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Optimizing UV-C irradiation for microbial safety and quality of Kinnow fruit juice

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The study analyzed UV-C irradiation as a non-thermal preservation method for Kinnow fruit juice (KFJ), aiming to reduce microbial contamination while preserving vitamin C content. By eliminating the need for thermal processing and chemical preservatives, the approach promotes energy-efficient and environmentally sustainable juice preservation. Using Response Surface Methodology (RSM), optimal UV exposure (25 minutes at 5 cm) predicted 34.056% vitamin C loss and 3.539 and 3.485 log reductions of AM and YM, respectively. The ANN model demonstrated superior performance over RSM, indicating that the ANN gives more accurate and reliable predictions. First-order kinetic modeling ($R^2 = 0.983$) shows better fittings than the Weibull model. Shelf-life studies revealed that KFJ stored at 4 °C in glass containers retained higher vitamin C and microbial stability, extending product shelf-life by nearly 6 days. These findings suggest that UV-C processing, in combination with proper packaging and storage conditions, holds the potential for juice preservation at the commercial level.

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Sustainability spotlight

This research advances sustainable food preservation by optimizing UV-C irradiation as a non-thermal method for enhancing the microbial safety and quality of Kinnow fruit juice. By reducing microbial load and maintaining vitamin C content without the use of heat or chemical preservatives, the process offers an energy-efficient and environmentally friendly alternative to conventional thermal pasteurization. The integration of modeling tools such as RSM and ANN ensures process precision, while shelf-life evaluation under refrigerated storage demonstrates extended product stability by nearly six days, effectively reducing post-harvest and processing losses. Through improved resource efficiency, waste reduction, and adoption of low-energy technologies, this study contributes to SDG 2 (Zero Hunger) by promoting food availability through longer shelf life, SDG 9 (Industry, Innovation, and Infrastructure) by applying innovative non-thermal and data-driven processing approaches, and SDG 12 (Responsible Consumption and Production) by encouraging sustainable food manufacturing practices that minimize environmental impact.

1 Introduction

Kinnow is a cross between 'King' (*Citrus nobilis*) and 'Willow Leaf' mandarin (*Citrus deliciosa*) and belongs to the Rutaceae family and is extensively cultivated in India and Pakistan. According to the Department of Agriculture & Farmers Welfare,¹ citrus fruit production in India reached 14.81 million metric tonnes, with cultivation over 1.095 million hectares. Major citrus fruits grown in India include Kinnow, mosambi, orange, lemon, lime, grapefruit, tangerine, and galgal, mainly suitable for Southeast Asian agro-climatic conditions. In India, its production is mainly in regions such as Punjab, Rajasthan, Himachal Pradesh, Jammu & Kashmir, Haryana, and Uttarakhand. Kinnow—a hybrid mandarin variety which is extensively

cultivated in Punjab, with annual yields reaching 1.2 million metric tonnes. Kinnow is recognized by its medium-sized, oblate fruits with smooth, glossy orange skin and deep orange flesh.^{2,3} The fruit is highly cherished for its pleasant flavor, vibrant color and therapeutic properties. Kinnow fruit consists of components such as flavedo, albedo, seeds and juice sacs (present in segments).

Kinnow is a seasonal fruit harvested from mid-December to mid-March, with peak yield in January and February. Kinnow fruit grows best at temperatures of 10–35 °C and with an annual rainfall of 500–1200 mm, moderate humidity levels (60–70%), and 6–8 hours of daily sunlight. However, the abundance of fruit during this period results in significant post-harvest losses if not managed properly. Due to its high water content, Kinnow is highly susceptible to microbial attacks.⁴ Harvest and post-harvest losses of Kinnow account for 25–30%.⁵

Various processing techniques, such as ultrafiltration, can be employed to minimize the losses and extend its availability.⁴ Kinnow juice is a primary processed product from a ripened fruit rich in vitamin C, folate, provitamin A, and other nutrients,

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such as asparagine, arginine, aspartic acid, proline and glutamine-like amino acids. It also provides essential minerals, meeting the body's calcium, magnesium, phosphorus, manganese, iron, zinc, and copper requirements. Moreover, the juice is abundant in bioactive components like phenols, flavones, flavonoids, and limonoids, along with carotenoids, which can contribute to its health benefits.⁶ The juice from the Kinnow fruit can be extracted using hand-reaming and mechanical juice extractors. The extracted Kinnow fruit juice (KFJ) can be consumed in its natural or raw state and processed for extended shelf-life and numerous applications. KFJ is easily prone to microbial spoilage, which can limit its market value due to its limited shelf life. For enhancing the safety and stability of KFJ, it is crucial to eliminate both microbes and inherent enzymes. The presence of inherent enzymes can result in loss of color, flavor, texture and nutrients in Kinnow.

Microbial growth and enzymatic activity play an important role in the spoilage of fruit juices, mainly due to aerobic mesophiles (AM) and yeasts and molds (YM). Both conventional and emerging preservation techniques have been assessed to address this issue. Studies have reported the use of ohmic heating, pasteurization⁷ and combinations involving preservatives,^{8,9} ultrafiltration,⁴ and ozonation^{10,11} in retaining the juice quality. While thermal treatments can effectively inactivate spoilage organisms, they often come at the cost of reduced nutritional and sensory attributes. In contrast, non-thermal treatments preserve these qualities, which makes them a preferable alternative for juice stabilization. In the era of novel non-thermal preservation techniques, ultraviolet (UV) irradiation stands out as an effective and promising method for preservation. UV treatment provides microbial safety and helps in retaining nutritional and sensory attributes, making it a low-energy, cost-effective alternative to conventional thermal methods without using preservatives.¹² Very limited literature is available on traditional heat treatment and non-thermal techniques like UV processing for KFJ.

The mechanism of microbial inactivation is associated with UV light through multiple pathways. DNA damage is the primary cause behind microbial cell inactivation, while lipid peroxidation and protein denaturation contribute to the same. UV-C radiation induces thymine–thymine dimer formation, which can alter the bioactive properties of microbial cells. In addition, UV-C generates cyclobutane-pyrimidine dimers with cytotoxic effects and 6–4 photoproducts, which are highly lethal and mutagenic. Reactive oxygen species produced during UV treatment can lead to lipid oxidation, disrupting the lipid bilayer by forming pores and disintegrating the membrane. The treatment induces oxidative changes in amino acids, resulting in conformational and compositional changes that inhibit critical enzymes and disrupt cellular functions.¹³ Fig. 1 illustrates the mechanism behind microbial inactivation using UV light.

