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Optimization of physical and enzymatic debittering methods for grapefruit juice

Darshana Sihmar* and Aradhita Barman Ray

*Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar-125001, Haryana, India. *E-mail: darshanaftgju@gmail.com*

Abstract

The acceptability of grapefruit juice is hindered by its inherent bitterness, primarily attributed to the presence of naringin as the main bitter component. To enhance consumer preference, two distinct methods, physical and enzymatic, were employed to mitigate the bitterness of grapefruit juice. In the physical method, the juice underwent treatment with the adsorbent Amberlite IR 400, utilizing a 3-level factorial design. The enzymatic method involved the application of naringinase enzyme, employing the Box-Behnken experimental design. Notably, the physical method revealed a significant linear and interaction effect of time and temperature on the naringin content, while the enzymatic method exhibited a significant linear effect of enzyme concentration, time and temperature on the same. Both debittering methods were optimized using a numerical multi-response optimization technique to determine the optimum variable levels. This ensured that both independent and dependent variables remained within the experimental range, achieving maximum desirability. For the physical debittering method, the optimized conditions were 96 minutes and 28 °C for time and temperature, respectively. Meanwhile, the enzymatic debittering method optimized conditions included 0.83 g/L enzyme concentration, 35 °C temperature, and 3 hours 50 minutes time. Under these optimized conditions, the enzymatic method demonstrated superior results, achieving a higher naringin reduction of 55.77%, compared to the 33.18% reduction achieved by the physical method using Amberlite IR 400.

Key words: Adsorbent; debittering; grapefruit juice; naringin; naringinase enzyme

Introduction

Citrus juices from oranges, grapefruits, kinnow, lemons, and limes are the most popular fruit juices with great flavour, colour, and aroma. Citrus juices are rich in vitamins, minerals, and antioxidants. The sweetness of the juice is attributed to the sugars, namely glucose, fructose, and sucrose. The consumer acceptability of citrus juices is affected by the development of bitterness that is primarily imparted by two compounds *i.e.*, limonin and naringin. In grapefruit, naringin is the main bitter component, which may range from 300 to 750 ppm in grapefruit juice (Purewal and Sandhu, 2021). To reduce the naringin in grapefruit juice below the threshold level for consumer acceptability, several physicochemical and enzymatic methods have been reported for debittering of grapefruit juice (Mishra and Kar, 2003; Ferreira *et al.,* 2008; Kranz *et al.,* 2011; Gupta *et al.,* 2020; Tran *et al.,* 2020; Ladole *et al.,* 2020; Gupta *et al.,* 2021; Muñoz *et al.,* 2022). Gupta *et al.* (2021) showed a reduced debittering time and enhanced adsorption and hydrolysis of naringin by 17% and 20% in resin and enzyme, respectively in a study on sonication-assisted debittering of pomelo fruit juice using resin and enzyme hydrolysis. The co-immobilized pectinase and naringinase on eco-friendly chitosan-coated magnetic nanoparticles showed an 85% reduction in the naringin content of grapefruit juice (Ladole *et al.,* 2021). Mishra and Kar (2003) treated the grapefruit juice with Amberlite IR 400, resulting in 69.23% naringin removal after 5 min exposure with significant clarification. They also treated the juice with alginate-entrapped naringinase, resulting in 83.84% naringin hydrolysis. Ferreira *et al. (*2008) observed 75% debittering of grapefruit juice under high pressure, with naringinase immobilized in calcium alginate beads. Gupta *et al. (*2020) optimized the resin concentration, time and stirring speed for debittering of Pomelo juice. Kranz *et al.* (2011) investigated the debittering of grapefruit juice using kinetic modelling and response surface methodology with XAD-7HP adsorbent resin. In the present study, grapefruit juice defoliation was done using two methods. The first was by the physical debittering method using Amberlite IR 400, and the second was by the enzymatic debittering method using the naringinase enzyme. Therefore, this work aimed to optimize and compare the physical and enzymatic debittering methods for reducing naringin content from grapefruit juice.

Materials and methods

Grapefruits (*Citrus paradisi*) of the Star Ruby variety with equal degrees of maturity were procured from the Ch. Hira Singh Wholesale Fruits and Vegetable Market, Azadpur, New Delhi, Inida. The naringin and enzyme used in the investigation were purchased from the Sigma-Aldrich Chemicals Private Limited, Bangalore, India.

Juice Extraction: After washing, the grapefruits were peeled with the help of a knife and juice was extracted by a screw-type juice extraction machine (Kalsi, Bhajan Singh and Sons, Ludhiana, India). The obtained juice was collected in the glass beaker and filtered through a sieve for further processing.

