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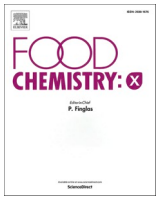

Valorization of faba bean peels for fungal tannase production and its application in coffee tannin removal

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ABSTRACT

This study describes the optimization of the production conditions of *Penicillium commune* tannase on unutilized food waste, green bean peels, using the central composite of the response surface methodology. It also focuses on applying purified tannase to reduce tannins in coffee. The proposed design recommended a temperature of 29.07 °C, pH of 6.74, a tannin level of 6.76%, and 3.31% bean peels for maximum tannase production (313.40 U/g/min) by solid-state fermentation. This waste can be used as a sustainable and low-cost substrate for tannase enhancement by ≈5 folds. Applying purified tannase in instant coffee beverage resulted in a ≈ 23% reduction in tannins and a ≈ 16% increase in reducing sugars, with no significant changes in caffeine and phenolic compound contents. Tannase had a detrimental effect on the volume and stability of the coffee foam. This study will pave the way for tannase industrial production and its promising use in low-bitter coffee production.

1. Introduction

Tannase (EC 3.1.1.20), also known as tannin acyl hydrolase, is an inducible glycoprotein esterase that degrades tannins to gallic acid and glucose (Prigione et al., 2018; Saeed et al., 2021). In foods, it is mostly used to remove tannins from tea, wine, beer, and Hibiscus tea (de Lima et al., 2018; Mostafa, 2023b), as well as to produce gallic acid for pharmaceutical applications (Pan et al., 2020). It is also used to clarify beers and fruit juices, reducing haze and bitterness (de Lima et al., 2014; Kumar et al., 2023). It is mostly produced by many fungal strains belonging to Aspergillus and Penicillium genera, such as *Aspergillus fumigatus* MA (Selwal & Selwal, 2023), *Aspergillus sydowii* (Ibrahim et al., 2023), and *Penicillium commune* (Mostafa, 2023a), as well as yeasts such as *Debaryomyces hansenii* (Song et al., 2023) and *Geotrichum cucujoidarum* (Thangavelu et al., 2024).

To diminish production costs, researchers tested different food-based wastes for tannase production, such as desiccated coconut residue, rice bran, brewer's rice, and spent coffee ground (Mansor et al., 2019), pomegranate peels (Ahmed et al., 2023), pomegranate rind, tea dust, and black gram husk (Varadharajan et al., 2017), potato peels (Mostafa, 2023a), as well as lemon peels, leaves of Indian gooseberry, and jamun (Thangavelu et al., 2024). They investigated these wastes in solid-state or submerged fermentation to save money and protect the environment from the health risks associated with the withdrawal of these wastes. Until now, green bean peels have not been examined for that purpose.

Green beans (*Vicia faba* L.) are said to have originated in the eastern Mediterranean region as a winter plant, with production reaching 1,642,153.15 tons in 2022, according to recent Food and Agriculture Organization statistics (FAOSTAT, 2022). According to the same statistics, Algeria, China, and Egypt are among the nations that primarily produce this plant for seeds. They are high in protein (>30% dry basis) and widely eaten in traditional cuisines (De Cillis et al., 2019). With such vast quantities harvested each season, there has been a significant difficulty in handling peel waste. Such waste accounts for 1,231,641 tons each year, accounting for 70–75% (*w*/w) of the fresh matter in the entire pod yield (Krenz et al., 2023). According to the literature, these empty pods contain condensed tannins that vary from 2.81 to 4.56 mg/g extract (Mejri et al., 2018). It is usually utilized for animal feeding due to its fibers and crude protein-rich content (Mateos-Aparicio et al., 2010); hence, it has not yet been valorized for enzyme production.

The removal of tannins (high-molecular weight polyphenols) is receiving a lot of interest these days since they are influencing the utilization of some important compounds in our foods. One of the antinutritive effects of these compounds is that they influence protein utilization by creating complexes with it when taken in a human diet (Wilhelmy et al., 2021), which cannot be broken down by digestive enzymes. Furthermore, the ortho-dihydroxyl groups in these compounds play a crucial role in the chelation of metal ions, particularly iron. Despite their high antioxidant capacity, these compounds are notable for

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their astringency (Catelani et al., 2016). These certain properties necessitate the elimination of these compounds to create more palatable foods. Tannase, as the eco-friendly, cost-effective, and most corporate tannin removal aid, was applied to decrease tannins in many types of tea, such as boldo tea (de Lima et al., 2018), roselle tea (Mostafa, 2023b), and recently in black and green tea (Aharwar & Parihar, 2023). No research studies have looked into removing tannins from coffee.

This study intends to maximize the value of green bean peels in tannase production by optimizing growth conditions using response surface methodology (RSM). The impact of the purified tannase on the chemical and physical properties of instant coffee was also investigated.