This study evaluates the impact of UV and thermal treatments on the quality characteristics of KFJ during storage. The juice was packaged in glass (GS) and polyethylene terephthalate (PET) and stored at two different temperatures (4 °C and room temperature). Key parameters, like ascorbic acid and microbial load, were evaluated to assess the quality degradation over storage time.

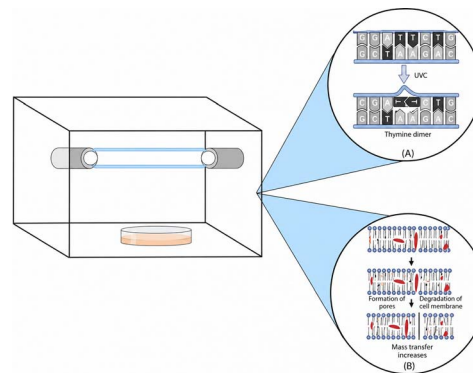


Fig. 1 Mechanism behind the inactivation of microbes using a UV lamp.

2 Materials and methods

2.1 Kinnow juice preparation

Fresh and ripe Kinnow fruit was bought from the local market in Bhubaneswar, Odisha, India. The fruits underwent cleaning and sorting, before storing the fruit at 4 °C until further analysis. The fruit peel was removed using a sanitized stainless-steel knife. The albedo portion was removed manually to separate the edible segments before juice extraction. The KFJ was extracted using a multifunctional food processor (Bajaj Electricals Limited, Model: FX11 600 Watts food processor). After extraction, the juice underwent filtration thrice using three-layered cheesecloth to separate juice from larger pulp particles and seeds.

2.2 Description of the ultraviolet treatment system

The UV chamber was designed to provide uniform exposure to UV rays. The system consists of a cuboid closed chamber constructed from 20 mm white acrylic sheets, chosen for their durability, ease of cleaning, and high visibility to detect stains or spilled juice, which can aid in sterilization and contamination prevention. The chamber dimensions are 48 cm in length, 15 cm in breadth, and 24 cm in height (Fig. 2). A UV-C lamp (GHO436T5L, Arklite Specialty Lamps Pvt. Ltd, India) of 48 W power was installed inside the chamber. The distance between the juice and the lamp can be regulated through a movable base, ensuring consistent UV exposure to the KFJ. To maintain sterile conditions, the chamber was sanitized with 80% ethanol

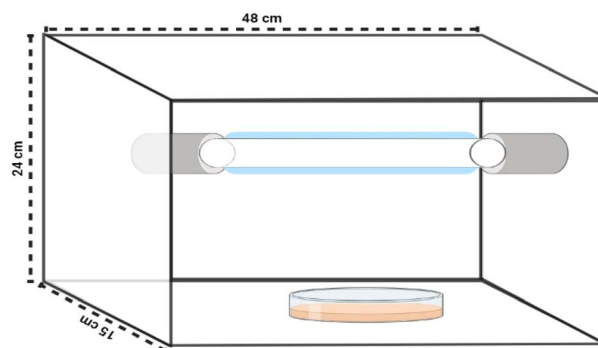


Fig. 2 Designed ultraviolet system.



before any experimental run. The operation of the lamp was controlled manually using a switch and stopwatch to ensure accurate treatment durations.

2.3 Experimental design and optimization

A face-centered central composite design optimized UV treatment conditions for maximum microbial reduction and minimizing vitamin C loss. The distance between the lamp and juice (Y_1) and treatment time (Y_2) were selected as the independent variables. In contrast, dependent variables assessed during the experiment design were total plate count (log reduction), yeast and mold (log reduction), and percentage loss of vitamin C. Independent parameters were evaluated at three distinct levels: distance at 3, 5, and 7 cm and treatment time at 15, 20, and 25 min. UV intensity was maintained constant throughout the experiment due to operational limitations of the UV system used; therefore, it was not included as an independent variable in the optimization study. The independent parameter limits were determined through preliminary trials. The experimental design and data analysis used Design-Expert software (version 13, Stat-Ease Inc., USA). 13 experimental runs were obtained, including 8 factorial runs, which represent different combinations of independent parameters, and 5 replicates at the center point to assess experimental error and ensure the reproducibility of the results.

2.4 Thermal treatment

Thermal treatment (TT) was conducted using a water bath (Meta Lab Scientific Industries Ltd, Mumbai, India). The KFJ sample was transferred to sterile stainless-steel containers covered with lids. The containers were placed in a preheated water bath at 90 °C. The KFJ was held at 90 °C for 2 minutes after the temperature reached 90 °C. After pasteurization, the juice was immediately cooled to room temperature by immersion in an ice bath. The juice was transferred to GS and PET bottles in a sterile environment.

2.5 Enumeration of microbial population

The AM and YM microbial populations in the KFJ were enumerated using the serial dilution-pour plate method. Fresh, UV and TT-treated KFJ samples were diluted with sterilized saline water (0.85% w/v sodium chloride in distilled water).¹⁴ The prepared dilutions were transferred as 1 mL of diluted inoculum to a sterile Petri plate, and 10–12 mL of agar was added. The Petri plate was rotated gently to ensure uniform mixing before the agar solidified. A second layer of 8–10 mL agar was poured to complete the plating process. Nutrient agar (HIMEDIA® M001-500 G) was used for AM, while potato dextrose agar (HIMEDIA® M096-500 G) was used for YM. The Petri plates were incubated for 24–36 hours at 37 °C for AM, while YM were incubated at 30 °C for 3–5 days. AM and YM can be expressed as colony-forming units per milliliter (CFU mL⁻¹). The reduction in microbial load can be calculated in the form of log cycles using the following equation:

$$n = \log\left(\frac{N_0}{N}\right) \quad (i)$$

where n represents the log cycle reductions, N_0 is the initial microbial count in untreated KFJ (CFU mL⁻¹), and N is the microbial count in treated KFJ (CFU mL⁻¹).⁷

2.6 Vitamin C

Vitamin C was determined spectrophotometrically using 2,6-dichlorophenolindophenol at 518 nm, in accordance with Challana *et al.*¹⁵ The ascorbic acid content was expressed as milligrams per 100 mL of juice (mg AA/100 mL).

