^2 of 0.9863 for both

Table 2
D-optimal runs and the corresponding responses.

Run	Time (min)	Calyces:water ratio	Preparation method	Hydrolyzable tannins	Protein-perceptible tannins	Condensed tannins	Anthocyanins
	A	B	C	Y1	Y2	Y3	Y4
1	15	2.5:100	Boiling	*170.05^{c} ± 0.77	109.41^{c} ± 11.41	200.96^{d} ± 14.57	1.74^{bcde} ± 0.50
2	25	4:100	Hot	176.50^{c} ± 2.82	97.20^{d} ± 1.69	284.60^{b} ± 2.05	2.58^{abc} ± 0.50
3	16.57	4:100	Cold	118.16^{f} ± 9.58	20.66^{ij} ± 0.49	238.10^{c} ± 7.21	1.74^{bcd} ± 0.05
4	25	2.24:100	Cold	82.66 ^{g} ± 4.24	28.39^{hi} ± 2.82	133.58^{f} ± 4.18	1.20^{def} ± 0.31
5	5	2.74:100	Cold	36.36 ^{k} ± 0.00	8.19 ^{k} ± 0.11	105.39 ^{g} ± 2.86	0.78^{ef} ± 0.04
6	23.13	1.46:100	Boiling	115.79^{e} ± 2.82	74.07^{e} ± 0.02	135.59^{ef} ± 3.67	1.17^{def} ± 0.01
7	25	4:100	Boiling	285.24^{a} ± 3.46	175.70^{a} ± 6.36	319.94^{a} ± 10.94	2.84^{a} ± 1.03
8	25	4:100	Boiling	285.24^{a} ± 3.46	175.70^{a} ± 6.36	319.94^{a} ± 10.94	2.84^{a} ± 1.03
9	15	2.5:100	Cold	66.85 ^{h} ± 0.86	11.69^{jk} ± 0.00	134.71^{ef} ± 1.41	0.98^{ef} ± 0.02
10	5	2.74:100	Cold	36.36 ^{k} ± 0.00	8.19 ^{k} ± 0.01	105.39 ^{g} ± 2.26	0.78^{ef} ± 0.04
11	25	1:100	Hot	44.13^{j} ± 1.23	24.30^{i} ± 1.06	71.15^{hi} ± 0.62	0.65^{f} ± 0.01
12	5	4:100	Boiling	207.44^{b} ± 3.21	154.7^{b} ± 4.77	317.86^{a} ± 1.88	2.72^{ab} ± 0.38
13	5	2.5:100	Boiling	129.65^{d} ± 1.48	96.68^{d} ± 0.84	198.66^{d} ± 7.88	1.70 ^{cd} ± 0.48
14	5	1:100	Boiling	51.86^{i} ± 1.35	38.68^{gh} ± 1.25	79.47 ^{h} ± 0.47	0.68^{f} ± 0.11
15	25	4:100	Hot	176.50^{c} ± 6.92	97.20^{d} ± 4.31	284.60^{b} ± 5.65	2.38^{abc} ± 0.82
16	15	1.83:100	Hot	62.38 ^{h} ± 0.11	42.87 ^{fg} ± 0.19	128.95^{f} ± 0.00	1.05^{ef} ± 0.07
17	5	2.5:100	Hot	51.54^{i} ± 0.49	51.69^{f} ± 2.32	148.04^{e} ± 9.95	1.31^{def} ± 0.29
18	5	4:100	Hot	82.46 ^{g} ± 2.24	82.70^{e} ± 7.49	236.86^{c} ± 13.64	2.10^{abcd} ± 0.00
19	5	1:100	Hot	20.62 ^{l} ± 0.86	20.68^{ij} ± 0.39	59.26^{ij} ± 1.55	0.53^{f} ± 0.11
20	13.34	1:100	Cold	23.78 ^{l} ± 0.29	4.16 ^{k} ± 0.05	47.92^{j} ± 0.35	0.35^{f} ± 0.00
21	16.57	4:100	Cold	118.16^{f} ± 9.58	20.66^{hj} ± 2.75	238.10^{c} ± 7.21	1.74^{bcde} ± 0.04
22	25	2.24:100	Cold	82.66 ^{g} ± 0.42	28.39^{hi} ± 0.30	133.58^{f} ± 2.37	1.21^{def} ± 0.14

*All values were expressed as the mean ± SD of three measurements. Different letters within rows denote statistically significant differences (p-value < 0.05) between the runs. All hydrolyzable, condensed, and protein-perceptible tannins were expressed as mg/100 ml of extract, whereas anthocyanins were calculated as cyanidin-3-glucoside.

Table 3
The Analysis of Variance (ANOVA) for various responses, including hydrolyzable, protein-perceptible, condensed tannins, and anthocyanins.

