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Degradation kinetics of betalains in red beetroot juices throughout fermentation process and storage

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 ORIGINAL ARTICLE

Abstract

Contemporary customers have increasingly heightened demands regarding their dietary preferences, favoring readily available and healthful ready-to-consume food and beverage alternatives. In order to fulfill this demand, the beverage industry has been exploring innovative products. Red beetroot juice (RBJ) is easily consumed and offers a beneficial advantage due to its betalain content. Both the traditional/spontaneous method and the industrial method are viable options for producing RBJ. The aim of this study was to examine the stability of betalains, the primary bioactive component in RBJ, throughout the fermentation process and during storage. The RBJ samples contained betalain pigments, as indicated by the infrared spectra. No decrease in betalain content was observed in either RBJ juice fermented by two different methods. The total betalain content of the RBJ samples was 594.068 and 535.152 mg/L at the beginning of fermentation through the spontaneous method and the method with the addition of *Lacticaseibacillus paracasei* 431 (*Lc. paracasei*), respectively, and 580.151 mg/L and 667.382 mg/L at the conclusion of fermentation, respectively. Mathematical models were employed to represent the degradation of betalains over time during storage. It was found that the sigmoidal model (R^2 = 0.974) and other models (R^2 = 0.957–0.969) demonstrated a greater potential to describe the degradation of betalains in RBJ samples produced by the spontaneous method compared to the first-order kinetics model (R^2 = 0.932). The models used in the study were successful, with R^2 values ranging between 0.927 and 0.932 in the RBJ samples produced with the addition of the probiotic *Lc. paracasei* for predicting betalain degradation.

Keywords: first-order kinetics, infrared spectrum, *Lacticaseibacillus paracasei* 431, mathematical model, spontaneous fermentation method

Introduction

A new diet-health paradigm has highlighted the positive aspects of diet, leading to nutritional studies that examine foods for their ability to protect and avoid disease (Nicoli, *et al.*, 1999). As a result, fruits and vegetables have gained prominence in the human diet as "functional foods" that can prevent or delay the onset of several types of chronic diseases due to the phytochemicals they contain (Kaur & Kapoor, 2001). The National Research Council (NRC) recommends that individuals consume at least five servings of vegetables and fruits every day to maintain optimal health (Jiratanan & Liut, 2004).

The rapid and chaotic contemporary lifestyle (characterized by quick adaptation to new inventions, dietary habits, and cultural changes) has led individuals to prefer beverages as simpler, more convenient options instead of natural forms of fruit and vegetables. Scientists have been investigating new, easily consumed, safe, and nutritious alternative products, while also suggesting optimal conditions for the beverage industry (Dimitrovski *et al.*, 2021; Durukan *et al.*, 2024; Mantzourani *et al.*, 2019, 2020; Mauro & Garcia, 2019; Sharma & Mishra, 2013; Usaga *et al.*, 2022; Wang *et al.*, 2022; Yoon *et al.*, 2004). For millennia, the production of fruit and vegetable juices has relied on the traditional or spontaneous fermentation methods. These methods allow for the efficient preservation of fruit and vegetable juices for extended periods, resulting in the production of appealing products. However, there is now increasing interest in industrial manufacturing due to potential concerns related to ensuring food safety of spontaneous methods. Additionally, people may have varied personal preferences for things produced using various methods.

Red beetroot (*Beta vulgaris* L.), is one of the selective vegetables that contains various compounds including carotenoids, phenolic substances, ascorbic acid, and flavonoids (de Oliveira *et al.*, 2021; Kujala *et al.*, 2001; Wootton-Beard & Ryan, 2011). Additionally, red beetroot is one of the sources of high concentration of betalains (Lee *et al.*, 2009). Betalains have been extensively studied for their nutritional and health benefits. Betalains and phenolic compounds enhance the resistance of low-density lipoproteins (LDL) to oxidation and help prevent cancer and heart disease by reducing the effect of free radicals on lipids (Hadipour *et al.*, 2020; Kanner *et al.*, 2001; Lever & Slow, 2010). Moreover, the consumption of beetroot has the potential to lower blood pressure and protect the liver from damage (Siervo *et al.*, 2013).

