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*Research Article*

# A Feasibility Study on Monitoring Shelf Life of Bottled Natural Fruit Juice Using Laser-Induced Autofluorescence

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Shelf life of bottled natural fruit juice (BNFJ) provides relevant information on quality and authenticity for consumer protection. However, existing techniques for monitoring the shelf life of BNFJ are destructive and time-consuming. We report on using laser-induced autofluorescence (LIAF) spectroscopic technique in combination with multivariate analysis for shelf life monitoring of BNFJ. The LIAF spectra data were acquired for nine (9) continuous days on three batches of BNFJ samples purchased from a certified retailer. Deconvolution of the LIAF spectra revealed underlying peaks representing constituents of the BNFJ. Principal component analysis (PCA) was able to monitor the trend in the changes of the BNFJ as it aged. Partial least square regression (PLSR) predicted the exact day from the production of the BNFJ accurately at 96.6% and 98.8% in the training and testing sets, respectively. We, therefore, propose the LIAF combined with multivariate analysis as a potential tool for nondestructive, rapid, and relatively inexpensive monitoring of the shelf life of BNFJ.

## 1. Introduction

Natural fruit juice (NFJ) contains a lot of nutritional ingredients such as vitamins, minerals, antioxidants, and fibres which are essential for human health [1]. Even though there are different kinds of NFJ in production today, bottled natural fruit juice (BNFJ) remains the most produced in the beverage industry [2]. Its shelf life provides vital information on quality and authenticity for consumer protection, explaining why expiring dates are relevant and provided. The shelf life for BNFJ is therefore an essential indicator for quality control in the industry and is known to be short [3]. Shelf life is the period of time under defined conditions of storage after manufacture or packaging, during which food products will remain safe and suitable for consumption [4]. During this period, food products retain their sensory, chemical, physical, functional, microbiological, and nutritional characteristics in the best conditions for acceptance by consumers [5]. Within this period, the products are expected to meet any label declaration of nutritional information when stored according to the recommended conditions. The shelf life of any food product depends on factors such as composition, processing methods, packaging, and storage conditions [4]. This can be determined by monitoring physical, chemical, microbiological, and sensory changes during storage by measuring deterioration characteristics [5]. Shelf life studies have previously been conducted on different fruits such as orange, carrot, apple, cider, cranberry, mango, and tomato, as well as a mixture of two or more of these fruits [6–12]. In these studies, physicochemical parameters (pH, titratable acidity, total soluble solids, etc.) and sensory tests are employed to determine the deterioration associated with the fruit juices as they aged. Methods of determining these parameters are cumbersome and destructive, and therefore there is a need for a nondestructive, sensitive, and convenient method of which

laser-induced autofluorescence (LIAF) offers the needed advantage.

Laser-induced autofluorescence is a nondestructive, noninvasive, and sensitive analytical technique based on light absorbance and emission intensities that can be used to rapidly identify the presence of fluorescent molecules in a sample [13]. Fluorescent molecules in food include aromatic amino acids, vitamins, polyphenolics, and a variety of flavouring compounds which are suitable and reliable to detect using the LIAF technique [14]. The application of this technique to food samples has been suggested for the analysis of sugar, yoghurt, cheese, oils, honey, and distilled beverages [15–19]. Despite such successful applications of the LIAF technique, its application to the study of the shelf life of BNFJ is yet to be fully exploited.

The LIAF measurements normally produce large sets of data which at times could be tricky to analyze by mere visual inspection. Multivariate statistical methods can be adopted for data exploration, data reduction, classification, calibration, regression, and wavelength selection to assist with this type of analysis. Multivariate analysis utilizes mathematics and statistics to extract relevant information from spectral data. Multivariate analysis methods such as principal component analysis (PCA) allow the extraction of principal components or eigenvectors of a correlation matrix in a dataset, finds the main sources of variability in the dataset, and establishes the relationship between/within objects and variables [20, 21], whereas partial least square (PLS) regression can be used to relate the values of predictive data to the studied response [22]. LIAF has been combined with PCA and PLS for several studies [23–25].

This research aimed to evaluate the feasibility of using LIAF and multivariate analysis methods to monitor the shelf life of BNFJ.