Source	Factor	Sum of squares	DF	Mean square	F value	p-value Prob > F	Remarks		
Hydrolyzable tannins (Quadratic model)									
Model		1.276E + 005	11	11596.33	807.92	< 0.0001	**Significant**	**R^2**	0.9989
A	**Time**	14295.49	1	14295.49	995.98	< 0.0001		**Adjusted R^2**	0.9976
B	**Calyces: water**	43527.93	1	43527.93	3032.62	< 0.0001		**Predicted R^2**	0.9893
C	**Preparation method**	40476.78	2	20238.39	1410.02	< 0.0001		**Adeq. Precision**	97.278
AB		1679.19	1	1679.19	116.99	< 0.0001		**Std. Dev.**	3.79
AC		27.54	2	13.77	0.96	0.4158		**Mean**	110.20
BC		4460.85	2	2230.42	155.39	< 0.0001		**C.V. %**	3.44
A^2		191.02	1	191.02	13.31	0.0045		**PRESS**	1367.81
B^2		2.92	1	2.92	0.20	0.6617			
Residual		143.53	10	14.35					
Lack of fit		143.53	5	28.71	14.55	0.0011			
Cor Total		1.277E + 005	21						
Protein-perceptible tannins (Quadratic model)									
Model		62389.04	11	5671.73	1157.25	< 0.0001	**Significant**	**R^2**	0.9992
A	**Time**	1096.23	1	1096.23	223.67	< 0.0001		**Adjusted R^2**	0.9984
B	**Calyces: water**	13728.80	1	13728.80	2801.21	< 0.0001		**Predicted R^2**	0.9951
C	**Preparation method**	31399.81	2	15699.91	3203.39	< 0.0001		**Adeq. Precision**	105.228
AB		1096.23	1	20.76	4.24	0.0666		**Std. Dev.**	2.21
AC		13728.80	2	51.68	10.54	0.0034		**Mean**	62.36
BC		4373.46	2	2186.73	446.18	< 0.0001		**C.V. %**	3.55
A^2		17.38	1	17.38	3.55	0.0890			
B^2		31.32	1	31.32	6.39	0.0300			
Residual		49.01	10	4.90				**PRESS**	304.95
Lack of fit		49.01	5	9.80	106.98	< 0.0001			
Cor Total		62438.05	21						
Condensed tannins (2FI model)									
Model		1.665E + 005	9	18502.37	168.86	< 0.0001	**Significant**	**R^2**	0.9922
A	**Time**	3893.19	1	3893.19	35.53	< 0.0001		**Adjusted R^2**	0.9863
B	**Calyces: water**	1.241E + 005	1	1.241E + 005	1132.87	< 0.0001		**Predicted R^2**	0.9515
C	**Preparation method**	21337.63	2	10668.81	97.37	< 0.0001		**Adeq. Precision**	41.589
AB		131.58	1	131.58	1.20	0.2947		**Std. Dev.**	10.47
AC		1489.50	2	744.75	6.80	0.0106		**Mean**	178.30
BC		846.66	2	423.33	3.86	0.0507		**C.V. %**	5.87
Residual		1314.89	12	109.57				**PRESS**	8143.45
Lack of fit		1314.89	7	187.84	10.86	0.0004			
Cor Total		1.678E + 005	21						
Anthocyanins (2FI model)									
Model		12.59	9	1.40	357.63	< 0.0001	**Significant**	**R^2**	0.9963
A	**Time**	0.30	1	0.30	75.53	< 0.0001		**Adjusted R^2**	0.9863
B	**Calyces: water**	8.65	1	8.65	2211.38	< 0.0001		**Predicted R^2**	0.9515
C	**Preparation method**	2.14	2	1.07	273.78	< 0.0001		**Adeq. Precision**	41.589
AB		4.968E-003	1	4.968E-003	1.27	0.2818		**Std. Dev.**	10.47
AC		0.059	2	0.030	7.57	0.0075		**Mean**	178.30
BC		0.21	2	0.10	26.39	< 0.0001		**C.V. %**	5.87
Residual		0.047	12	3.912E-003				**PRESS**	8143.45
Lack of fit		0.027	7	3.841E-003	0.96	0.5385			
Cor Total		12.64	21						

responses. With an adequate signal > 4, the adequate precision ratio for both condensed tannins and anthocyanins was 41.589, indicating an adequate signal. The probability > F for these models was < 0.0500, indicating that model terms are significant. A, B, C, and AC are significant terms in condensed tannins, whereas all terms plus BC are significant in anthocyanins. The final predictive equations for both responses are as follows:

Condensed tannins = +165.90 + 16.46* A + 102.12* B − 35.86* C[1] − 0.83* C[2] + 3.98* AB + 13.30* AC[1] − 0.68* AC[2] − 7.61* BC[1] − 4.61* BC[2]

Anthocyanins = +1.42 + 0.14* A + 0.84* B − 0.36* C[1] + 0.019* C[2] + 0.024* AB + 0.088* AC[1] − 0.012* AC[2] − 0.19* BC[1] + 0.021* BC[2]

where A, B, and C are the time, calyces-to-water ratio, and preparation method, respectively.

Three-dimensional response surface plots

Based on the model equations, three-dimensional (3-D) response surface plots (Fig. 1) were created to investigate the interaction between different parameters and the optimal level of each. The 3-D plots demonstrated that the anthocyanins, hydrolyzable, PP, and condensed tannins in *Hibiscus* tea prepared by any method increased with time and calyces-to-water ratio. Except for hydrolyzable and PP tannins extracted at 25 °C, the calyces-to-water ratio affected all tannin and anthocyanin levels in the extract, regardless of extraction method. Contrarily, the extraction time, especially at 25 °C affected all tannins and anthocyanins.

