

Effect of Pretreatments on Enzyme Inactivation Kinetics, Quality Attributes, and Microbial Stability of Amla (*Emblica officinalis*) Juice

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Abstract

Amla, highly valued for its exceptional nutritional and medicinal properties, has limited shelf-life and its astringent flavor limits its fresh consumption, thereby, necessitating processing into value-added products such as juice. This study evaluated the influence of different pretreatments - hot water (HB), steam (SB), and microwave blanching (MB) - on enzyme inactivation juice recovery, physicochemical and phytochemical quality, and microbial stability during ambient storage. The Polyphenol oxidase (PPO) and Peroxidase (POD) inactivation during each blanching was fitted to First order, Biphasic and Weibull model. In both the cases of enzyme inactivation, Weibull model was found to best fit the data. The blanching criterion was 90% reduction of enzyme activity; and it was achieved upon treatment for 180s, 120s, and 150s in case of HB, SB, and MB, respectively. Moreover, SB significantly improved juice yield but caused marked browning and substantial degradation of phytochemicals. In contrast, MB and HB produced juice yields comparable to the untreated control, although the latter resulted in greater losses. During seven days of ambient storage, all juice samples exhibited a decline in ascorbic acid and phenolic content, accompanied by increased browning and microbial growth, whereas pH and total soluble solids remained relatively stable. Also, SB juice exhibited the highest degradation rate constant, while MB exhibited lowest for ascorbic acid and HB juice exhibited higher degradation of phenolic compounds during storage in comparison to SB and MB. Spoilage occurred within six days for all treatments, with the control deteriorating fastest (within four days), followed by microwave- and hot water-blanching juices, which spoiled within five days. Overall, the findings demonstrate that the selection of appropriate pretreatment strategies can enhance processing efficiency, improve product quality, and reduce post-processing losses, thereby contributing to more sustainable amla juice production.

Categories: Food processing, Food biochemistry, Food engineering

Keywords: ppo and pod, inactivation kinetics, weibull model, juice recovery, storage study

Introduction

Amla, also referred to as Indian gooseberry (*Emblica officinalis*), is a significant fruit crop that is farmed throughout the Indian subcontinent. Amla is celebrated for opulence of ascorbic acid content, as it is considered to be one of the greatest sources of Vitamin C among all fruits, secondary only to Barbados cherry [1]. Besides, amla fruits have ample amounts of bioactive compounds, such as polyphenols, tannins, flavonoids, etc., that imparts a plethora of health benefits such as anti-oxidants, immunomodulatory, antimicrobial, anticancer, antidiabetic, cardioprotective, gastroprotective activities,

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and so on. Thence owing to its medicinal benefits, it is frequently employed in Ayurvedic and Unani systems [2]. However, fresh amla fruits have limited shelf-life, and its sour & astringent flavor limits its use as fresh fruits. As a result, it is processed into a variety of products, such as juice, squash, preserve, jam, sauce, powder, etc. Out of which amla juice is widely consumed [3].

The conventional method for amla juice preparation involves deseeding of the manual fruits followed by juice extraction by passing the amla flesh through juicing machine and subsequently through muslin cloth or sieves to remove the foreign particles from the juice [4]. However, the high amount of ascorbic acid and acidic pH of amla juice could not prevent enzymatic browning and flavor loss, making it perishable at ambient conditions. Hence, preprocessing of amla fruits seems crucial for preserving the juice quality. Thus, blanching could be used to inactivate the enzymes and reduce microbial load that would in turn have positive impact on the juice quality [5]. Blanching can be done by employing traditional methods such as hot water blanching (HB) and steam blanching (SB) or by improved techniques. As, conventional blanching techniques involve leaching of valuable nutrients and would generate waste waters with high organic load [6]. Hence, to meet the increasing demands of sustainable and minimal processed products, novel heating methods such as microwave, ohmic, etc., might be employed for blanching fruits and vegetables [7].

Microwave blanching (MB) is a novel blanching technology that works on dielectric heating principle and results in better retention of nutrients as compared to traditional methods. Microwave treatment results in faster heating rates and shorter processing time, as compared to conventional blanching, therefore, would lower leaching of water-soluble compounds into water in addition to hastened processing [8]. Furthermore, many studies have employed blanching as a pretreatment before extracting juice from fruits and vegetables such as carrot juice [9], apple juice [10], bottle gourd juice [11], etc. However, limited work has been reported on the effect of blanching pre-treatment on the quality of amla juice. In addition, no study to best of our knowledge has explored the kinetic insights on enzyme inactivation during blanching pretreatment. Therefore, this study is focused on evaluating the effect of various blanching techniques on the enzyme inactivation kinetics, juice yield and quality attributes such as its physico-chemical characteristics, phytochemical characteristics and microbial load of the amla juice during storage at ambient temperature of 25°C.

Materials And Methods

Raw materials and chemicals

Fresh Indian gooseberry fruits (c.v. Banarasi) were obtained from the farms of Chandra Shekhar Azad University, Kanpur, India. Mature fruits (3 kg: 80-90 fruits) were sorted for uniform size (35 ± 2 g) and washed under running water to get rid of adhering dirt particles and to reduce the microbial load on the fruit. The fruits were stored at the refrigerated conditions ($5^{\circ}\text{C} \pm 1^{\circ}\text{C}$; 90% relative humidity) until they were processed. All the chemicals and reagents used in the experimentation were of analytical grade.

Pretreatments of amla fruit

Amla fruits were subjected to HB, SB, and MB in batches of 200 g amla fruits for 180 seconds and the enzyme activity was recorded at the 30-second interval. For HB, the fruits were blanched in hot water for 30-180 seconds maintained at 85°C , taking fruit: water ratio of 1:2. In case of SB, amla fruits were steam blanched for 30-180 seconds, using a blanching vessel. In MB, the fruits placed in water (1:2) were treated for 30-180 seconds in microwave at 4.5 W/g power density (900W output power, Model MC900W, LG, India). The blanched fruits were immediately cooled for 60 seconds in ice to prevent cooking

Determination of enzyme activity

Polyphenol oxidase (PPO) activity was assessed following the procedure outlined in available literature [12], with slight adjustments. Briefly, 1.5 mL of amla juice, initially centrifuged at $10000\times g$ for 10 min at 4°C , was mixed with 0.5 mL of pyrocatechol (0.5 mol/L) and 3 mL of potassium phosphate buffer (pH 7.0). The increase in absorbance was monitored at 420 nm at an interval of 30 seconds over a period of 180 seconds using a double-beam UV-visible spectrophotometer (Cary 60 UV-Vis, Agilent,) calibrated against phosphate buffer. One unit of PPO activity was defined as an increase in

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absorbance of 0.001 per min; and the enzyme activity was expressed as residual activities (%). Furthermore, for assessment of Peroxidase (POD) activity the reaction mixture consisted of 2 mL of amla juice (initially centrifuged at 10,000×g for 10 min at 4°C), 1 mL of 0.2M guaiacol solution and 2 mL of phosphate buffer (pH 6.5). Absorbance changes at 420 nm were recorded every 30 seconds for 180 seconds using the same spectrophotometer as described above. One unit of POD activity was defined as an absorbance change of 0.001 per min. The residual activity was calculated from the linear section of the absorbance-time curves, and it was expressed using the respective calculation equation.

$$\text{Residual Activity (\%)} = \frac{\text{Current activity}}{\text{Initial activity}} \cdot 100 \quad (1)$$

Enzyme inactivation kinetics

The inactivation behavior of PPO and POD during blanching pretreatments was evaluated using three commonly employed kinetic models: first-order, biphasic, and Weibull models [13], Eq. 2-4. These models were chosen as they represent different degradation mechanisms, ranging from simple exponential inactivation to more complex, multi-phase responses, thus allowing a more holistic interpretation of enzyme thermal sensitivity. Traditionally, thermal inactivation of enzymes is described by first-order kinetics, which assumes a single-step, irreversible conversion of active enzymes into their inactive form. The biphasic model, in contrast, postulates that the enzyme population comprises two isoenzymes that inactivate simultaneously, both following first-order decay but at different rates. The Weibull model, proposed as an alternative to linear decay kinetics, incorporates variability in enzyme resistance, where inactivation rates are affected by treatment severity and possible resistance development [14]. This model is defined by two parameters: the scale factor (n), which influences the reaction rate constant ($k = 1/n$, min^{-1}), and the shape parameter (b), which determines the curve's form. Together, these models provide a robust framework for describing enzyme inactivation kinetics under thermal processing, accommodating both simple and heterogeneous degradation pathways.

