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Optimization of blending ratios in asparagus-lemon juice using response surface methodology

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Abstract

In this study, the effects of degradation on ascorbic acid (AA) in asparagus-lemon juice with different blending ratios were investigated during storage. The R^2 values of the first-order kinetic model for predicting AA loss were 0.8216-0.9990, indicating that the model described well the degradation of AA in asparagus-lemon juice with different blending ratios. Response surface methodology (RSM) was applied to optimize the blending ratio of asparagus-lemon juice during storage. The optimum blending ratios that selected by the RSM were: 23%, 26%, 27%, 28% and 29% of diluted asparagus juice blended with 85% of diluted lemon juice, and the desirability were 0.820. The RSM correlation coefficients (R^2) between experimental and model predicted values ranged from 0.6394 to 0.9975. Therefore, RSM and the first-order model may be effective tools to optimize the blending ratio of blended juice and AA degradation in juices.

Keywords: asparagus juice; lemon juice; blending ratio; ascorbic acid degradation; kinetic model; response surface methodology.

Practical Application: Use RMS to optimize the blending ratio of fruit juice and vegetable juice for improving storage ability and enhancing the nutritional value of blended juice.

1 Introduction

There is a growing interest in minimally processed fruits and vegetables due to their freshness, convenience, and characteristics closer to those of fresh foods (Moura & Vialta, 2022). The industry searches for market opportunities and tends to diversify its products by preparing new foods and enhancing sensorial, nutritional, and functional attributes (Mena et al., 2014). Many vegetable juices have a grassy smell and astringent taste, making them unpalatable. Since fruit juices have a sweet smell and good taste, they are usually used to mask the smell and taste of vegetable juice. Accordingly, new beverages based on fruit and vegetable juice are increasingly promoted and consumed. Recent research has attempted to demonstrate that blended juice are good alternatives to new products, which combines new taste and improvement in the quality of nutrition (Bamidele & Fasogbon, 2017). However, information that regards the stability of blended juice during storage is still scarce.

Asparagus (*Asparagus officinalis* L.) is popular in many countries because of its good sensory attributes and high nutritional values. Several studies have demonstrated that asparagus is rich in ascorbic acid (AA), phenols, flavonoids, polysaccharides, amino acids, and steroidal saponins (Guo et al., 2020). Asparagus is mainly used as fresh vegetable for cooking, but it is not common in the processing form. Lemon [*Citrus limon* (L.) Burm. f.] are rich in AA, dietary fiber, phenolic compounds, and flavonoids (Oikeh et al., 2015), and possess a variety of health functions including antioxidant capacity, anti-inflammatory, anti-tumor activity, cancer prevention activity, cardiovascular disease inhibition, lipid-lowering, *etc* (İnan et al., 2018). When lemon juice and asparagus juice are mixed, blended juice helps to improve flavor, slow the rate of degradation of AA, and increase antioxidant activity. AA is naturally found in most fruits and can be a quality indicator in foods since it is generally observed that if AA is well preserved, the other nutrients are also well retained (Megías-Pérez et al., 2014). Furthermore, AA has numerous biological functions, such as preventing free radical damage to DNA and cell, increasing water solubility and oil-entrapping ability, and resisting lipase activity (Hong et al., 2019). Significant AA losses occur during food processing and storage, reflecting the effects of food processing temperature and time, pH, packing material, and storage conditions (light, temperature, oxygen, *etc.*) (Chotyakul et al., 2014).

The advantages of response surface methodology (RSM) include the reduction in the number of experimental trials by evaluating multiple parameters and the function as a statistical tool to identify interactions (Mirani & Goli, 2021). RSM has been used to focus on the effect of binder and AA loading rates on improving AA retention (Nizori et al., 2018). Nonetheless, few studies have reported on using RSM to optimize the blending ratio of juice for enhancing the stability of AA during storage.