The stability of pigments is significantly influence by factors such as pH, heat, light, enzymes, oxygen, and aw values, in addition to the concentration of pigments and the particular structures of betalains (Martínez-Parra & Muñoz, 2001). To preserve the optimal pigment and color stability in betalainic foods, it is essential to carefully regulate specific time-temperature conditions throughout the food manufacturing process. Furthermore, external factors that arise during storage, such as fluctuations in temperature, exposure to light, and interaction with oxygen, must be taken into account (Herbach *et al.*, 2006).

Mathematical models have proved valuable for describing the experimental fermentation conditions of various substances (Cheng *et al.*, 2011; Dammak *et al.*, 2018; Germec *et al.*, 2018; Karaoglan *et al.*, 2021; Silva *et al.*, 2017). Furthermore, investigations have been conducted on the kinetics of degradation of particular bioactive chemicals under various food processing conditions, including temperature (Chew *et al.*, 2019; Peleg, 2019; Serris & Biliaderis, 2001; Thao *et al.*, 2023; Vieira *et al.*, 2000), light (Wong & Siow, 2015), moisture (Peleg, 2019), and other preservation techniques (Karaoglan *et al.*, 2019; Odriozola-Serrano *et al.*, 2009; Polydera *et al.*, 2005; Zhou *et al.*, 2016). In addition to bioactive compounds, betalains from various sources have been used in degradation-kinetic investigations, with most of these experiments finding that first-order kinetics were successful in describing degradation (Chew *et al.*, 2019; Guneser, 2021; Laqui-Vilca *et al.*, 2018; Rodríguez-Mena *et al.*, 2021; Tobolková *et al.*, 2020; Yang *et al.*, 2021). However, no research has been conducted to evaluate the degradation kinetics of betalains from the start of the fermentation process to the end of the storage period. In this study, two distinctive fermentation methods were used to produce RBJ, and the degradation was observed. Furthermore, many mathematical models were employed to demonstrate the degradation kinetics of betalains in RBJ during storage, rather than focusing on a single model.

Materials and Methods

Material

Fresh red beetroot and other components were purchased from local markets and used immediately. *Lacticaseibacillus paracasei* 431 (*Lc. paracasei*) was used as probiotic bacteria in the experiments and was obtained from CHR-HANSEN (Horsholm, Denmark).

Production of RBJ

As detailed in a previous study by our team (Duyar *et al.*, 2024), two different methodologies were selected to produce RBJ. The methods chosen were as follows: (i) the spontaneous method, which included neither temperature application nor a starter culture; and (ii) the industrial method, which included the addition of *Lc. paracasei* as a probiotic culture along with temperature application.

The spontaneous method involved combining the extracted vegetable juice mixture with 25% yogurt juice and allowing it to ferment at ambient temperature (24–25 °C) without any thermal treatment. In the method addition of *Lc. paracasei*, the vegetable juice mixture was pasteurized as part of the procedure before the precultured probiotic *Lc. paracasei* was added. Duyar *et al.* (2024) delineated a comprehensive protocol for inoculum preparation. For this methodology, our team established the parameters for pasteurization temperature, duration, and incubation temperature based on a previous optimization study (Durukan *et al.*, 2024). The optimum conditions for pasteurization at 60 °C for 22 minutes and incubation at 31 °C, which resulted in the highest total betalain content (TBC), lactic acid bacteria (LAB) count, and overall acceptability score of the RBJ samples. According to the article, the pH levels of the samples of RBJ produced spontaneously and with the addition of *Lc. paracasei* were average 4.00 and 3.83, respectively (Duyar *et al.*, 2024).

Total betalain content

The total betalain content (TBC) of the RBJ samples were assessed at different time points for fermentation and storage periods. The TBC were conducted in triplicate without adjusting the pH. The molar attenuation coefficients of betacyanin (ε=60,000 L/mol cm H_2O; λ=538 nm; MW=550 g/mol) and betaxanthin (ε = 48,000 L/mol cm H_2O; λ = 480 nm; MW = 308 g/mol) were used. The samples were prepared in various buffers and deionized water. For this purpose, 0.05 mol/L pH 6.5 phosphate buffer, pH 4.5 and pH 5.0 buffers were prepared. RBJ samples were sufficiently diluted with these buffers and deionized water to obtain absorption values of 0.8 ≤ A ≤ 1.0. The TBC of the samples was calculated using the following equation (Stintzing *et al.*, 2003).

$$BC[mg/L] = \frac{A \times DF \times MW \times 1000}{\varepsilon \times L},$$

where A is listed above for betacyanin and betaxanthin, respectively.