First-order kinetics model:

$$\frac{A_t}{A_o} = \exp(-kt) \quad (2)$$

Biphasic model:

$$\frac{A_t}{A_o} = A_L \exp(-k_L t) + A_S \exp(-k_S t) \quad (3)$$

Weibull model:

$$\frac{A_t}{A_o} = \exp\left(-(kt)^b\right) \quad (4)$$

here, A_t is the enzyme activity at time t , A_o is initial enzyme activity, A_L is the proportion of thermolabile enzyme and A_S is the fraction of thermostable enzyme; k is the inactivation rate constant (min^{-1}), k_L is the inactivation rate of labile fraction (min^{-1}) and k_S is the inactivation rate of stable fraction (min^{-1}); and b is the shape parameter.

Extraction of amla juice

The pretreated fruits were manually de-seeded and its juice was extracted using a commercial juicer (Bechtop Equipment Inc., India). The extracted juice was subsequently filtered through a double-layer muslin cloth to remove coarse fibrous particles and residual seed fragments. No centrifugation or further clarification was performed. The filtered juice was immediately transferred and sealed into sealed polyethylene terephthalate (PET) bottles at 25°C for 7 days. An identical extraction and filtration procedure was followed for all treatments to ensure consistency in juice yield determination and quality evaluation. The juice samples were further analyzed for physico-chemical and phytochemical characteristics at an interval of 1 day.

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Quality analysis

Juice Yield

Juice recovery, in percentage basis was calculated in terms of juice extracted from the treated fruits subsequent to manual deseeding, using the following equation:

$$\text{Juice. yield (\%)} = \frac{\text{Weight. of. juice. recovered}}{\text{Weight. of. fruit} - \text{Weight. of. seed}} \times 100 \quad (5)$$

Physico-chemical characteristics

pH and Total Soluble Solids

pH of the juice samples was determined by using a pH meter, while the total soluble solids (TSS) were measured by using hand refractometer (Erma, Japan). The TSS values were expressed in degree brix.

Titrateable Acidity

Titrateable acidity (TA) and reducing sugar were estimated by titrimetric methods using 0.1N NaOH and Fehling A & B solutions, respectively [15].

Nonenzymatic Browning

Non-enzymatic browning in juice was measured in terms of optical density of ethanol extracted samples at 420 nm using a UV-VIS spectrophotometer (Agilent Cary 3500) [16].

Phytochemical characteristics and kinetic considerations

Ascorbic Acid

Ascorbic acid content of juice was determined by titrimetric method using 2,6-dichloro indophenol dye solution, [15].

Total Phenolic Content

The total phenolic content (TPC) was estimated spectrophotometrically against methanol as blank, at 760 nm using Follin and Ciocalteu's phenol reagent [17]. The TPC values were expressed in terms of gallic acid equivalents (mg GAE/L).

Phytochemical degradation kinetics

First-order model in its exponential form was used to estimate the kinetic behavior of degradation of ascorbic acid and total phenolic content of amla juice during storage.

$$\frac{C_t}{C_o} = \exp(-kt) \quad (6)$$

here, C_t corresponds to concentration of phytochemical (ascorbic acid or total phenolic content) at time t ; C_o corresponds to initial concentration of phytochemical and k (min^{-1}) corresponds to degradation rate constant.

Microbial enumeration

The effects of blanching pretreatments on the microbial load of amla juice were evaluated in terms of total plate count and yeast & mold count using the pour plate method [4]. The standard plate count agar and yeast & mold agar was used as respective growth media which was incubated for 48 hours at $25 \pm 1^\circ\text{C}$. The detection limit was set as 10 colony-forming units (cfu)/mL of juice.

Statistical analysis

All the experiments were conducted in triplicates and the data were expressed as mean $\pm$ standard deviation. Nonlinear regression was used to fit the mathematical model to the experimental data in order to estimate the parameters. The correlation coefficient (R^2), the reduced Chi square (χ^2), the Root Mean Square Error (RMSE), and the analysis of residual

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plot were the criterion analyzed to assess the adequacy of the models in fitting the effect of blanching pretreatment on enzyme residual activity. The goodness of fit can be indicated by the higher R^2 and lower χ^2 values, while RMSE value provides the deviation between the experimental and anticipated values. Data were statistically analyzed with Minitab software version 17.1.0 (Minitab LLC, USA). The difference in the means was determined using Tukey's test at $P < 0.05$ by one-way analysis of variance (ANOVA).

Results And Discussion

Effect of blanching pretreatment on enzyme inactivation

The efficiency of HB, SB, and MB in inactivating PPO and POD in amla fruit was assessed for 180 seconds, with enzyme activities measured at 30 seconds intervals (Table 1). In HB, PPO activity showed a gradual decline from 100% to 89.21% at 30 seconds, 49.44% at 90 seconds, and finally 9.90% at 180 seconds. POD was comparatively less sensitive, decreasing from 100% to 93.58%, within the first 30 seconds and further reducing to 9.99% after 180 seconds. MB demonstrated better enzyme inactivation, although was found less effective than SB. MB could decrease the PPO and POD activity to 8.12 and 8.23%, respectively. While SB demonstrated greatest enzyme inactivation efficiency, with PPO activity decreasing from 100% to 6.26%, and POD reaching 7.87% at 180 seconds. Overall, PPO exhibited significantly greater susceptibility to all blanching methods than POD, confirming its thermolabile nature. Besides, among the treatments, steam blanching proved to be the most effective in suppressing enzymatic activity, achieving the highest level of inactivation for both enzymes. It resulted in reduction of residual enzyme activity below the widely accepted blanching threshold of 10% within 120 seconds [4]. In comparison, microwave blanching required up to 150 seconds, while hot water blanching took 180 seconds to achieve a similar level of inactivation. Furthermore, the superior efficacy of steam blanching maybe attributed to its higher heat transfer efficiency and the rapid penetration of moist heat into tissue matrices, which accelerates protein denaturation and disruption of catalytic sites. Optimizing blanching conditions for amla fruits, can play a critical role in enhancing enzyme stability, preserving product quality, and minimizing post-processing browning in amla fruit and the juice extracted thereafter. Henceforth, for further juice extraction, fruits were steamed blanched for 180 seconds, 120 seconds, and 150 seconds in case of HB, SB, and MB, respectively.

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Time (s)	Residual activity (%)					
	PPO			POD		
	HB	SB	MB	HB	SB	MB
0	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
30	89.21 ± 0.19	80.46 ± 0.17	85.24 ± 0.21	93.58 ± 0.24	89.76 ± 0.15	90.87 ± 0.21
60	68.47 ± 0.14	51.30 ± 0.15	64.97 ± 0.18	75.49 ± 0.19	56.24 ± 0.16	73.52 ± 0.16
90	49.44 ± 0.13	20.96 ± 0.14	33.26 ± 0.17	52.33 ± 0.14	29.37 ± 0.11	48.61 ± 0.14
120	31.06 ± 0.12	9.62 ± 0.09	21.35 ± 0.15	36.82 ± 0.13	9.94 ± 0.08	26.47 ± 0.13
150	20.21 ± 0.11	7.23 ± 0.08	9.26 ± 0.10	23.27 ± 0.11	8.34 ± 0.09	9.94 ± 0.09
180	9.90 ± 0.08	6.26 ± 0.07	8.12 ± 0.08	9.99 ± 0.03	7.87 ± 0.07	8.23 ± 0.07

TABLE 1: Changes in enzyme residual activity during blanching of amla fruits.