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Asparagus juice and lemon juice have aroused great interest in the area of food science and food processing, but studies of asparagus-lemon juice need more attention. Thus, the purposes of this research are (1) to evaluate the kinetics of AA degradation in different blended asparagus and lemon juice during different storage temperatures (15, 25, and 35 °C), and (2) to optimize the blending ratio of asparagus juice and lemon juice for alleviating the loss of AA during storage using RSM. As a result, an effective method has been provided for the juice industry to optimize the blending ratio of fruit and vegetable juice, aiming to improve the storage capacity and nutritional value of the blended juice.

2 Materials and methods

2.1 Raw material and juice extraction

Fresh asparagus was purchased from the local market in Jinhua (Zhejiang, China). Fresh ripe lemons were harvested from the lemon growing base in Anyue (Sichuan, China). The collected asparagus and lemons samples were immediately transported to laboratory by using a shock-proof box with an ice pack. Both asparagus and lemons were peeled, washed, and cut into pieces before the single strength juice extract using a juice extractor (Midea, JP351, China). Both single strength juices of asparagus and lemons were filtered using a double layer of cheesecloth to obtain clear juice, respectively.

2.2 Blended juice preparation

Both clarified asparagus juice and lemon juice were mixed with distilled water, respectively. Blended juice samples containing varying amounts of diluted asparagus juice (AJ) and diluted lemon juice (LJ), as shown in Table 1, using a central composite design, were prepared (Chauhan et al., 2014; Ya'acob & Zainol., 2020). Each sample of blended juice was packed in 100 mL glass bottles and pasteurized in a thermostatic water bath (Boxun, HH. S21-8, Shanghai, China) at 90 °C for 90 s before storage (Zhang et al., 2020). After that, samples were rapidly cooled in an ice bath and stored at 15 °C, 25 °C, and 35 °C in the dark in temperature-controlled storage cabinets, respectively (Fuyilian, FYL-YS-150L, Beijing, China).

Table 1. Central composite design for sampling (design factors are: blended juice of diluted asparagus juice and lemon juice).

Run	*Coded variables*		*Uncoded variables*	
	x_1	x_2	X_1: Diluted asparagus juice (%)	X_2: Diluted lemon juice (%)
1	0	0	50	50
2	-1	-1	25	25
3	0	0	50	50
4	1.41	0	100	50
5	0	1.41	50	100
6	0	0	50	50
7	0	0	50	50
8	1	1	75	75
9	0	-1.41	50	0
10	-1.41	0	0	50
11	0	0	50	50
12	-1	1	25	75
13	1	-1	75	25

2.3 Determination of Ascorbic Acid (AA)

AA content in the blended juice was determined based upon the quantitative discoloration of 2, 6-dichlorophenol indophenol (Merck KgaA, Darmstadt, Germany) titrimetric method as described in AOAC methodology No. 967.21 (Association of Official Analytical Chemists, 2000). Results of AA content were expressed as milligram AA per 100 mL juice. All determinations were performed in triplicate.

2.4 First-order kinetic model

It is generally assumed that the degradation of AA follows first-order kinetics in most biological materials of the food system. The loss of AA in the blended juice was calculated by using the first-order reaction given below

(Equation 1) (Chani-Paucar et al., 2021):

$$C = C_0 exp^{\left(-K^* t\right)} \quad (1)$$

where C, the AA content at time t; C_0, the AA content at time 0; K, the rate constant; t, the storage time.

2.5 Arrhenius equation

The Arrhenius equation is the most acceptable expression to account for the effect of temperature on the rate of AA destruction in food systems. The Arrhenius relationship for the temperature dependence for the rate constant degradation K is as follows (Equation 2) (Wang et al., 2022):

$$K = A\exp(-\mathrm{Ea}/\mathrm{RT}) \quad (2)$$

Where Ea, the activation energy of the reaction; R, the gas constant; T, the absolute temperature; A, the pre-exponential constant.