2. Materials and methods

2.1. Raw materials and reagents

Fresh and mature faba bean green pods (*Vicia faba* L.) were purchased from a local market. After washing and collecting the seeds, the empty pods were dried at 50 °C for 6 h until the moisture content was <5%. After milling, they were stored at 4 °C until used. DNSA (3, 5-dinitrosalysilic acid) reagent, gallic acid, vanillin, KIO_3, rhodanine, and tannic acid were purchased from Sigma-Aldrich, Steinem, Germany. Dichloromethane and methanol (HPLC grade), as well as Folin-Ciocalteu phenol reagent, were acquired from Merck, Germany.

2.2. Optimization of tannase production by RSM

Temperature, initial pH, tannin, and faba bean peel (% *w/v*) concentrations were statistically optimized for their interaction effect on tannase synthesis using the central composite design (CCD) of RSM (Mostafa, 2023a). Table 1 shows the evaluation of each independent factor at four levels: -α, −1, 1, and + α. Based on 30 experimental runs, tannase activity (U/g dw) was monitored as a response.

2.3. Solid state fermentation

Tannase from the fungal strain *Penicillium commune* HS2 (Gene Bank identification number: MT084558) was produced in the following manner: Green bean peels were pre-autoclaved at 121 °C for 15 min and cooled before being used as a solid substrate for tannase production. The green peel quantity, pH value, temperature, and tannin level were set according to the Central Composite Design runs (Table 2). In 250-mL Erlenmeyer flasks, a specific amount of green bean peels was moistened with 5 mL of a solution (filter sterilized by a 0.45 μm filter membrane) containing 0.5% NaCl and various concentrations of pure tannin at defined pH values. Each flask was inoculated with one ml of the spore suspension (35×10^7 CFU/mL), properly mixed, and incubated for four days at the specified temperature (Bhoite & Murthy, 2015). The spore suspension was prepared by adding a volume of a solution containing 0.85% NaCl and 0.01% Tween 80 *v/v* to a fully sporulated slant (7 days old).

2.4. Tannase purification and activity determination

Tannase was extracted from the culture flasks by adding 20 ml of distilled water to each, which was then pooled and filtered using Whatman No. 1 filter paper. The crude enzyme was purified as described in detail by Mostafa (2023a). Its activity was evaluated by estimating the amount of gallic acid generated using the rhodanine reagent (Sharma et al., 2000). In brief, 500 μL of tannic acid in sodium acetate buffer (10 $mmolL^{-1}$, pH 5) was mixed with a defined volume of enzyme extract. The same buffer was used to bring the total volume to one milliliter. After 20 min at 30 °C, 1.5 mL of methanolic rhodanine (0.667%) was added, followed by 5 min of adding 0.5 mL of KOH solution (0.5 $molL^{-1}$). The absorbance of the final solution was recorded at 520 nm using a T60-UV visible spectrophotometer (PG, Leicestershire, LE17 5BH, UK). The enzyme unit (U) was defined as the quantity of tannase required to liberate 1 $nmolL^{-1}$ gallic acid/min/g substrate dw after plotting the standard curve of fresh gallic acid solution (5–50 $nmolL^{-1}$).

Table 1
Experimental factors and their range for statistical screening using Central Composite Design (CCD).

Factor code	Independent factor	Unit	Range			
			-α	-1	+1	+α
A	Temperature	°C	10	20	40	50
B	Initial pH	–	1	4	10	13
C	Tannin	% (w/v)	−2.5	0	12	18
D	Green bean peel	% (w/v)	−6	0	5	7.5

Table 2
Central Composite Design (CCD) runs and the experimental tannase activity (U/g/min) values. Mean ± SD.

Run	Factors				Tannase activity
	Temperature	Initial pH	Tannin	Green bean peel	Experimental value
1	20	10	0	5.0	2.77 ± 0.24
2	20	4	12	5.0	146.25 ± 0.25
3	30	7	6	2.5	308.88 ± 1.23
4	30	13	6	2.5	0.00 ± 0.00
5	30	7	18	2.5	112.03 ± 3.70
6	40	10	0	5.0	8.71 ± 1.73
7	30	7	6	−2.5	11.77 ± 0.37
8	40	10	0	0.0	3.81 ± 0.24
9	30	7	−6	2.5	35.63 ± 0.98
10	40	4	0	5.0	19.67 ± 0.37
11	30	7	6	2.5	308.88 ± 1.23
12	40	10	12	0.0	3.64 ± 1.23
13	10	7	6	2.5	35.31 ± 3.58
14	50	7	6	2.5	9.76 ± 0.00
15	20	10	12	5.0	103.33 ± 1.23
16	30	1	6	2.5	15.02 ± 1.35
17	40	4	12	5.0	70.89 ± 1.23
18	30	7	6	7.5	109.93 ± 4.94
19	30	7	6	2.5	308.88 ± 1.23
20	20	4	12	0.0	10.19 ± 1.11
21	20	4	0	5.0	20.42 ± 0.98
22	40	10	12	5.0	75.31 ± 1.23
23	20	4	0	0.0	9.14 ± 0.12
24	40	4	0	0.0	6.26 ± 1.47
25	20	10	12	0.0	10.28 ± 3.21
26	40	4	12	0.0	23.64 ± 0.49
27	30	7	6	2.5	308.88 ± 1.23
28	30	7	6	2.5	308.88 ± 1.23
29	20	10	0	0.0	1.01 ± 0.85
30	30	7	6	2.5	308.88 ± 1.23