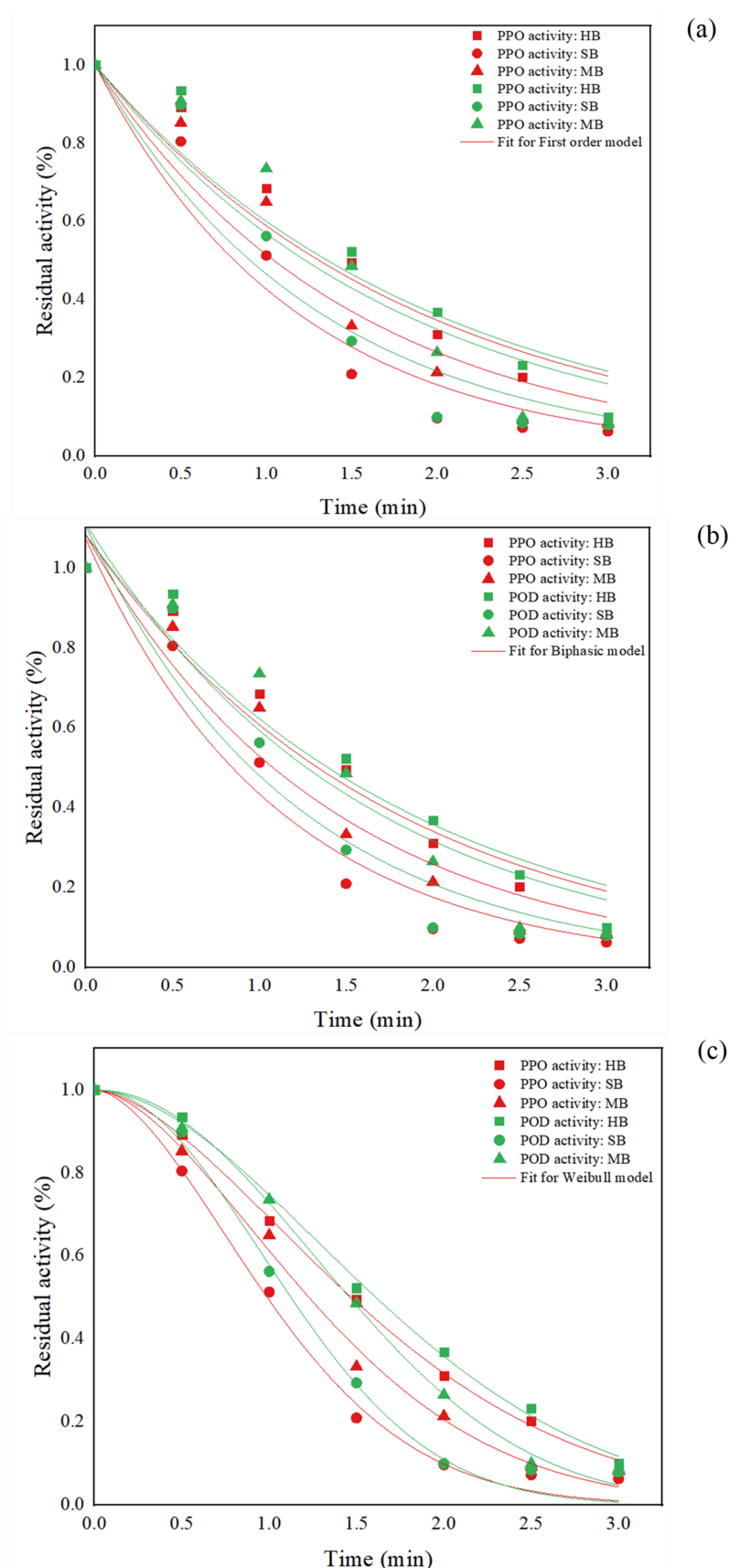
PPO, Polyphenol Oxidase; POD, Peroxidase; HB, Hot Water Blanching; MB, Microwave Blanching; SB, Steam Blanching

Mathematical modeling for enzyme inactivation kinetics

The kinetic behavior for inactivation of PPO and POD during different blanching treatments were evaluated. Residual activity data for the enzymes were fitted into three kinetic models and the corresponding kinetic constants and coefficients under different pretreatments are given in Table 2. These models were evaluated on the basis of correlation coefficient (R^2), reduced Chi square (χ^2), the Root Mean Square Error (RMSE) value, and residual plots. The PPO and POD residual activities of amla decreased with increasing time for each blanching treatment (Figure 1). This decrease in residual activity of PPO was observed to decline abruptly in case of steam blanching, as the curve was steeper at the beginning and became nearly constant in the latter phase. In contrast, the curve was less stepper in case of microwave blanching and hot water blanching, consecutively. Furthermore, the first-order model assumes a constant inactivation rate proportional to the residual enzyme activity (Figure 1a). It recorded reasonably high R^2 values (0.9367-0.9482) and (0.8947-0.9342) for PPO & POD, nonetheless χ^2 and RMSE were higher, suggesting deviations between experimental and predicted values, besides, the residual plots also showed random distribution. On the other hand, the biphasic model which accounts for two isoenzyme fractions with different thermal resistances, was associated with R^2 values 0.8297-0.9107 lesser and higher χ^2 ($1.11-2.47 \times 10^{-3}$) and RMSE (0.0332-0.3555) when compared with the first-order model. In addition, values of the model parameters k_1 and k_s were found to be equal, thereby nullified the presence of isoenzymes (Figure 1b).

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FIGURE 1: PPO and POD residual activity of amla juice obtained from different treatments of amla fruits. Continuous lines represent the (a) First-order model, (b) Biphasic model, and (c) Weibull model.

PPO, Polyphenol Oxidase; POD, Peroxidase; HB, Hot Water Blanching; MB, Microwave Blanching; SB, Steam Blanching

Furthermore, the enzyme inactivation data was fitted to Weibull model. The Weibull model consistently provided best fit for both PPO and POD inactivation for different blanching techniques (Figure 1c). It recorded highest R^2 values of 0.9928-0.9994 for PPO inactivation and 0.9975-0.9978 for POD inactivation; along with lowest χ^2 and RMSE values of 1.10×10^{-3} - 6.42×10^{-5} and 3.16×10^{-3} - 4.13×10^{-3} and 0.0034-0.0050 and 0.0014-0.0021 for PPO and POD inactivation. Its efficacy lies in its flexibility, as the shape (b) and scale (n) parameters allow adaptation to both the sharp initial decline and the slower tailing phase of enzyme inactivation. This dual-phase representation is particularly important in PPO and POD systems, which often display rapid thermal denaturation of labile fractions followed by the persistence of more resistant isoenzymes. The random distribution of residuals further confirmed Weibull's superiority in capturing the kinetics. Overall, although first-order and other discrete models gave acceptable statistical fits, they were unable to represent the complex thermal behavior of enzymes. The Weibull model emerged as the most reliable approach, offering both statistical accuracy and biological plausibility in describing PPO and POD inactivation during blanching pre-treatment.