In addition, temperature quotients (Q_{10}) were calculated from the following Equation 3 (Ordonez-Santos & Martinez-Giron, 2020):

$$Q_{10} = K_{(T+10)} / K_{(T)} \quad (3)$$

where $K_{(T+10)}$, the rate constant at (T+10); $K_{(T)}$, the rate constant at T.

2.6 Calculation of the half destruction time ($T_{1/2}$)

The half destruction time $T_{1/2}$ (the time required for the degradation of 50% AA) of AA loss was calculated as Equation 4 (Moon et al., 2015):

$$T_{1/2} = ln2 / K \quad (4)$$

Where K, the first-order rate constant.

2.7 Central composite design (CCD)

CCD based RSM was employed to optimize the level of independent parameters and their effect on response factors. A central composite design with two variables was applied to study the response pattern and to determine the optimum combination of the variables (Ya'acob & Zainol., 2020). In this study, the water-based diluted juice of asparagus juice (X_1:0-100%) and lemon juice (X_2:0-100%) were used as two independent variables (Zaman et al., 2016). The codes(x) for the two independent variables were -1.41, −1, 0, 1, and +1.41 (Rosida et al., 2022). According to the following Equation 5 (Zhao et al., 2011), the variables were coded:

$$X_i = \frac{\left(X_i - \overline{x_i}\right)}{\Delta x_i} \tag{5}$$

Where x_i, the coded value of an independent variable; X_i, the real value of an independent variable; $\overline{x_i}$, the real value of an independent variable at the center point; Δx_i, the step change. The experimental design matrix in coded (x) form and at the actual level (X) of variables is given in Table 1.

2.8 Statistical analysis

Analysis of variance (ANOVA) was carried out by using the software SAS (version 9.2). Mean values were considered significantly different at $p < 0.05$. General linear and nonlinear fitting procedures for the analyses were determined using OriginPro software version 9.0 (OriginLab Inc., Northampton, MA). Optimal blending ratio was estimated by regression analysis and three-dimensional (3D) response surface plots of the independent variables with each dependent variable.

3 Results and discussion

3.1 Kinetic model of AA degradation in the blended juice

Figure 1 showed that the AA content in all blended juice was significantly decreased with time at a rate depending on the storage temperature. Compared with the blended juice at 15 °C