2.7 Artificial neural network

2.7.1 Artificial neural network modeling. For developing and assessing different Artificial Neural Network (ANN) models, MATLAB software (R2018a, MathWorks, USA) was employed to validate the responses obtained from RSM. There are three layers on the ANN surface, *i.e.*, input, hidden, and output. These were constructed using a feed-forward topology. The input layers consist of two independent variables: distance between the lamp and juice and treatment time, with total plate count (log reduction), yeast and mold (log reduction), and percentage loss of vitamin C considered as output-dependent variables (Fig. 3).

The number of neurons in the hidden layer was determined through a trial-and-error approach to optimize network performance. The number of neurons varied from 1 to 20. A small number of neurons could result in underfitting, whereas excessive neurons can cause overfitting and prolong the training time. Before splitting the dataset into training (70%), validation (15%), and testing (15%) subsets, the datasets underwent randomization to ensure a representative distribution. The available dataset consists of 13 observations per variable, constrained by experimental data availability. To address these constraints and strengthen the model validation, a k-fold cross-validation strategy was employed. The strategy ensured that each data point contributed to training and testing, which can help overcome the effects of limited datasets and enhance the model's reliability and prediction accuracy. A comparative analysis was conducted between the ANN and RSM models to assess better fitting, predictive capability, and accuracy.^{15,16}

2.7.2 Comparison between RSM and ANN models. Different statistical indices like the coefficient of regression

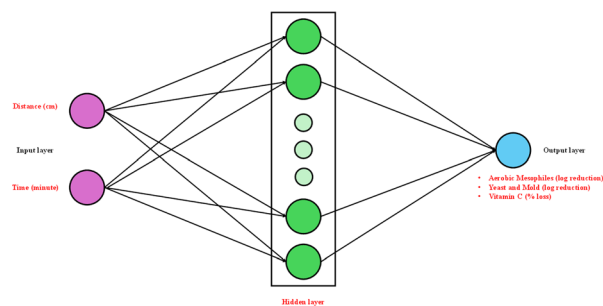


Fig. 3 ANN model configuration.



(R^2), mean square error (MSE), and root mean squared error (RMSE) were used to evaluate the performance of the ANN model.^{15,17} The statistical parameters were analyzed using the following equations:

$$\text{MSE} = \frac{1}{n} \sum_{j=1}^n (Y_j - \hat{Y}_j)^2 \quad (\text{ii})$$

$$\text{RSME} = \left\{ \frac{1}{n} \sum_{j=1}^n (Y_j - \hat{Y}_j)^2 \right\}^{1/2} \quad (\text{iii})$$

$$R^2 = \frac{\sum_{j=1}^n (Y_j - \hat{Y}_j)^2}{\sum_{j=1}^n Y_j^2 - \sum_{j=1}^n \hat{Y}_j^2} \quad (\text{iv})$$

where n is the observation number, Y_j is the predicted output variable for the i th observation, and \hat{Y}_j is the experimental output variable for the i th observation.

2.8 Curve fitting for microbial inactivation

Microbial inactivation during UV treatment was described using first-order kinetics and the Weibull model.

2.8.1 First-order kinetics. Microbial reduction followed by first-order kinetics can be expressed using equation (v). This model can be applied only when the microbial reduction increases proportionally with treatment intensity.

$$\log\left(\frac{N_i}{N_t}\right) = kt \quad (\text{v})$$

N_i is the initial microbial count (CFU mL⁻¹), N_t is the microbial count after treatment time t , and k is the first-order inactivation rate constant (s⁻¹).

2.8.2 Weibull model. The Weibull model can be applied to the non-linear microbial inactivation, which can be described by eqn (vi)

$$\ln\left(\frac{N_i}{N_t}\right) = \left(\frac{t}{\delta}\right)^\beta \quad (\text{vi})$$

where N_i is the initial microbial count (CFU mL⁻¹), N_t is the microbial count after treatment time t , δ is the scale parameter (characteristic time), and β is the shape parameter.¹⁸

β is the deciding factor for the Weibull distribution curve.

- $\beta < 1$ represents upward concavity, indicating microbial resistance and adaptation to UV stress over time.

- $\beta > 1$ represents downward concavity, suggesting accelerated microbial inactivation and increasing susceptibility with prolonged UV treatment.

- $\beta = 1$ represents a linear curve and reflects a constant microbial inactivation rate where the microbial inactivation is independent of exposure duration.

2.9 Shelf-life estimation

PET and GS bottles were the two packaging materials for storing and processing. A storage analysis was performed for fresh (JF), UV, and TT samples (JU and JT), with a primary focus on ensuring the microbiological safety of KFJ. The KFJ samples were unacceptable if the microbial count exceeded 6 log counts.¹⁹ Fresh samples (untreated juice), UV-treated samples, and TT samples were analyzed after 48 hours to evaluate the effect of different treatments. Fresh samples refer to untreated juice with no processing applied. UV-treated samples were those exposed to the optimized UV treatment conditions determined in this study. TT samples were processed at 90 °C for 2 minutes prior to storage. The shelf-life was determined by evaluating the critical parameters such as AM, YM, and vitamin C.

3 Results and discussion

3.1 Effect of independent parameters on responses

The effect of independent variables and their interactions on dependent variable observations is provided in Table 1. The fresh KFJ has an AM count of 4.38 log CFU mL⁻¹, a YM count of

Table 1 Actual values and coded values for the factors that are variable, and experimental values of responses collected during UV using FCCD

	Factor 1	Factor 2	Response 1	Response 2	Response 3
	Distance [cm]	Time [minute]	Aerobic mesophiles [log reduction]	Yeast and mold [log reduction]	Vitamin C [% loss]
Run	A	B	Y_1	Y_2	Y_3
1	7	15	2.279	2.159	21.842
2	5	20	3.169	2.987	32.554
3	7	25	2.371	3.415	25.127
4	5	20	3.315	3.214	29.895
5	5	15	2.314	2.359	29.639
6	5	20	3.478	3.150	30.588
7	7	20	2.314	2.987	27.856
8	3	15	3.059	2.538	38.430
9	5	25	3.812	3.536	34.295
10	5	20	3.515	3.090	27.845
11	3	20	3.840	3.369	43.799
12	3	25	3.989	3.599	50.235
13	5	20	3.418	2.895	31.114



5.12 log CFU mL⁻¹, and a vitamin C concentration of 35.65 ± 2.75 mg of ascorbic acid/100 mL.

