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Degradation Kinetics of the Physicochemical and Phytochemical Properties of MD2 Pineapple Juice during Heat Treatment and Storage

(Kinetik Degradasi Sifat Fisikokimia dan Fitokimia Jus Nanas MD2 semasa Rawatan dan Penyimpanan Haba)

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ABSTRACT

Thermal processing is crucial for preserving fruit juice while minimising nutritional loss. A comprehensive study of heat treatment and storage effects on a popular pineapple hybrid, MD2, is required to enhance juice marketability. This study examined the thermal degradation kinetics of MD2 pineapple juice on the physicochemical and phytochemical properties by heat treatment at different temperatures (40-90 °C for up to 150 min), and storage conditions (-20, 1, 4, 7 and 27 °C). Physicochemical properties were determined based on the total soluble solids (TSS) content and browning index (BI). Meanwhile, phytochemical properties were evaluated on the pineapple juices' vitamin C and protein contents. Results showed that TSS and BI remained constant, while vitamin C and protein contents declined during the heat treatment. Pineapple juices' thermal degradation followed first-order kinetics, with vitamin C degrading faster at higher rate constants (4.38×10^3/min during heat treatment and 6.23×10^3/min during storage) as compared to protein content (4.30×10^3/min and 5.38×10^3/min, respectively). These findings may provide information for food manufacturers in optimising temperature and time for preservation and safety of pineapple juice.

Keywords: Degradation kinetics; physicochemical; phytochemical; storage; thermal treatment

ABSTRAK

Pemprosesan termal adalah penting untuk memelihara jus buah-buahan sambil meminimumkan kehilangan nutrisi. Kajian menyeluruh tentang rawatan haba dan kesan penyimpanan pada hibrid nanas yang popular, MD2 diperlukan untuk meningkatkan kebolehpasaran jus. Penyelidikan ini mengkaji kinetik degradasi haba jus nanas MD2 pada sifat fizikokimia dan fitokimia dengan rawatan haba pada suhu berbeza (40-90 °C sehingga 150 min) dan keadaan penyimpanan (-20, 1, 4, 7 dan 27 °C). Sifat fizikokimia ditentukan berdasarkan kandungan jumlah pepejal larut (TSS) dan indeks keperangan (BI). Sementara itu, sifat fitokimia dinilai pada kandungan vitamin C dan protein jus nanas. Keputusan menunjukkan bahawa TSS dan BI kekal malar, manakala kandungan vitamin C dan protein menurun semasa rawatan haba. Degradasi haba jus nanas mengikuti kinetik urutan pertama dengan vitamin C merosot lebih cepat pada pemalar kadar yang lebih tinggi (4.38×10^3/min semasa rawatan haba dan 6.23×10^3/min semasa penyimpanan) berbanding kandungan protein (masing-masing 4.30×10^3/min dan 5.38×10^3/min). Keputusan ini boleh memberi maklumat kepada pengeluar makanan dalam mengoptimumkan suhu dan masa untuk pemeliharaan dan keselamatan jus nanas.

Kata kunci: Fitokimia; fizikokimia; kinetik degradasi; penyimpanan; rawatan haba

INTRODUCTION

Malaysia has been one of the main pineapple producers, contributing to 1.1% of the world's production in Southeast Asia (Zaini et al. 2024). Pineapple (*Ananas comosus*) is one of the most popular tropical fruits due to its natural taste and nutritional benefits. The pineapple variety MD2 is recognised as 'golden ripe' due to its golden yellow skin and flesh colour when ripened (Vollmer et al. 2020). The variety has gained interest among consumers due to its sweeter flesh, higher vitamin C, lower acidity, and thinner peel compared to other varieties (Ali et al. 2020). Meanwhile, the sensory qualities of pineapple,

including sweetness and visual appeal, are influenced by its physicochemical properties, such as total soluble solids (TSS) and colour (Shourove et al. 2020). In addition to its deliciousness, pineapples are rich in vitamins, minerals, secondary phytochemical metabolites, and health-promoting compounds. Bromelain, total phenolic compounds, and vitamin C are among the key bioactive molecules that enhance the nutritional and therapeutic value of pineapples (Gopalsamy, Latifah & Hamid 2024; Sasongko et al. 2024).