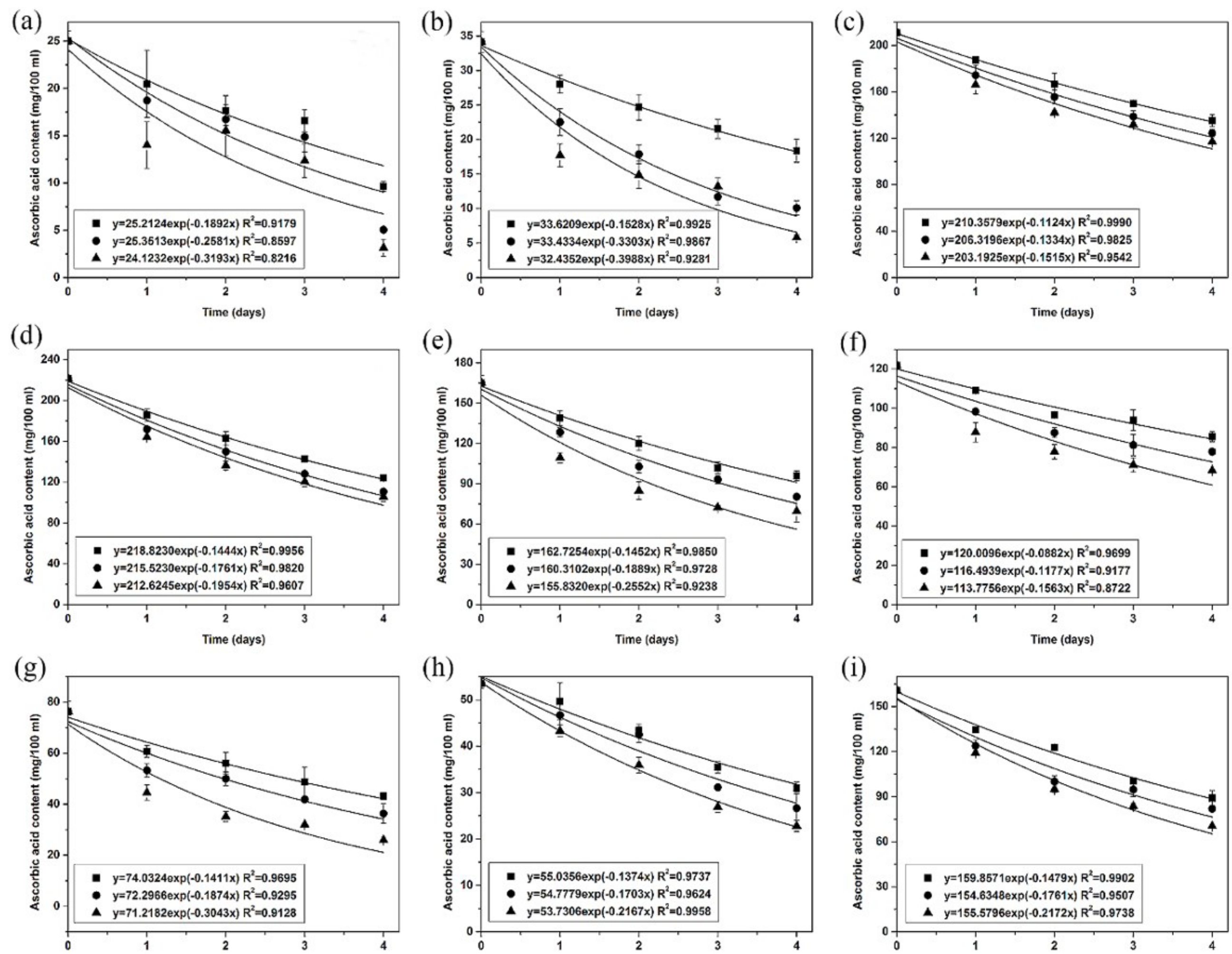

Figure 1. Degradation kinetics of AA in blended juice during storage at different temperatures (■15 °C, ●25 °C and ▲35 °C) and different blending ratios: (a) 50% AJ and 0% LJ, (b) 25% AJ and 25% LJ, (c) 50% AJ and 100% LJ, (d) 75% AJ and 75% LJ, (e) 50% AJ and 50% LJ, (f) 25% AJ and 75% LJ, (g) 75% AJ and 25% LJ, (h) 0% AJ and 50% LJ, (i) 100% AJ and 50% LJ.

and 25 °C, the blended juice stored at 35 °C was more likely to lose AA, which indicated that the loss of AA in the blended juice was decreased at lower temperatures. The results of this experiment were consistent with the results of several studies on the kinetics of thermal degradation of AA in food, all of which found that the variation of each physicochemical parameters of the samples stored at higher temperatures was greater than that of the samples stored at lower temperatures (Menevseoglu et al., 2020). The first-order kinetic model has been applied by many researchers to assess the AA degradation of biomaterials in food systems (Remini et al., 2015). The R^2 values ($R^2=0.8216$-0.9990) obtained from the fitting using the first-order kinetic model indicated that the first-order kinetic model appeared to be appropriate to explain the loss of AA in this study.