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Blanching treatment	Blanching treatment	R^2	χ^2	RMSE	k	b	A_L	A_S	K_L	K_S
PPO inactivation										
First-order model	HB	0.9401	7.17×10^{-3}	0.0043	0.52905					
	SB	0.9482	7.86×10^{-3}	0.0472	0.85104					
	MB	0.9367	8.83×10^{-3}	0.0530	0.66305					
Bi phasic	HB	0.9076	1.11×10^{-2}	0.0332			0.5413	0.5413	0.5781	0.5781
	SB	0.9107	1.35×10^{-2}	0.0406			0.5362	0.5362	0.9041	0.9041
	MB	0.8955	1.46×10^{-3}	0.0437			0.5413	0.5413	0.7165	0.7165
Weibull	HB	0.9994	6.66×10^{-5}	0.0034	0.5444	1.6385				
	SB	0.9958	6.42×10^{-4}	0.0032	0.8171	1.7141				
	MB	0.9928	1.01×10^{-3}	0.0050	0.6561	1.6916				
POD inactivation										
First order model	HB	0.9342	9.02×10^{-3}	0.0451	0.5099					
	SB	0.9152	1.39×10^{-3}	0.0839	0.7643					
	MB	0.8947	1.53×10^{-3}	0.0917	0.5633					

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Bi phasic	HB	0.8706	1.77×10^{-3}	0.0355			0.5416	0.5416	0.5540	0.5539
	SB	0.8594	2.31×10^{-3}	0.0695			0.5524	0.5524	0.8335	0.8335
	MB	0.8297	2.47×10^{-3}	0.0742			0.5552	0.5552	0.6273	0.6273
Weibull	HB	0.9975	3.48×10^{-4}	0.0014	0.5075	1.1818				
	SB	0.9975	4.13×10^{-4}	0.0021	0.7415	2.0155				
	MB	0.9978	3.16×10^{-4}	0.0016	0.5735	2.0581				

TABLE 2: Statistical regression results and kinetic parameters for models fitted to amla blanching data.

HB, Hot Water Blanching; SB: Steam Blanching; MB: Microwave Blanching; PPO, Polyphenol Oxidase; RMSE, Root Mean Square Error R^2 is the correlation coefficient, χ^2 is the reduced Chi square, k is the inactivation rate constant (min^{-1}); b is the shape parameter; A_L is the proportion of thermolabile enzyme; A_S is the fraction of thermostable enzyme; k_L is the inactivation rate of labile fraction (min^{-1}) and k_S is the inactivation rate of stable fraction (min^{-1}).

Effect of blanching pretreatment on juice yield

The amount of fruit juice collected was affected by blanching the fruits before extracting juice. Blanching softens the fruit tissue and makes juice extraction easier, and therefore result in a higher juice yield, however, depending on the blanching circumstances used, the results might vary [18]. As established earlier, fruits were given HB treatment for 180 seconds; SB treatment for 120 seconds and microwave blanching treatment for 150 seconds; and their juice recovery was calculated. In agreement with the previously indicated fact, juice recovery from amla fruits was found to increase in all pretreated fruit samples as compared to the raw control (Figure 2). SB resulted in maximum recovery percentage (61.93%); this was considerably greater than in case of MB (58.09%) and HB (57.41%) ($P < 0.05$). Nonetheless, for the latter processes, the juice recovery was almost equal to that of the control samples (56.79%). Steam when applied to fruit during the blanching process, softens the fruit's outer layers and forms microchannels within the fruit tissue, which facilitates more effective juice release [19]. However, HB and MB both improve juice extraction; the former being somewhat more effective than the latter because it uses microwave radiation to boost heating rates. For this reason, juice recovery was slightly greater in the former [8]. Similar findings on the impact of SB and HB on amla fruit juice recovery have been published [20].

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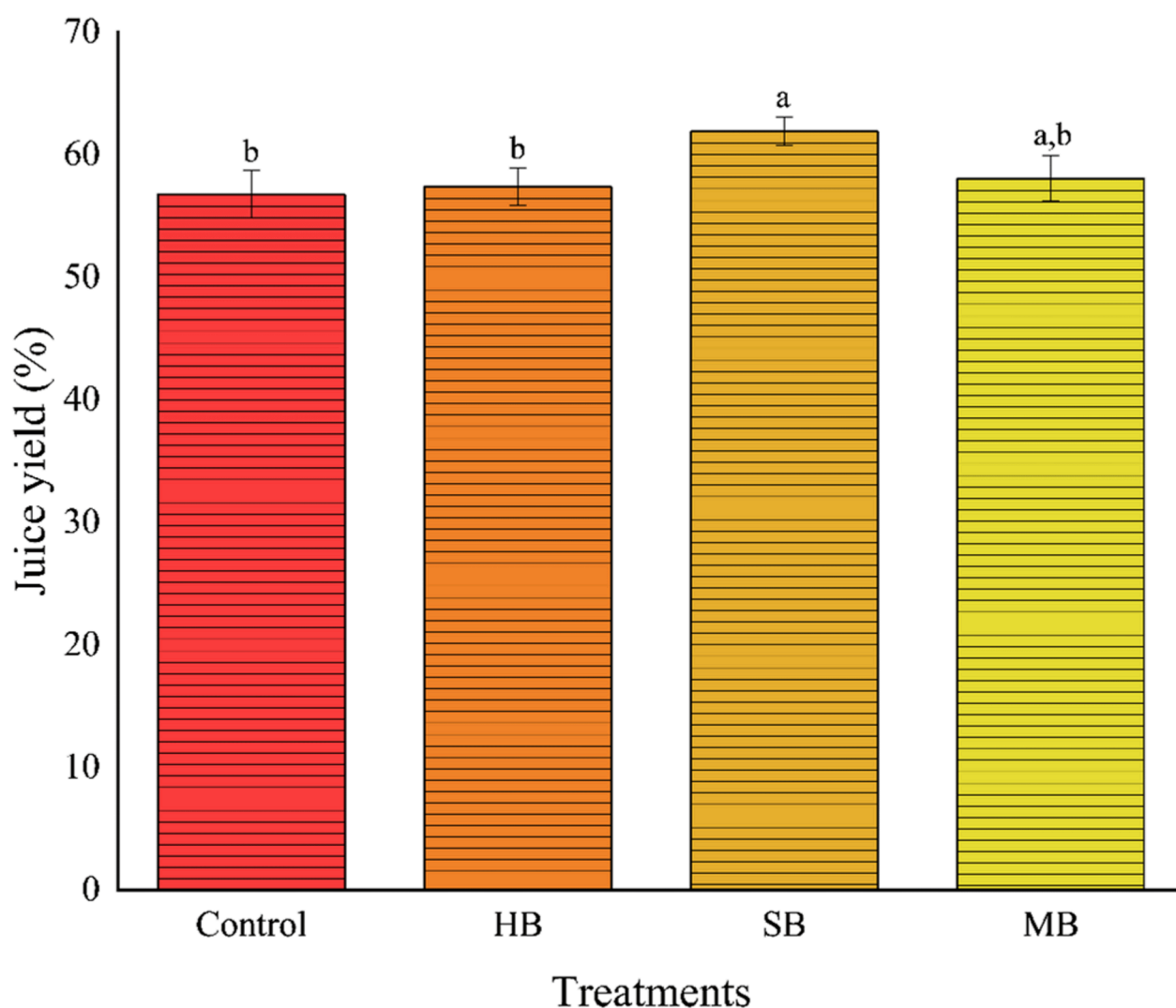


FIGURE 2: Effect of pretreatment on juice recovery from amla fruit.

HB, Hot Water Blanching; SB, Steam Blanching; MB, Microwave Blanching; Means with a different letter are significantly different, determined by Tukey's test ($P < 0.05$).