Optimization and model validation

Supplementary 1 depicts the predicted and actual values of the optimized preparation conditions following their application and testing. Except for the method of preparation factor, which was

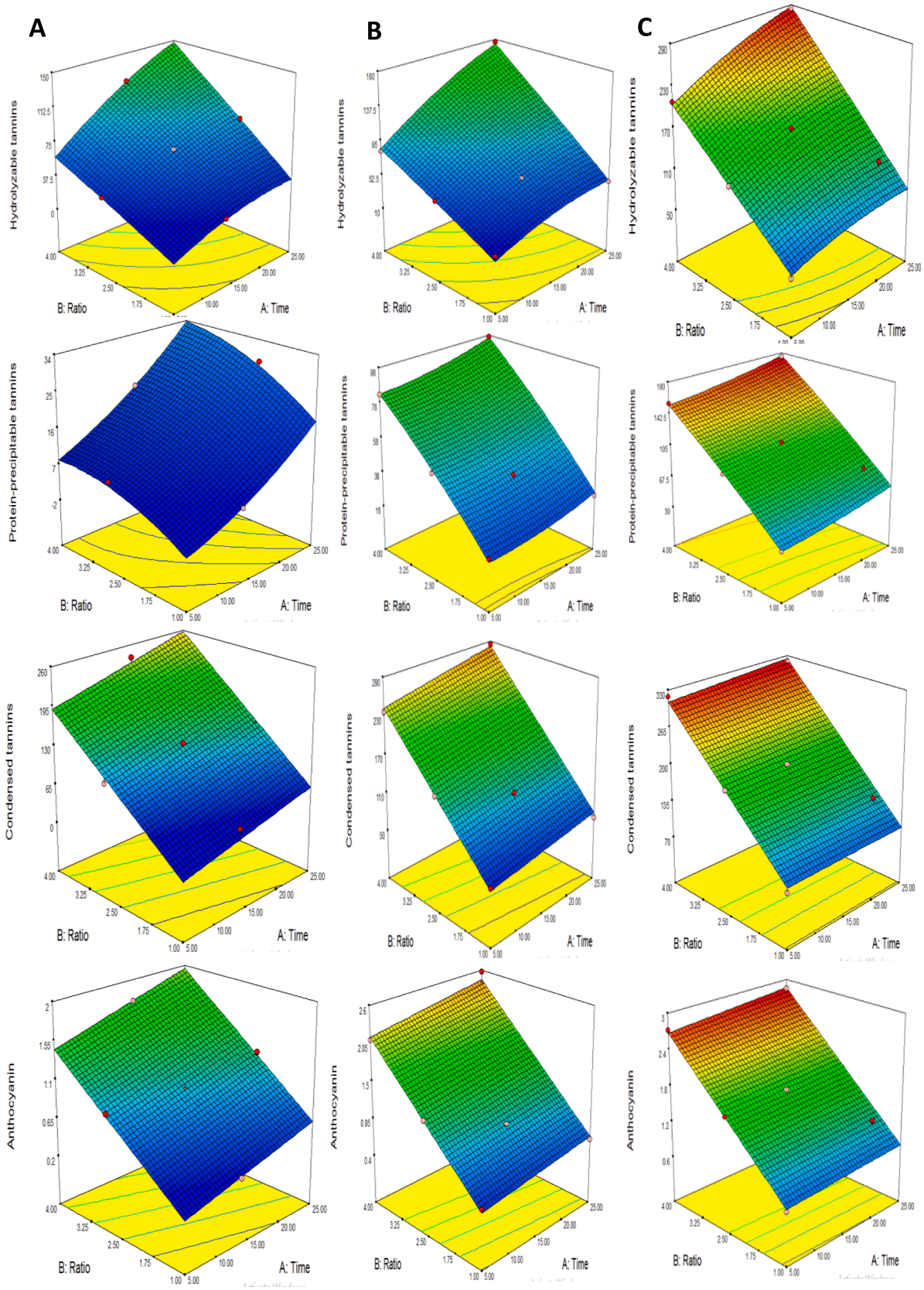

Fig. 1. Response 3-D plots of the interaction of three factors affecting the tannin (hydrolyzable, protein-perceptible, and condensed) and anthocyanin content of *Hibiscus* tea extracted by various methods. **A**: Cold at 25 °C, **B**: Hot at 90 °C, and **C**: Boiling at 100 °C.

maintained at its range, all variables were kept at their minimum level. Tannin responses were also maintained at a minimum, whereas anthocyanins were maintained at a maximum. After performing computer optimization, the suggested condition was boiling 2 g of calyx powder for 5 min to obtain a *Hibiscus* tea rich in anthocyanins and low in tannins. As can be seen, the observed amounts of all tannins and anthocyanins correspond to the predicted values suggested by the RSM, indicating model significance and predictability. These results also confirmed that the D-optimal design could be applied successfully to optimize the conditions for producing anthocyanin-rich and tannin-low *Hibiscus* tea.

Our findings are consistent with those of Sindi, Marshall, and Morgan (2014), who discovered that increasing the extraction time led to a significant increase in the total phenolic content and who suggested 10 min as the optimal extraction time for bioactive molecules from *H. sabdariffa*. They also suggested extracting these compounds at the boiling temperature and with water (among other solvents). Higher extraction temperatures also increased the anthocyanin content of this plant extract (Cissé et al., 2012). Cissé et al. (2012) emphasized the importance of the solid-to-solvent ratio in the extraction procedure. A calyces-to-water ratio of 1:30 yielded more anthocyanins than a ratio of 1:10, which is lower than that applied in the current study. In contrast, RSM optimization yielded the highest phenolic compound content (21.47 mg GAE/100 ml) in the *Hibiscus* extract following 291 min of extraction at 82 °C with 4.9 g calyces/100 ml water (Preciado-Saldaña et al., 2019).

Tannin degradation by Penicillium commune tannase

Given the economic significance of tannase at the industrial level, the objective of the present study was to hydrolyze the tannins of *Hibiscus sabdariffa* tea using *P. commune* tannase and to examine the enzyme's effect on the tea's physicochemical properties.