DF is the dilution factor.

L, 1 cm, is the cuvette's path length.

MW and ε are given above separately for betacyanin and betaxanthin.

Storage of the RBJ

The fermented juice samples are stored at 4°C in the freezer.

Spectroscopy

The infrared spectra were obtained by conducting measurements at a regulated temperature using the Bruker ALPHA ATR Platinum system, which equipped with a diamond crystal and a DTGS detector (Bruker Optics GmbH, Germany). Each measurement comprised of 16 scans within the spectral range of 4000 cm^{-1} to 400 cm^{-1}, with a resolution of 4 cm^{-1}.

Mathematical Modeling of degradation of betalains

The time-dependent TBC acquired during storage of samples were quantified using various equations. Samples were produced in triplicate for each methodology, and the average value for each sampling time was utilized in the modeling studies.

Equations employed:

1. Zero-order kinetics
   $C_t = C_0 - K \times t$
   C_t: the TBC of the samples at a specific time t (mg/L).
   C_0: initial concentration of TBC of the samples at time t=0 (mg/L)
   K: the kinetic constant, measured in units of per week
   t: time (week)

2. First-order kinetics
   $C_t = C_0 \times e^{K*t}$
   C_t: the TBC of the samples at a specific time t (mg/L).
   C_0: initial concentration of TBC of the samples at time t=0 (mg/L)
   K: the kinetic constant (per week)
   t: time (week)

3. The Weibull model
   $$C_t = C_0 \times \exp[-\left(\frac{t}{\alpha}^{\beta}\right)$$
   C_t: the TBC of the samples at a specific time t (mg/L).
   C_0: initial concentration of TBC of the samples at time t=0 (mg/L)
   α and β are the parameters that determine the shape of an estimation curve
   t: time (week)

4. Sigmoidal or modified Gompertz function equation (Lambert & Pearson, 2000)
   $C_t = A + X \times \exp[-\exp(k \times (t - M))]$
   C_t: the TBC of the samples at a specific time t (mg/L).
   A: minimum value that a function approaches as 't' approaches infinity
   K: the constant (per week)
   X: the difference between the high and low asymptotes.
   M: location where the fracture is observed

5. Exponential function (Arroyo-López *et al.*, 2008)
   $C_t = D + S \times \exp[-K \times t]$
   C_t: the TBC of the samples at a specific time t (mg/L)
   D: the specific value that the variable approaches as time approaches infinity.
   S: the anticipated value of the change
   K: the kinetic constant (per week)

6. The cubic regression
   $C_t = \beta_0 + \beta_1 \times t + \beta_2 \times t^2 + \beta_3 \times t^3 + E$
   C_t: the TBC of the samples at a specific time t (mg/L)
   β_0: constant of regression

β_1, β_2, β_3: the regression coefficients
t: time (week)
ε: residual term

7. Quadratic regression
$C_t = \beta_0 + \beta_1 \times t + \beta_2 \times t^2 + E$
C_t: the TBC of the samples at a specific time t (mg/L)
β_0: constant of regression
β_1, β_2: the regression coefficients
t: time (week)
ε: residual term

The adequacy of the models in describing this process was assessed using the regression coefficient (R^2) and root mean square error (RMSE) values, which indicate the degree of correlation between the expected and observed values. The RMSE value was computed using the following equation:

$$\mathrm{RMSE} = \left[\frac{1}{n}\sum_{i}^{n}\left|\left(C_{\mathrm{exp.}} - C_{\mathrm{obt.}}\right)\right|^{2}\right]^{1/2}$$

N: number of data points,
C: TBC of the samples (mg/L)

A RMSE score approaching 0 indicates that the model accurately predicts values that are either identical or very similar to the observed values. Equations for each model were developed to represent the rate of degradation of betalains over time, using the most appropriate model parameters. Subsequently, these equations were used to obtain the half-life values—the time required for 50.0% degradation of betalains ($t_{1/2}$).

Statistical analyses

The statistical analyses were performed using the MiniTab (20th Edition) program (MiniTab, State College, Pennsylvania). Tukey's pairwise tests of the Analyzes of variance (ANOVA) were used to determine the differences between each treatment. The *p*-values less than or equal to 0.05 indicated a significant difference in the means.