Physical debittering method for grapefruit juice: Grapefruit juice was subjected to treatment by Amberlite IR 400. A batch process was adopted for the juice treatment and 50 mL of juice was mixed with 10 g of Amberlite IR 400 in a beaker. Constant

stirring was done, and juice was filtered by centrifugation. The effect of independent variables (time and temperature) was observed on the naringin content reduction of juice (Table 1). The obtained data were analyzed by fitting a two-factor interaction model to assess the applicability of process variables (time and temperature) on the naringin content of the juice. Plots were obtained using the Design Expert trial version (State-Ease, Minneapolis, MN) to facilitate the visual presentation of responses with respect to variables.

Table 1. Three level factorial experimental design and response of physical debittering of grapefruit juice

Experiment	Variables		Response	
	Time (min) (A)	Temperature (°C) (B)	Naringin (ppm)	Naringin reduction (%)
1	60	20	552.10	19.83
2	90	20	522.14	24.18
3	120	20	539.45	21.67
4	60	30	464.92	32.49
5	90	30	448.34	34.90
6	120	30	489.84	28.87
7	60	40	548.38	20.37
8	90	40	540.32	21.54
9	120	40	578.36	16.02
10	90	30	442.60	35.73
11	90	30	453.80	34.10
12	90	30	450.10	34.64
13	90	30	452.52	34.29

Enzymatic debittering method for grapefruit juice: Grapefruit juice was subjected to treatment with naringinase enzyme. A batch process was adopted for the juice treatment and 10 mL of juice was mixed with the enzyme in a flask. The enzyme treatment was carried out as per Box-Behnken experimental design (Table 2). Variation in the naringin content of debittered grapefruit juice was observed as dependent variables with respect to desired variable combinations of enzyme concentration, time, and temperature. Response surface quadratic model was fitted to assess the applicability of process variable on naringin content. Plots were obtained using the Design Expert trial version (State-Ease, Minneapolis, MN) to facilitate the visual presentation of responses with respect to variables.

Naringin content estimation: The naringin content of juice samples was estimated using Davis's (1947) spectrophotometric method with slight modifications. The absorbance was recorded at 420 nm against a blank in a UV spectrophotometer (Evolution One UV-Vis Spectrophotometer, Thermo Fisher Scientific, United States), and naringin content was calculated by drawing a standard curve of naringin (10-200 µg).

Naringin reduction: The reduction in naringin content was calculated in reference to the naringin content of untreated juice (688.67 ppm) and expressed in per cent.

Data collection and analysis: The experimental data was noted down in Microsoft excel worksheet 365 (Microsoft Corporation, New Maxico, US), calculated and interpreted with the help of Microsoft excel worksheet (Kaushik *et al.,* 2015; Sharma *et al.,* 2016).

Table 2. Three-level Box-Behnken experimental design and response of enzymatic debittering of grapefruit juice

Experiment	Variables			Response	
	(A) Enzyme (g/L)	(A) Time (min)	(C) Temperature (°C)	Naringin (ppm)	Naringin reduction (%)
1	0.5	30	2.5	477.60	30.65
2	1	30	2.5	297.98	56.73
3	0.5	50	2.5	492.62	28.47
4	1	50	2.5	423.26	38.54
5	0.5	40	1	431.80	37.30
6	1	40	1	297.10	56.86
7	0.5	40	4	423.16	38.55
8	1	40	4	257.50	62.61
9	0.75	30	1	383.70	44.28
10	0.75	50	1	436.26	36.65
11	0.75	30	4	353.34	48.69
12	0.75	50	4	437.78	36.43
13	0.75	40	2.5	336.25	51.17
14	0.75	40	2.5	343.10	50.18
15	0.75	40	2.5	351.65	48.94
16	0.75	40	2.5	342.20	50.31
17	0.75	40	2.5	345.41	49.84

Results and discussion

Effect of physical debittering on naringin content of grapefruit juice: The naringin content of debittered juice by physical method varied from 442.60 to 578.26 ppm with an average value of 498.68 ppm (Table 1). The maximum reduction in the naringin content of grapefruit juice was found at the combination of time and temperature of 90 min and 30 °C, respectively. The model F-value indicates that the model is significant (Table 3). Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case A, B, A^2, B^2 and AB are significant model terms.