Pineapples are commonly processed into juice to extend the products' availability and market potential (Roslan et al. 2020). However, fresh pineapple juice without a preservation technique has a reduced shelf life (8 h), even at a storage temperature of 4 °C (Lan et al. 2021). Therefore, introducing a preservation technique to minimise microbial load and enzymatic actions could prevent physical and chemical changes during the storage of pineapple juice. Although modern and advanced methods have shown promising preservation techniques in pineapple juices, the impact on the nutritional value of the juice is detrimental (Szczepaska et al. 2021). Therefore, heat treatment remains a practical and cost-effective solution in the industry (Mênouwesso et al. 2020). According to Vollmer et al. (2020), heat treatment could improve the safety and quality of the pineapple juice by eliminating spoilage microorganisms and enzymes that pose health risks to consumers.

High temperatures (105 °C) are effective in inhibiting pathogens' growth in pineapple juice. However, high temperature treatments could degrade nutrients and flavours due to the heat-sensitive properties of the juice. Nevertheless, low-temperature treatments may not effectively inhibit pathogens (Fonteles et al. 2021). Given that physicochemical and phytochemical properties vary by variety, it is crucial to analyse how MD2 pineapple juice can be heat-treated to inhibit harmful pathogens and spoiling agents while preserving its nutritional and physicochemical quality (Rashima et al. 2019). In addition, pineapple juice properties may change during storage (Li et al. 2020). Therefore, this study aims to evaluate the effect of heat treatment and storage on the physicochemical and phytochemical properties of MD2 pineapple juice.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

All reagents purchased were of analytical grade (AR). Metaphosphoric acid (HPO_3), ascorbic acid ($C_6H_8O_6$), and 2,6-dichlorophenol-indophenol, $C_{12}H_7NCl_2O_2$ (DCPIP) were purchased from HmbG Chemicals, Germany. Bradford reagent (Coomassie Brilliant Blue G-250, CBBG) and sodium carbonate (Na_2CO_3) were acquired from R&M, Malaysia and bovine serum albumin (BSA) was purchased from Nacalai Tesque, Japan.

PREPARATION OF MD2 PINEAPPLE JUICE

Ripe and overripe MD2 pineapples were donated by Smart KJ Agro (Asia) PLT farm in Bukit Tangga, Kedah. The maturity index of pineapple fruits was categorised based on the classification by the Malaysian Federal Agricultural Marketing Authority (FAMA) (FAMA 2019). The pineapples used in this study were mature-green (Index 3), ripe (Index 5) and overripe (Index 6 and 7). Ripened MD2 pineapples used in this study had an average weight of 1.5 ± 0.3 kg, a length of 20 ± 2 cm, and a diameter of 12 ± 1 cm. Overripe fruits weighed an average of 1.0 ± 0.2 kg, with lengths of 22 ± 2 cm and diameters of 11 ± 1 cm. For both heat treatment and storage trials, pineapple juices were prepared based on the standard juicing method. Firstly, pineapple crowns were removed manually, and the skin was peeled using a kitchen knife. Then, juicing was performed using a slow juicer (BioChef Synergy BCSYN, Australia). Finally, the pineapple juice was strained through a 40-mesh filter cloth to remove large pulp particles.

HEAT TREATMENT OF MD2 PINEAPPLE JUICE

Heat treatment was performed on the same day as pineapple juice preparation. An amount of 10 mL of pineapple juice was placed in a glass screw-capped test tube and subjected to heat treatment using a thermostatic water bath (Julabo TW12, Germany). The effect of heat treatment was done at different temperatures (40, 50, 60, 70, 80 and 90 °C) and time (0-150 min) on the physicochemical content (TSS and BI). During this period, sub-samples were removed from the water bath at 10-min intervals and immediately cooled in an ice bath to halt further thermal effects. For the phytochemical degradation of vitamin C and total protein content, the same heat treatment was conducted at different temperatures (50-90 °C) with a time of 0-30 min. During the phytochemical degradation sampling, samples were withdrawn from the water bath at 2-min intervals and rapidly cooled in an ice bath (Sattar et al. 2019). Pineapple juice samples were stored in a freezer (Midea, Malaysia) at -20 °C and analysed on the following day.

STORAGE TRIAL AND SHELF-LIFE ASSESSMENT

The storage trials were conducted to assess the shelf-life stability of pasteurised pineapple juice under different temperatures designed to simulate real-world storage environments. Firstly, the juice was pasteurised at 90 °C for 30 s to reflect standard industrial processing practices and to ensure microbiological safety during the storage period. Then, pasteurisation was performed on the same day as juice preparation using a custom-built continuous-flow pasteurizer. Before pasteurisation, boiled water was circulated through the tubing and coil system to sterilise the equipment. The prepared pineapple juice was then pumped through the pasteurizer coil at a flow rate of 0.55 L/min, dispensed into ultraviolet (UV)-sterilised 100 mL PET bottles with twisted caps, and immediately cooled in an ice bath (Akyildiz, Mertoglu & Agcam 2021).