2.5. Tannin removal from instant coffee

Twenty grams of instant coffee powder (Misr Café Company, 10th of Ramadan City, Egypt) were re-dissolved in 500 mL of distilled water at 50 °C. The purified tannase (180 U/mL) was added to the coffee, which was then diluted to 1500 mL with distilled water and divided into two equal parts in closed bottles. The first, as a control, was inactivated by heating at 90 °C for 5 min then cooled. The second was incubated for tannase treatment for three hours at 45 °C until no more gallic acid was detected. Later, as previously stated, tannase was inactivated, and the coffee was cooled to room temperature before being stored at −20 °C until analysis (de Lima et al., 2018).

2.6. Effect of tannase on instant coffee characteristics

The physical and chemical parameters of tannase-treated instant coffee beverage were compared to those of untreated coffee, as shown below. Acidity was determined in the diluted sample (1:1 *v/v*) by

titration with NaOH (0.05 N) until pH 8, while pH was monitored by a digital pH meter (Adwa AD1030, Romania) (Wang & Lim, 2023). Konica Minolta (CR-410, Tokyo, Japan) recorded the color attributes (L*, a*, and b*) of the coffee before and after tannase.

The reducing sugars were examined using the DNSA method, followed by an absorbance measurement at 540 nm with the previous spectrophotometer (Miller, 1959). To determine the total phenolic content (TPC), 200 µL of coffee sample was mixed with 2.5 mL of a 10% Folin-Ciocalteu reagent. After 5 min, two millimeters of sodium carbonate (7.5%) were added, following the protocol of Mostafa (2023b). The blank was prepared like the sample, but with distilled water. TPC was calculated as mg gallic acid equivalents/100 mL coffee. To quantify hydrolyzable tannins, a diluted coffee sample (1:4 distilled water) was mixed with five ml of KIO_3 (2.5%) at 30 °C for 2 min (Choi & Koh, 2017), while the condensed tannins were determined by the vanillin-HCl method (Mak et al., 2013). Briefly, the coffee beverage sample was diluted with the same volume of distilled water, and 1 mL of the diluted coffee sample was combined with 2.5 mL vanillin (4% in methanol) and 2.5 mL HCl (8% in methanol). The absorbance at A500 was measured after half an hour of dark incubation, and the blank was a diluted coffee sample combined with 5 mL of distilled water. Gallic acid was measured using methanolic rhodanine, as described in detail in the tannase activity determination, and expressed as ng/mL coffee. The caffeine concentration was measured by dichloromethane extraction (Belay et al., 2008). In brief, the caffeine content of five-millimeter coffee sample was extracted with 15 mL of dichloromethane using a glass-separating funnel and repeated three times. The final volume was completed to 50 mL by the same solvent, and the absorbance was recorded at 270 nm in comparison to pure dichloromethane (blank).

The foaming characteristics of instant coffee before and after the tannase treatment were investigated by measuring the maximum foam volume (foamability) and foam stability. The foam was created by mixing the coffee beverage with a coffee frother mixer (GE810HA0SPKLZNAFAMZ, China) for one minute. The foam volume was measured after shaking 50 mL of coffee in a graded cylinder of 100 mL and expressed in milliliters. According to Shankaran and Chinnaswamy (2019), foam drainage was determined by the rate of decrease in foam height, which corresponded to the line's slope in the graph between foam height and time over two hours at 10 °C. The foam stability was calculated using Eq. 1:

$$\text{Foam stability} = \frac{F(z) - F(2h)}{F(z)}\, x\, 100 \quad (1)$$

Where F (z) is the foam volume at zero time (z) and F (2 h) is the remaining volume after 2 h at 10 °C.

2.7. Statistical analysis

Statistical analysis of the experimental design to optimize tannase production on faba bean pod peels was performed using the software Design-Expert V7 (Stat-Ease, Inc., Minneapolis, MN, 55418, USA). On the other side, CoStat software (Berkeley, CA, USA) was used to compare tannase-untreated and treated coffee samples using t-Student test (Gauderman, 1988). The means of three replicates were statistically compared at a significant level of $p \leq 0.05$.

