AATCC Review

26 February 2025: Received
21 April 2025: Revised
28 April 2025: Accepted
30 May 2025: Available Online

https://aatcc.peerjournals.net/

Original Research Article | Open Access

Impact of adsorbent Amberlite XAD-16 in reducing bitterness of Pummelo juice

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ABSTRACT

Pummelo [Citrus grandis (L.)] Osbeck, is an important underutilized fruit crop grown mainly in the states of the North Eastern region of India. The juice of pummelo fruit is an excellent source of antioxidant and health-promoting compounds. However, it is not very popular among consumers due to its unbearable bitterness. This bitter taste is due the presence of bittering compounds (limonin and naringin) which gets extracted during juice extraction. It is the main reason for the poor organoleptic attributes and acceptability of pummelo juice among the consumers. It is also one of the limiting factors for commercial production and processing of pummelo based fruit juices. This investigation was therefore, aimed at reducing bitterness by reducing the concentration of bittering compounds (limonin and naringin) by using an adsorbent, Amberlite XAD-16, and optimization of the same in terms of quantity (mg/100 mL) and reaction time (seconds) for debittering purposes using response surface methodology (RSM). The impact of independent variables (quantity of adsorbent and reaction time) on dependent variables (quantity of limonin and naringin) and the extent of reduction in bitterness of pummelo juice were studied. Effects of quantity of adsorbent and reaction time were found to be significant ($p < 0.05$) for dependent variables. The Amberlite XAD-16 resin was found to be very effective in reducing limonin and naringin as it reduced limonin by 88% and naringin by 40% in the juice resulting in reduced bitterness in pummelo fruit juice.

Keywords: *Pummelo fruit, Juice extraction, Bitterness, adsorbent, Amberlite XAD-16, limonin, naringin, organoleptic attributes, acceptability*

Introduction

Due to changing lifestyles and increased health awareness, people's affection towards the health benefits of pure fruit juices and fruit-based beverages increased significantly. Consumers' preference to consume more drinks with high-fruit juice, increased the demand for fruit-based beverages as compared to non-fruit-based beverages [1, 2]. There are many fruit juices available for human consumption all over the world. Among them, citrus juices are most popular due to their health benefits, refreshing and a rich source of health-promoting phytochemicals [3]. In the orange group, pummelo is an important fruit crop grown in many countries of the world. The world production of pummelo and grapefruit together is about 6.9 million metric tons [4, 5]. It is now one of the most popular fruits in the world [6]. In India, citriculture is the 3rd most important fruit crop grown with an annual production of 13.40 million MT, covering an area of about 1.034 million hectares [7]. Pummelo is also grown in many states of the country but the major growing areas fall in the North Eastern region of India [8]. It is known by different names like pomelo, shaddock, or Chinese grapefruit [9] and is commonly grouped on the basis of segment colors such as white, pink, and red. Consumption of fresh or processed pummelo juice in India is very low which limits its popularization and commercial production. This is due to the bitter taste of the fresh juice (immediate bitterness) which is not liked by the consumers. The bitterness rapidly increases after juice extraction and exceeds the tolerance level within a few hours after extraction (delayed bitterness). The reasons behind the immediate bitterness are the presence of flavonoids (naringin) in the fruit itself that gets mixed with juice during juice extraction (Fig 1) and delayed bitterness is the rapid synthesis of limonoids (limonin) after juice extraction [10].

The debittering process in citrus juice by using resin-based adsorbents like XAD-4, XAD-7, and XAD-16 was tried by many workers, in grapefruit juice [11], in citrus fruits [12], in citrus juice [13], in kagzi lime [14], in kinnow mandarin [15], in

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DOI: https://doi.org/10.21276/AATCCReview.2025.13.03.113

grapefruit seed oil [16] and reported reduction in bitterness. However, debittering of pummelo juice in India using adsorbents XAD-16 was found negligible in the literature. The present investigation is, therefore, undertaken to study the impact of adsorb and, Amberlite XAD-16 in reducing the bitterness of pummelo juice.