3.2 Response surface methodology of AA degradation in the blended juice

The obtained model coefficients were shown in Table 2, and the correlation coefficients (R^2) between experimental and model-predicted values ranged from 0.6394 to 0.9975. The response surfaces obtained from the determined models are represented in Figure 2 and Figure 3. The initial AA content of the blended juice has a linear and quadratic function relationship with the concentration of asparagus juice and lemon juice. As shown in Figure 2a, the increase in initial AA content depended on the increase in the concentration of asparagus juice and lemon juice. Figure 2b showed that Ea decreased as lemon juice concentration increased. One possible explanation for the result is that lemon juice showed a relatively low pH value, which lead to a decrease in Ea. Burdurlu et al. (2006) reported that the Ea of AA degradation was found higher in orange (pH 3.20), tangerine (pH 3.23) and grapefruit (pH 2.56) juice concentrate than in lemon (pH 1.82) juice concentrate.

Graphics of the response surface function analysis of the effect of asparagus juice and lemon juice concentration on Q_{10} ($Q_{10\ (15\text{-}25)}$, $Q_{10\ (25\text{-}35)}$, and $Q_{10\ (15\text{-}35)}$) of AA loss in the blended juice during storage were given in Figure 3a-3c. There was a strong negative linear relationship between the lemon juice concentration and Q10 in the blended juice ($p < 0.05$; Table.2). In addition, at $Q_{10\ (25\text{-}35)}$, the interaction effect between asparagus juice concentration and lemon juice concentration reached an extremely significant level ($p < 0.001$). Temperature is a factor that affects the degradation of AA. In general, AA is negatively

Table 2. Estimated coefficients of the fitted second-order polynomial for the eleven responses and their signification based on t-statistic.

		Coefficient [a]						*P*	*R*^{2}
		Intercept	X_1	X_2	X_1*X_2	X_1^2	X_2^2		
AA (mg 100 mL^{-1})		165.13	36.70***	62.03***	14.38***	-28.77***	-23.31***	< 0.0001	0.9975
Ea (kcal mol^{-1})		4.92	-0.66	-1.33**	0	0	0	0.0167	0.6590
Q_{10}	$Q_{10\ (15\text{-}25)}$	1.35	-0.13	-0.15*	0.18	0	0	0.0435	0.6770
	$Q_{10\ (25\text{-}35)}$	1.30	0.018	-0.067*	-0.16***	0	0	0.0102	0.6981
	$Q_{10\ (15\text{-}35)}$	1.32	-0.047	-0.098**	0	0	0	0.0207	0.6394
K	$K_{(15)}$	0.14	0.007	-0.021***	0.017**	0	0	0.0007	0.8355
	$K_{(25)}$	0.19	-0.010	-0.050***	0.050***	0	0	< 0.0001	0.9220
	$K_{(35)}$	0.25	-0.007	-0.074***	0.033*	0	0	< 0.0001	0.8975
T	$T_{(15)}$	5.04	-0.40*	0.84***	-0.86***	0	0	0.0008	0.8321
	$T_{(25)}$	3.67	-0.068*	0.95***	-0.89***	0.15***	0.12***	< 0.0001	0.9962
	$T_{(35)}$	2.72	-0.045*	0.92***	-0.36**	0.17**	0.26**	< 0.0001	0.9734

[a] *$P<0.05$, **$P<0.01$ and ***$P<0.001$.