## 2. Experimental Methods and Procedure

*2.1. Bottled Natural Fruit Juice Samples.* Nine (9) BNFJ samples comprising 3 samples from 3 different production batches were purchased from a certified retailer for the study. These samples had been prepared from a mixture of mango, pineapple, and passion fruits with no preservatives added. The shelf life as indicated on the BNFJ samples was seven (7) days. Each batch of the three samples was bought fresh on the first day of production and kept in a refrigerator at 4°C in the same laboratory with the LIAF setup. The samples were brought out one after the other each day for their LIAF spectra measurement to be conducted and kept back in the refrigerator within five minutes.

*2.2. Laser-Induced Autofluorescence Measurements.* The LIAF setup used in this study is shown in Figure 1. It comprises mainly of a laser source (O-Like, China) emitting at 445 nm, a bifurcated fibre optic probe (R400-7, Ocean Optics, USA), high-pass absorptive edge filter (GG445; $\lambda > 445$ nm, Ocean Optics, USA), and a spectrometer (USB 2000, Ocean Optics, USA).

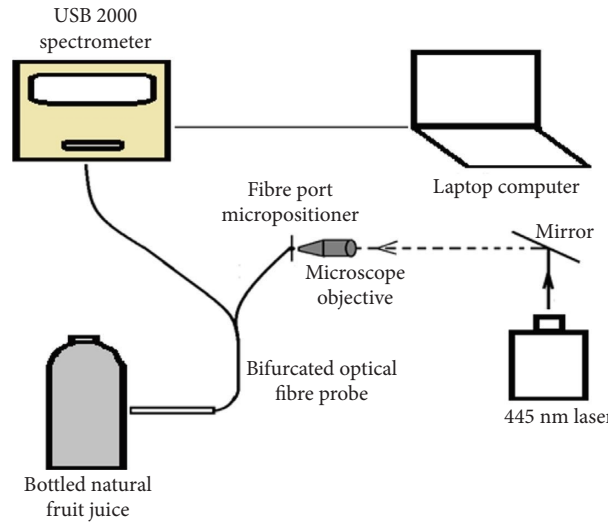


Figure 1: Schematic diagram of laser-induced autofluorescence (LIAF) setup used for data acquisition. Light via one arm of the bifurcated fibre probe excites the sample from which the emitted light is collected through the other arm of the probe. A high-pass filter allows only fluorescence light into the spectrometer to be visualized and saved on the computer.

The laser source was coupled to one arm of the bifurcated fibre optic probe using a fibre port micro-positioner (PAF-SMA-5-B, Thorlabs). This arm of the bifurcated fibre optic probe was incident on the sample, and the backscattered fluorescence light was collected by the other arm of the bifurcated fibre into the detection system which consisted of the high-pass absorptive edge filter and spectrometer. The filter was placed before the spectrometer to cut off the excitation wavelength, and the spectrometer was interfaced to an HP laptop computer (Intel (R) Core i3-2310M CPU @ 2.10 GHz, 796 MHz, and 2.94 GB of RAM) to display and record the LIF spectra using OOIBase32 software, an interface for the USB 2000 Ocean Optics spectrometer.

The LIAF spectral data were first obtained for an emptied BNFJ bottle and then on the NFJ filled samples. The spectral data collection on each sample was done within a space of five minutes in 24-hour intervals for nine (9) continuous days using the LIAF setup. For each measurement, 92 LIAF spectra data were recorded for 60 s using an integration time of 300 ms at ambient temperature. In all 276 spectra, data (i.e., 92 spectra × 3 replicates) were recorded and averaged for each BNFJ sample. A total of 81 spectra were obtained comprising spectra from 3 samples × 3 batches of production × 9 days of measurement. The recorded data were then exported into MATLAB (R2019a MATLAB 9.6, MathWorks Inc., USA) for analysis.

*2.3. Data Processing and Analysis*

*2.3.1. Deconvolution of the Laser-Induced Autofluorescence Spectra.* PeakFit software (4.12 version, Jandel Scientific, Germany) was used to deconvolve each of the spectra into separate bands as was done in a previous study [26]. The

PeakFit software combined the Loess smoothing function and Marquardt–Levenberg and Lorentzian spectral functions for analyzing the LIAF spectra. The Lorentzian spectral function helped the choosing of a reasonable corresponding fit of the spectra. This enabled the determination of the peak amplitude, centre wavelength, and full width at half maximum (FWHM) for further analysis. The individual peak wavelength positions of the peak were compared with the literature to identify corresponding molecular constituents of the BNFJ.