MATERIALS AND METHODS

Plant material and treatments

Pummelo Fruits were procured from the fruit orchard, Uttar Banga Krishi Viswavidyalaya (UBKV), Pundibari, Cooch Behar, (W.B.) and brought to the laboratory of the Department of Pomology and Postharvest Technology (PPHT), Faculty of Horticulture. After sorting and grading, the juice was extracted from uniform ripened fruits with the help of a screw-type juice extractor and filtered by muslin cloth. After filtration, a resin-based adsorbent, "Amberlite XAD-16" was used for debittering pummelo juice. The quantity of resin (mg/100 mL) and treatment time (seconds) were designed in combinations by D-Optimal Design using Response Surface Methodology (RSM). Thirteen (13) combinations were formulated by the designs which were termed as 13 runs (Table 1). The thirteen runs were the thirteen different combinations of adsorbent Amberlite XAD-16 resin treated for different time intervals (treatment time) that were used for debittering purposes. Each run was replicated thrice and all axial points were replicated twice for precision. The combinations used for debittering were evaluated based on the reduction in limonin and naringin content of juice and sensory properties. The evaluation of sensory properties was based on a nine-point hedonic scale. The quantity of adsorbent used was in the range of 200-500 mg and treatment time ranged between 120 -300 seconds (Table 1). Limonin content was estimated by the colorimetric method of Vaks and Lifshitz [17] and Naringin content in the fruit juice was estimated by the method of Davis [18].

Table 1. The quantity of adsorbent XAD-16 (mg/100 mL) and treatment time (seconds)

Std	Run	Factor 1 A: XAD-16 (mg)	Factor 2 B: Treatment Time (s)	Response 1 Naringin (mg/100ml)	Response 2 Limonin (mg/100ml
11	1	350.00	210.00		
5	2	137.87	210.00		
2	3	500.00	120.00		
6	4	562.13	210.00		
1	5	200.00	120.00		
8	6	350.00	337.28		
4	7	500.00	300.00		
3	8	200.00	300.00		
12	9	350.00	210.00		
13	10	350.00	210.00		
10	11	350.00	210.00		
7	12	350.00	82.72		
9	13	350.00	210		

The treatments were carried out on a batch basis. The juice was treated separately as per thirteen combinations (runs) in a beaker of 100 mL. The fractions in the quantity of adsorbent and reaction time were considered one when the fraction was above 0.5 in numerical value. After the debittering process, the juice was analyzed for the content of limonin and naringin. All thirteen lots were replicated thrice for better accuracy. The response of two independent variables (quantity of Amberlite XAD-16 and treatment time) was studied on three dependent variables (limonin, naringin, and organoleptic quality). The optimized best treatment was judged in terms of the lowest limonin and naringin content.

All the necessary chemicals that were used for analysis were purchased from Loba and Merck Millipore, India.

Statistical analysis

The data with three replications were collected for each parameter and analyzed statistically by using factorial Completely Randomized Design (factorial CRD). The data were analyzed by using SPSS Version 26. Differences between the means at a 5 % level of significance were considered significant.

RESULTS AND DISCUSSION

Limonin content

The results of limonin content as adsorbed by Amberlite XAD-16 resin were recorded minimum (3.01 µg/mL) in run no. 12 while, maximum (23.49 µg/mL) in run no. 02 (Table 2). This is almost an 87% reduction in limonin content. Nas and Karatas, [19] also reported a 90% reduction in limonin content in orange juice at 30°C by using Amberlite XAD-16 resin. This reduction in limonin content might be due to the adsorption of limonin by Amberlite XAD-16 through its large surface area and high pore radius. Guneser and Yilmazi [16] also reported similar results with the adsorbent Amberlite XAD-7 (A very similar adsorbent XAD-16) which exhibited high adsorption capacities in the reduction of bitterness of cold pressed grapefruit seed oil due to its large surface area and high pore radius. Lee and Kim (2003) also observed a linear relationship between Amberlite resins and with adsorption of limonin through its physical properties such as cross-linking ratios, pore diameters, and specific surface areas. Similar results have been also reported by Kola et.al. [20] in Washington navel orange juice when treated with different adsorbents (Amberlite XAD16 and Dowex Optipore L285) at different temperatures (20ºC, 35ºC and 50ºC).

Table 2. Impact of different combinations (runs) of adsorbent XAD-16 (mg/100 mL) with treatment time (s) and their response on limonin (µg/mL) content

Std	Run	Factor A: XAD-16 (mg/100 mL)	Factor B: Treatment Time (s)	Limonin (µg/mL)
5	2	137.87	210.00	23.49
1	5	200.00	120.00	7.86
3	8	200.00	300.00	7.39
11	1	350.00	210.00	10.82
12	9	350.00	210.00	12.36
13	10	350.00	210.00	6.56
10	11	350.00	210.00	3.36
7	12	350.00	82.72	3.01
8	6	350.00	337.28	3.98
2	3	500.00	120.00	7.74
4	7	500.00	300.00	6.58
6	4	562.13	210.00	4.78