The pasteurised juice samples were stored for 84 days under four different temperatures representing common storage scenarios: frozen (-20 °C), chilled (4 °C and 7 °C), and ambient temperature (27 °C). The sampling frequency varied based on the expected rate of spoilage. Finally, samples stored at 7 °C and 27 °C were analysed daily due to the higher likelihood of degradation at elevated temperatures. Meanwhile, samples stored at -20 °C and 4 °C were analysed weekly, given the slower expected rate of change at lower temperatures.

Due to the limitations in laboratory equipment, the shelf-life assessment focused only on the physical stability of the juice during storage. The physical appearance of the samples was visually examined before each phytochemical analysis. Samples exhibiting signs of spoilage, such as cloudiness, sediment formation, or the presence of white precipitates, were deemed microbiologically or physically unstable and were excluded from further analysis. Therefore, these spoiled samples were discarded to ensure the accuracy, reliability, and consistency of the analytical data.

PHYSICOCHEMICAL ANALYSIS OF MD2 PINEAPPLE JUICE

Total soluble solids

The total soluble solid (TSS) value of pineapple juice was measured using a handheld digital refractometer (Atago PAL-1, Japan) in terms of Brix degree (°Brix). Measurements were done at ambient room temperature (25 °C), and the device was calibrated using distilled water (Mênouwesso et al. 2020).

Browning index assessment

A sample of 15 mL of pineapple juice was poured into a 3.5 cm Petri dish. Then, the image of each pineapple juice sample was captured using a smartphone camera (Oppo CPH2239, Malaysia) at a distance of 10 cm. Once the images of the samples were imported into ImageJ software (1.49v, USA, 2015), the colour L* (lightness), a* (redness), and b* (yellowness) values were analysed. After analysing the colour, the browning index (BI) of the pineapple juice sample was calculated using Equation (1) according to the methods of Dhar, Bhalerao and Chakraborty (2021).

$$BI = \frac{31}{0.172}\left[\frac{\alpha^* + 1.75 \chi L^*}{5.645 \chi L^* + \alpha^* - 3.012 \chi b}\right] \quad (1)$$

PHYTOCHEMICAL ANALYSIS OF MD2 PINEAPPLE JUICE

Total protein content

An amount of 0.1 mL of pineapple juice was thoroughly mixed with 1 mL of Bradford reagent. Then, the samples were incubated at room temperature for 5 min. After incubation, the absorbance of the samples was measured at 595 nm using a UV-visible spectrophotometer (Genesys 20 ThermoSpectronic, USA). Finally, the protein content in the pineapple juice samples was determined using a standard curve developed using bovine serum albumin (BSA) (Li et al. 2022). Results were expressed in terms of the BSA equivalent (mg of BSA/100 mL of pineapple juice).

Vitamin C content

Vitamin C content was determined using 2,6-dichlorophenolindophenol (DCPIP) visual titration method. In brief, a solution of 2,6-dichlorophenolindophenol (DCPIP) was prepared by dissolving 0.25 g of DCPIP and 0.21 g of sodium carbonate in 1 L of distilled water. Next, 2 mL of the juice sample was mixed with 8 mL of metaphosphoric acid solution (3% w/v). After obtaining the mixtures, it was then titrated with DCPIP solution until a light pink colour persisted for at least 15 s, indicating the endpoint. Results of vitamin C concentration were expressed as milligrams of ascorbic acid per 100 mL of pineapple juice and calculated using Equation (2), according to the methods of Hassan and Joshi (2020).

$$\text{Vitamin C (mg ascorbic acid / 100 mL)} = \left[\frac{\text{Dye factor} \times \text{Volume of DCPIP}}{\text{Pineapple juice volume (mL)}}\right] \quad (2)$$

DEGRADATION KINETICS OF MD2 PINEAPPLE JUICE

The thermal degradation kinetics of the pineapple juice compositions were modelled using a first-order rate law, based on the Arrhenius equation. Experimental data were fitted to the model using SigmaPlot software (version 14, Systat Software Inc., USA). The reaction rate constant (k) was determined using Equation (3), and calculations were performed in Microsoft Excel (version 2019).

$$C_t = C_0 e^{-kt} \quad (3)$$

where C_0 is the concentration of the properties of the pineapple juice at 0 s; C_t is the concentration at time t; and k is the kinetic constant (min^{-1}) (Sarkis et al. 2019).