*2.3.2. Principal Component Analysis of Laser-Induced Autofluorescence Spectra.* Principal component analysis (PCA) was applied to the averaged LIAF spectra data of the BNFJ samples using self-written MATLAB codes. The PCA technique is useful for reducing the dimensionality and exploring underlining patterns in the spectral data [20]. In this work, the fluorescence spectra matrix is represented as $Q$ with $i$ rows (81 observations) and $j$ columns (2048 variables). PCA decomposes $Q$ as a sum of series combinations of scores ($u_i$) and the loadings ($v_i$) as in equation (1). The scores ($u_i$ vectors) contain information on how the fluorescence spectra relate to each other in the principal component (PC) space, whereas the loadings ($v_i$ vectors) contain information on how the wavelengths relate to one another.

$$Q = u_1 v_1^T + u_2 v_2^T + \ldots + u_k v_k^T + F. \tag{1}$$

*2.4. Partial Least Square Regression Analysis.* PLS is a very popular algorithm used for regression analysis. The dataset of 81 observations (predictors) used in this study was partitioned into training and test sets using the Kennard–Stone algorithm. Thus, 70% samples were used for training, and 30% samples were used for testing. The training set was used to develop the model, while the testing set was used for evaluating the predictive ability of the developed model. The PLS algorithm basically captures variations from both the predictive ($X$) and response ($Y$) parameters in equations (2) and (3) to construct a regression model to predict the response for unknown sample $X_{\text{test}}$ using equation (4).

$$X = MP^T, \tag{2}$$

$$Y = NQ^T, \tag{3}$$

$$Y_{\text{test}} = X_{\text{test}} U Q^T, \tag{4}$$

where $M$ and $N$ are the scores and $P$ and $Q$ are the loadings of $X$ and $Y$, respectively. $U$ is a diagonal matrix with the regression weights.

The accuracy of prediction model was measured using Pearson's correlation coefficient ($R^2$), rroot mean square error of calibration (RMSEC), and root mean square error of prediction (RMSEP) with equations (5)–(7), respectively. An acceptable robust model should have a high correlation coefficient, a low root mean square error (RMSE), and a very low prediction bias [27].

$$\text{RMSEC} = \sqrt{\frac{\sum_{i=1}^{n} (y_i - \widehat{y}_i)^2}{n}}, \tag{5}$$

$$\text{RMSEP} = \sqrt{\frac{\sum_{1=1}^{n} (y_i - \widehat{y}_i)^2}{n}}, \tag{6}$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n} (y_i - \widehat{y}_i)^2}{\sum_{i=1}^{n} (y_i - \overline{y})^2}, \tag{7}$$

where $n$ is the number of samples (in the training set and test set), $y_i$ is the reference measurement results for sample $i$, $\widehat{y}_i$ is the estimated result for sample $i$ when the model is constructed with sample $i$ removed, $y_i$ is the estimated results of the model for the sample $i$, and $\overline{y}$ is the average of the reference measurement results for all.

In addition, the systematic error of the average difference between the actual and predicted values known as bias was calculated using the following equation.

$$\text{Bias} = \sum_{i=1}^{n} \frac{\widehat{y}_i - y_i}{n}, \tag{8}$$

where $\widehat{y}_i$ is the prediction of the removed sample.

## 3. Results and Discussion

*3.1. Laser-Induced Autofluorescence Spectra.* The LIAF spectra of the BNFJ samples for the 9 continuous days are shown in Figure 2. Each day's spectra represent the averaged spectra from all samples (9) of the 3 batches. 9 days were chosen to enable two (2) extra days of observation of the LIAF spectra from the BNFJ upon expiry after the recorded shelf life of 7 days. Initial measurement on the fluorescence spectra of the empty NFJ bottle only (not presented) shows that the bottle had no effect on the LIAF measurements. Visual inspection of Figure 2 shows variations that can be observed in the normalized intensities of the LIAF spectra for the different days in different wavelength regions of the spectra. For instance, a reduction in spectral intensity is seen each day within the spectral region of 520–560 nm (inset).

The observed wavelengths in this study are closely related to the spectral signatures of chemical compositions in fruits [28–31]. Molecules that exhibit numerous conjugated double bonds, for instance, carotenoids, chlorophylls, and porphyrins, show light absorption in these spectral regions. Their absorption properties could be used to assess food products and may be used to predict shelf life [32]. This observation suggests that once BNFJ is freshly prepared, there are variations in the soluble solid substances with time, resulting in weakening in absorption and re-emission of incident light by endogenous fluorophores in the BNFJ. This phenomenon can be used as a basis for monitoring the shelf life of BNFJ.