3. Results and discussion

3.1. RSM-based optimization of tannase production

Temperature, initial pH, and tannin content have been identified as the most effective variables in tannase production (Kumar et al., 2016). Taking this into account, these independent parameters, in addition to the green bean peels (% *w/v*) concentration, were chosen to determine the optimal conditions for maximum tannase synthesis using CCD of RSM analysis. An overall total of thirty experiments were suggested and carried out using various combinations, with the software suggesting a quadratic model (Table 2).

The multiple regression study of tannase as a response yielded the quadratic equation:

$$\begin{aligned}\text{Tannase activity } (U/g/min) = {} & +308.88 - 5.94^{*}\,A - 5.32^{*}\,B + 21.86^{*}\,C \\ & + 23.99^{*}\,D + 2.48^{*}\,A^{*}\,B - 6.35^{*}\,A^{*}\,C \\ & - 6.56^{*}\,A^{*}\,D - 1.20^{*}\,B^{*}\,C - 2.29^{*}\,B^{*}\,D \\ & + 19.79^{*}\,C^{*}\,D - 73.0^{*}\,A^{2} - 76.84^{*}\,B^{2} \\ & - 60.26^{*}\,C^{2} - 63.50^{*}\,D^{2}. \end{aligned} \quad (2)$$

Where A, B, C, and D are the codes of temperature, initial pH, tannin, and green bean peal concentrations, respectively.

3.2. Model validation

According to the ANOVA table (Table 3) and Fisher's test, the model has an F-value of 217.4, implying that it is significant. The coefficient R^2 was used to verify the model's best fit. In this study, $R^2 = 0.9951$ means that the independent factors account for 99.51 of the total variation in tannase yield. The predicted R^2 of 0.9718 closely matches the adjusted R^2 of 0.9905 sensibly. This demonstrates the reliability of the experiment in predicting precise conditions. When the values of Prob > F are <0.05, it indicates that model terms are significant. In accordance, Table 3 displays the significant model terms in this design, where the four variables, A, B, C, and D, as well as the quadratics AC, AD, and CD, and the squared terms A^2, B^2, C^2, and D^2 are significant. This suggests that the interaction of those factors strongly affects tannase production. Values >0.10 imply that the model term is not significant. That means the quadratics; AB, BC, and BD are non-significant. An adequate precision of 39.228, as it is greater than four, suggests an appropriate signal, and the model might be used to navigate the design.

3.3. Three-dimensional (3-D) response surface plots

The 3-D three-dimensional response graphs for the response (tannase production) depict the interaction of two parameters when all other parameters are set to zero (Fig. 1 **A-F**). It was developed using the model equation to investigate the interaction of the aforementioned factors and identify the optimum level of each one for maximum tannase synthesis on green bean wastes. According to these 3-D plots, increasing the pH value >4 and the incubation temperature over 20 °C leads to increased tannase productivity, with optimal values of 7 and 30 °C (Fig. 1 **A**). Enzyme synthesis declined with a further increase in temperature or at basic conditions. Changes in pH can cause deprotonation or protonation of amino acids and the active site of any enzyme, resulting in altered tannase activity (Zhao et al., 2024). **Fig. (1 B)** also demonstrates that the interaction between green bean peels and temperature confirmed tannase productivity decreased at temperatures <25 °C or >35 °C, while it increased by increasing the peels until reaching the highest between 2.50 and 3.75%, then decreased again. In addition, the highest tannase production occurred when fermentation was performed in a medium containing a tannin level of 6.0–9.0% at the optimum temperature and pH value (Fig. 1 **C & F**). A further increase in tannin content beyond the optimum level resulted in decreased enzyme synthesis. Tannase activity dropped, most likely due to tannins in that waste (2.81 to 4.56 mg/g extract) and tannin itself, which restrict fungal development and hence enzyme production (Mejri et al., 2018).

3.4. Optimization and model validation

Following testing, Table 4 displays the optimal levels of the four components as well as the proposed value for each one, with zero being the least desired amount of response and 1.0 representing the most

Table 3
ANOVA results for response surface quadratic model of Central Composite Design for *P. commune* tannase production.