The Arrhenius equation (Equation 4) was used to calculate the activation energy to describe the temperature dependence of the reaction during heat treatment.

$$k_t = Ae^{\frac{-E_a}{RT}} \quad (4)$$

where k is the degradation rate constant at absolute temperature T (K); A is the frequency factor or the Arrhenius constant (min^{-1}); E_a is the activation energy (J/mol); and R is the universal gas constant (8.314 J/mol·K) (Hardinasinta et al. 2019).

The half-life of degradation ($t_{1/2}$) for a first-order reaction was calculated using Equation (5) (Sarkis et al. 2019).

$$t_{1/2} = \frac{ln2}{k} \quad (5)$$

STATISTICAL ANALYSIS

All experimental measurements were conducted in triplicate, and the data were reported as means ± standard deviations. The data were subjected to a one-way analysis of variance, followed by Tukey's test for multiple comparisons, with $p < 0.005$ considered significant. The analysis was performed using Minitab software (version 17, Minitab LLC, 2010).

RESULTS AND DISCUSSION

EFFECT OF HEAT TREATMENT ON THE PHYSICOCHEMICAL PROPERTIES OF MD2 PINEAPPLE JUICE

The effect of heat treatment on the physicochemical properties (TSS and BI) of the pineapple juice is shown in Figure 1. Both TSS and BI remained constant at temperatures of 40-80 °C during durations from 0 to 150 min. As observed, soluble solids (mainly sugar) and coloured substances in pineapple juice were not affected by heat at those temperatures. However, as the temperature reached 90 °C, TSS and BI values were observed as significantly different. The TSS content was observed as the lowest at a temperature of 90 °C (Figure 1(A)). Meanwhile, BI exhibited the highest value when treated at 90 °C as compared to the other temperatures (Figure 1(B)). The slight decrease in TSS at 90 °C (12%) showed that prolonged exposure to high temperatures (above 90 °C) might degrade sugar compounds, thus reducing the TSS. Nonetheless, these results are comparable to other fruit and vegetable juices (Gonçalves et al. 2020; Makroo, Srivastava & Jabeen 2022).

Moreover, temperature and time could influence the colour of pineapple juice. Exposure to high temperatures could also lead to browning, a process known as non-enzymatic browning. However, Fellows (2017) highlighted that heat treatments have a lower effect on the juice colour as compared to enzymatic browning. Meanwhile, the BI value of juice was influenced by the non-enzymatic pathway than enzymatically when heated at 90 °C. In this study, a maximum increase of 19% in BI was observed during the heating temperature and time of 90 °C and 150 min, respectively. As a result, a change in deeper yellow colour was observed in pineapple juices. Considering these two factors, the stability of the BI at 40 °C and 50 °C suggested that neither heating nor enzymatic browning significantly affects the BI of the pineapple juice at lower temperatures. A possible explanation for this might be that, during heat treatment, the elevated temperatures might have caused an increase in browning by inactivating polyphenol oxidase (PPO) and peroxidase (POD), thus stabilising the colour of pineapple juice (Mênouwesso et al. 2020).

Several factors could explain this observation. Firstly, reactions that occur during fruit processing and storage could cause the pineapple juice to develop browning as measured in BI. The browning reaction in pineapple juices is caused by the activity of PPO and peroxidase POD. These oxidoreductases catalyse the oxidation of phenolic compounds, leading to the formation of brown pigments in pineapple juice. Alternatively, high temperatures could inactivate the activity of these enzymes (above 60 °C for POD and above 80 °C for PPO), by controlling the browning effect in the pineapple juice (Vollmer et al. 2020).

EFFECT OF HEAT TREATMENT AND DEGRADATION KINETICS ON THE PHYTOCHEMICAL PROPERTIES OF MD2 PINEAPPLE JUICE

Vitamin C and protein in pineapple juice are susceptible to heat. As shown in Figure 2, the holding time of heat treatment for analysing these properties was reduced to 30 min, with temperatures at 50-90 °C. The total protein content and vitamin C concentration in pineapple juice decreased with increasing temperature and heating time, as shown in Figure 2(A) and 2(B), respectively. Meanwhile, the degradation kinetics of pineapple juices' phytochemicals are according to the corresponding Arrhenius plots presented in Figure 2(C) and 2(D). According to Hounhoigan et al. (2014), the reduction in vitamin C concentration during heat treatment is attributed to vitamin C being oxidised into dehydroascorbic acid or diketogulonic acid.