The SPSS software package (SPSS Inc, Chicago, USA) was used to identify the most suitable model for estimating the degradation curve in regression analysis. Among the variety of models, only those with high the (R^2) values were selected. For this purpose, the following models were selected: zero-order kinetics, first-order kinetics, the Weibull model, the sigmoidal or modified Gompertz function equation, the exponential function, the cubic regression model, and quadratic regression. Subsequently, the parameters associated with each model were calculated using the Microsoft Office Excel solver.

Results

This study evaluated the stability of betalains, which are crucial components of RBJ. Samples were produced using two different methods, and the quantities of TBC of the samples were assessed during both the fermentation process and the storage period. The data collected from the storage were presented using suitable mathematical models.

Characterization of the betalains in the RBJ samples

The infrared spectrum in Figure 1 displays distinct peaks at specific wave numbers, including 3315 and 3218 cm^{-1}, 1634 cm^{-1}, and 580 and 577 cm^{-1} for RBJ produced by the methods spontaneously and addition of *Lc. paracasei*, respectively.

Stability of betalains in RBJ samples during fermentation

Figure 2 illustrates the changes in TBC of RBJ samples during the two different fermentation methods periods. For the spontaneous method, TBC of samples obtained at the beginning and the end of fermentation was 594.068 and 580.151 mg/L, respectively. In the samples produced with the addition probiotic *Lc. paracasei*, the TBC at the beginning and end of fermentation was determined to be 535.152 and 667.382 mg/L, respectively.

According to the ANOVA test results, the effect of time on TBC was found to be significant for both methods ($p \leq 0.05$). Despite fluctuations in the TBC level during spontaneous fermentation, no statistically significant difference was observed between the beginning and the end of the fermentation ($p > 0.05$). The TBC of the samples reached its highest level at the end of fermentation (44th hour) in the samples produced with the addition of *Lc. paracasei* (Figure 2). The absence of a decrease in TBC during fermentation in both production methods indicates that betanin's exhibited resistance to degradation.

Betalain degradation of RBJ samples during storage

Both production methods involved sampling at various intervals over an 8-week period at 4°C, followed by the determination of the samples' TBC values. Based on the available data, mathematical models were used to express the degradation of betalains. The SPSS program conducted nonlinear regression to estimate curves and identify the most accurate models for data estimation. An Excel solver was used to optimize the parameters and the equations estimated with these models. Mathematical models tested include zero-order kinetics, first-order