Effect on the content of gallic acid and catechin

Catechins are categorized into two types: a) free or non-esterified catechins that have an OH group at carbon 3 (i.e., GC, C, EGC, and EC), and b) esterified catechins in which the OH group is esterified with gallic acid (i.e., EGCG, GCG, ECG, and CG). Table 4 displays the changes in catechin concentrations in *Hibiscus* tea before and after tannase treatment. Supplementary 2 shows the peak chromatograms of the standards, untreated, and treated *Hibiscus* tea. Non-treated *Hibiscus* tea contains various non-esterified and esterified catechins, of which catechin gallate (CG) is the most abundant. Al-Yousef et al. (2020) also identified gallocatechin (GC) and methyl gallate in an aqueous extract of *Hibiscus deflersii* by utilizing UPLC-ESI-MS. In this study, *P. commune* tannase treatment increased degallated catechins (non-esterified) including GC, C, EGC, and EC by 19.78 %. Simultaneously, gallated catechins (esterified) decreased by 8.91 %, with EGCG exhibiting the greatest decrease at 11.05 %. Additionally, the gallic acid content increased 3.94-fold. There was no significant difference between total catechin levels before (227.74 mg/ml) and after (224.42 mg/ml) tannase treatment. These findings demonstrate that *P. commune* tannase successfully hydrolyzed and converted the esterified gallated catechins (EGCG, GCG, ECG, and CG) to their non-esterified isomers (EGC, GC, EC, and C, respectively). The bitterness and astringency of *Hibiscus* tea are primarily associated with EGCG and ECG catechins (Li et al., 2017), which explain its taste. The degallolation of these catechins may improve the taste of this potential tea, as the ester catechins are responsible for the tea infusion's bitter taste (Shao et al., 2020). By the action of *Aspergillus tubergensis* tannase, Li et al. (2017) decreased the esterified gallocatechins in Oolong tea leaves by 23.51 %, which is greater than that reached in our study, whereas de Lima et al. (2018) using *Aspergillus ficuum* tannase decreased EGCG 2.5-fold and increased EGC content 2-fold in Boldo tea. These differences may be attributable to the tannase structure, the source strain, and the biochemical conditions. Tannase can break down complex natural tannins such as polyphenolic esters of gallic acid and tannic acid, producing a simpler mixture of compounds with gallic acid as the predominant product, which explains the varying percentages of increase and decrease between compounds (Saeed et al., 2021).

Table 4
Quantity of individual catechin detected by HPLC in *Hibiscus* tea (mg/g) and gallic acid as affected by tannase treatments.

Component†	Non-treated *Hibiscus* tea	Tannase-treated *Hibiscus* tea
GA	*3.24^{b} ± 0.197	12.79^{a} ± 0.098
GC	6.28^{b} ± 0.019	8.58 ^{a} ± 0.014
C	12.80^{b} ± 0.028	14.87^{a} ± 0.055
EGC	18.88^{b} ± 0.042	23.08^{a} ± 0.052
EC	19.62^{b} ± 0.026	22.92^{a} ± 0.020
Total non-esterified	57.58^{b}	69.45^{a}
EGCG	27.97^{a} ± 0.025	24.88^{b} ± 0.047
GCG	37.15^{a} ± 0.015	33.40^{b} ± 0.047
ECG	43.87^{a} ± 0.064	39.10^{b} ± 0.046
GC	61.17^{a} ± 0.024	57.59^{b} ± 0.040
Total esterified	170.16^{a}	154.97^{b}
Total catechins	227.74 ± 0.079	224.42 ± 0.015

*All results were expressed as the mean ± SD. Different letters within columns indicate statistically significant differences between the two treatments (p-value < 0.05). †gallic acid (GA), (−)-gallocatechin (GC), (+)-catechin (C), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), (−)-epigallocatechin gallate (EGCG), (−)-gallocatechin gallate (GCG), (−)-epicatechin gallate (ECG), and (−)-catechin gallate (CG).

Effect on the physicochemical properties

The physicochemical properties of untreated and tannase-treated *Hibiscus* tea are summarized in Table 5. A comparison of the main quality variables, namely: pH, acidity, anthocyanin content, total

Table 5
A comparison between *Hibiscus* tea properties before and after tannase treatment.

Physicochemical properties	Unit	Before tannase	After tannase	Changes in treated *Hibiscus* tea
pH		*2.51^{b} ± 0.007	2.91^{a} ± 0.14	↑
Acidity	% w/v as hibiscus acid	0.707^{a} ± 0.09	0.534^{b} ± 0.12	↓
Reducing sugars	mg/100 ml	69.59^{b} ± 5.28	223.52^{a} ± 4.10	↑
Anthocyanin content	†mg C-3-G/ 100 ml	1.360^{a} ± 0.01	0.895^{b} ± 0.04	↓
Color density		3.280^{a} ± 0.01	2.480^{b} ± 0.02	↓
Total phenolic content	mg GAE/ 100 ml	8934.62^{b} ± 100.00	9703.85^{a} ± 145.04	↑
DPPH inhibition	%	55.42^{a} ± 9.46	40.27^{a} ± 1.78	
Tannins				
Hydrolyzable tannins	mg/100 ml	91.43^{a} ± 4.50	71.84^{b} ± 0.34	↓
Protein-perceptible tannins	mg/100 ml	58.23^{a} ± 1.17	48.65^{b} ± 0.17	↓
Condensed tannins	mg catechins/ 100 ml	107.18^{a} ± 2.35	41.93^{b} ± 1.41	↓
α-amylase inhibition activity	% of inhibition	68.48^{a} ± 1.22	49.32^{b} ± 4.89	↓

*All results were expressed as the mean ± SD. Different letters within columns indicate statistically significant differences between the two treatments (p-value < 0.05). †C-3-G = Cyanidin-3-glucoside.

phenolic compounds, tannin content, and antioxidant activity, revealed that tannase treatment significantly altered the tea's characteristics.