Furthermore, to explore the constituents of the BNFJ, PeakFit analysis, as shown in Figure 3, revealed six (6) hidden peaks in the LIAF spectra of the samples. Peak 1 ranges from 475 nm to 500 nm; peak 2 overlaps peak 1 and

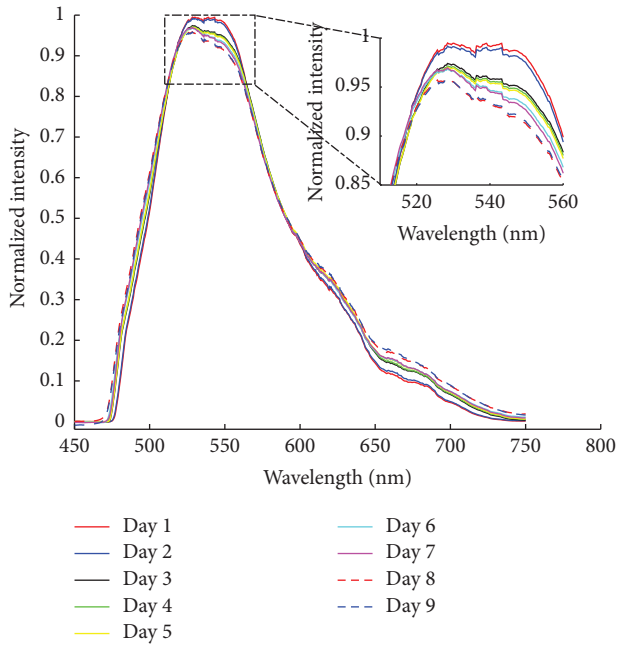


Figure 2: Mean LIAF spectra of BNFJ monitored for nine (9) consecutive days. Inset: spectral region (520–560 nm) showing reduction of LIAF intensity in 9 days.

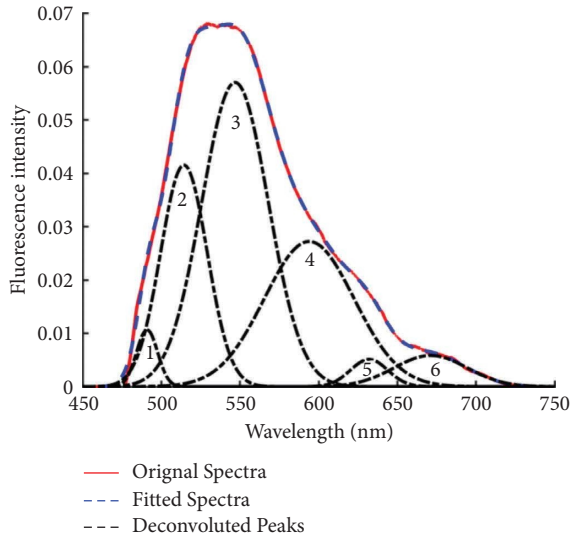


Figure 3: Deconvoluted LIAF spectra of BNFJ showing individual peak contributions.

ends at 550 nm; peak 3 exhibits the highest fluorescence intensity and spread from 500 nm to 600 nm. The remaining peaks are peak 4 (525 nm–650 nm), peak 5 (600 nm–650 nm), and peak 6 (625 nm–725 nm). Peak 5 shows the lowest fluorescence intensity. Peaks 1 to 4 (400–600 nm) show the presence of antioxidants mainly carotenoids which are essential for the delay of oxidation in fruits [31, 32]. The antioxidants present in the BNFJ samples deteriorate with the increasing number of days causing a rise in oxidation as observed in Figure 2, whereas peaks 5 and 6 (600–750 nm) show the presence of chlorophyll pigments [28].

*3.2. Principal Component Analysis.* Variance plot describing the contribution of two (2) principal components (PCs): PC1 (85%) and PC2 (7%), and loading plot showing wavelength-dependent factors influencing the variations in the 2 PCs are presented in Figures 4(a) and 4(b), respectively. Figure 4(a) reveals variations, significance, and contributions by ten (10) PCs to the LIAF data. It shows that PC1 is the linear combination of the LIAF data with maximum variance and PC2 is the linear combination with the next maximum variance orthogonal to PC1. The variation in the LIAF data can be further interpreted by inspecting the loadings [20] in this case corresponding to PC1 and PC2 (Figure 4(a)). Loading plot of PC1 and PC2 (Figure 4(b)) can be related to the deconvoluted peaks 1 and 2 (Figure 3). Besides, this plot shows part of the visible region (450 nm–750 nm) of the electromagnetic spectrum where electronic transitions occur. In addition, some peaks can be observed at specific wavelengths (475, 478, 480, 495, 520, 550, 630, 660, 754, and 760 nm) which are considered useful for PCs to be used for predicting shelf life of BNFJ.