Source	Factor	Sum of squares	DF	Mean square	F value	*p*-value Prob > F	Remarks
Model		4.000E+005	14	28,571.94	217.40	< 0.0001	Significant
A	Temperature	847.23	1	847.23	6.45	0.0227	Significant
B	Initial pH	678.69	1	678.69	5.16	0.0382	Significant
C	Tannin	11,464.11	1	11,464.11	87.23	< 0.0001	Significant
D	Green bean peel	13,809.81	1	13,809.81	105.08	< 0.0001	Significant
AB		98.15	1	98.15	0.75	0.4011	
AC		646.08	1	646.08	4.92	0.0425	
AD		687.79	1	687.79	5.23	0.0371	
BC		23.09	1	23.09	0.18	0.6811	
BD		83.80	1	83.80	0.64	0.4370	
CD		6268.85	1	6268.85	47.70	< 0.0001	
A^2		1.465E+005	1	1.465E+005	1114.68	< 0.0001	
B^2		1.619E+005	1	1.619E+005	1232.23	< 0.0001	
C^2		99,595.49	1	99,595.49	757.82	< 0.0001	
D^2		1.106E+005	1	1.106E+005	841.65	< 0.0001	
							Value
R^2							0.9951
Adjusted R^2							0.9905
Predicted R^2							0.9718
Adeq Precision							39.228
Std. Dev.							11.46
Mean							89.93
C.V. %							12.75
PRESS							11,354.94

desired response. All the factors were maintained within their range, and only tannase activity was at its maximum. To maximize tannase synthesis on green bean peels, Design Expert V 7 recommended growing medium parameters of 29.07 °C, an initial pH of 6.74, a tannin content of 6.76%, and 3.31% green bean peels to achieve tannase production of 313.40 U/g/min. The CCD design used in this investigation increased tannase titers by ≈5 folds than un-optimized conditions (62.44 U/g/min). The current results are substantially comparable to those of de Lima et al. (2014). *Penicillium montanense* produced 41.64 U/mL of tannase when cultivated on Barbados cherry wastes with 3.5% tannic acid and 70% moisture content.

As shown in Table 5, green bean peels yielded more tannase (313.40 U/g dw/min) than most other reported food-based wastes, even when the same producing strain was cultivated on potato peel wastes (Mostafa, 2023a). Compared to the wastes documented for tannase production in the literature, green bean peels are promising for enzyme production. Validation of the response surface model was confirmed using the model's optimum conditions. The experimental value of 300 U/g dw/min was very close to the anticipated values, indicating that the model was successfully verified and it is reliable for forecasting synthesis tannase by SSF from *P. commune*.

3.5. Effect of tannase on the physicochemical attributes of coffee

More than three-quarters of the world's population drinks a caffeinated beverage every day. Coffee (*Coffea* L.) is one of the primary caffeinated beverages, after tea. *C. arabica* L. (Arabica) is the most popular and desired coffee variety globally. The coffee business is predicted to be worth >100 billion USD globally, with exports alone accounting for >20 billion USD (Acidri et al., 2020). It has numerous health benefits, primarily as a stimulant due to caffeine, and it displays its anti-proliferative action and decreases cancer and diabetes due to its content of potent bioactive compounds (Rawangkan et al., 2022). On the other hand, its chemical composition is a significant limiting factor due to the presence of anti-physiological substances such as caffeine, polyphenolic compounds, and tannins (Hakil et al., 1998), including tannic acid at high concentrations (3–4%) (Aguilar et al., 2000). For this importance, instant coffee was chosen, and to our knowledge, no previous study has investigated the influence of tannase on its properties. Instant coffee is a soluble coffee powder or granule that has been spray-dried or freeze-dried. This beverage can be prepared by just adding hot water, enabling consumers to prepare coffee very quickly and in a convenient way (Shankaran & Chinnaswamy, 2019).

Table 6 summarizes the tested physicochemical parameters of both untreated and tannase-treated instant coffee. A comparison of the key factors affecting quality, namely: reducing sugars, gallic acid and tannin content, color, and foam features, demonstrated that tannase significantly altered the coffee's characteristics at $p \leq 0.05$ level.

Green coffee contains acids such as chlorogenic, quinic, citric, and malic acids, whereas acetic, lactic, formic, and glycolic acids are generated by the heat degradation of soluble carbohydrates during coffee roasting. Each acid concentration may vary depending on the cultivar and roasting degree (Rune et al., 2023). Here, the tannase-treated instant coffee had increased titratable acidity (acetic acid) and a lower pH than the untreated coffee, with no significant difference ($p > 0.05$). Tannase-treated green tea leaves (Cao et al., 2019), as well as black and green tea infusions (Aharwar & Parihar, 2023), had lower pH values than the untreated samples. The acidity (inverse of pH) of apple juice was also low after the tannase treatment (Jana et al., 2015). Monteiro et al. (2021) showed that tannase did not significantly alter the measured characteristics of pitanga juice, such as pH, acidity, and TSS. This indicates that pH and acidity changes following tannase may be influenced by the beverage or fruit juice composition, as well as the reactions that may occur during tannase treatment or its inactivation.

Tannase from *Penicillium commune* increased the TPC of instant coffee in a non-significant manner ($p > 0.05$). Polyphenols have physiological and pharmacological benefits, such as anti-mutagenic and anti-carcinogenic effects (Laskar & Mazumder, 2020). According to Shao et al. (2020) and Mostafa (2023b), tannase increased the amounts of TPC in green and Hibiscus teas, respectively.