Depending on the cultivar and harvesting conditions, the fresh calyces of *Hibiscus* can contain up to 60 % organic acids. In *Hibiscus* aqueous extract, hibiscus acid (13 %–24 %) is the most abundant acid, followed by hydroxycitric acid, citric acid, and malic acid (Portillo-Torres et al., 2019). Here, the untreated *Hibiscus* tea had a higher titratable acidity, as hibiscus acid, and a lower pH value than the tannase-treated tea. The reason for this is unknown, but catechins and the formed gallic acid may undergo complex reactions with coexisting substances such as anthocyanins, producing more basic substances. Similarly, the dimerization and polymerization of catechins and anthocyanins have been demonstrated in a wine-like model system due to its high anthocyanin content (Es-Safi, Fulcrand, Cheynier, & Moutounet, 1999). After tannase treatment, Jana et al. (2015) observed a drop in the titratable acidity of Jamun and apple juice. However, the pH of tannase-treated black and green tea was lower than that of untreated samples (Aharwar & Parihar, 2021).

The content of anthocyanins in non-treated *Hibiscus* tea was 1.36 mg of cyanidin-3-glucoside/100 ml, which was significantly reduced to 0.895 mg C-3-G/100 ml after tannase treatment. Anthocyanins are highly unstable molecules, in the food matrix. Solvents, pH, light, temperature, and enzymes have a substantial impact on the color stability of these compounds (Shruthi & Ramachandra, 2019). Moreover, pH is one of the most important factors in anthocyanin stability. In general, they are more stable in acidic solutions with a low pH than they are in basic solutions. This could explain the decrease in anthocyanin content following tannase treatment. Under acidic pH ($pH < 2$), anthocyanins exist predominantly as a dark red. Due to the fact that the pH of tannase-treated tea is increased to ≈ 3 and its acidity is significantly decreased, a few anthocyanin degradations may occur. Cruz et al. (2019) showed with a photograph how C-3-G degrades when the pH is shifted from 2.5 to 3.14 in the presence or absence of gallic acid. Another explanation is that thermal treatment accelerates the oxidation rate of EGCG, which originally had a red color, and thus enhances the browning of the EGCG-flavonol mixture (Dai et al., 2017). In fact, anthocyanin content correlated with color density, which also decreased after tannase treatment.

Tannase from *Penicillium commune* increased the total phenolic compound content (TPC) of *Hibiscus* tea by 8.6 %, as determined by the Folin-Ciocalteu assay. The physiological and pharmacological benefits of polyphenols include anti-carcinogenic and anti-mutagenic properties (Laskar & Mazumder, 2020). This suggests that enzymatic treatment of *Hibiscus* calyces tea is a promising approach for enhancing this beverage's health benefits. According to Shao et al. (2020), tannase also increased the levels of TPC in green tea. The radical scavenging activity of the *Hibiscus* extract, as measured by the DPPH assay, was not significantly decreased ($p \geq 0.05$) in this study after tannase treatment, hence TPC increased significantly. This may be due to a reduction in anthocyanin content. Anthocyanins, a chief source of antioxidant capacity in *Hibiscus* extracts, are positively correlated with the DPPH assay ($r = 0.978$, $p < 0.01$) (Monteiro et al., 2017), and monomeric anthocyanins such as cyanidin-3-glucoside correlate with DPPH ($r = 0.718$). (Sindi et al., 2014).

The calyces of *Hibiscus* naturally contain between 3 % and 5 % sugar by dry weight. Fructose, glucose, and sucrose were identified as the free sugars in *Hibiscus* extracts, with glucose being the most abundant, followed by fructose (Laskar & Mazumder, 2020). Following the tannase experiment, the amount of reducing sugars as glucose significantly increased by 3.2 times (Table 5). This is to be expected since tannase cleaves the di-ester bond in tannins, releasing gallic acid and glucose (Saeed et al., 2021). This indicates that tannase treatment may enhance the sweetness and acceptability of such a traditional beverage. In agreement, sugars such as glucose increased significantly after tannase treatment, while fructose and xylose remained constant in Jamun and cashew apple juice (Jana et al., 2015).

The most important impact of *P. commune* tannase was the reduction of hydrolyzable and condensed tannin contents by 21.42 % and 60.87 %, respectively, resulting in lower BSA-binding ability. This is a result of catechin elimination and gallic acid liberation. Condensed tannins play an important role in the product's sensory characteristics, particularly bitterness and astringency (Ju et al., 2021). The BSA assay is the most common method with a good linear correlation coefficient ($r = 0.47$ to 0.9) with sensor astringency because the interaction between tannins and the precipitant agent is similar to the astringency mechanism (Wilhelmy et al., 2021). Therefore, reducing these tannins may enhance the final product's taste and mouth feel. de Lima et al. (2018) used 170 U/ml of *Aspergillus ficuum* tannase to remove 22 % of total tannins from Boldo tea after 2 h at 40 °C, whereas the immobilized enzyme removed 31 % under the same conditions. Aharwar and Parihar (2021) achieved the greatest reduction in tannin content by 120 U tannase/ml in black tea (78 %) and green tea (59 %) infusions at 60 °C after 20 min.