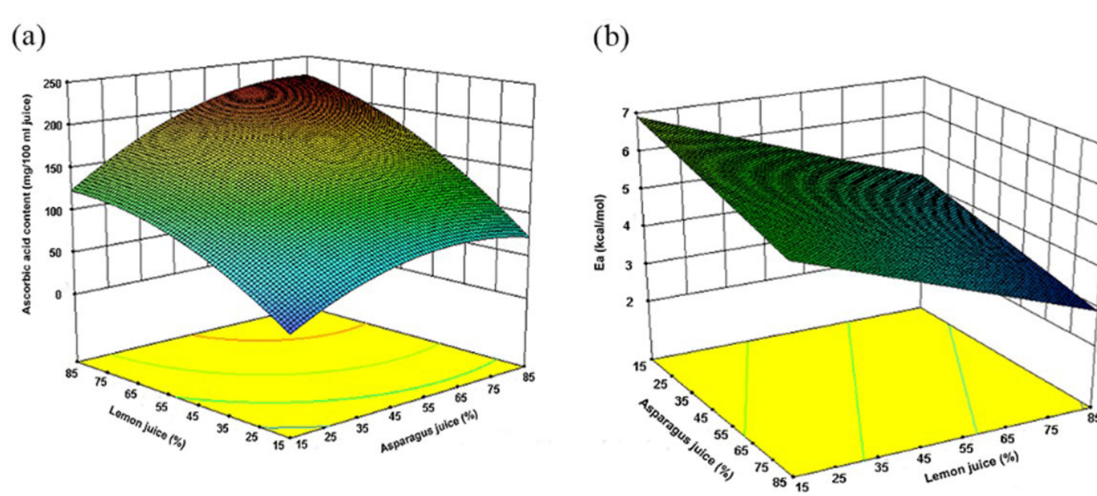

Figure 2. Response surfaces showing effect of blended juice on (a) AA content and (b) Ea.