Effect of blanching pretreatment on physico-chemical characteristics of amla juice during storage

The effect of HB, SB, and MB treatment given for optimum time on the physico-chemical characteristics of amla juice has been tabulated in Figure 3.

pH and Total Soluble Solids

Blanching result in minimal changes in pH and total soluble solids (TSS) (Figure 3a and b). pH values of amla juice ranged between 2.22 and 2.30, indicates its highly acidic nature. Besides, the TSS values for amla juice samples were recorded to vary between 12.5 and 13.9, with SB producing the highest TSS (13.9 °Brix) due to tissue softening, which allows easier extraction of soluble solids and sugars into the juice [21]. HB and MB involve leaching of sugars and soluble solids into the immersed water resulting in lower TSS, compared to steam blanching, although still higher than compared to control due to the heating effect during blanching [22]. Furthermore, the leaching phenomena is more pronounced in the former process [8]. Furthermore, the storage study revealed that the pH and TSS of the juice did not change significantly throughout the storage period. Although, pH increases, rather slightly, upon storage. The slight increase in pH implies

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reduction in acidity of the juice, which might be a consequence of degradation of acidic compounds during ambient storage. In addition, the TSS content of amla juice also increases over the time. This can be due to the biochemical processes, such as breakdown of more complex carbohydrates into simple sugars, which contribute to higher soluble solids content in the juice [23]. Similar observations has been reported in case of amla juice [24] and amla juice-based beverages [23].

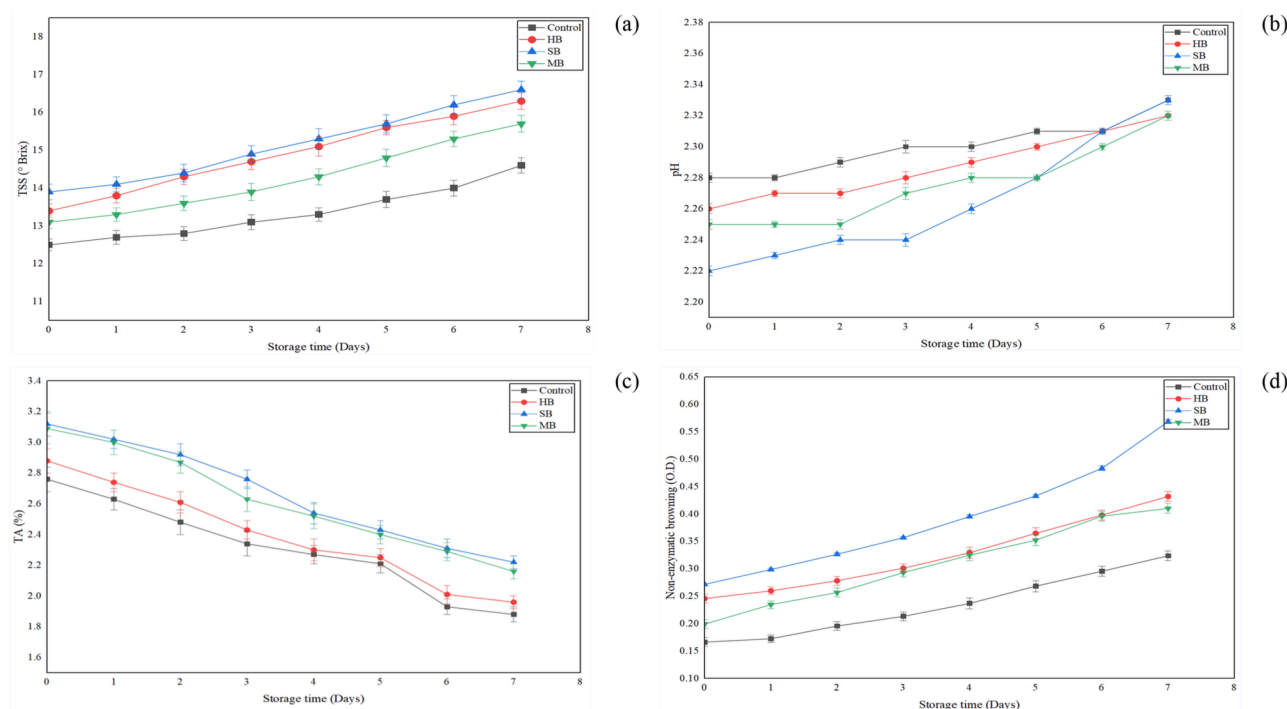


FIGURE 3: Effect of pretreatment on (a) pH, (b) TSS, (c) TA, and (d) nonenzymatic browning of amla juice during storage at room temperature.

HB: Hot Water Blanching; SB: Steam Blanching; MB: Microwave Blanching; TSS, Total Soluble Solids; TA, Titratable Acidity

Titratable acidity

Additionally, the titratable acidity (TA) of juice ranged between 2.76 to 3.12%, with SB resulting in the highest value, though not significantly different for the other treatments (Figure 3c). Similar to carrot juice, wherein the blanching increased its titratable acidity to 0.09-0.015%, slightly higher than unblanched carrot juice (0.08%) [25]. Besides, the titratable acidity of amla juice decreases during storage, seconded by the increase in pH, as pH of fruit juice is a negative function of its acidity [26]. The primary reason behind the decrease in titratable acidity of juice is the degradation of organic acids within the juice during storage, in addition to the compositional changes including potential loss of volatile acids that causes subsequent altering acidity levels. Furthermore, the microbial growth during storage also plays a significant role in affecting the acidity of the juice. As the microbes can metabolize organic acids thereby contributing in exacerbating the decline in acidity as storage time extends [27].

Nonenzymatic browning

Blanching pretreatment significantly affected the physical appearance of the extracted amla juice, particularly, in terms of browning caused by the thermal process. The notable effect of blanching can be seen in the Figure 4. Furthermore, the nonenzymatic browning increased in all blanched juice samples as indicated by Figure 3d, with SB resulting in most prominent browning (0.2783 OD), followed by HB (0.2458 OD), and MB (0.1993 OD). Control samples recorded least

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browning (0.1664 OD). The higher degree of browning in SB and HB is likely due to greater heat exposure. A similar observation was reported in amla fruits [7]. Besides, prominent changes in terms of browning were observed in amla juice during storage. Nonenzymatic browning increased in all juice samples over the time; however, it was significantly high in case of SB, due to initial surge in browning during blanching that continued to rise with storage time period. While comparable browning elevation was observed in case of HB and MB, which were again comparatively higher than in control samples. Similar observations regarding the increase in NEB in amla juice during storage at room temperature have also been reported earlier [24].

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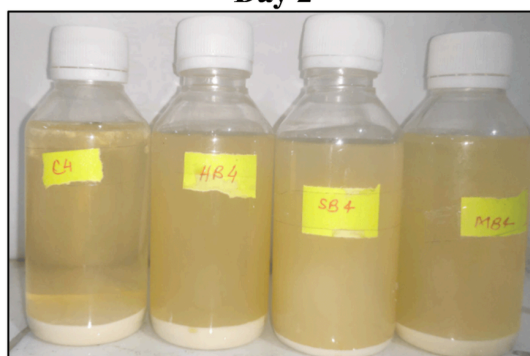
Day 1



Day 2



Day 3



Day 4



Day 5



Day 6



Day 7

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FIGURE 4: Effect of blanching pre-treatment on amla juice during 7 days of storage.