Table 1 presents the experimental first-order rate constants (k), half-life values ($t_{1/2}$) and activation energy (E_a) of vitamin C and protein degradation obtained directly from kinetic modelling at each temperature treatment. The k (min^{-1}) increased, while the $t_{1/2}$ (h) decreased as the temperature increased from 50 °C to 90 °C, further confirming that vitamin C and protein degradation are temperature-dependent.

The Arrhenius degradation kinetic model for total protein content and vitamin C during heat treatment is presented in Equations (6) and (7), respectively. Through the first-order kinetic model, determination coefficients (R^2) for total protein content and vitamin C concentration exhibited values of 0.97 and 0.99, respectively. Table 2 summarises the Arrhenius model parameters, which were derived by plotting the natural logarithm of the rate constants (ln k) from Table 1 against the reciprocal of absolute temperature (1/T), following the Arrhenius equation. The higher k value for vitamin C indicated that it degrades more rapidly than protein at the studied temperatures. Consequently, a higher activation energy (E_a) suggested that the degradation rate for vitamin C is strongly influenced by an increase in temperature, making it more thermally sensitive than protein.

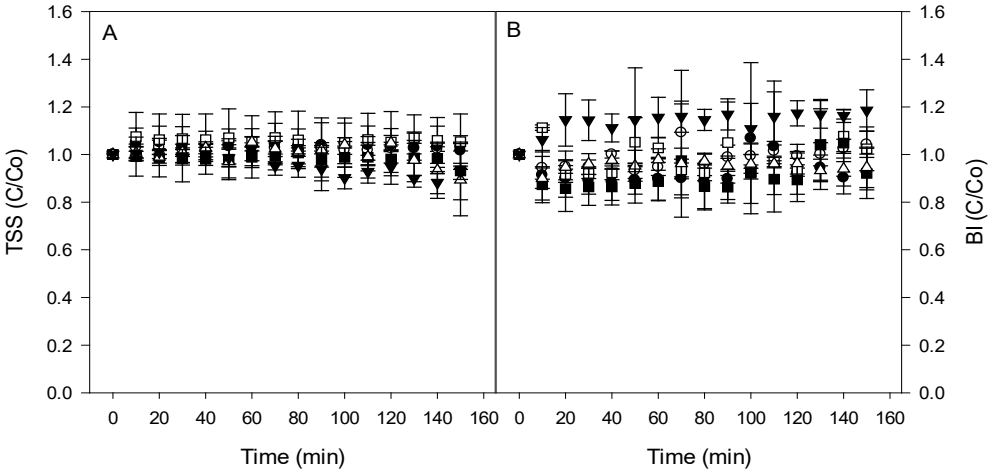

FIGURE 1. Effect of heating at 40 °C (○), 50 °C (●), 60 °C (□), 70 °C (■), 80 °C (Δ), and 90 °C (▼) for 150 min on TSS (A) and BI (B) in MD2 pineapple juice