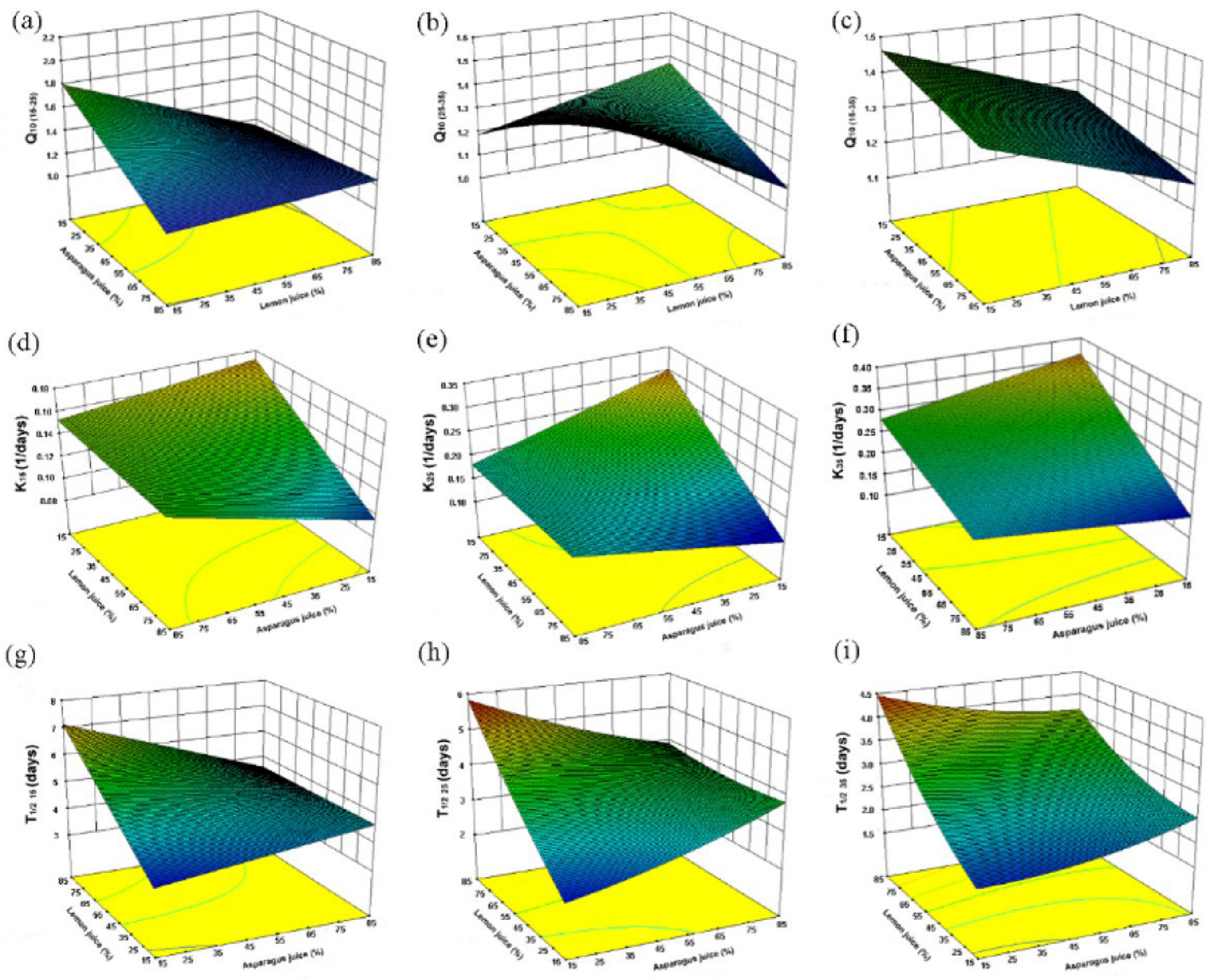

Figure 3. Response surfaces showing effect of blended juice on (a) $Q_{10\ (15\text{-}25)}$, (b) $Q_{10\ (25\text{-}35)}$, (c) $Q_{10\ (15\text{-}35)}$, (d) $K_{(15)}$, (e) $K_{(25)}$, (f) $K_{(35)}$, (g) $T_{1/2\ (15)}$, (h) $T_{1/2\ (25)}$, and (i) $T_{1/2\ (35)}$.

impacted by high temperatures (Mazur et al., 2014). At a higher concentration of lemon juice in the blended juice, it may be inferred that the degradation of AA was less impacted by the rise of temperature. The AA decrease in K ($K_{(15)}$, $K_{(25)}$, and $K_{(35)}$) was demonstrated in Figures 3d–3f, which inferred that the degradation of AA was impacted by the increase of lemon juice concentration in the blended juice. There was a strong negative linear relationship between the lemon juice concentration and K in the blended juice (*P <0.001*). There was a strong negative linear relationship between $T_{1/2}$ ($T_{1/2\ (15)}$, $T_{1/2\ (25)}$, and $T_{1/2\ (35)}$) and asparagus juice concentration in the blended juice (p <0.05), while there was a strong positive linear relationship between $T_{1/2}$ and lemon juice concentration in the blended juice (p <0.001). Moreover, Table.2 also showed that the quadratic term of $T_{1/2\ (25)}$ and $T_{1/2\ (35)}$ was positively and significant related to the concentration of asparagus juice and lemon juice (p <0.01). $T_{1/2}$ increased with increasing lemon juice concentration and decreasing asparagus juice concentration. The stability of AA could be partially attributed to the low pH value. A higher ratio of orange juice (70% orange juice: 30% pineapple juice) in the orange-pineapple juice can slow down the degradation of AA in the blended juice (Akusu et al., 2016). Thus, the blended juice with more lemon juice and less asparagus juice was more likely to inhibit the degradation of AA.

3.3 Optimization using desirability function

The optimum blending ratio of blended juice for alleviating the loss of AA during storage was developed based on the following objectives: maximizing the initial AA content and $T_{1/2}$, and minimizing Ea, Q_{10} and K. These objectives were combined into an overall desirability function. Desirability is an objective function that ranges from zero outside of the limits to one at the goal. The main purpose of optimization is to find a good set of conditions that satisfy all the objectives rather than achieving a desirability value of 1.0, which is a simply mathematical approach to finding the optimum (Kumar et al., 2020). Figure 4 showed a ramp desirability that was generated

Table 3. Optimum blending ratios and their initial AA content and kinetic parameters (Ea, Q_{10}, K and $T_{1/2}$) during storage.