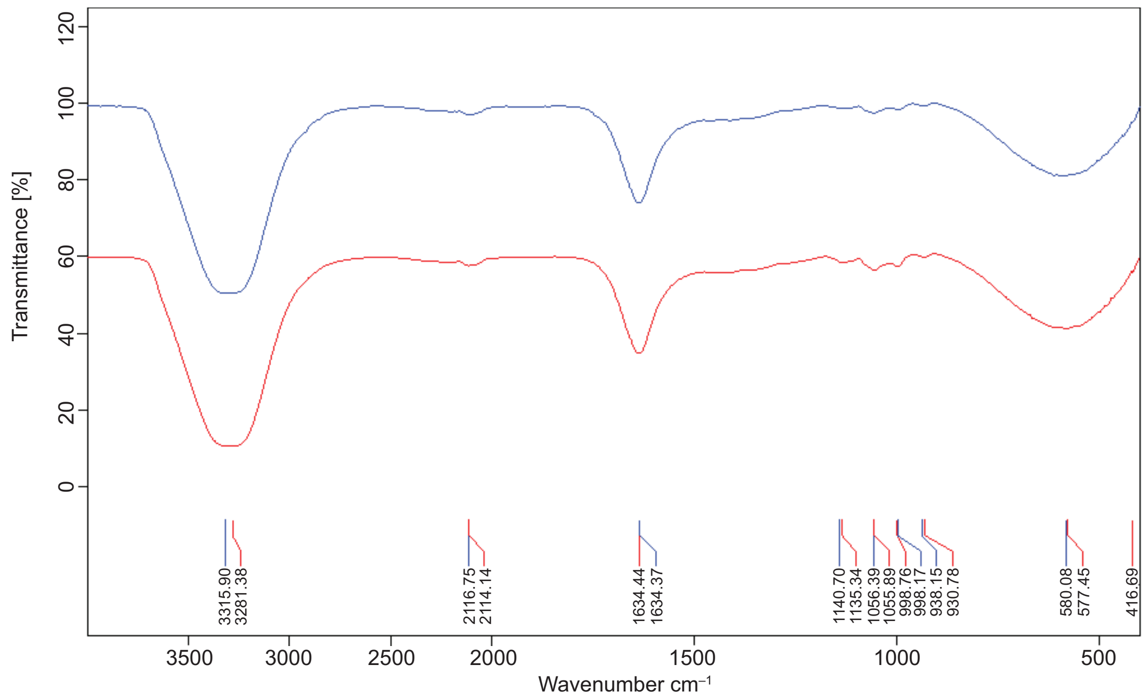

Figure 1. ATR results obtained at the end of fermentation of the samples produced by two different methods. *Blue lines represent the samples produced by spontaneous methods; red lines represent the samples produced by the addition of *Lc. Paracasei*.

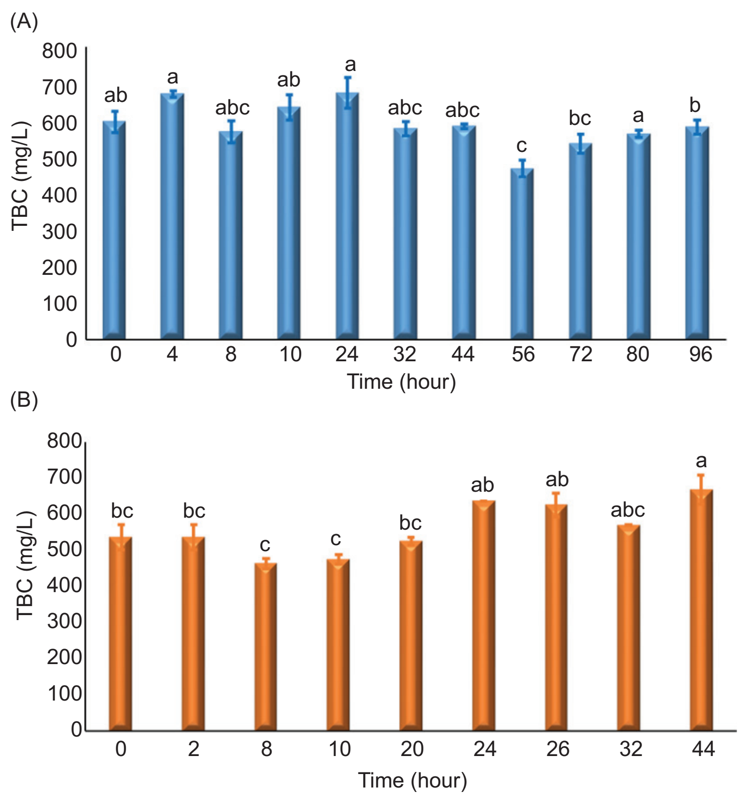

Figure 2. Time-dependent pH and TBC variation during fermentation of RBJ samples produced by different methods. (A) spontaneous method, (B) by the addition of *Lc. paracasaei*. TBC; total betalain content, RBJ; red beetroot juice. **Lowercase letters represent statistical differences of TBC within each production method.

kinetics, the Weibull model, the sigmoidal or modified Gompertz function equation, the exponential function, the cubic regression model, and quadratic regression. To select the model that most accurately reflected these data, R^2 and RMSE values were used. A well-estimated model is expected to have an R^2 value as close to 1 as possible and a lower RMSE value (Karaoglan *et al.*, 2019).

The betalains in the RBJ samples produced by the spontaneous method showed an increasing degradation trend throughout the storage period (Figure 3). Among all tested models, the first-order equation had the lowest R^2 value of 0.932 and the highest RMSE value of 21.416. The R^2 values of the other models range from 0.962 to 0.980. The findings indicated that all the models successfully predicted the degradation of betalain in RBJ over the 8-week storage period at a temperature of 4°C. The half-life of betalains in RBJ varies from 6.831 to 7.479 weeks, as presented in Table 1.

The betalains in the RBJ samples produced by the addition of *Lc. paracasei* displayed degradation when stored at a temperature of +4 °C. Figure 3 illustrates the degradation trends in the samples. Findings have indicated that, unlike samples produced by spontaneous methods, the degradation did not consistently increase on a weekly basis. Instead, specific intervals of stability were observed in weeks 3-5 and 6–8, within which degradation occurred. This suggests that the betalains in RBJ produced with the addition of *Lc. paracasei* exhibit improved stability compared to those produced by the spontaneous method.