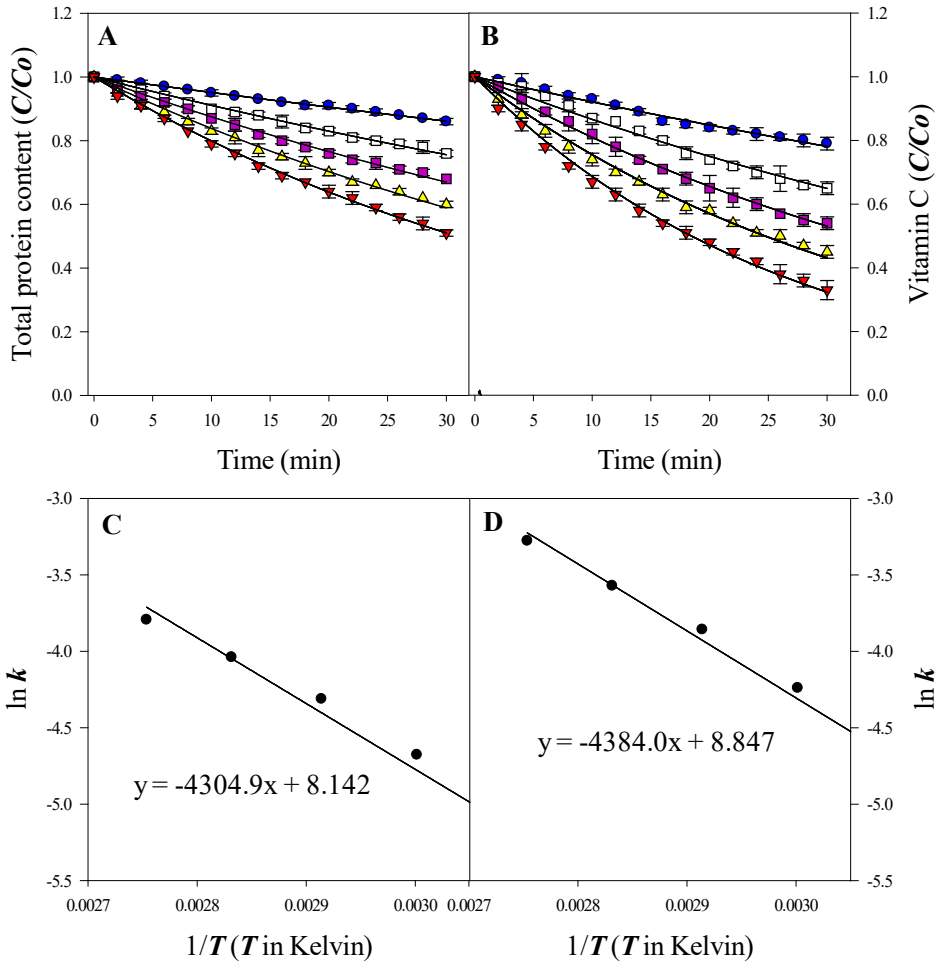

FIGURE 2. Effect of heating at 50 °C (●), 60 °C (□), 70 °C (■), 80 °C (▲), and 90 °C (▼) for 30 min on protein content (A) and vitamin C (B) in pineapple juice. The corresponding Arrhenius plots are shown in (C) for protein content and (D) for vitamin C

TABLE 1. First-order kinetic parameters for the thermal degradation of total protein content and vitamin C in pineapple juice during heat treatment

Property	T (°C)	k (min^{-1})	$t_{1/2}$ (h)
Total protein content	50	0.0050	2.31
	60	0.0093	1.24
	70	0.0134	0.86
	80	0.0176	0.66
	90	0.0225	0.51
Vitamin C	50	0.0082	1.41
	60	0.0144	0.80
	70	0.0211	0.55
	80	0.0281	0.41
	90	0.0377	0.31

T is temperature, k is kinetic constant, and $t_{1/2}$ is half-life

$$y = -4304.9x + 8.142 \quad (6)$$

$$y = -4384.0x + 8.847 \quad (7)$$

In another context, most proteins and enzymes denature at high temperatures (40 °C as optimal functioning temperature). An enzyme found in pineapple, such as bromelain, demonstrates stability to withstand temperatures at 60 °C due to its optimal activity between 40 and 60 °C (de Lencastre Novaes et al. 2015). Since bromelain constitutes part of the protein content in pineapple juice, it might not be significantly affected by heating at 50 °C. Therefore, bromelain concentrations may remain stable, while other protein concentrations might decrease. These relationships may be explained by Omotoyinbo and Sanni (2017), in which protein degradation rates at 50 °C and 60 °C may appear to be attributed to other proteins.

In evaluating the reliability of the constructed model, a validation test was performed to analyse the effect of pasteurisation on total protein content and vitamin C at a point within the model's range. Table 3 shows the prediction and validation data of the thermal model for both total protein content and vitamin C concentration after heating the pineapple juice samples at 90 °C for 30 s. As the difference between the prediction and validation data is statistically insignificant ($p > 0.05$), the developed model is considered capable of predicting the total protein and vitamin C concentration in pineapple juice when heated between 50 and 90 °C for 30 min. In addition to statistical testing ($p > 0.05$), model performance was further assessed using root mean square error (RMSE) and mean absolute error (MAE). The low values of both metrics (< 5% of the observed mean) indicate a high degree of accuracy in predicting vitamin C and protein degradation under the tested storage conditions.

PHYSICAL STABILITY AND DEGRADATION KINETICS OF TOTAL PROTEIN CONTENT AND VITAMIN C IN MD2 PINEAPPLE JUICE DURING STORAGE

The effect of storage duration (84 days) at different temperatures (-20, 1, 4, 7, and 27 °C) was evaluated on the protein content and vitamin C in MD2 pineapple juice (Table 4). As presented in Table 4, the physical stability in preserving the quality of pasteurised MD2 pineapple juice was observed at low-temperature storage (-20 to 4 °C). Meanwhile, phytochemical properties examined in the heat treatment section were also analysed on the effect of low-temperature storage, as shown in Figure 3(A) and 3(B). Based on the results, the total protein content and vitamin C in pineapple juice decreased over an 84 days storage period.