C, Control; HB, Hot Water Blanching; SB, Steam Blanching; MB, Microwave Blanching

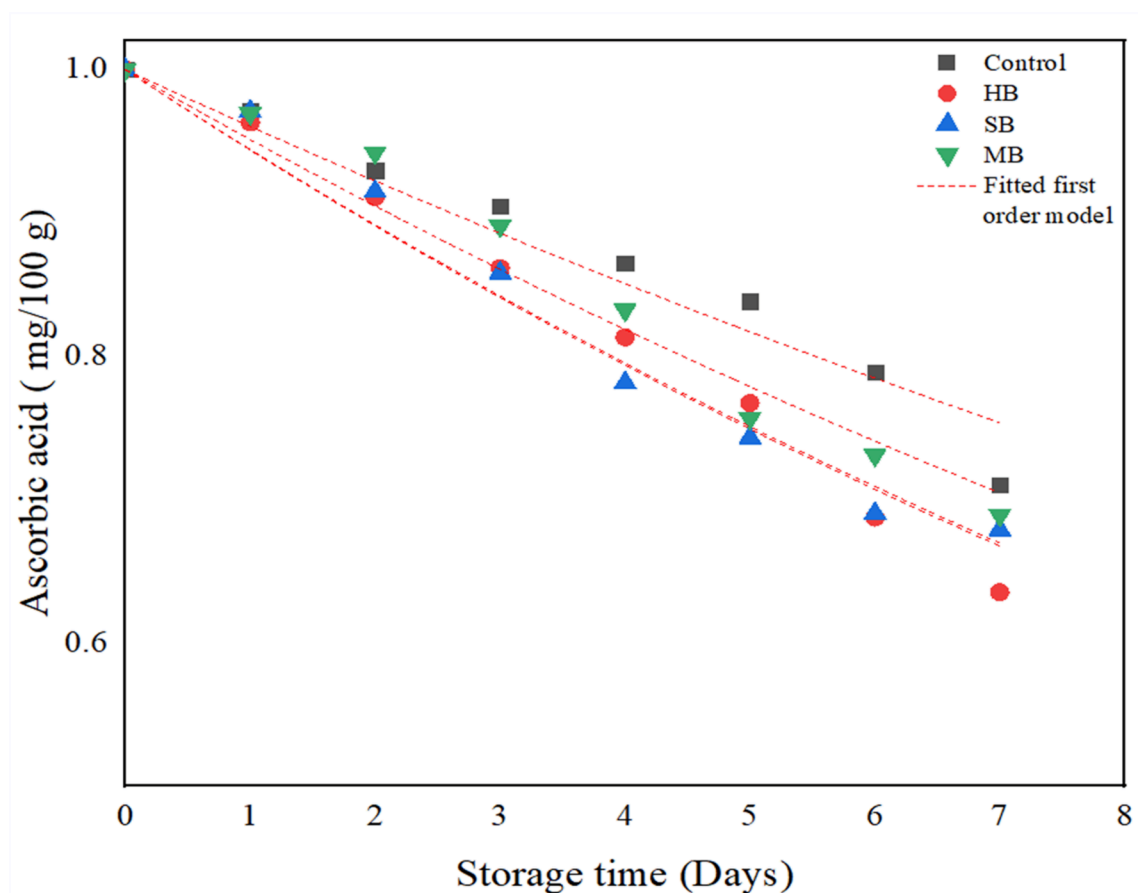
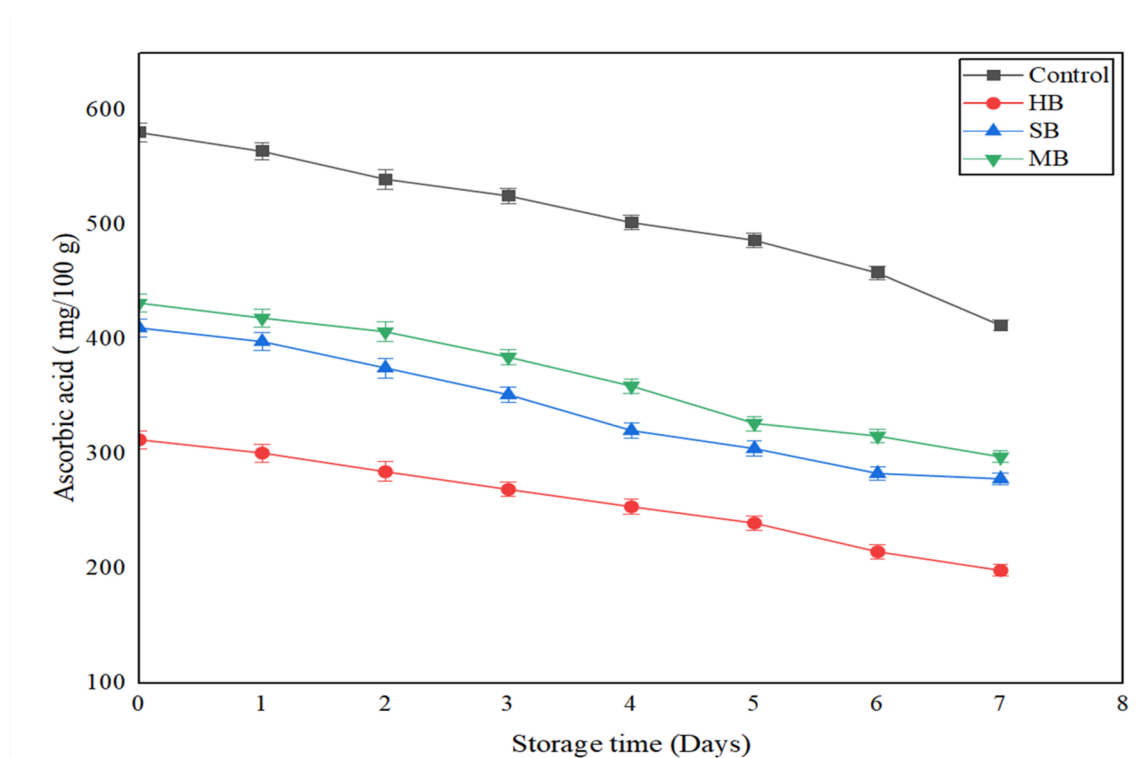
Kinetic consideration of the effect of blanching pretreatment on phytochemical characteristics of amla juice during storage

Ascorbic Acid

A significant reduction in the ascorbic acid content of amla juice was observed upon blanching, due to its heat sensitive and water-soluble properties [2]. MB resulted in 25.62% loss while SB and HB caused 29.37% and 46.18% degradation, respectively, compared to control (580.71 mg/100 g) (Figure 5a). MB retained more ascorbic acid due to internal heat generation, while in contrast SB caused more degradation due to higher heat. On the other hand, HB was associated with prominently higher destruction of ascorbic acid, which might be attributed to thermal destruction of ascorbic acid along with high amounts of leaching losses upon exposure to high temperature and oxygen [28]. A similar observation, of significantly higher degradation of ascorbic acid (AA) in case of HB (53.45%), when compared to MB (24%), has been recorded in amla fruits [7]. Besides, lower decrease in ascorbic acid content of amla juice was recorded in case of amla fruits that underwent SB when compared to water-blanching fruits for the same time duration has been observed [20]. Additionally, upon storage, the AA content in amla juice declined significantly, as represented in Figure 5a. This might be due to its oxidation to dehydroascorbic acid by the oxygen dissolved in the juice, during room temperature storage [24]. Furthermore, the degradation of ascorbic acid during storage was observed to be most pronounced in case of HB (36.47%), closely followed by SB (32.15%) and MB (31.09%) samples, in comparison to values at day 0. While a loss of 30.99% was recorded in case of control samples. Significant loss in the ascorbic acid content of amla juice during room temperature storage has also been reported previously [24].

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FIGURE 5: (a) Effect of pretreatment on ascorbic acid and (b) degradation kinetics of ascorbic acid content of amla juice during storage at room temperature.