Effect on α-amylase inhibition activity

α-Amylase and α-glucosidase catalyze the intestinal breakdown of ingested starch into glucose and other mono- and disaccharides. In vitro screening or non-animal model-based strategies targeting these enzymes is an inexpensive and highly efficient method for identifying or comparing functional ingredients with optimal anti-hyperglycemic benefits among numerous samples. The α-amylase inhibitory activity is a fast assay for determining whether novel plant extracts have potential anti-hyperglycemic properties (Banwo et al., 2022). *Hibiscus* has been demonstrated as an inhibitor of α-amylase and α-glucosidase, decreasing carbohydrate metabolism and blood insulin levels (Portillo-Torres et al., 2019). Different *Hibiscus* extracts inhibited α-amylase by a range of 63.58 % to 78.85 %, and the aqueous extract made of 3 g/100 ml inhibited its activity by 37 % (Al-Yousef et al., 2020). In this study, non-enzymatically treated *Hibiscus* tea inhibited 68.48 % of that enzyme, while tannase-treated tea inhibited 49.32 %. Yang et al. (2020) found a link between the extract's α-amylase inhibitory potency and the concentration, composition, and structure of its phenolic compounds, which could explain this reduction. The amount and position of a hydroxyl group in a phenolic compound's structure also influences its inhibitory activity. Tannic acid has more hydroxyl groups (25 OH) than gallic acid (3 OH), thus interacts with amylase's active site to achieve a stable inhibition state. Furthermore, acarbose and tannic acid had IC_{50} values of 10.40 and 3.46 g/ml, respectively, indicating that tannic acid is more effective at inhibiting α-amylase activity. In contrast, when Zaharudin, Salmeán, and Dragsted (2018) examined the effects of epigallocatechin (EGC) with a Mw of 458.37, epicatechin (EC) with a Mw of 290.26, and gallic acid with a Mw of 170.12, on porcine pancreatic α-amylase activity, they determined that the compound with the lowest molecular weight, gallic acid, is the most potent.

Sensory evaluation

Supplementary 3 displays the mean values of the sensory characteristics of tannase-treated and untreated *Hibiscus* tea samples. Consumers found both *Hibiscus* tea samples to be acceptable; however, there were significant differences between them in some sensorial attributes. Taste, bitterness, and overall acceptability improved significantly ($p < 0.05$) after tannase treatment. This could be explained by lowering the tannin content, which is the main cause of bitterness. In contrast, no significant difference ($p \geq 0.05$) in the color or acidity exists between the two samples. That means it is technically possible to improve the sensory characteristics of *Hibiscus* beverage by the action of tannase..

Conclusion

In this study, a low-tannin *Hibiscus* tea was developed to meet the demand for nutritional beverages. The effects of the preparation conditions (time, plant: water ratio, and extraction temperature) on the

tannin and anthocyanin contents of this tea were investigated using a D-optimal experimental design. The proposed models accurately represent the relationship between responses and the variables employed in this study. The best way to obtain *Hibiscus* tea rich in anthocyanins and low in tannins is to boil 2 g of calyces in 100 ml of water for 5 min. In addition, a novel *Penicillium commune* tannase was able to successfully biotransform *Hibiscus* tea in order to reduce its tannin content and astringency effect. Consequently, the final product could be considered a new member of the functional beverage family, hence the anti-diabetic effect should be considered.

CRediT authorship contribution statement

Heba Sayed Mostafa: Conceptualization, Formal analysis, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Data availability

Data can be obtained from the corresponding author.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100562.

References

Aharwar, A., & Parihar, D. K. (2021). *Talaromyces verruculosus* tannase immobilization, characterization, and application in tea infusion treatment. *Biomass Conversion and Biorefinary, 2021*. https://doi.org/10.1007/s13399-020-01162-6

Al-Yousef, H. M., Hassan, W. H. B., Abdelaziz, S., Amina, M., Adel, R., & El-Sayed, M. A. (2020). UPLC-ESI-MS/MS profile and antioxidant, cytotoxic, antidiabetic, and antiobesity activities of the aqueous extracts of three different *Hibiscus* Species. *Journal of Chemistry, 2020*, 1–17. https://doi.org/10.1155/2020/6749176

Amaya-Cruz, D., Peréz-Ramírez, I. F., Pérez-Jiménez, J., Nava, G. M., & Reynoso-Camacho, R. (2019). Comparison of the bioactive potential of Roselle (*Hibiscus sabdariffa* L.) calyx and its by-product: Phenolic characterization by UPLC-QTOF MS and their anti-obesity effect in vivo. *Food Research International, 126*, Article 108589. https://doi.org/10.1016/j.foodres.2019.108589

Andrade, P. M., Baptista, L., Bezerra, C. O., Peralta, R. M., Góes-Neto, A., Uetanabaro, A. P., … da Costa, A. M. (2021). Immobilization and characterization of tannase from *Penicillium rolfsii* CCMB 714 and its efficiency in apple juice clarification. *Journal of Food Measurement and Characterization, 15*, 1005–1013. https://doi.org/10.1007/s11694-020-00705-9

Banwo, K., Sanni, A., Sarkar, D., Ale, O., & Shetty, K. (2022). Phenolics-linked antioxidant and anti-hyperglycemic properties of edible Roselle (*Hibiscus sabdariffa* Linn.) calyces targeting type 2 diabetes nutraceutical benefits in vitro. *Frontiers in Sustainable Food Systems, 6*, Article 660831. https://doi.org/10.3389/fsufs.2022.660831

Catelani, T. A., Bittar, D. B., Pezza, L., & Pezza, H. R. (2016). A rapid and eco-friendly method for determination of hydrolysable tannins and its application to honey samples. *Food Analytical Methods, 9*, 2552–2559. https://doi.org/10.1007/s12161-016-0454-1

Choi, B., & Koh, E. (2017). Spent coffee as a rich source of antioxidative compounds. *Food Science and Biotechnology, 26*, 921–927. https://doi.org/10.1007/s10068-017-0144-9