The linear effect as well as the quadratic effect of different quantities of Amberlite XAD-16 resin and reaction time on limonin content were found to be significant at a 95% level of confidence (Table 3)

Table 3. Analysis of variance (ANOVA) for limonin content as affected by quantity of resin and treatment

Source	Sum of Squares	df	F-value	Coefficient of Estimate	p-value
Model	45.77	5	1370.93	6.47	< 0.0001
A-XAD	19.77	1	2961.35	-1.11	< 0.0001
B-Time	23.55	1	3527.55	-1.21	< 0.0001
AB	0.99	1	148.87	0.35	< 0.0001
A^2	0.84	1	125.29	-0.28	< 0.0001
B^2	0.166	1	24.76	0.13	0.0002
Residual	0.10	15			
Lack of Fit (LOF)	0.02	3	1.14		0.37
Pure Error	0.08	12			
Corrected Total	45.87	20			
Std. Dev.	0.08			R^2	0.99
Mean	6.30			Adjusted R^2	0.99
C.V. %	1.30			Predicted R^2	0.99
PRESS				Adequate Precision	106.71

The linear effect of Amberlite XAD-16 resin on the reduction of limonin content was less pronounced than the reaction time as can be evidenced from the less negative value of the coefficient of estimate of Amberlite XAD-16 resin (-1.11) than reaction time (-1.21). This could be visualized from the graph presented in Fig. 1 where the concentration of limonin in the final juice has a positive correlation with the concentration of Amberlite XAD-16 resin. Thus, with an increase in the amount of Amberlite XAD-16 resin when reaction time is kept constant, a gradual decline in limonin concentration was recorded. This can also be visualized from the response surface in Fig. 2 that at a definite period if we increase the amount of XAD there is a gradual decline of limonin in treated pummelo juice.

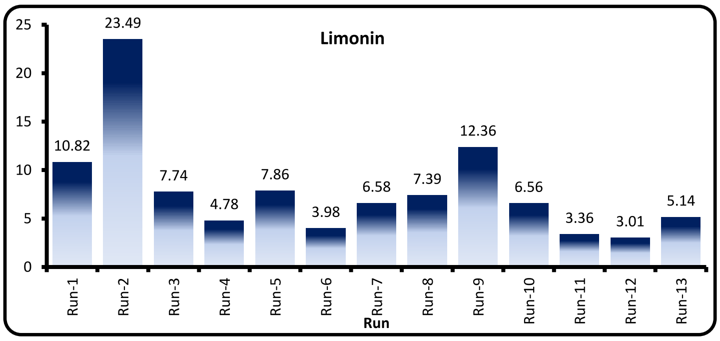

Figure 1. Experimental runs with independent variables (quantity of resin and reaction time)and their response to limonin

Similarly, increasing in reaction time while keeping quantity of Amberlite XAD-16 resin constant, a decline in limonin content was evident from the same Fig. 2. Thus, Amberlite XAD-16 resin, when kept constant, say at a minimum value (200mg/100mL); it was observed that limonin concentration was declining from 7.86 µg/mL for 120 seconds of reaction time to 7.39 µg/mL for 300 seconds of reaction time.

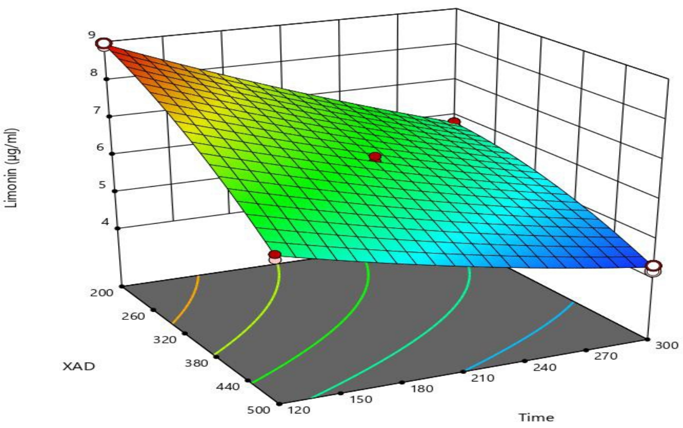

Fig 2. Limonin content as affected by quantity of adsorbent Amberlite XAD-16 concerning reaction time

The adjusted R^2 and predicted R^2 of the model were in close adjustment with each other and adequate precision was much higher than 4, indicating the model developed through this experiment can effectively be used for predicting the limonin removal capacity of Amberlite XAD-16 resin within the space of the design. Adequate precision greater than 4 is desirable and indicates an adequate signal [21]. In this case, the ratio is 106.71 which indicates an adequate signal.