3.1.1 Aerobic mesophiles. The actual values and coded values of the independent variables, along with the responses, are given in Table 4. The log reduction value of AM varies from 2.28 to 3.99 across the different experimental conditions. Based on the ANOVA results (Table 2), the model's overall F-value is 13.09 and has a *p*-value of 0.002, which signifies that the model is statistically significant. The model has an *R*² value of 0.903, which means that there is a 90.3% variability in AM reduction that the model can explain. There was a non-significant lack of fit, suggesting that the model accurately predicts the AM. The factors, *i.e.*, distance (*A*) and time (*B*), significantly impact AM reduction, whereas *AB*, *A*² and *B*² are factors that do not substantially alter the AM count reduction.

Eqn (vii) gives the correlation between the AM log reduction and independent parameters

$$\text{AM (log reduction)} = +3.36 - 0.654 A + 0.42B - 0.21AB - 0.223 A^2 - 0.237B^2 \quad (\text{vii})$$

From the above equation, the negative coefficient of distance suggests that distance and reduction in AM are inversely proportional to each other, which means a decrease in distance between the UV lamp and KJF resulted in higher AM count reduction (Fig. 4A and B). Similarly, the positive coefficient of time suggests that exposure time is directly linked with a reduction in AM count. The trends align with the findings reported by Kim *et al.*,²⁰ where reducing the sample's distance from the UV lamp and increasing the exposure time resulted in improved microbial reduction in lettuce. The trend could be ascribed to an increase in the distance, resulting in a lower intensity of UV, which diminished the antimicrobial effect, while prolonged treatment enhances the antimicrobial impact. The decrease in effectiveness with longer distances can be explained by the "inverse square law," which states that

doubling the distance resulted in a four-fold decrease in irradiance.^{21,22} As UV intensity diminishes with distance, prolonged treatment is required to achieve the same effect as at a closer distance.

3.1.2 Yeast and mold. The YM value varied from 2.16 to 3.6 log reduction (Table 1). Based on the ANOVA results (Table 2), the model value was found to be highly significant (*p* < 0.0001) with an *R*² value of 0.966, which depicts 96.6% variability in the data. The lack of fit was non-significant, which confirmed that the model adequately describes the data. The factors *A*, *B* and *B*² have been found to have a significant (*p* < 0.05) influence on the reduction of YM. At the same distance and time, other factors like *AB* and *A*² are found to be statistically insignificant (*p* > 0.05).

The correlation between YM reduction and independent parameters is given below:

$$\text{YM (log reduction)} = +3.09 - 0.158A + 0.582B + 0.049AB + 0.048A^2 - 0.183B^2 \quad (\text{viii})$$

From the above equation, the negative coefficient indicates that reducing the gap between the UV lamp and KJF enhances YM reduction, demonstrating an inverse relationship as illustrated in Fig. 4(C and D). On the other hand, the positive coefficient of time suggested that longer exposure leads to a higher YM reduction. The negative coefficient of *B*² indicates that while increasing treatment time improves the YM reduction, the effect decreases after a certain time, indicating a plateau or decline in effectiveness with longer exposure.

The reduction in YM is less pronounced than AM, indicating that UV treatment is more effective against AM, which can be attributed to the reduced pyrimidine nucleosides (cytosine and thymine) in the YM genetic material, along with the thicker cell wall of the YM in comparison to AM.^{23,24} These factors contribute to the higher resistance of yeast cells to UV treatment. Similar findings were reported by Torkamani and

Table 2 Summary of the estimated coefficients of the quadratic model for dependent variables^a

Factors	Aerobic mesophiles [log reduction]		Yeast and mold [log reduction]		Vitamin C [% loss]	
	(Y ₁)		(Y ₂)		(Y ₃)	
	Coeff	<i>p</i> -Value	Coeff	<i>p</i> -Value	Coeff	<i>p</i> -Value
Intercept	3.356		3.085		30.880	
<i>A</i>	-0.654 ^a	<0.001 ^a	-0.158 ^a	0.009 ^a	-9.607 ^a	<0.001 ^a
<i>B</i>	0.420 ^a	0.005 ^a	0.582 ^a	<0.001 ^a	3.291 ^a	0.065 ^a
<i>AB</i>	-0.210 ^b	0.145 ^b	0.049 ^b	0.393 ^b	-2.13 ^b	0.083 ^b
<i>A</i> ²	-0.223 ^b	0.190 ^b	0.048 ^b	0.483 ^b	3.745 ^a	0.021 ^a
<i>B</i> ²	-0.237 ^b	0.167 ^b	-0.183 ^a	0.025 ^a	-0.115 ^b	0.930 ^b
Lack of fit	Not significant		Not significant		Not significant	
ANOVA of the model						
<i>F</i> -values	13.09		39.90		30.61	
<i>p</i> -value _(model)	0.002		<0.0001		0.001	
<i>p</i> -value _(LOF)	0.052		0.817		0.238	
<i>R</i> ²	0.903		0.966		0.956	

^a Superscript a represents a *p*-value less than 0.05 (*p* < 0.05), whereas superscript b represents a *p*-value greater than or equal to 0.1 (*p* ≥ 0.1).



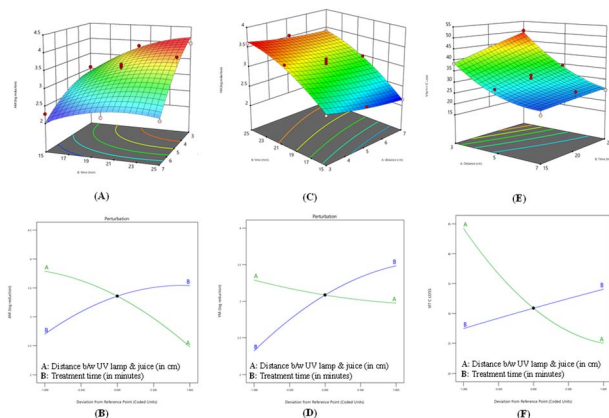


Fig. 4 Response surface plots (A, C and E) and perturbation plots (B, D and F) depicting the effect of independent parameters after UV treatment on (A and B) total plate count log reduction; (C and D) yeast and mold log reduction; (E and F) vitamin C loss.