Following the tannase trial, the amount of reducing sugars, specifically glucose, significantly rose by 15.94% (Table 6). This is anticipated given that tannase cleaves the diester link in tannins, releasing glucose and gallic acid (Saeed et al., 2021). This suggests that tannase treatment could improve the sweetness and acceptability of such a popular beverage. In agreement, sugars such as glucose increased significantly following tannase treatment, although fructose and xylose remained constant in cashew and Jamun apple juice (Jana et al., 2015). Mostafa

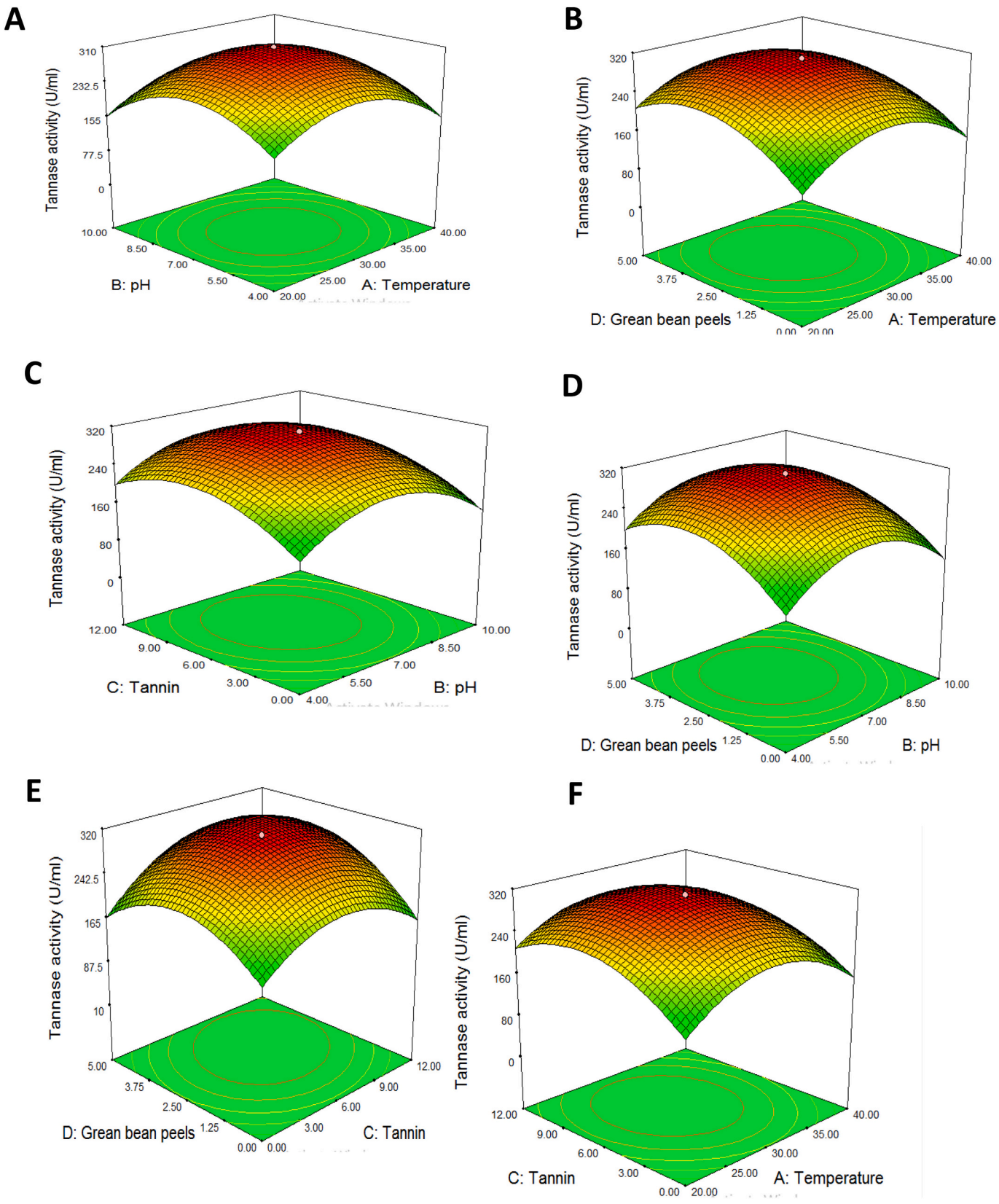

Fig. 1. Response 3-D plot of the interaction of 4 factors affecting tannase productivity. **A** Temperature and initial pH, **B** Green bean peels and temperature, **C** Tannin content and initial pH, **D** Green bean peels and initial pH, **E** Green bean peels and tannin content, and **F** Tannin content and temperature. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
Numerical optimization and the suggested value for each factor for optimum tannase production by *P. commune*.