In order to select the most suitable model for describing the observed degradation, the fit data, R^2, and RMSE

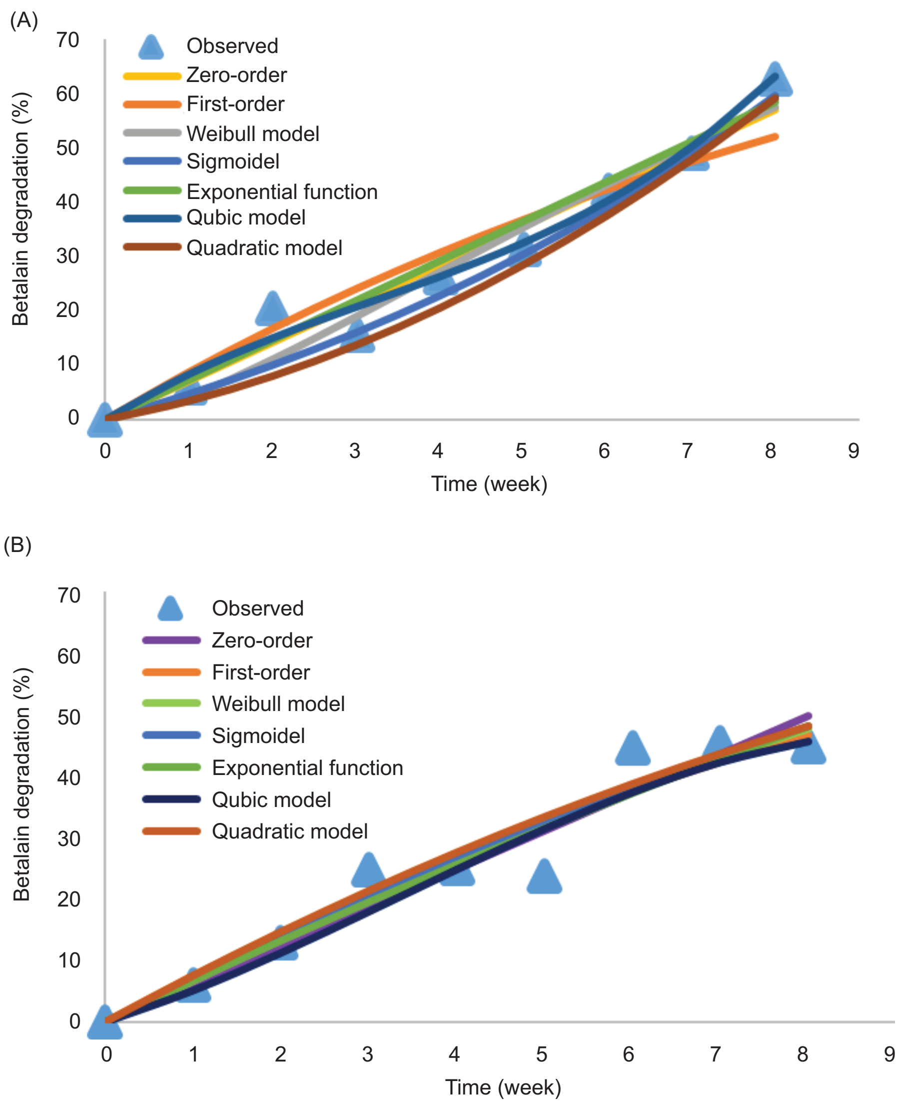

Figure 3. The description of the degradation kinetics of betalains in RBJ with various mathematical models. (A) spontaneous method, (B) by the addition of *Lc. paracasaei*.

Table 1. Fitting data of the models used for betalain degradation of RBJ samples produced by different methods.