The following empirical equation was developed to predict the removal of bittering compound, limonin within the design space.

Limonin= $1.3 \times 10^{1} - 4.03\times10^{-3}A - 2.9\times10^{-2}B + 2.61\times10^{-5}A\times B - 1.27\times10^{-5}AA^{2} + 1.56\times10^{-5}BA^{2}$

Where, A= Amberlite XAD-16 resin & B= Reaction time

Naringin content

The results of naringin content as adsorbed by Amberlite XAD-16 resin are shown below in Table 4 and Figure 3 and the graph showed that the minimum level of naringin (1284.31 µg/mL) was recorded in run no. 07, while naringin content was maximum (1964 µg/mL) in run no. 05. This might be due to adsorption of naringin by Amberlite XAD-16 through its physical properties, like large surface area for adsorption and cross-linking with naringin molecules. Lee and Kim [22] reported debittering in grapefruit juice and claimed that 78% of naringin content was removed by the XAD-16 adsorption column. Adsorption is a physico-chemical process that involves the mass transfer of a solute (adsorbate) from the fluid phase to the adsorbent surface till the thermodynamic equilibrium of the adsorbate concentration is attained, with no further net adsorption [23]. Lee and Kim (2003) observed a linear relationship between Amberlite resins and with adsorption of naringin through its physical properties such as cross-linking ratios, pore diameters, and specific surface areas. The same authors observed a 78% reduction in bitterness of grapefruit juice based on naringin content.

Table 4. Influence of different combinations (runs) of adsorbent XAD-16 (mg/100 mL) with treatment time (s) and their response on naringin (µg/mL) content

Std	Run	Factor A: XAD-16 (mg/100mL)	Factor B: Treatment Time (s)	Naringin (µg/mL)
11	1	350.00	210.00	1234.84
5	2	137.87	210.00	1541.27
2	3	500.00	120.00	1374.60
6	4	562.13	210.00	1340.85
1	5	200.00	120.00	1964.00
8	6	350.00	337.28	1523.21
4	7	500.00	300.00	1284.31
3	8	200.00	300.00	1669.15
12	9	350.00	210.00	1649.62
13	10	350.00	210.00	1754.71
10	11	350.00	210.00	1591.89
7	12	350.00	82.72	1443.58
9	13	350.00	210.00	1489.17

The linear effect of both quantities of Amberlite XAD-16 resin as well as reaction time on naringin content was found to be significant at a 95% level of confidence (Table 5). The quadratic effect of different quantities of Amberlite XAD-16 resin on naringin content was also found to be significant at a 95% level of confidence. However, the interaction effect of Amberlite XAD-16 resin and reaction time on naringin content was found to be insignificant. The quadratic effect of reaction time was also found to be insignificant.

The linear effect of Amberlite XAD-16 resin on the reduction of naringin concentration was more pronounced than the reaction time as can be evidenced from the more negative value of the coefficient of estimate of Amberlite XAD-16 resin (-169.92) than reaction time (-143.66). This could be visualized from the graph presented in Figure 3. where the concentration of naringin in the final juice has a negative correlation with the concentration of Amberlite XAD-16 resin. Thus, with an increase in the amount of Amberlite XAD-16 resin when reaction time is kept constant, a gradual decline in naringin concentration was recorded. This can also be visualized from the response surface in Figure 4. At a definite period if we increase the amount of XAD there is a gradual decline of naringin in treated pummelo juice. Similarly, increasing in reaction time while keeping the quantity of Amberlite XAD-16 resin constant, a decline in naringin content was evident from Figure 4. Thus, Amberlite XAD-16 resin, when kept constant, say at a minimum value (200mg/100mL); it was observed that naringin concentration declined from 1964 µg/mL for 300 seconds of reaction time to 1669.15 µg/mL for 120 seconds of reaction time.

Table 5. Analysis of variance (ANOVA) for naringin content as affected by quantity of resin and treatment time

Source	Sum of Squares	Df	F-value	Coefficient of Estimate	p-value
Model	836400	5	41.78	1496.05	< 0.0001
A-XAD	462000	1	115.39	-169.92	< 0.0001
B-Time	330200	1	82.47	-143.66	< 0.0001
AB	241.78	1	0.0604	5.5	0.8092
A^2	23050.01	1	5.76	47.25	0.0299
B^2	6608.39	1	1.65	-25.3	0.2184
Residual	60054.88	15			
Lack of Fit	18249.44	3		1.75	0.2108
Pure Error	41805.43	12			
Corrected Total	8.96E+05	20			
Std. Dev.	63.27			R^2	0.93
Mean	1512.78			Adjusted R^2	0.91
C.V. %	4.18			Predicted R^2	0.87
PRESS				Adequate Precision	18.54