Niakousari²⁵ in UV-treated orange juice, where bacteria were more affected than YM. This was likely due to their smaller size, simpler cell wall composition, and higher pyrimidine content, resulting in bacterial DNA being more prone to UV-induced damage.

3.1.3 Vitamin C. Vitamin C loss after treatment ranged from 21.84 to 50.23% (Table 1). The ANOVA results (Table 2) indicate that the model was found to be statistically significant ($p < 0.01$). The distance plays a dominant role over exposure time in contributing to the % vitamin C loss during UV treatment. The quadratic model fits the vitamin C loss well, demonstrating no significant lack of fit, which is desirable for evaluating the impact of independent variables. The model's R^2 value was 0.956, which signifies strong predictive accuracy. The correlation between % vitamin C loss and independent parameters is given below:

$$\% \text{ Vitamin C loss} = +30.88 - 9.61A + 3.29B - 2.13AB + 3.75A^2 - 0.116B^2 \quad (\text{vi})$$

The perturbation plot (Fig. 4(F)) highlights that distance was found to have a greater influence on vitamin C degradation than exposure time. The plot clearly illustrates that increasing the distance between the UV lamp and the sample reduces the vitamin C loss as the effectiveness of UV reduces with an increase in the gap. On the other hand, longer treatment time was directly linked with higher vitamin C degradation. The positive coefficient of A^2 indicates an inverted parabolic response, which means that the loss of vitamin C initially increases with an increase in distance, but beyond a certain limit, the loss of vitamin C decreases.

Spectrophotometric analysis of KFJ (Fig. 5) revealed maximum absorption peaks mainly in the UV region (200–400 nm), with minimal absorption beyond 400 nm in the visible spectrum, which signifies that most of the light is transmitted in the visible range. This suggests that vitamin C degradation is more rapid in the UV region, with minimal impact from visible light. Similar findings were observed by de Souza *et al.*,²⁶ who reported a decrease of nearly 23% in vitamin C content in citrus juice after UV-C treatment. The degradation could be attributed to the absorbance of ascorbic acid near 260 nm, which overlaps with the UV-C region, thereby accelerating its degradation. As vitamin C is very sensitive to light and temperature,²⁷ prolonged exposure to UV accelerates its degradation when the distance between the juice and the minimum distance to the higher intensity of UV light results in more significant vitamin C loss.

The results show that distance is important in microbial inactivation and vitamin C retention. The increase in distance not only resulted in lower microbial inactivation but also helped in better retention of vitamin C, whereas the increase in treatment time improved the microbial inactivation and diminished the vitamin C concentration. These responses indicate an inverse relation between nutrient retention and microbial inactivation.

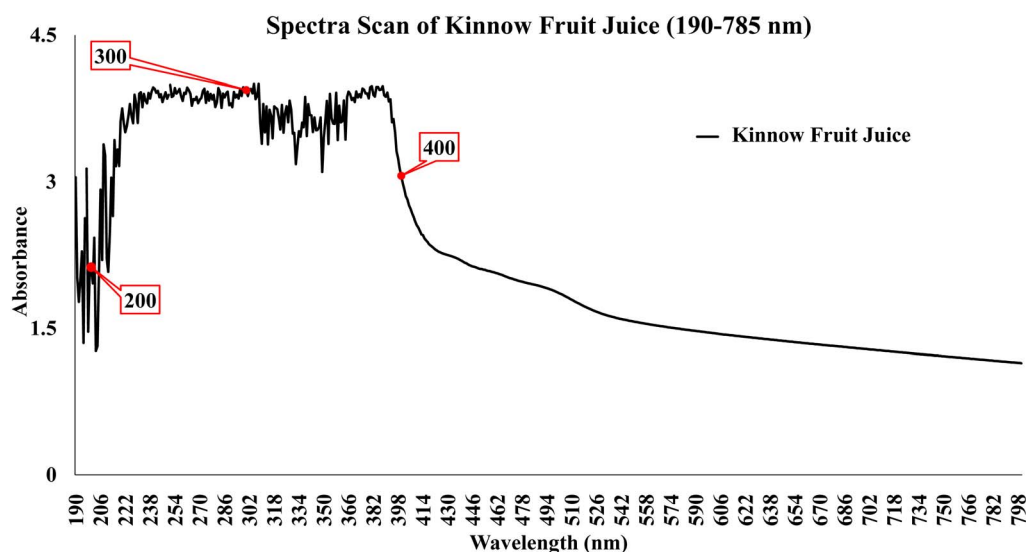


Fig. 5 Spectral scan of Kinnow fruit juice (190–785 nm).



Table 3 Validation of response, i.e., % increase in yield and % loss of vitamin C of KFJ under optimized conditions

Optimized process	Predicted ^a	Experimental ^b	Accuracy (%)
Aerobic mesophiles [log reduction]	3.539	3.311 ± 0.186	93.56
Yeast and mold [log reduction]	3.485	3.219 ± 0.168	92.37
Vitamin C [% loss]	34.056	30.874 ± 0.657	90.66

^a Predicted value of dependent parameters with the help of quadratic equations. ^b Data are the average of 6 experiments under the expected optimum conditions of the independent variables.

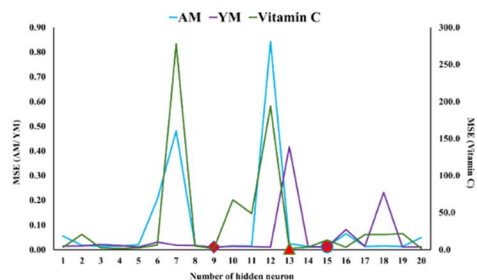


Fig. 6 Comparison of ANN performance based on the least RMSE values for AM, YM (log reduction), and vitamin C loss with different hidden neuron numbers.

Torkamani and Niakousari²⁵ also reported that enhancing UV-C treatment resulted in microbial reduction but also caused loss of vitamin C. These findings highlight the inverse relationship between microbial load and nutrient retention.