Method for RBJ production	Model	R^2	RMSE	$t_{1/2}$ (week)	Equations
Spontaneous method	Zero-order kinetics	0.962	21.416	7.012	$Y = 560.185 - 39.942 \times t$
	First-order kinetics	0.932	30.328	7.271	$Y = 560.185 \times e^{-0.0920*t}$
	Weibull	0.957	22.825	6.976	$Y = 560.185 \times e^{-\left(\frac{t}{8.9099}\right)^{1.429}}$
	Sigmoidal	0.974	16.441	7.271	$Y = -59844.4 + 60585.59 \times e^{-e^{0.1196*(t-47.6814)}}$
	Exponential function	0.962	20.95	6.831	$Y = -59929.7 + 60500.27 \times e^{-0.0007*t}$
	Cubic regression	0.980	15.421	6.925	$Y = -0.74517 \times t^3 + 7.1066 \times t^2 - 53.648 \times t + 563.589$
	Quadratik	0.969	16.566	7.479	$Y = -2.95 \times t^2 - 15.27 \times t^2 + 526.706$
Addition of *Lc. paracasei*	Zero-order kinetics	0.927	28.725	7.551	$Y = 654.592 - 40.679 \times t$
	First-order kinetics	0.932	27.887	7.600	$Y = 654.592 \times e^{-0.079 \times t}$
	Weibull	0.932	27.525	8.133	$Y = 654.592 \times e^{-\left(\frac{t}{8.9012.003}\right)^{1.077}}$
	Sigmoidal	0.932	27.555	8.090	$Y = 15.026 + 65517.388 \times e^{-e^{0.0342(t+22.4086)}}$
	Exponential function	0.931	27.721	8.165	$Y = -709.447 + 1361.535 \times e^{-0.033 \times t}$
	Cubic regression	0.932	27.239	8.350	$Y = -0.74517 \times t^3 + 7.1066t^2 - 53.648 \times t + 563.589$
	Quadratik	0.931	27.475	7.977	$Y = -2.95 \times t^2 - 15.27 \times t^2 + 526.706$

RBJ: red beetroot juice.

values of the models were used (Table 1). Among all the models, the zero-order equation exhibited the lowest R^2 value of 0.927 and the highest RMSE value of 28.725. The R^2 values of the other models ranged from 0.931 to 0.932, indicating that all of these models were appropriate for predicting the degradation of betalains in RBJ produced by adding *Lc. paracasei* (Table 1). The half-life of RBJ produced with the addition *Lc. Paracasei,* as predicted by the models, ranged from 7.551 to 8.350 weeks (Table 1).

Discussions

The researchers conducted infrared spectral analyses to identify the primary functional groups present in the vegetables (De Souza *et al.*, 2003). The assignment of functional groups and their corresponding vibration modes was conducted in accordance with the existing literature. The presence of wide and intense peaks at 3315 and 3218 cm^{-1} indicates the occurrence of stretching vibrations in the hydroxyl groups (O–H) bond (Rodríguez-Félix *et al.*, 2022). Betalains' structures contain O-H groups that cause charge polarization and hydrogen bond formation (Calva-Estrada *et al.*, 2022). The wave numbers in the range of 1700–1600 cm^{-1} can be primarily attributed to the stretching vibrations of the amine I (C=O) bonds (Mecozzi *et al.*, 2012), and the wave number 1634 cm^{-1} confirming the presence of amine group. The peaks within the range of 1724 to 775 cm^{-1} primarily correspond to the functional groups of betalains (Güzel *et al.*, 2018). A less intense peak was detected in the RBJ samples at 2116 and 2114 cm^{-1} for RBJ produced by the methods spontaneously and the addition *Lc. paracasei*, respectively. According to Kumar *et al.* (2017) the wave number of 2116 cm^{-1} indicates the existence of stretching vibrations in carbonyl groups (C=O). Also, the identification of O–H and C=O functional groups, among others, provides evidence for the presence of betalains in RBJ.

There was no noticeable decrease in the samples' TBC level during the fermentation period. Betalains shows overall insensitivity to both external and internal factors within the pH range of 3–7 (Janiszewska-Turak *et al.*, 2022). A lower pH helps maintain betacyanin stability and prevents oxidating by polyphenoloxidases (PPO) and peroxidase (POD) (Strack *et al.*, 2003). If these enzymes are not properly inactivated, they might degrade betalain and lead to a loss of color (Czyzowska *et al.*, 2006; Paciulli *et al.*, 2016; Strack *et al.*, 2003). Previous research has shown that temperature treatment significantly impacts the inactivation of PPO and POD activity in various fruits and vegetables (Keenan *et al.*, 2012). Paciulli *et al.* (2016) found in their study that subjecting beetroot slices to a temperature treatment of 90°C for 7 min. resulted in an average PPO and POD activity of 30 and 25%, respectively. Additionally, an increase in betalain levels may be attributed to the extraction of betalains through thermal treatment performed during the method procedure.