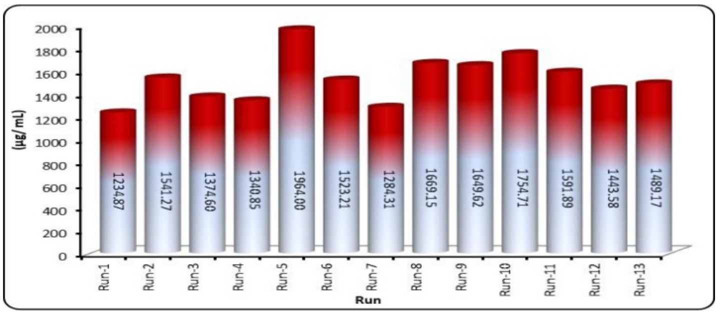

Figure 3 Influence of different combinations (runs) of adsorbent XAD-16 (mg/100 mL) with treatment time (s) and their response on naringin (µg/mL) content

The adjusted R^2 and predicted R^2 of the model were in close adjustment with each other and adequate precision was much higher than 4, indicating the model developed through this experiment can effectively be used for predicting the naringin removal capacity of Amberlite XAD-16 resin within the space of the design. Adequate precision greater than 4 is desirable and indicates an adequate signal [21]. In this case, the ratio is 18.54 which indicates an adequate signal. The following empirical equation in terms of actual value was developed to predict the concentration of naringin within the design space was developed to predict naringin content within the design space.

Naringin = $2.38\times10^{3} - 2.68\times A - 0.42\times B + 4.07\times10^{-4}\times A\times B + 2.1\times10^{-3} - 3.12\times10^{-3}$
Where, A= Amberlite XAD-16 resin and B= Reaction time

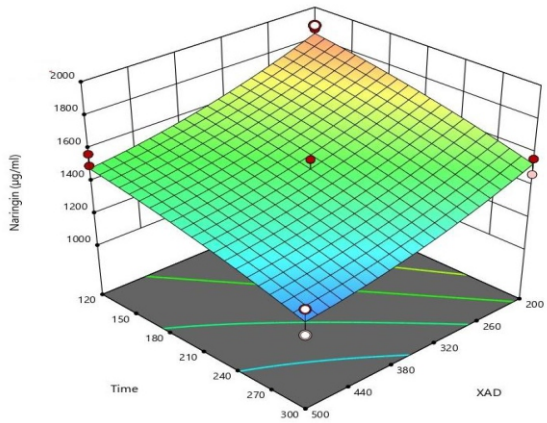

Figure 4. Naringin (µg/mL) content as affected by quantity of adsorbent Amberlite XAD-16 in relation to reaction time

CONCLUSION

The impact of adsorbent Amberlite XAD-16 resin was found to be highly effective in reducing the bitterness of pummelo fruit juice. The pummelo juice was treated in a batch process with different combinations of Amberlite XAD-16 resins (mg/100mL) and reaction time (seconds) formulated by using Response Surface Methodology (RSM). There were 13 different combinations formulated for debittering purposes. The combination of 426.40 mg/100ml Amberlite XAD-16 resin treated for 300 seconds was found to be optimum and very effective in removing the bitterness in the juice. However, the adsorbent Amberlite XAD-16 resin was found to have a more prominent impact in reducing delayed bitterness (limonin 88%) as compared to immediate bitterness (naringin 40%).

Acknowledgment

We acknowledge the help extended by the scientists of Bihar Agricultural University (BAU), Sabour particularly the Department of Food Science and Postharvest Technology and Plant Pathology, BAU, Sabour. We wish to express our sincere thanks to all who helped us directly or indirectly in harvesting, and transportation of pummelo fruits and during analysis.

CONFLICT OF INTEREST

There is no conflict of interest among the author(s) with respect to the research, authorship, and/or publication of this article.

FUTURE SCOPE

The future scope of pummelo juice is immense. It is due to richness of pummelo juice in phytochemicals and health promoting compounds. However, acceptability and commercial success depends on low cost and effective debittering technology.

FUNDING

Funding for this research was partially supported by the Department of Pomology and Postharvest Technology, Faculty of Horticulture, UBKV, Pundibari, Cooch Behar, W.B. (India) and partially supported by Bihar Agricultural University, Sabour, Bhagalpur under faculty development programme by allowing me to pursue PhD as an in-service candidate with full salary.

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