Factor	Goal	Lower limit	Upper limit	Importance	Suggested value	Desirability
Temperature	Is in range	20	40	5	29.07 °C	1.000
Initial pH	Is in range	4	10	5	6.74	
Tannin	Is in range	0	12	5	6.76%	
Grean bean peel	Is in range	0	5	5	3.31%	
Tannase activity	Maximize	0	308.88	5	313.40 U/g/min	

Table 5
Comparison of tannase yields produced by different microbial strains on various wastes as substrates.

Substrate	Microbial strain	Production conditions	Tannase yield (U)	Reference
Green bean peel	*Penicillium commune*	[a] 6.86, [b]29.6 °C, [c]6.46%	313.40	**This study**
Potato peel	*Penicillium commune*	5.00, 25.1 °C, 9.99%	288.48	Mostafa (2023a)
Pomegranate peel	*Bacillus velezensis* TA3	7.00, 30.0 °C. 0.86%	32.00	Lekshmi et al. (2020)
Black plum leaves	*Aspergillus oryzae* FCBP-PTF-1202	5.50, 30.0 °C, 0.00%	179.95	Saeed et al. (2021)
Tea stalk extract in urethane sponge	*Aspergillus tubingensis*	5.00, 30.0 °C, 7.49%	245.00	Wu et al. (2018)
Gooseberry leaves	*Klebsiella pneumoniae*	5.52, 39.7 °C, 2.17%	30.00	Kumar et al. (2016)
Tea stalk	*Aspergillus tubingensis*	6.00, 30.0 °C, 0.00%	84.24	Xiao et al. (2015)
Coffee pulp	*Penicillium verrucosum*	5.00	115.99	Bhoite and Murthy (2015)

a = pH value, b = Temperature, c = Tannin content. U = nM gallic acid/g dry substrate.

Table 6
Comparison between instant coffee beverage properties before and after tannase treatment.

Physicochemical properties	Unit	Instant coffee		Change
		Before tannase	After tannase	
†**pH**		4.84 ± 0.000	4.80 ± 0.007	↓
Acidity	% w/v as acetic acid	0.108 ± 0.00	0.150 ± 0.02	↑
Total phenolic content	μg GAE/100 mL	116.6 ± 3.88	119.1 ± 1.62	↑
Reducing sugars	mg/mL	*6.9[b] ± 0.12	8.0[a] ± 0.10	↑
Tannins				
Hydrolyzable	mg/100 mL	511.15[a] ± 15.62	393.9[b] ± 5.51	↓
Condensed	mg catechins/100 mL	141.5 ± 17.44	83.2 ± 6.59	↓
Gallic acid	ng/mL	28.52[b] ± 0.049	43.05[a] ± 1.31	↑
Color attributes				
L*		42.73[b] ± 1.42	49.96[a] ± 0.63	↑
a*		17.43[a] ± 0.56	15.33[b] ± 0.45	↓
b*		33.57[b] ± 1.60	39.70[a] ± 0.20	↑
Caffeine content	mg/mL	10.3 ± 1.20	10.0 ± 0.38	–
Foam				
Volume	mL	25[a] ± 2.3	15[b] ± 1.4	↓
Drainage	mL/s	−0.1533[b] ± 0.0002	−0.0567[a] ± 0.0001	↑
Stability	(After 2 h)	72[a] ± 0.93	47[b] ± 0.58	↓

†All results were expressed as the mean ± SD. * Different letters within the same row indicate significant difference at ($p \leq 0.05$) as calculated by *t*-test.

(2023b) also observed a 3.2-fold rise in reducing sugars in tannase-treated *Hibiscus sabdariffa* tea.