Table 3. Analysis of variance for naringin content of physically debittered grapefruit juice (Level of significance: $P \leq 0.05$)

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	28841.6	5	5768.32	224.96	< 0.0001	Sig.
A	297.51	1	297.51	11.60	0.0113	Sig.
B	474.72	1	474.72	18.51	0.0036	Sig.
A^2	1824.77	1	1824.77	71.16	< 0.0001	Sig.
B^2	17479.64	1	17479.64	681.70	< 0.0001	Sig.
AB	454.32	1	454.32	17.71	0.0040	Sig.
Residual	179.48	7	25.64			
Lack of Fit	102.56	3	34.18	1.77	0.2903	NS
Pure Error	76.92	4	19.23			
Cor Total	29021.08	12				
R-Squared		0.99				
Adjusted R-Squared		0.98				
Adeq Precision		38.32				

The predicted R-square of 0.9618 was in reasonable agreement with the adjusted R-squared of 0.9893. The coefficient of coded factors obtained by analysis of variance is presented in the equation as

$$\text{Naringin content} = 450.10 + 7.041A + 8.895B + 25.703A^2 + 79.553B^2 + 10.657AB$$

Where A and B are the value of time and temperature, respectively. The visual presentation for naringin content is given in a 3D surface plot (Fig. 1) by comparing the effects of temperature and time by setting the reference point at the center point coded as 0 for both time and temperature in the design space. The naringin content reduction in grapefruit juice during the debittering process was due to the adsorption of naringin by adsorbent Amberlite IR 400 from the grapefruit juice and consequently, there was a reduction in the bitterness of grapefruit juice. In a study on the adsorption of naringin, Singh *et al.* (2008) observed that most of the adsorption takes place during the first 90 min. Mishra and Kar (2003) treated the grapefruit juice with Amberlite IR 400 and reported 69.23% naringin content removal. Similarly, Gupta *et al.* (2020) studied the effect of process variables like resin concentration (Amberlite IRA-400), and time exposure for the removal of naringin content in pomelo juice. The extent of naringin reduction depends on the resin concentration, exposure time, and speed of agitation of juice (Scordino *et al.,* 2003).

Optimization and validation of the physical debittering method: The optimization of responses was done by the numerical optimization technique (Saklani *et al.,* 2021). The optimum condition of responses (time and temperature) was obtained based on the desirability function. The optimum solution with a desirable 1 was selected for validation at actual laboratory conditions. The experiment was conducted at nearly adjusted values of time (96 min) and temperature (28 °C) to validate the predicted values of naringin content. There was a 1.61 % deviation in actual values from the predicted values of naringin content and hence these values of time (96 min) and temperature (28 °C) were considered as an optimized condition for the physical debittering method with 33.18 % reduction in naringin content.

Effect of enzymatic debittering on naringin content of grapefruit juice: The naringin content of debittered juice by enzymatic method varied from 257.50 to 492.62 ppm with an average value of 378.28 ppm (Table 2). The model F-value indicates that the model is significant (Table 4). Values of "Prob > F" less than 0.05 indicate model terms are significant. A, B, C, A2, B2 and AB are significant model terms in this case. The predicted R-squared of 0.9121 is in reasonable agreement with the adjusted R-squared of 0.9838. The coefficient of coded factors obtained by analysis of variance is presented in the equation as

Naringin content= 343.722 -68.66A + 34.66B -9.63C +14.38A^2 + 64.76B^2 – 5.71C^2 +27.56AB -7.74AC +7.97BC

A, B, and C are the enzyme concentration, temperature, and time values, respectively. The visual presentation for naringin content is given in a 3D surface plot by comparing the effects of enzyme and temperature (Fig. 2), enzyme and time (Fig. 3), and temperature and time (Fig. 4). There was a significant (P<0.05) linear effect of enzyme concentration, temperature, and time on the naringin content. The significant (P<0.05) quadratic effect of enzyme concentration and time showed that the optimum condition was towards the center point of experimental conditions of enzyme concentration and time. Prakash *et al.* (2002) reported a maximum reduction of 75 % in naringin content at an enzyme concentration of 1g/L in a study on enzymatic debittering of grapefruit juice. Ladole *et al.* (2021) reported an 85% reduction in the naringin content in grapefruit juice by the naringinase enzyme. Gao *et al.* (2021) conducted experiments on Ougan juice debittering using ultrasound aided enzymatic hydrolysis and reported enhanced hydrolysis of naringin (89.90 %) after sonication. Similarly, Mishra and Kar (2003) reported 83.84% naringin hydrolysis with 1.98 enzyme units/mL of juice by alginate-entrapped naringinase treatment of the grapefruit juice.