Concentration (%)		The optimum blending ratios				
	Asparagus juice	23	26	27	28	29
	Lemon juice	85	85	85	85	85
	AA (mg 100 mL^{-1})	147.906	157.107	157.939	160.373	162.748
	Ea (kcal mol^{-1})	4.0872	4.0217	4.0516	3.9975	3.9794
Q_{10}	$Q_{10\,(15\text{-}25)}$	1.1600	1.1652	1.1657	1.1671	1.1686
	$Q_{10\,(25\text{-}35)}$	1.3408	1.3268	1.3255	1.3216	1.3177
	$Q_{10\,(15\text{-}35)}$	1.2560	1.2513	1.2509	1.2496	1.2483
K	$K_{(15)}$	0.1016	0.1040	0.1042	0.1049	0.1056
	$K_{(25)}$	0.1106	0.1147	0.1150	0.1162	0.1173
	$K_{(35)}$	0.1550	0.1576	0.1579	0.1586	0.1593
T	$T_{1/2\,(15)}$	6.8474	6.7220	6.7102	6.6755	6.6409
	$T_{1/2\,(25)}$	5.5555	5.4387	5.4279	5.3961	5.3648
	$T_{1/2\,(35)}$	4.3001	4.2360	4.2302	4.2131	4.1964
Desirability		0.820	0.820	0.820	0.820	0.820

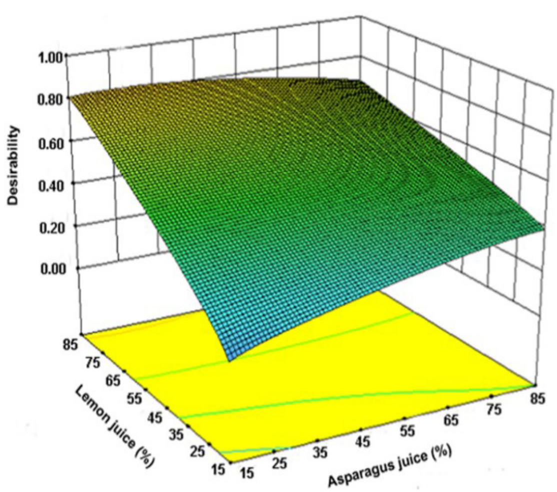

Figure 4. Response surface corresponding to the desirability function when the blended juice was optimized analyzing eleven responses simultaneously.

from 11 optimum points via numerical optimization. Through the optimization of RSM, the optimum blending ratio of blended juice in this study was obtained, as shown in Table 3. In addition, the initial AA content, $T_{1/2}$, Ea, Q_{10} and K were calculated under these optimum conditions as shown in Table 3. The optimum blending ratios selected by the RSM were: 23%, 26%, 27%, 28%, and 29% of diluted asparagus juice blended with 85% of diluted lemon juice. Under these conditions, the desirability value for multiple responses were 0.820, which meant that the predicted results matched well with the experimental results, verifying the validity of the RSM.

4 Conclusion

The results from this study showed that the thermal degradation of AA content presented in blended juice depends on time, temperature, and pH. The thermal degradation of the AA content in blended juice followed first-order kinetics. This study concentrated on the blending of asparagus juice and lemon juice and developed a method to assess the kinetics of AA degradation during storage at different temperatures. The findings indicated that temperature has an accelerated impact on AA degradation, which suggests that blended juice should be maintained at a lower temperature to prevent AA degradation. The optimum blending ratios selected by the RSM, were: 23%, 26%, 27%, 28% and 29% of diluted asparagus juice blended with 85% of diluted lemon juice, with a desirability of 0.820. In conclusion, asparagus-lemon juice with a higher concentration of lemon juice and a lower concentration of asparagus juice were a suitable choice for enhancing the stability of AA during storage. The RSM correlation coefficients (R^2) between these experimental and model-predicted values ranged from 0.6394 to 0.9975, which indicated that RSM may be an effective tool to optimize the blending ratio of blended juice.

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