HB, Hot Water Blanching; SB: Steam Blanching; MB: Microwave Blanching

Furthermore, the degradation kinetics of AA in amla juice obtained from different pretreatments were evaluated throughout the 7-day ambient storage period using a first-order kinetic model. The estimated degradation rate constants (k) and goodness-of-fit statistics are presented in Table 3, while the experimental and predicted degradation profiles are illustrated in Figure 5b. The first-order model adequately described AA degradation for all treatments, with high coefficients of determination ($R^2 = 0.9523$ - 0.9799) and low reduced chi-square values, indicating good agreement between the experimental and predicted data. Ascorbic acid content declined progressively during storage irrespective of the pretreatment; however, the degradation rate varied among treatments. Steam-blached juice exhibited the highest degradation rate constant ($k = 0.0577 \text{ day}^{-1}$), followed closely by hot water-blached juice ($k = 0.0573 \text{ day}^{-1}$), indicating a relatively faster loss of AA during storage. In contrast, microwave-blached juice showed a comparatively lower degradation rate ($k = 0.0500 \text{ day}^{-1}$), whereas the untreated control exhibited the lowest apparent degradation rate ($k = 0.0404 \text{ day}^{-1}$). Although the control displayed the smallest rate constant, its initial ascorbic acid content was substantially lower due to the absence of pretreatment-induced enzyme inactivation and the greater extent of oxidative deterioration during storage. The steeper decline observed in steam- and hot water-blached juices during the initial storage period suggests that thermal processing accelerated AA degradation, possibly due to heat-induced oxidation and the increased susceptibility of residual ascorbic acid to nonenzymatic oxidative reactions. Conversely, MB better preserved AA during storage, likely because of its shorter heating duration and reduced thermal exposure, thereby retaining higher vitamin C levels while still ensuring effective enzyme inactivation.

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Phytochemical attribute	Blanching treatment	Statistical parameters			Model coefficients
		R^2	χ^2	RMSE	k
AA	Control	0.9523	4.43×10^{-4}	0.0031	0.0404
	HB	0.9715	4.74×10^{-4}	0.0033	0.0573
	SB	0.9799	3.13×10^{-4}	0.0021	0.0577
	MB	0.9622	5.18×10^{-4}	0.0036	0.0500
TPC	Control	0.9349	5.28×10^{-4}	0.0037	0.0348
	HB	0.9334	6.99×10^{-4}	0.0049	0.0401
	SB	0.9349	5.49×10^{-4}	0.0039	0.0352
	MB	0.9514	4.24×10^{-4}	0.0030	0.0370

TABLE 3: Statistical regression results and kinetic parameters for first-order model fitted to phytochemical degradation data during storage.

HB, Hot Water Blanching; SB: Steam Blanching; MB, Microwave Blanching; TPC, Total Phenolic Content

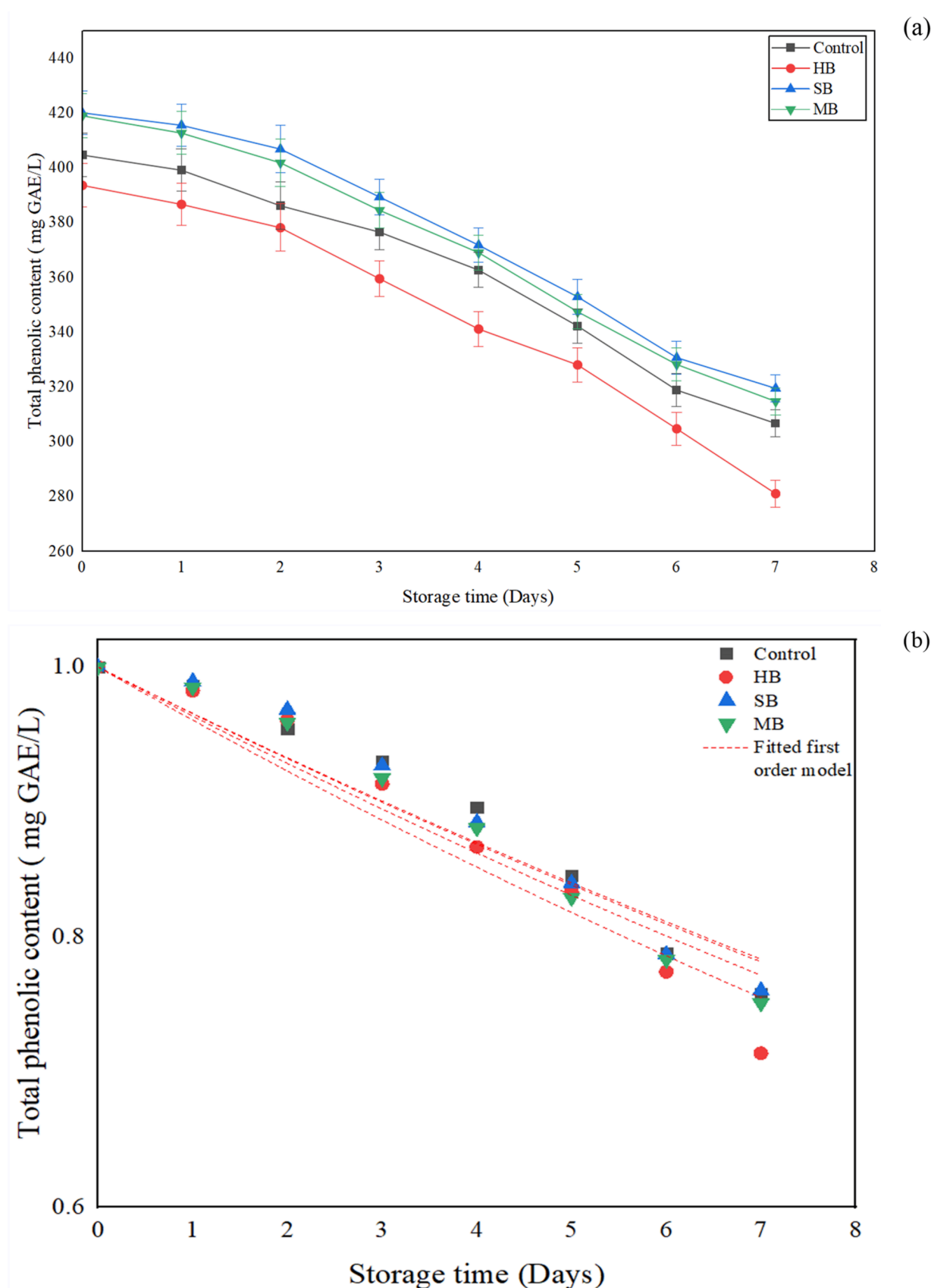
R^2 is the correlation coefficient, χ^2 is the reduced chi square, RMSE is the Root Mean Square Error; k is the inactivation rate constant (min^{-1}).

Total phenolic content

The total phenolic content (TPC) in control juice was 404.74 ± 9.56 mg GAE/L. The blanched samples had a notable increasing trend in total phenolic content (Figure 6a) following nearly, 3.81 and 3.54% in SB and MB, respectively, in relation to the untreated sample. Nonetheless, juice extracted from hot water-blanched samples showed decreasing trend (2.73%). This rise in phenolic content in case of SB and MB might be attributed to the release of certain bound phenolic acids from the cellular breakdown in plant cell walls during the blanching process [29]. Besides, the inactivation of polyphenol oxidase during the blanching, which is responsible for the destruction of polyphenols, may also result in the rise in phenolics [30]. Analogous results were documented for amla fruits [7], tomato [31], apple, orange, and banana peels [29]. However, in case of HB, the decrease in polyphenolic compounds might be due to their thermal breakdown, diffusion, and leaching processes [32]. A similar observation has been reported in carrot juice extracted from water blanched carrots ($300.17 \mu\text{g GAE/g}$), wherein, 3.69 % decrease was observed in comparison to control unblanched carrots ($311.69 \mu\text{g GAE/g}$) [32]. Furthermore, irrespective of the trend observed in juice samples extracted post various blanching pretreatments, a decreasing trend in the total phenolic content was noticed during storage, since polyphenols are water-soluble in addition to being sensitive to oxidation. The greatest degradation of polyphenols was observed in case of hot water-blanched samples (28.59%), followed by microwave blanched (24.88%), control (24.20%), and steam-blanched samples (23.95%). The amla juice in case of the latter three samples was observed to have comparable fall during the 7 days of storage.

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FIGURE 6: (a) Effect of pretreatment on total phenolic content and (b) degradation kinetics of total phenolic content of amla juice during storage at room temperature.