3.1.4 Optimization of ultraviolet treatment as a non-thermal technique for preservation. After conducting RSM, numerical optimization was performed to maintain the desired balance between microbial safety and vitamin C retention. The goal was to minimize the vitamin C loss to retain nutritional value while maximizing the log reduction value of AM. For the YM reduction, no specific preference was set.

The relative importance (r) values were assigned to prioritize the outcomes; $r = 5$ was set to minimize the vitamin C loss and $r = 3$ was set to maximize AM reduction. The overall desirability value (D) was used to evaluate the fitness of each solution. A higher D value means closer alignment with the desired objectives. The solution yielding the highest D value was selected. The optimized conditions were 5 cm distance between juice and the UV lamp and 25 minutes of exposure time with a D value of 0.826. The predicted responses were 34.056% vitamin C loss, 3.539 log reduction of AM, and 3.485 log reduction of YM. Hakguder Taze *et al.*²⁸ reported that the UV-treated orange juice at an intensity of 1.32 mW cm⁻² for 20 minutes resulted in a YM reduction of 1.76 log count at a dose of 108.42 mJ cm⁻².

The experimental values for the log reduction of AM and YM were 3.311 ± 0.186 (93.56%) and 3.220 ± 0.170 (92.37%), respectively. Similarly, vitamin C loss was observed at 30.874 ± 0.657%, with an accuracy of 90.66%. Validation of responses under optimized conditions is presented in Table 3. These results confirm the reliability of the predictive models used for process optimization. Minor deviations between predicted and experimental values were within acceptable limits for practical applications.

3.2 Artificial neural network

3.2.1 Artificial neural network modeling. A multi-layer feed-forward topology ANN was used, and different configurations were estimated by varying the number of neurons. The MSE of the output layer was plotted against the varying neuron counts. This analysis demonstrated the impact of a hidden number of neurons on network performance.²⁹ The optimal models were determined based on the lowest MSE and highest R^2 , which were identified as 2-15-1 for AM, 2-9-1 for YM, and 2-13-1 for vitamin C loss (Fig. 6).

Fig. 7A–C illustrates the ANN model's training progress, including MSE across training, validation and test data sets over the 7 epochs for AM, 20 for YM, and 13 for vitamin C loss. The best validation performance was observed with MSE values of 0.0202 (at epoch 1) for AM, 0.0199 (at epoch 16) for YM, and 7.431 (at epoch 7) for vitamin C loss. An overview of the training process for all models is illustrated in Fig. 7D–F. The ANN-predicted correlation coefficients (R) for the training, validation and testing phases were 0.9838, 0.8166, and 0.9897 for AM; 0.9668, 0.9631, and 0.9736 for YM; and 0.9108, 0.9880, and 0.9951 for vitamin C loss, respectively (Fig. 7G–I).

3.2.2 Comparison of RSM and ANN models. The predictive performance of RSM and ANN models for AM and YM (log reduction) and % vitamin C loss was compared using statistical parameters such as MSE, RMSE and R^2 values, as presented in Table 4. The results demonstrated that the ANN outperformed RSM for AM and vitamin C loss parameters. For AM, the ANN model has lower MSE (0.012) and RMSE (0.110) in contrast to RSM (MSE: 0.035; RMSE: 0.187) with a higher R^2 value (0.967), which indicates more reliable and precise predictions of the model. In addition to vitamin C loss, the ANN gives better results with reduced MSE (1.470), RMSE (1.210), and a higher value of R^2 (0.973), which demonstrates better modeling of quality degradation.

Although the RSM model showed a marginal advantage in predicting YM (log reduction), it had a lower MSE (0.006) and RMSE (0.079). In addition, the ANN model retained a strong R^2 value (0.946 vs. 0.966), which signifies that the ANN is still a reliable approach. It can be concluded that the ANN model demonstrated superior performance in handling complex, non-linear associations³⁰ and offers enhanced predictive accuracy for microbial and quality parameters and RSM is limited to second-order polynomial approximations.