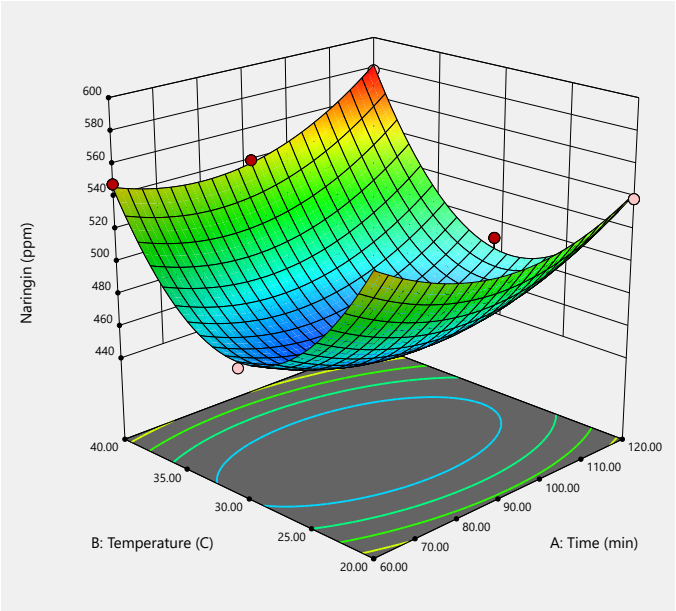

Fig. 1: 3D surface plot for effect of time (A) and temperature (B) on the naringin content of physically debittered grapefruit juice

Table 4. Analysis of variance for naringin content of enzymatically debittered grapefruit juice (Level of significance: P≤0.05)

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	70612.19	9	7845.80	109.27	< 0.0001	Sig.
A	37721.80	1	37721.80	525.34	< 0.0001	Sig.
B	9611.91	1	9611.91	133.86	< 0.0001	Sig.
C	742.67	1	742.67	10.34	0.0147	Sig.
A^2	870.85	1	870.85	12.13	0.0102	Sig.
B^2	17659.17	1	17659.17	245.93	< 0.0001	Sig.
C^2	137.45	1	137.45	1.91	0.2090	NS
AB	3039.32	1	3039.32	42.33	0.0003	Sig.
AC	239.63	1	239.63	3.34	0.1105	NS
BC	254.08	1	254.08	3.54	0.1020	NS
Residual	502.63	7	71.80			
Lack of Fit	378.39	3	126.13	4.06	0.1046	NS
Pure Error	124.24	4	31.06			
Cor Total	71114.82	16				
R-Squared		0.99				
Adjusted R-Squared		0.98				
Adeq Precision		35.74				

Optimization and validation of the enzymatic debittering method: The optimization of responses (enzyme concentration, temperature, and time) was done by a numerical optimization technique (Saklani *et al.,* 2021). The optimum condition of responses was obtained based on the desirability function. The optimum solution having a desirability of 1 was selected for validation at actual laboratory conditions. The experiment was conducted at nearly adjusted values of enzyme concentration (0.83 g/L), temperature (35 °C), and time (3 h 50 min) to validate

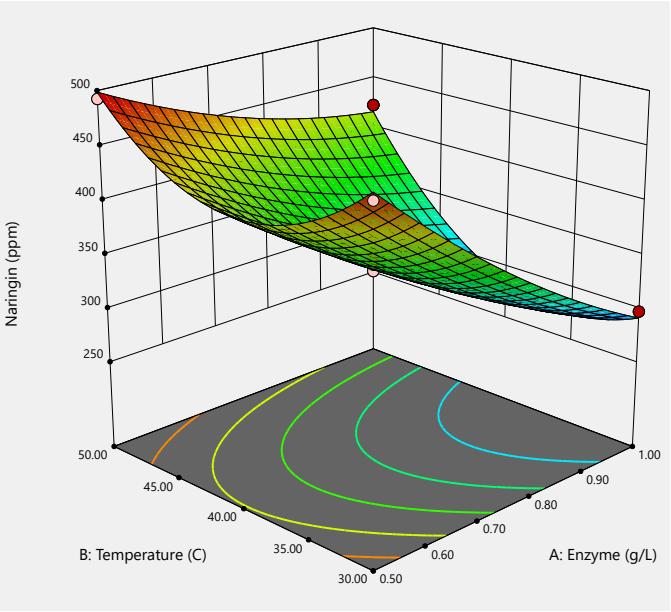

Fig. 2. 3D surface plot for effect of enzyme (A) and temperature (B) on the naringin content of enzymatically debittered grapefruit juice