The greatest health impact of the tested tannase was the reduction of hydrolyzable and condensed tannin levels by 22.93% and 41.20%, respectively. This was also detected by a 1.5-fold increase in gallic acid levels. This is the result of gallic acid liberation and catechin elimination. Tannins are high-molecular-weight polyphenolic compounds (500 to 3000 Da) found in two principal classes: hydrolyzable tannins and condensed tannins. The main responsible factor for the product's sensory characteristics, particularly its astringency and bitterness, is the condensed tannins (Ju et al., 2021). Hydrolyzable tannins contain either ellagitannins or gallotannins. The ellagitannins contain hydroxydiphenoyl residues that produce ellagic acid, while gallotannins degradation yields gallic acid and glucose. Condensed tannins, otherwise known as proanthocyanidins, are compounds consisting of oligomers and polymers of flavan-3-ol units, usually linked via C4-C6 or C4-C8 bonds (Catelani et al., 2016). That means that the hydrolysis of tannins in coffee is advantageous and effective for lowering its astringency. de Lima et al. (2018), in agreement, employed *Aspergillus ficuum* tannase (170 U/mL) to remove 22% of the tannins from boldo tea, although the immobilized form of the same enzyme removed 31% under similar conditions. Mostafa (2023b) found that after 2 h, the hydrolyzable and condensed tannin levels of *Hibiscus sabdariffa* tea fell by 21.42% and 60.87%, correspondingly, by the action of the same strain. Kumar et al. (2023) reduced tannins in tea by 55% after 3 h using 6.0 units of enzyme/ml at 35 °C. Aharwar and Parihar (2023) achieved the largest reduction in tannin content by 120 U tannase/ml in black tea (78%) and green tea (59%) infusions at 60 °C for 20 min. No previous study has examined the effect of tannase on any type of coffee. The coffee wastes were solely evaluated for tannase production. Bhoite and Murthy (2015) employed coffee pulp as the solitary carbon source to produce tannase from *Penicillium verrucosum*, yielding 28.173 U/gd. On the other side, spent coffee ground, which contained 155 mg tannin/g substrate, exhibited poor fungal growth and tannase production; however, when added to rice bran, it enhanced tannase activity by 1.8-fold and 3-fold in comparison to rice bran alone or with tannic acid, respectively, with *Aspergillus niger* PN1 producing 260.39 U/g according to Mansor et al. (2019).

The tannase treatment significantly changed the color attributes of instant coffee. L* and b* values increased dramatically, while a* value declined. That indicates that the tannase-treated coffee appears lighter and more yellowish. This could be owing to the color of the formed gallic acid. Its crystals are white, yellowish-white, or pale fawn-in color (Pal Singh & Gupta, 2018). The color intensity and anthocyanin concentration of red Hibiscus tea were likewise reduced after tannase treatment for two hours (Mostafa, 2023b).

Caffeine, a xanthine alkaloid compound, is a central nervous system stimulant found in some plants, including coffee and tea. Due to its excitatory effects, caffeine can elevate spirits, reduce fatigue, eliminate sleepiness, and improve thinking and memory function (Yamamoto et al., 2019). As a result, its existence is extremely significant to coffee consumers. In this study, the caffeine level of instant coffee did not change appreciably following treatment with tannase. That is because caffeoyl esterase is an enzyme that cleaves caffeine, which is an alkaloid

rather than a substrate for tannase (Acidri et al., 2020). Nonetheless, caffeine retention in coffee is desirable since it is often regarded as a powerful bioactive coffee component with the potential to provide those physiological advantages. This means that tannase has no detrimental effect on the excitatory properties of that beverage.

Aside from color, smell, and taste, most consumers regard the existence of persistent foam to be a key quality attribute in coffee (Shankaran & Chinnaswamy, 2019). The layer of foam above instant coffee is a sign of a super-quality coffee cup (Ishwarya & Nisha, 2021). Despite this fact, detailed research into its physicochemical characteristics and the effect of tannase on its structure is limited. The foam behavior is quantified using two indices: "foamability" and "foam stability." While foamability is described as the ease and extent of foam formation, foam stability refers to the capacity of the continuous phase to maintain the gas for a certain period of time. Foam stability has also been characterized as an index of how quickly the foam structure degrades after formation (Prins, 1988). Table 6 shows that the foam volume of tannase-untreated coffee decreased dramatically from 25 mL to 15 mL, and foam stability dropped from 72% to 47%, as shown by the increase in foam drainage (slope) following tannase treatment. This indicates that tannase activity had a detrimental effect on the foam properties of instant coffee, such as foamability and stability. Foamability and foam stability were found to be connected to the polysaccharides and protein content, of which the proteins play a prominent role in foaming owing to their greater hydrophobicity imparted by the chemical nature of the side chains of their constituent amino acids. It could also be related to changes in the pH of the coffee after tannase, which affects some foaming agents such as egg white protein and hydroxypropylmethylcellulose (Sadahira et al., 2018). The findings of the current study indicate that tannins may also play a role in foam development and persistence in instant coffee and that the degradation of these high-molecular-weight molecules into gallic acid beside liberating glucose negatively affected it. Prior studies have not fully understood the chemical nature of surface-active compounds of the coffee foam, and new research is strongly encouraged.

4. Conclusion

Bean pod waste should be considered a byproduct for enzyme production as it is rich with protein and other important constituents. One conceivable use for such waste is tannase synthesis by the fungus *Penicillium commune* under the optimized conditions. As a novel application of the purified tannase, it was utilized to reduce the tannin content of coffee. The greatest impact of tannase was a considerable increase in reducing sugars and a decrease in tannins and coffee foam stability. Given the industrial importance of tannase, we describe a new functional beverage made from low-bitter coffee that may have health benefits such as reducing protein-complex formation, which influences protein utilization in the human diet.

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Heba Sayed Mostafa: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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Data availability

All data generated or analyzed during this study are included in this published article.

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