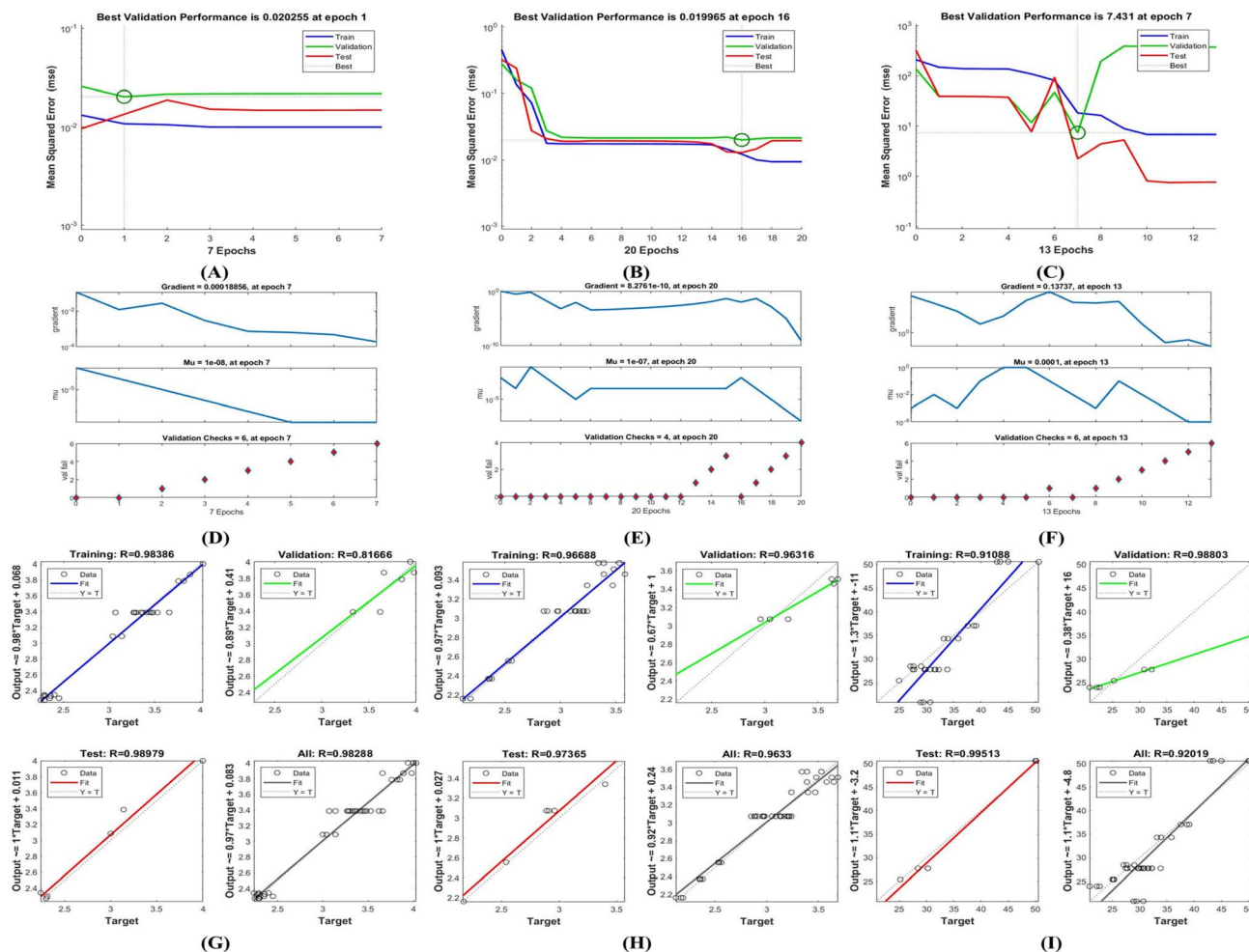


Fig. 7 Training and performance evaluation of the ANN model for (A) AM log reduction, (B) YM log reduction, and (C) % vitamin C loss, with corresponding performance plots (D–F) and comparison between experimental and predicted values (G–I).

Table 4 Predictive accuracy comparison between RSM and ANN for model outputs

Parameters	AM (log reduction)		YM (log reduction)		% Vitamin C loss	
	RSM	ANN	RSM	ANN	RSM	ANN
MSE	0.035	0.012	0.006	0.010	2.400	1.470
RMSE	0.187	0.110	0.079	0.101	1.549	1.210
R ²	0.903	0.967	0.966	0.946	0.956	0.973

3.3 Microbial inactivation modeling for ultraviolet treatment

The microbial inactivation modeling for UV treatment was performed using first-order kinetics and the Weibull distribution model. Table 5 shows that the first-order kinetics model ($R^2 = 0.983$) fits better than the Weibull distribution model ($R^2 = 0.979$). The inactivation rate constant (k) for first-order kinetics was determined to be 0.108, suggesting a steady microbial inactivation over time. In the Weibull distribution model, α (the scale parameter) was 14.14 and β (the shape parameter) was

1.403. $\beta > 1$ predicts downward concavity, suggesting accelerated microbial inactivation and increasing susceptibility with prolonged UV exposure. This suggests that the microorganisms become more vulnerable with time when exposed to UV due to cumulative damage induced by UV light. Both models effectively describe the inactivation of microbes, but the Weibull model provides an extra edge in varying resistance patterns.

3.4 Shelf-life

3.4.1 Effect on microbial quality during storage. The AM and YM are the naturally occurring microflora commonly present in fruit juices and have been recognized as the main contributors to spoilage and decreased shelf-life.³¹ So, retaining microbial stability is a key requirement for retaining the safety and quality of fruit juices during storage. The maximum allowable limit for AM or YM counts in acidic fruit juice and purees should not exceed 6.0 log CFU mL⁻¹.³² The microbial stability of control, thermally treated, and UV-treated KFJ stored in glass and PET bottles under refrigerated (4 °C) and room temperature conditions were monitored to assess the shelf-life of KFJ (Fig. 8A–D).



Table 5 R^2 value and constant values of both the model's first-order kinetics and the Weibull model

Parameters	First-order kinetics	Weibull distribution model
R^2	0.983	0.979
Equation	$y = -kx$	$y = -(x/\alpha)^\beta$
Parameters	$k = 0.108$	$\alpha = 14.14$ $\beta = 1.403$

The AM and YM counts of untreated KFJ were 4.29 and 5.12 log CFU mL⁻¹, respectively. In the JF (control) sample, AM exceeded 6.0 log CFU mL⁻¹ after 8 days (at 4 °C) and >2 days (at 25 °C), while YM crossed the threshold level after 2 days (at 4 °C) and <2 days (at 25 °C) during storage. Under refrigeration, UV-treated juice samples exhibited a shelf life of >18 days in PET and <20 days in glass bottles, while TT extended the shelf life to nearly 24 days in both PET and glass bottles. In comparison, at room temperature, UV-treated juice demonstrated reduced microbial stability, lasting only >6 days in glass bottles. TT juice demonstrated comparatively higher stability, with a shelf life of >8 days in PET and <8 days in glass bottles for AM. The

thermally treated juice showed higher stability for YM. The KFJ lasts for nearly 20 days (at 4 °C) and up to 8 days (at 25 °C) while stored in glass bottles. In contrast, UV-treated KFJ has lower stability, whereas juice has nearly 16- and 8-day stability under refrigeration and room temperature storage in *b*. The findings demonstrated that storage at ambient temperature compromises treatment efficacy, which could be attributed to enhanced proliferation of yeast under these conditions in contrast to refrigerated storage.³³

The juice samples in glass bottles have lower microbial counts than those in PET bottles, ascribed to GS's excellent oxygen and moisture barrier properties.³⁴ The reduced water vapor and transmission rate of glass effectively retards microbial growth by minimizing the external contamination, which is essential for microbial growth. In addition to that, UV treatment has been shown to extend the shelf-life of fruit juices like starfruit,³⁵ watermelon,³⁶ orange,³⁷ and apple and grape juices.³⁸ The effect becomes more pronounced when the juice is stored under refrigerated conditions, suppressing microbial metabolism.³⁹ Thus, the combination of glass packaging and UV treatment, along with optimal storage conditions, plays an important role in enhancing the shelf life of KFJ.