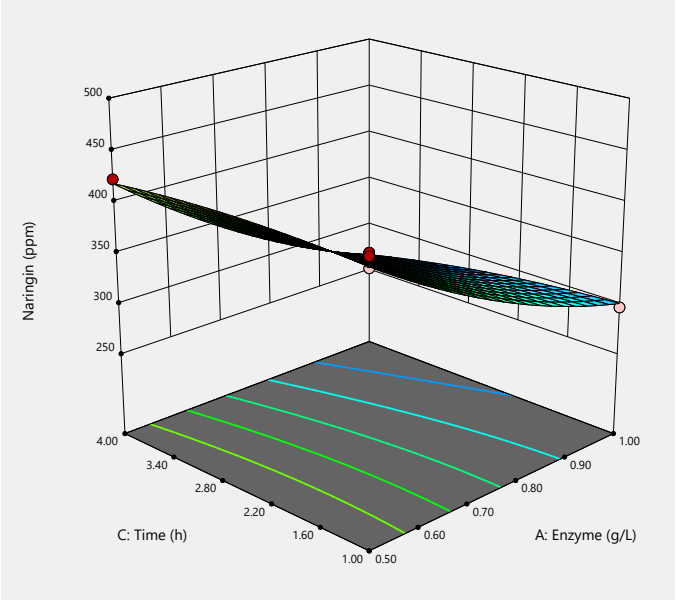

Fig. 3. 3D surface plot for effect of enzyme (A) and time (C) on the naringin content of enzymatically debittered grapefruit juice

the predicted values of naringin content. There was a 2.69 % deviation in actual values from the predicted values of naringin content. Hence these values of enzyme concentration (0.83 g/L), temperature (35 °C), and time (3 h 50 min) were considered as an optimized condition for the enzymatic debittering method with 55.77 % reduction in naringin content.

The physical and enzymatic debittering methods were optimized by using a 3-level factorial design and Box-Behnken experimental design, respectively. A numerical multi-response optimization technique was utilized to optimize both physical and enzymatic debittering processes, ensuring that independent and dependent variables remained within the experimental range with maximum desirability. For physical debittering, the optimal conditions were determined as 96 minutes at 28°C. In contrast, for enzymatic debittering, the optimal conditions were found to be an enzyme concentration of 0.83 g/L, a temperature of 35°C, and a duration of 3 hours and 50 minutes. The enzymatic method demonstrated

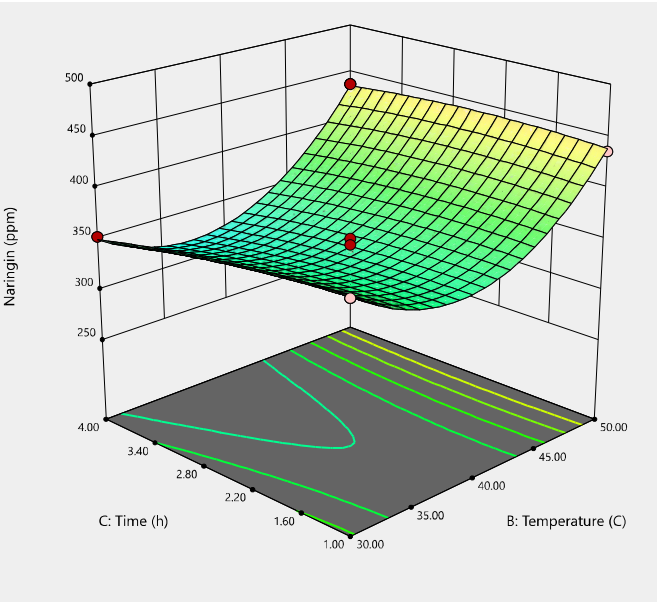

Fig. 4. 3D surface plot for effect of temperature (B) and time (C) on the naringin content of enzymatically debittered grapefruit juice

a higher naringin reduction of 55.77% compared to 33.18% achieved by the physical method.

The study compared physical and enzymatic debittering methods for reducing naringin content in grapefruit juice. Optimal conditions for physical debittering were identified as 96 minutes at 28 °C, resulting in a 33.18% reduction. For enzymatic debittering, the optimal conditions were 0.83 g/L enzyme, 35 °C, and 3 hours 50 minutes, leading to a higher reduction of 55.77%. The enzymatic method proved more effective in reducing naringin content, highlighting its potential for commercial grapefruit juice production.

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