The most noticeable change in the composition of pineapple juice was the reduction in vitamin C concentration during storage. Consequently, a significant reduction of Vitamin C in the pineapple juices stored at 7 °C and 27 °C was observed until day 16 and 7, respectively. Meanwhile, vitamin C in the pineapple juices stored at 1 °C and 4 °C decreased significantly from day 1 to approximately day 30. Therefore, a slower rate of declination occurred during storage, which conforms to the first-order degradation kinetics model. Furthermore, the least degradation of vitamin C was observed in the sample stored at -20 °C, where the lowest C/C_o concentration was 0.58.

The linear curves showed R^2 values of 0.81 and 0.92 for total protein content and vitamin C, respectively, indicating a good fit to the first-order kinetic model (Table 6). In a complex system such as fruit juices, obtaining an ideal R^2 is unpredictable due to the natural variability in a biological system, such as various phytochemical substances present in the juice. The Arrhenius degradation kinetic model for total protein content and vitamin C during storage at the studied temperature and time is presented in Equations (8) and (9), respectively.

TABLE 2. Temperature dependence of total protein content and vitamin C degradation in pineapple juice during heat treatment

Property	k (min^{-1})	$t_{1/2}$ (s)	E_a (kJ mol^{-1})	R^2
Total protein content	4.30×10^3	0.0097	35.8	0.97
Vitamin C	4.38×10^3	0.0095	36.4	0.99

k is the kinetic constant, $t_{1/2}$ is half-life, E_a is activation energy, and R^2 is determination coefficient value

TABLE 3. Prediction and validation values of total protein content and vitamin C concentration in pineapple juice samples heated at 90 °C for 30 s using the thermal model

	Total protein content (mg BSA/100 mL)			Vitamin C (mg ascorbic acid/100 mL)		
	Concentration	RMSE	MAE	Concentration	RMSE	MAE
Prediction	94.57 ± 2.22[a]	1.27	1.13	52.97 ± 2.18[a]	0.64	0.56
Validation	93.43 ± 2.93[a]			52.41 ± 1.88[a]		

Data are presented as means ± standard deviations (N = 3). Values followed by different letters within each column are significantly different ($p < 0.05$) using Tukey's honest significant test

TABLE 4. Physical stability of pasteurised pineapple juice at different storage temperatures

Temperature (°C)	-20	1	4	7	27
Day	84	84	76	16	7

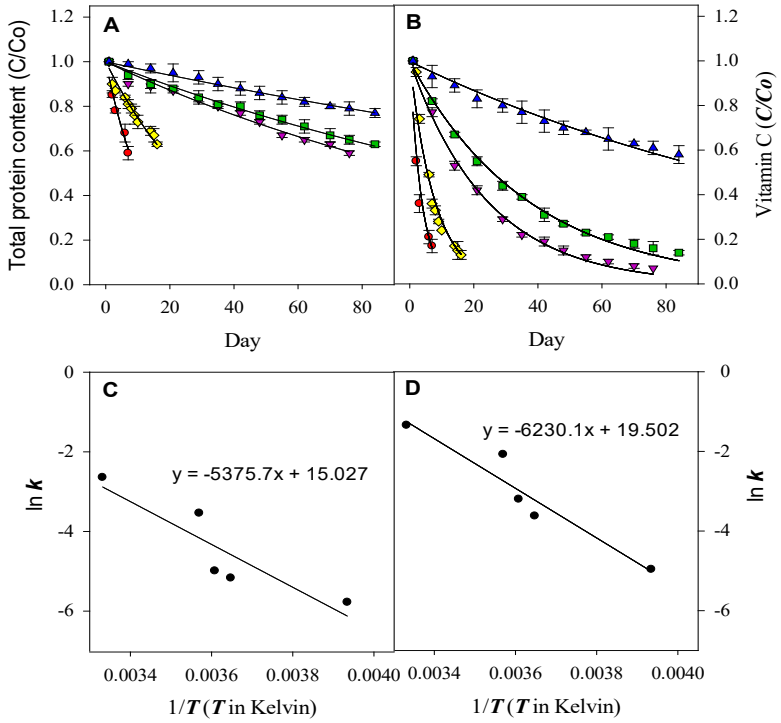

FIGURE 3. Effect of storage at -20 °C (▲), 1 °C (■), 4 °C (▼), 7 °C (♦), and 27 °C (●) on the total protein content (A) and vitamin C (B) in pineapple juice. The corresponding Arrhenius plots are shown in (C) for total protein content and (D) for vitamin C. Sampling frequency and total storage duration varied by temperature due to earlier spoilage at higher temperatures, which resulted in the exclusion of visibly spoiled samples