3.4.2 Vitamin C. Vitamin C is a main constituent within the nutritional composition of KFJ, which significantly contributes to its antioxidant capacity and scavenges free radicals. Ascorbic acid is highly sensitive to processing and storage conditions.¹⁵ Fig. 8E and F demonstrates the impact of the processing method, packaging, and storage conditions on vitamin C concentration in KFJ. The ascorbic acid (AA) content of control, TT, and UV-treated samples was 34.20, 23.78, and 25.71 mg ascorbic acid/100 mL on the 0th day of storage. The AA content of each treated sample dropped throughout the storage time. The UV-GS-treated sample retained 65.7% of its initial ascorbic acid content on the 24th day of storage, which was higher than that of the TT-treated sample (63.9%) at 4 °C. The effect became more pronounced during storage at room temperature, where the JU-GS sample retained 15.60 units (65.6% retention) on day 24 compared to 16.43 units (63.9% retention) in the TT-treated sample. The glass bottle exhibited higher vitamin C retention than PET across all treatments. At 25 °C, the JU-GS sample retained 15.60 units compared to 15.30 units in PET, which can be attributed to glass's superior oxygen barrier properties. This effect was more pronounced in fresh juice, where glass bottles showed 12.7% higher retention than PET bottles on the 10th day of storage. Juice stored under refrigerated conditions exhibited greater stability than samples stored at room temperature.

The juice samples stored in glass bottles exhibited higher AA values, which could result from their superior barrier characteristics. This protective effect hindered oxygen permeation and minimized the conversion of ascorbic acid to dehydroascorbic acid. In contrast, juice stored in PET bottles had lower concentrations of vitamin C due to the limited barrier properties of PET against moisture and gases, which allowed oxygen migration into the product, thereby promoting the oxidative degradation of vitamin C.^{34,40}

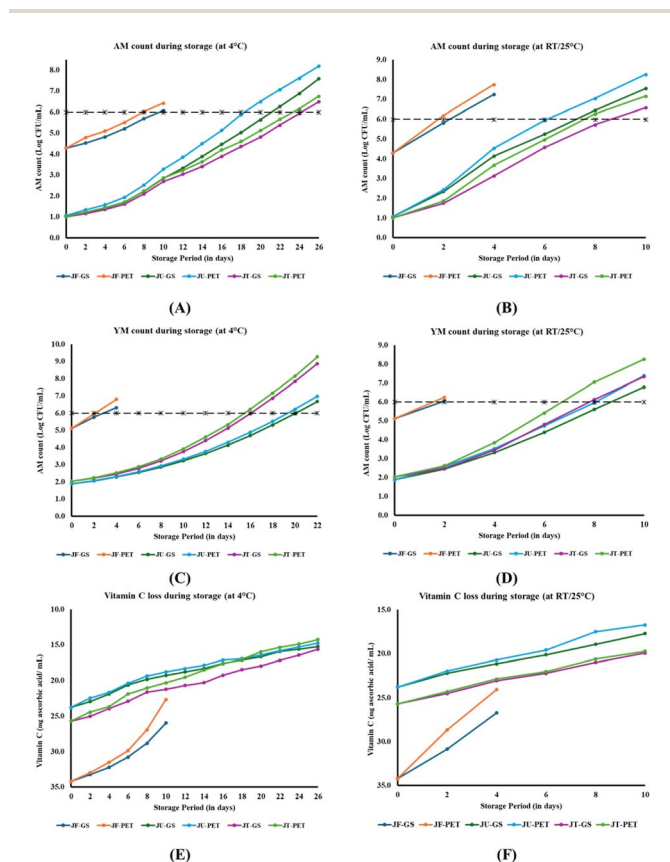


Fig. 8 Shelf-life evaluation of control, thermally treated, and UV-treated KFJ stored in glass and PET bottles under refrigerated and room temperature conditions: Aerobic mesophile count at (A) 4 °C and (B) room temperature, yeast and mold count at (C) 4 °C and (D) room temperature; and Vitamin C concentration at (E) 4 °C and (F) room temperature.



Conclusion

The study optimized UV irradiation as a non-thermal treatment to lower the vitamin C loss and maximize AM's log reduction. The optimal conditions chosen were 25 minutes of UV exposure at a distance of 5 cm between juice and the UV lamp, which resulted in a predicted vitamin C loss of 34.06%, 3.54, and 3.49 log reduction of AM and YM, respectively. Experimental validation showed accuracies of 90.66% for vitamin C loss, 92.37% for YM reduction, and 93.56% for AM reduction. The ANN model (2-15-1 for AM and 2-13-1 for vitamin C loss) has better predictive ability, fitting, and accuracy than RSM. The Weibull distribution model provided a superior fit ($R^2 = 0.979$) compared to the first-order kinetics model ($R^2 = 0.983$). The shelf-life study indicated that KFJ stored at 4 °C in glass bottles exhibited enhanced microbial and vitamin C stability compared to room temperature in PET bottles. Notably, the shelf-life of the JU-GS sample was improved by nearly six days compared to the untreated sample.

However, some limitations should be acknowledged. Microbial analysis was limited to aerobic mesophiles and yeast and mold counts, and further evaluation of specific foodborne pathogens such as *E. coli*, *Salmonella*, and *L. monocytogenes* would provide a more comprehensive assessment of microbial safety. In addition, shelf-life assessment mainly considered microbial stability and vitamin C retention, while inclusion of other quality attributes such as phenolic compounds, flavonoids, antioxidant activity, and sensory characteristics could provide a broader understanding of the impact of UV treatment on juice quality.

Although the present work was conducted using a laboratory scale batch UV system, the optimized treatment conditions provide a useful basis for the development of pilot scale or continuous flow UV processing systems. Future studies should therefore focus on evaluating additional quality parameters, assessing pathogen inactivation, optimizing reactor design for continuous processing, and examining the industrial feasibility of UV treatment for commercial juice processing.

Author contributions

Vimal Challana: conceptualization, methodology, investigation, formal analysis, software, writing – original draft. Ankita Naykodi: writing – review & editing, supervision. Shivanand S. Shirkole: software, formal analysis, methodology, writing – review & editing. Akshaya K. Sahoo: conceptualization, methodology, supervision, project administration, validation, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

Abbreviations

KFJ Kinnow fruit juice

AM	Aerobic mesophiles
YM	Yeast and molds
UV	Ultraviolet
GS	Glass
PET	Polyethylene terephthalate
CFU mL ⁻¹	Colony-forming units per milliliter
JU	UV-treated juice
JT	Thermally treated juice
TT	Thermal treatment
AA	Ascorbic acid

Data availability

The datasets used, analyzed, and generated during this study are included in the article.

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