Cissé, M., Bohuon, P., Sambe, F., Kane, C., Sakho, M., & Dornier, M. (2012). Aqueous extraction of anthocyanins from *Hibiscus sabdariffa*: Experimental kinetics and modeling. *Journal of Food Engineering, 109*, 16–21. https://doi.org/10.1016/j.jfoodeng.2011.10.012

Cruz, L., Basilio, N., Mendoza, J., Mateus, N., de Freitas, V., Tawara, M. H., Correa, J., & Fernandez-Megia, E. (2019). Impact of a water-soluble gallic acid-based dendrimer on the color-stabilizing mechanisms of anthocyanins. *Chemistry A European Journal, 25*, 11696–11706. https://doi.org/10.1002/chem.201901912

Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I., & Heinrich, M. (2014). *Hibiscus sabdariffa* L. – A phytochemical and pharmacological review. *Food Chemistry, 165*, 424–443. https://doi.org/10.1016/j.foodchem.2014.05.002

Dai, Q., He, Y., Ho, C., Wang, J., Wang, S., Yang, Y., Gao, L., & Xia, T. (2017). Effect of interaction of epigallocatechin gallate and flavonols on color alteration of simulative green tea infusion after thermal treatment. *Journal of Food Science and Technology, 54*, 2919–2928. https://doi.org/10.1007/s13197-017-2730-5

de Lima, J. S., Cabrera, M. P., Casazza, A. A., da Silva, M. F., Perego, P., de Carvalho, L. B., … Converti, A. (2018). Immobilization of *Aspergillus ficuum* tannase in calcium alginate beads and its application in the treatment of Boldo (*Peumus boldus*) tea. *International Journal of Biological Macromolecules, 118*, 1989–1994. https://doi.org/10.1016/j.ijbiomac.2018.07.084

Es-Safi, N., Fulcrand, H., Cheynier, V., & Moutounet, M. (1999). Studies on the acetaldehyde-induced condensation of (-)-epicatechin and malvidin 3-*o*-glucoside in a model solution system. *Journal of Agriculture and Food Chemistry, 47*, 2096–2102. https://doi.org/10.1021/jf9806309

Gauderman, W. J. (1988). Review of Costat 2.03. *Computer Statistics and Data Analysis, 6*, 189–192. https://doi.org/10.1016/0167-9473(88)90050-3

Gong, X., Li, Y., & Qu, H. (2014). Removing tannins from medicinal plant extracts using an alkaline ethanol precipitation process: A case study of danshen injection. *Molecules, 19*, 18705–18720. https://doi.org/10.3390/molecules191118705

Handali, S., Moghimipour, E., Rezaei, M., Saremy, S., & Dorkoosh, F. A. (2019). Co-delivery of 5-fluorouracil and oxaliplatin in novel poly(3-hydroxybutyrate-co-3-hydroxyvalerate acid)/poly(lactic-co-glycolic acid) nanoparticles for colon cancer therapy. *International Journal of Biological Macromolecules, 124*, 1299–1311. https://doi.org/10.1016/j.ijbiomac.2018.09.119

Ifie, I., Ifie, B. E., Ibitoye, D. O., Marshall, L. J., & Williamson, G. (2018). Seasonal variation in *Hibiscus sabdariffa* (Roselle) calyx phytochemical profile, soluble solids and α-glucosidase inhibition. *Food Chemistry, 261*, 164–168. https://doi.org/10.1016/j.foodchem.2018.04.052

Jana, A., Halder, S. K., Ghosh, K., Paul, T., Vágvölgyi, C., Mondal, K. C., & Das Mohapatra, P. K. (2015). Tannase immobilization by chitin-alginate based adsorption-entrapment technique and its exploitation in fruit juice clarification. *Food and Bioprocess Technology, 8*, 2319–2329. https://doi.org/10.1007/s11947-015-1586-9

Ju, Y., Yang, L., Yue, X., He, R., Deng, S., Yang, X., … Fang, Y. (2021). The condensed tannin chemistry and astringency properties of fifteen *Vitis davidii* Foex grapes and wines. *Food Chemistry: X, 11*, Article 100125. https://doi.org/10.1016/j.fochx.2021.100125

Laskar, Y. B., & Mazumder, P. B. (2020). Insight into the molecular evidence supporting the remarkable chemotherapeutic potential of *Hibiscus sabdariffa* L. *Biomedicine & Pharmacotherapy, 127*, Article 110153. https://doi.org/10.1016/j.biopha.2020.110153

Li, J., Xiao, Q., Huang, Y., Ni, H., Wu, C., & Xiao, A. (2017). Tannase application in secondary enzymatic processing of inferior Tieguanyin oolong tea. *Electronic Journal of Biotechnology, 28*, 87–94. https://doi.org/10.1016/j.ejbt.2017.05.010

Maciel, L. G., do Carmo, M. A., Azevedo, L., Daguer, H., Molognoni, L., de Almeida, M. M., … Rosso, N. D. (2018). *Hibiscus sabdariffa* anthocyanins-rich extract: Chemical stability, in vitro antioxidant and antiproliferative activities. *Food and Chemical Toxicology, 113*, 187–197. https://doi.org/10.1016/j.fct.2018.01.053

Mak, Y. W., Chuah, L. O., Ahmad, R., & Bhat, R. (2013). Antioxidant and antibacterial activities of hibiscus (*Hibiscus rosa-sinensis* L.) and Cassia (*Senna bicapsularis* L.) flower extracts. *Journal of King Saud University – Science, 25*, 275–282. https://doi.org/10.1016/j.jksus.2012.12.003

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry, 31*, 426–428. https://doi.org/10.1021/ac60147a030

Miranda, R. F., Silva, J. P., Machado, A. R. F., Silva, E. C., Souza, R. C., Marcolino, V. A., … Barao, C. E. (2019). Impact of the addition of *Lactobacillus casei* and oligofructose on the quality parameters of orange juice and hibiscus tea mixed beverage. *Journal of Food Processing and Preservation, 43*, e14249.