The values of k, $t_{1/2}$, and E_a of total protein content and vitamin C degradation are presented in Table 5. As the k value decreased, the $t_{1/2}$ value increased when the storage temperature decreased from 27 to -20 °C. Referring to the overall k, $t_{1/2}$, and E_a in Table 6, it was found that vitamin C is more susceptible to degradation during storage compared to the total protein content.

$$y = -5375.7x + 15.027 \quad (8)$$

$$y = -6230.1x + 19.502 \quad (9)$$

These higher rates of protein and vitamin C degradation at elevated storage temperatures were due to increased Maillard reaction rate. A probable explanation is that proteins reacted with sugars in the Maillard reaction, particularly under high-temperature conditions. Therefore, the reaction led to the reduction of amino acids, causing vitamin C to degrade rapidly as the Maillard reaction rate increases. Intermediates produced during the reaction may catalyse vitamin C oxidation. Consequently, the reaction progresses to form brown pigments known as melanoidins, contributing to the browning of the spoiled pineapple juice. The reduction in protein content and vitamin C concentration during storage may be caused by the presence of oxygen in the headspace of the pineapple juice bottle, known as oxidation. According to Pipliya, Kumar and Srivastav (2024), oxidation could increase the chemical reaction rate caused by heat treatment. Research findings indicated that lower storage temperatures effectively preserve the properties of fruit juice compared to higher temperature conditions. In addition to maintaining the physicochemical and phytochemical quality of the product, low-temperature storage aids in controlling microbial activity, such as heat-resistant fungi (Ali et al. 2024; Souza et al. 2017).

A validation test was conducted to evaluate the degradation kinetic model for total protein content and vitamin C concentration during storage. The validation points were conducted at week 1 on vitamin C concentration and protein contents at 1 °C and 4 °C, respectively. Table 7 shows the experimental means of the properties and the predicted values from the model. As the differences between the prediction and validation data are statistically insignificant ($p > 0.05$), the developed model is able to predict the total protein and vitamin C concentration in pineapple juice during storage within the studied durations and temperatures. The RMSE and MAE values represent less than 5% of the observed mean, indicating high predictive accuracy of the model.

TABLE 5. First-order kinetic parameters of total protein content and vitamin C in MD2 pineapple juice during storage at different temperatures

Property	T (°C)	k (day^{-1})	$t_{1/2}$ (day)
Total protein content	-20	0.0031	223.6
	1	0.0057	121.6
	4	0.0068	101.9
	7	0.0290	23.9
	27	0.0712	9.7
Vitamin C	-20	0.0070	99.0
	1	0.0267	26.0
	4	0.0408	17.0
	7	0.1256	5.5
	27	0.2604	2.7

T is temperature, k is kinetic constant, and $t_{1/2}$ is half-life

TABLE 6. Temperature dependence of total protein content and vitamin C degradation in MD2 pineapple juice during storage

Property	k (min^{-1})	$t_{1/2}$ (s)	E_a (kJ mol^{-1})	R^2
Total protein content	5.38×10^3	11.15	44.69	0.81
Vitamin C	6.23×10^3	9.61	51.80	0.92

k is the kinetic constant, $t_{1/2}$ is half-life, E_a is activation energy, and R^2 is determination coefficient value

TABLE 7. Prediction and validation values of total protein content and vitamin C concentration in pineapple juice during storage using the thermal model

	Protein content (mg BSA/100 mL)			Vitamin C (mg ascorbic acid/100 mL)		
	Concentration	RMSE	MAE	Concentration	RMSE	MAE
Prediction	63.17 ± 0.75[a]	0.42	0.36	12.32 ± 0.26[a]	0.47	0.47
Validation	62.95 ± 0.69[a]			12.16 ± 0.30[a]		

Data are presented as means ± standard deviations (N = 3). Values followed by different letters within each column are significantly different ($p < 0.05$) using Tukey's honest significant test

CONCLUSION

The degradation kinetics of MD2 pineapple juice were analysed on its physicochemical and phytochemical properties during heat treatment and over an 84 days storage period. The effect of different temperatures between 40-90 °C demonstrated pineapple juices' thermal stability as observed from TSS and BI. Meanwhile, vitamin C and protein degradation were observed to be temperature-dependent, showing decreased concentrations according to the first-order degradation kinetics. Commercial pineapple juice exhibited low phytochemical loss when stored at 4 °C, providing lower maintenance costs and convenience for ready-to-drink customers. The prediction and estimation of the phytochemical compounds (total protein content and vitamin C) in MD2 pineapple juice were successfully conducted using the kinetic models established in this study.

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