HB, Hot Water Blanching; SB, Steam Blanching; MB, Microwave Blanching

Furthermore, the degradation kinetics of total phenolic content in amla juice subjected to different pretreatments were evaluated during the 7-day ambient storage period using a first-order kinetic model. The estimated degradation rate constants (k) together with the model-fitting statistics are presented in Table 3, while the experimental and predicted degradation profiles are shown in Figure 6b. The first-order model adequately described the degradation behavior of TPC for all treatments, as indicated by satisfactory coefficients of determination ($R^2 = 0.9334-0.9514$) and low reduced chi-square values, demonstrating good agreement between the experimental and predicted data. A gradual decline in TPC was observed throughout storage irrespective of the pretreatment, reflecting the progressive oxidation and degradation of phenolic compounds. Among the treatments, hot water blanching exhibited the highest degradation rate constant ($k = 0.04010 \text{ day}^{-1}$), indicating the fastest loss of phenolic compounds during storage. Microwave-blanching juice showed an intermediate degradation rate ($k = 0.0370 \text{ day}^{-1}$), whereas steam-blanching juice ($k = 0.0352 \text{ day}^{-1}$) exhibited comparatively lower apparent degradation rates. Although the control displayed the lowest rate constant, its initial phenolic content was substantially lower than that of the pretreated juices owing to the absence of blanching-induced enzyme inactivation and the greater extent of oxidative deterioration. The higher degradation rate observed in HB juice may be attributed to the prolonged exposure to heat, which accelerates oxidation and polymerization of phenolic compounds during storage. In contrast, SB and MB were comparatively more effective in preserving phenolic compounds, likely because of rapid enzyme inactivation and reduced oxidative degradation, thereby contributing to improved retention of antioxidant constituents throughout storage.

Effect of blanching pretreatment on microbial enumeration of juice during storage

Microbial load increased in all the amla juice samples, irrespective of the pretreatment given (Table 4), over the 7 days of room temperature. However, the highest rate of microbial growth was observed in case of control, which reached 4.30 log CFU/mL total plate count and 3.18 log CFU/mL yeast & mold count surpassing the limit standard for juice beverage [33]. Thus, indicating that the control juice spoiled in 4 days of storage, besides, the microbial growth could also be observed visually in the juice samples (Figure 4). The reason for the higher microbial growth in the control group may be traced back to the lack of initial heat treatment, which facilitated unrestricted microbial development. The fact was also supported by the lowered acidity levels in control that was comparatively favorable for microbial growth (Table 4) [27]. Furthermore, on the other hand, juice samples that underwent blanching techniques, specifically steam blanching, exhibited a markedly decreased microbial burden ($P < 0.05$). The juice that had been steam-blanching showed the least amount of microbial growth out of all of these, indicating that the high heat used in SB successfully rendered more microorganisms inactive and improved microbial control during storage [33]. While hot water-blanching and microwave-blanching samples were also able to control the microbial growth, although somewhat lesser than that of the steam-blanching samples. Thus, the microwave-blanching and hot water-blanching samples could restrict microbial growth below standard limit for 5 days of storage. However, all the four samples spoiled post 5 days of storage.

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Days of storage	Total plate count (log CFU/ml)				Yeast and mould count			
	Control	HB	SB	MB	Control	HB	SB	MB
Day 0	ND	ND	ND	ND	ND	ND	ND	ND
Day 1	1.78±0.03 ^a	1.27±0.04 ^b	1.21±0.02 ^b	1.25±0.03 ^b	0.92±0.03 ^a	0.61±0.02 ^b	0.55±0.02 ^b	0.59±0.03 ^b
Day 2	2.29±0.02 ^a	2.11±0.05 ^b	1.94±0.02 ^c	2.03±0.04 ^{b, c}	1.48±0.03 ^a	1.21±0.03 ^b	0.96±0.03 ^c	1.06±0.04 ^{b, c}
Day 3	3.20±0.07 ^a	2.87±0.08 ^b	2.24±0.04 ^d	2.69±0.06 ^c	2.21±0.07 ^a	1.83±0.06 ^b	1.47±0.04 ^d	1.69±0.05 ^c
Day 4	4.30±0.08 ^a	3.69±0.10 ^b	3.26±0.07 ^c	3.48±0.09 ^{b, c}	3.18±0.08 ^a	2.61±0.07 ^b	2.12±0.06 ^c	2.46±0.07 ^{b, c}
Day 5	5.31±0.11 ^a	4.12±0.12 ^b	3.88±0.10 ^b	4.01±0.11 ^b	4.07±0.11 ^a	3.22±0.08 ^b	2.91±0.08 ^b	3.10±0.09 ^b
Day 6	6.14±0.12 ^a	4.91±0.17 ^b	4.47±0.15 ^c	4.86±0.13 ^b	5.02±0.12 ^a	3.94±0.10 ^b	3.48±0.10 ^c	3.81±0.11 ^b
Day 7	7.58±0.15 ^a	6.05±0.13 ^b	5.13±0.14 ^d	5.94±0.17 ^c	6.18±0.14 ^a	4.89±0.13 ^b	4.12±0.12 ^d	4.73±0.13 ^c

TABLE 4: Effect of pretreatment on microbial growth in amla juice during storage.

Mean ± S.D. with different superscripts in a row indicate significantly different values determined by Tukey's test ($P < 0.05$). SB, Steam Blanching; MB, Microwave Blanching; HB, Hot Water Blanching; CFU, Colony Forming Units; ND, Not Determined

Conclusions

This study examined how different blanching methods, hot water, steam, and microwave, used as pretreatments before extracting juice from amla fruits, affects the enzyme inactivation kinetics, juice recovery, and the quality of the extracted juice. Blanching was accomplished upon treatment for 180 seconds, 120 seconds, and 150 seconds in case of HB, SB, and MB, respectively. Besides, Weibull model was found to best fit the PPO and POD inactivation during each blanching treatment. Furthermore, SB improved the juice recovery from fruits significantly; however, it also resulted in prominent browning and degradation in AA and phenolic content. While juice recovery post-HB and MB of fruits were at par with control (unblanched) fruits. Furthermore, the former led to pronounced browning and loss in the phytochemicals, which was comparatively higher than in latter. Moreover, kinetic considerations revealed highest degradation rate constant AA and TPC during storage was observed in case of SB juice and HB juice, respectively. While lowest degradation rate constant of AA and TPC was observed in case of MB and SB juice, respectively. Besides, the microbial load increased in all juice samples, irrespective of treatment, however, was slowest in case of SB. Control juice spoiled in 4 days, while hot water and microwave samples spoiled by day 5, whereas steam-blanching juice spoiled by day 6. Furthermore, upon storage, irrespective of the blanching pretreatments, acidity, AA content, and TPC depreciated; browning and the microbial load increased; while no significant changes were observed in the pH and TSS of the amla juice samples. Overall, SB enhanced the juice yield but resulted in greater quality deterioration, while MB appeared to be a more

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balanced option, minimizing both browning and nutrient loss; however, it did not improve juice recovery considerably. Thus, the study thus highlights that the choice of blanching method plays a critical role in determining the trade-off between juice recovery and retention of nutritional quality in amla juice. Besides, storage further affected the juice quality, suggesting that blanching treatments alone are not sufficient to maintain quality attributes over time.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Vivek Kumar, Sumaiya Fatima

Acquisition, analysis, or interpretation of data: Vivek Kumar, Sumaiya Fatima

Critical review of the manuscript for important intellectual content: Vivek Kumar, Sumaiya Fatima

Supervision: Vivek Kumar

Drafting of the manuscript: Sumaiya Fatima

Disclosures

Plant subjects: All authors have confirmed that this study did not involve any herbarium specimen. **Human subjects:** All authors have confirmed that this study did not involve human participants or tissue. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Data Availability Statements

All data generated or analyzed during this study are included in this published article and/or its appendices.

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