Monteiro, M. J., Costa, A. A., Fliedel, G., Cissé, M., Bechoff, A., Pallet, D., … Pintado, M. M. (2017). Chemical-sensory properties and consumer preference of hibiscus beverages produced by improved industrial processes. *Food Chemistry, 225*, 202–212. https://doi.org/10.1016/j.foodchem.2016.11.127

Mostafa, H. S. (2022). Potato peels for tannase production from *Penicillium commune* HS2, a high tannin-tolerant strain, and its optimization using response surface methodology. *Biomass Conversion and Biorefinary, 2022*. https://doi.org/10.1007/s13399-021-02205-2

Portillo-Torres, L. A., Bernardino-Nicanor, A., Gómez-Aldapa, C. A., González-Montiel, S., Rangel-Vargas, E., Villagómez-Ibarra, J. R., … Castro-Rosas, J. (2019). Hibiscus acid and chromatographic fractions from *Hibiscus sabdariffa* calyces: Antimicrobial activity against multidrug-resistant pathogenic bacteria. *Antibiotics, 8*, 218. https://doi.org/10.3390/antibiotics8040218

Preciado-Saldaña, A. M., Domínguez-Avila, J., Ayala-Zavala, J., Villegas-Ochoa, M. A., Sáyago-Ayerdi, S. G., Wall-Medrano, A., … Gonzalez-Aguilr, G. A. (2019). Formulation and characterization of an optimized functional beverage from hibiscus (*Hibiscus sabdariffa* L.) and green tea (*Camellia sinensis* L.). *Food Science and Technology International, 25*, 547–561. https://doi.org/10.1177/1082013219840463

Saeed, S., Aslam, S., Mehmood, T., Naseer, R., Nawaz, S., Mujahid, H., … Sultan, A. (2021). Production of gallic acid under solid-state fermentation by utilizing waste from food processing industries. *Waste and Biomass Valorization, 12*, 155–163. https://doi.org/10.1007/s12649-020-00980-z

Saito, S. T., Welzel, A., Suyenaga, E. S., & Bueno, F. (2006). A method for fast determination of epigallocatechin gallate (EGCG), epicatechin (EC), catechin (C) and caffeine (CAF) in green tea using HPLC. *Food Science and Technology, 26*, 394–400. https://doi.org/10.1590/S0101-20612006000200023

Shao, Y., Zhang, Y. H., Zhang, F., Yang, Q.-M., Weng, H.-F., Xiao, Q., & Xiao, A. F. (2020). Thermostable tannase from *Aspergillus niger* and its application in the enzymatic extraction of green tea. *Molecules, 25*, 952. https://doi.org/10.3390/molecules25040952

Sharma, S., Bhat, T. K., & Dawra, R. K. (2000). A spectrophotometric method for assay of tannase using rhodanine. In *Analytical Biochemistry* (pp. 85–89). Academic Press Inc.

Shruthi, V. H., & Ramachandra, C. T. (2019). Roselle (*Hibiscus sabdariffa* L.) calyces: A potential source of natural color and its health benefits. In *Food Bioactives* (pp. 169–190). Apple Academic Press.

Siegień, J., Buchholz, T., Popowski, D., Granica, S., Osińska, E., Melzig, M. F., & Czerwińska, M. E. (2021). Pancreatic lipase and α-amylase inhibitory activity of extracts from selected plant materials after gastrointestinal digestion in vitro. *Food Chemistry, 355*, Article 129414. https://doi.org/10.1016/j.foodchem.2021.129414

Sindi, H. A., Marshall, L. J., & Morgan, M. R. A. (2014). Comparative chemical and biochemical analysis of extracts of *Hibiscus sabdariffa*. *Food Chemistry, 164*, 23–29. https://doi.org/10.1016/j.foodchem.2014.04.097

Singh, M., Thrimawithana, T., Shukla, R., & Adhikari, B. (2021). Extraction and characterization of polyphenolic compounds and potassium hydroxycitrate from *Hibiscus sabdariffa*. *Future Foods, 4*, Article 100087. https://doi.org/10.1016/j.fufo.2021.100087

Wilhelmy, C., Pavez, C., Bordeu, E., & Brossard, N. (2021). A review of tannin determination methods using spectrophotometric detection in red wines and their ability to predict astringency. *South African Journal of Enology and Viticulture, 42*, 2021. https://doi.org/10.21548/42-1-3852

Yang, Z., Qin, C., Weng, P., Zhang, X., Xia, Q., Wu, Z., … Xiao, J. (2020). In vitro evaluation of digestive enzyme inhibition and antioxidant effects of naked oat phenolic acid compound (OPC). *International Journal of Food Science and Technology, 55*, 2531–2540. https://doi.org/10.1111/ijfs.14504

Zaharudin, N., Salmeán, A. A., & Dragsted, L. O. (2018). Inhibitory effects of edible seaweeds, polyphenolics and alginates on the activities of porcine pancreatic α-amylase. *Food Chemistry, 245*, 1196–1203. https://doi.org/10.1016/j.foodchem.2017.11.027

Zhang, Y., Li, Y., Ren, X., Zhang, X., Wu, Z., & Liu, L. (2023). The positive correlation of antioxidant activity and prebiotic effect about oat phenolic compounds. *Food Chemistry, 402*, 134231. https://doi.org/10.1016/j.foodchem.2022.134231