A scatter plot of PC1 and PC2, which together accounted for 92% of the total variability in the LIAF spectral data for 9 days, is presented in Figure 5. The coefficients of PC1 are more significant as the number of days increases and also show a pattern of distribution of the BNFJ samples. The negative coefficients of PC1 show the scores of BNFJ samples monitored for the first 4 days while the positive coefficients display the scores of those monitored for the last 5 days. PC2 coefficients show a majority of the scores above the origin with a marginal separation of the BNFJ samples. These observations indicate that changes in BNFJ samples followed a common trend for 9 days in PC space, especially when PC1 scores are considered. Thus, PCA could help shelf life monitoring of BNFJ.

However, as seen in Figure 5, even though the PCA (unsupervised algorithm) follows a trend with regard to the shelf life, it did not give clear boundary between some of the days (e.g., day 1 and day 2). Therefore, a supervised regression algorithm was used to investigate the feasibility of LIAF for BNFJ shelf life monitoring.

*3.3. PLS Regression Analysis.* The PLS regression analysis for predicting the shelf life of BNFJ on both training and testing datasets is shown in Figure 6. The optimal PLS model was obtained on 6 latent factors. Prediction performance of 0.98 and 0.99 for $R^2$, 0.36 and 0.22 for RMSE (C/P), and 0.047 and 0.046 for prediction bias of the training set and test set was observed, respectively. The prediction performance demonstrates the suitability of the PLS model for shelf life monitoring of BNFJ. The PLSR analysis showed that each day of the BNFJ life span can correctly be predicted. Previous work on fruit juice was mostly destructive and not as simple and straight forward as compared to the LIAF method

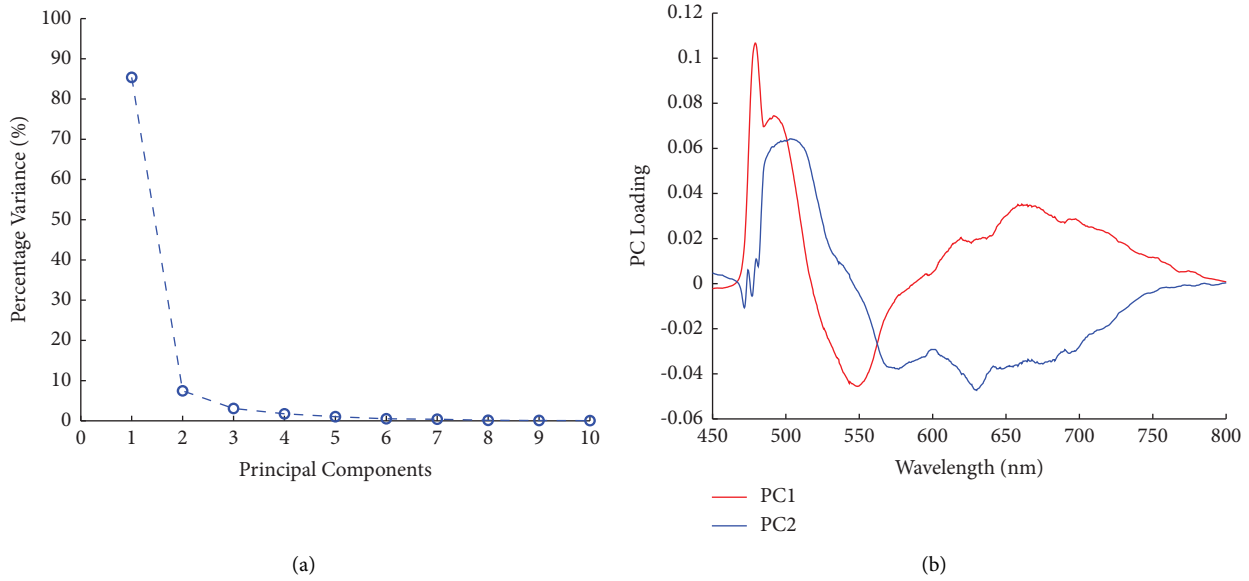


Figure 4: Variance plot describing the contribution of PC1 (85%) and PC2 (7%) (a) and loading plot showing wavelength-dependent factors influencing the variations in PC1 and PC2 (b).