Ravichandran *et al.* (2013) have found that preservation techniques such as microwave, vacuum, and high-pressure can enhance the concentration of betalains in red beet. The extraction process could lead to an elevation in the level of TBC due to the extraction of betalains, in the method by adding *Lc. paracasei*.

Beetroot pigments are derived from betalains, which consists of yellow-orange betaxanthins and red-violet betacyanins. The primary pigments present are betacyanins with betanin, which contribute 75–95% of the red color, while yellow betaxanthins with vulgaxanthin-I, account for 95% of the yellow color (Lacid & Ndez, 2003). In our study, we examined the degradation of TBC (total of red and yellow colored pigments). However, other research assessed the degradation of yellow-orange pigments, noting approximately 9.4% degradation after 12 hours of fermentation in Cactus pear juice (Turker *et al.* 2001).

Studies conducted on betalain extracts from sources such as red beetroot (Aztatzi-Rugerio *et al.*, 2019), quinoa peel (Laqui-Vilca *et al.*, 2018), purple pitaya drink (K. M. Herbach *et al.*, 2004) and red beetroot extracts added to yoghurts (Guneser, 2021) have also found that first-order reaction kinetics is an appropriate model for demonstrating degradation kinetics. Our results similarly show that first-order kinetics performs well for modelling degradation of betalains. However, the effectiveness of alternative models should not be overlooked. Particularly in the modeling of the degradation of RBJ samples produced by the spontaneous methods, the sigmoidal model (R^2=0.974) and other models (R^2=0.957-0.969) were more found to be more effective than the first-order kinetics model (R^2=0.932).

Along with pH, temperature application is a crucial factor in the degradation of bioactive compounds. Multiple studies have examined the effect of temperature variations on the degradation of bioactive compounds, demonstrating that higher temperatures lead to increased degradation rates (Vieira *et al.*, 2000; Serris & Biliaderis, 2001; Chew *et al.*, 2019; Peleg, 2019; Karaoglan *et al.*, 2019; Thao *et al.*, 2023). Therefore, beverages rich in bioactive compounds, such as RBJ, should be preserved at low temperatures.

Therefore, it was determined that the method involving the addition of *Lc. paracasei* required a longer time for the degradation of betalains compared to the spontaneous method. In a study conducted by Tobolková *et al.* (2020), the degradation of betalains in apple-RBJ during storage was examined revealing the half-life ($t_{1/2}$) values of betacyanin and betaxanthin to be 94.9 and 108.3 days, respectively, when held at a temperature of 2°C. Simultaneously, it was found that the $t_{1/2}$ of betacyanin and betaxanthin to be 30.3 and 59.2 days, respectively, when held at a temperature of 7°C. The study conducted by Yang *et al.* (2021) revealed that the $t_{1/2}$ for the degradation of betalains in white currant juice to be 29 days. By comparing our observed $t_{1/2}$ values with those reported in earlier studies, we may conclude that our findings align with those of Tobolková *et al.* (2020). In addition, our samples exhibit reduced sensitivity to betalains compared to the study conducted by (Yang *et al.* 2021). It is noteworthy that the TBC of the samples can vary based on the specific beet variety and the geographical location of cultivation (Janiszewska-Turak *et al.*, 2022).

Conclusions

RBJ is a trendy beverage known for its numerous health benefits due to its betalains content. However, betalains can be affected by a variety of factors, which may lead to quick degradation. Therefore, degradation kinetic studies are extremely helpful for identifying betalain degradation, understanding the impact of internal or external factors, optimizing process conditions, and minimizing degradation.

In this study, we found that the fermentation process did not cause degradation in the TBC of the samples for either of the fermentation methods. This highlights the significance of consuming beverages with a lower pH, as they inhibit betalain degradation. Additionally, maintaining stable, lower storage temperature is crucial for preserving betalains.

Based on the observed betalain levels throughout storage, variations were found between the two procedures. RBJ samples produced with *Lc. paracasaei* showed greater resistance to degradation in certain weeks and required a longer time for degradation to occur. The findings indicated that all the models successfully predicted the degradation of betalain in RBJ throughout an 8-week storage period at a temperature of 4°C. Furthermore, this study underscores the value of employing alternative models, particularly the Sigmoidal model, for investigating processes that were inadequately described by first-order models.

Conflict of Interest

The authors declare no conflict of interest.

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Author Contributions

H.A.K.: conceptualization; project administration; methodology, writing. F.S.: conceptualization; methodology; investigation. S.M.D.: methodology; investigation.

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