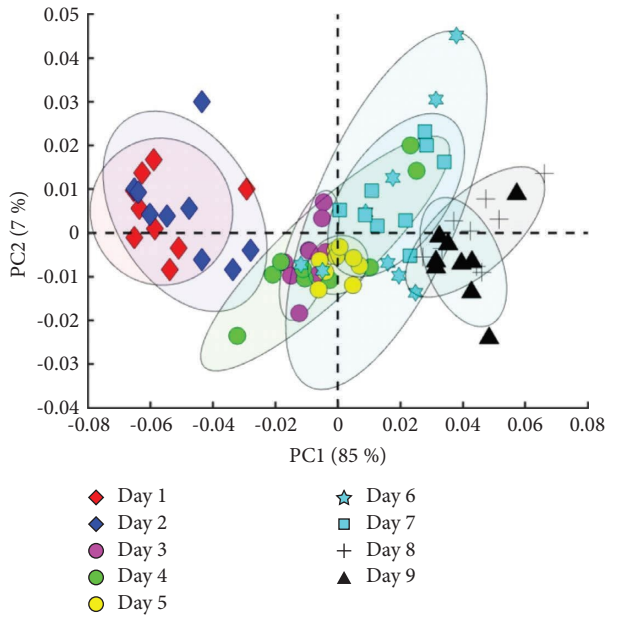


Figure 5: Scatter plot of first two principal components (PCs), PC1 and PC2, of LIAF spectra data showing shelf life monitoring for 9 days.

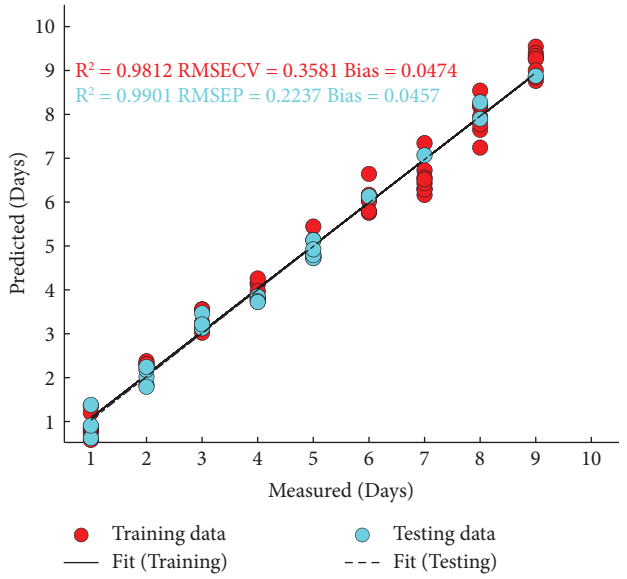


Figure 6: PLSR model plot of training and testing performance for LIAF spectra for BNFJ shelf life monitoring.

[12, 33]. Our results using the LIAF combined with PLS regression produced a clear indication of shelf life of BNFJ.

## 4. Conclusion

The feasibility of the LIAF technique for monitoring the shelf life of BNFJ has been evaluated. The LIAF spectra revealed fluorophores in the BNFJ at different emission wavelengths, mainly antioxidants (450–600 nm) as well as chlorophyll pigments (600–750 nm). The LIAF spectra of BNFJ were found to decrease within the spectral range of 520 nm to 560 nm from the day of production. Also, with PCA, the shelf life of the BNFJ could be monitored along the PC1 axis relating to wavelength around 480 nm semi-qualitatively. PLSR, however, was capable of predicting the shelf life of the BNFJ on a daily basis. The LIAF technique, in combination with the multivariate methods (PCA and PLSR), therefore provides satisfactory results for determining the shelf life of the BNFJ. The LIAF with the multivariate methods shows that the shelf life of BNFJ can be monitored independent of the usual physical, chemical, or sensory measurement. Even though this study considered

BNFJ in clear containers, further studies on colored and opaque containers are needed. Also, the study can be extended to other types of bottled natural fruit juices. This LIAF setup is simple and can be developed into a compact miniaturized system for mobility and easy use by regulatory bodies, BNFJ producers, and consumers.

## Data Availability

The laser-induced autofluorescence spectra data from the bottled natural fruit juice samples used to support the findings of this research are available from the corresponding author upon reasonable request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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