



Shelf-life prediction and degradation kinetics of bioactive compounds in biscuits fortified with *Ehretia asperula* leaf powder under different packaging materials

Predicción de la vida útil y cinética de degradación de los compuestos bioactivos en galletas fortificadas con polvo de hojas de *Ehretia asperula* bajo diferentes materiales de envasado

U.N. Le¹, M. V. Kieu¹, T. D. Luu², N. M. P. Nguyen¹, T. T. Chau¹, T. T. N. Do³, C. N. Tran^{1*}

¹Institute of Food and Biotechnology, Can Tho University, Can Tho City, Viet Nam.

²College of Agriculture, Can Tho University, Can Tho City, Viet Nam.

³Can Tho University of Technology, Can Tho City, Viet Nam.

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Abstract

Biscuits were developed by supplementing *E. asperula* leaf powder and were packaged in three different materials: paper laminate (PAPER/PET/PE), aluminum laminate (Al/PET/PE), and polypropylene (PP). The study was conducted to investigate changes in biscuit quality during storage through various parameters, which were moisture content, water activity (a_w), hardness, color values (L^* and a^*), total polyphenol content (TPC), total flavonoid content (TFC), and total counts of aerobic microorganisms (AMBC) and yeasts/molds (YMC). Results showed that the texture of the biscuits deteriorated rapidly after 8 weeks of storage in all packaging types. In particular, it was indicated that moisture increased (1.66-2.81 times) and hardness decreased (1.67-2.18 times). However, although a_w varied notably (0.4852-0.6328), the reached levels permissive for microbial growth (>0.60), the product consistently maintained low microbial counts (<10 CFU/g) throughout storage. It was suggested that other inhibitory factors played a dominant role in microbial control. Meanwhile, the degradation of TPC and L^* in the biscuits followed a biphasic kinetic model, while TFC and a^* followed a first-order kinetic model ($R^2 > 0.95$). The estimated shelf-life indicated that biscuits packed in Al/PET/PE maintained better quality than those packed in PAPER/PET/PE and PP. Therefore, Al/PET/PE packaging appears to be the most effective material for maintaining the quality of biscuits supplemented with *E. asperula* leaf powder during storage.

Keywords: Biscuits, degradation kinetics, *Ehretia asperula*, packaging and storage.

Resumen

Se desarrollaron galletas suplementadas con polvo de hojas de *E. asperula* y se envasaron en tres materiales diferentes: laminado de papel (PAPER/PET/PE), laminado de aluminio (Al/PET/PE) y polipropileno (PP). El estudio monitoreó los cambios en la calidad de las galletas durante el almacenamiento mediante varios parámetros, incluyendo el contenido de humedad, la actividad de agua (a_w), la dureza, los valores de color (L^* y a^*), el contenido total de polifenoles (TPC), el contenido total de flavonoides (TFC) y los recuentos totales de microorganismos aeróbicos mesófilos (AMBC) y de levaduras/hongos (YMC). Los resultados mostraron que la textura de las galletas se deterioró rápidamente después de 8 semanas de almacenamiento en todos los tipos de envase. En particular, se indicó que la humedad aumentó (1.66-2.81 veces) y la dureza disminuyó (1.67-2.18 veces). Sin embargo, aunque el a_w varió notablemente (0.4852-0.6328) y alcanzó niveles permisivos para el crecimiento microbiano (>0.60), el producto mantuvo consistentemente bajos recuentos microbianos (<10 UFC/g) durante todo el almacenamiento. Se sugirió que otros factores inhibitorios desempeñaron un papel dominante en el control microbiano. Mientras tanto, la degradación del TPC y de L^* en las galletas siguió un modelo cinético bifásico, mientras que el TFC y a^* siguieron un modelo cinético de primer orden ($R^2 > 0.95$). La vida útil estimada indicó que las galletas envasadas en Al/PET/PE mantuvieron una mejor calidad que las envasadas en PAPER/PET/PE y PP. Por lo tanto, el envase Al/PET/PE parece ser el material más eficaz para mantener la calidad de las galletas suplementadas con polvo de hojas de *E. asperula* durante el almacenamiento.

Palabras clave: galletas, cinética de degradación, *Ehretia asperula*, empaque y almacenamiento.

*Corresponding author. E-mail: tcnhan@ctu.edu.vn ;

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1 Introduction

Biscuits are among the most widely consumed snack foods, produced in almost all countries worldwide, with the United Kingdom recognized as a pioneer in the industrialization of the biscuit manufacturing sector (Manley, 2011). Biscuits are high in calories and are favored by consumers for their pleasant flavor and convenience. The quality attributes of biscuits are sensitive to moisture, oxygen, and microorganisms in the environment; therefore, they are prone to changes during storage, which significantly affect their sensory properties and usability (Manley, 2011). Accordingly, Pasqualone *et al.* (2014) reported a significant reduction in phenolic content during storage of grape-pomace-enriched biscuits. The hardness of biscuits made from germinated *Chenopodium album* flour also decreased significantly over storage time in both packaging types, namely low-density polyethylene (LDPE) and metalized polypropylene (MET-PPE) (Jan & Singh, 2017). Previously, Tran *et al.* (2025) investigated the formulation and baking conditions to develop biscuits supplemented with powder from *E. asperula* leaves, which are rich in TPC=2.65±0.07 mg GAE/g and TFC=1.72±0.12 mg QE/g. The study indicated that the product has high commercial potential; however, changes in biscuit quality during storage were not evaluated. Meanwhile, *E. asperula* leaves are traditionally used in Vietnam as a medicinal herb to treat the following related diseases as gastritis, hepatitis, cirrhosis, and cancer. In addition, the leaves can serve as a substitute for synthetic antioxidants, helping to prevent lipid oxidation and enhance the storage stability of biscuits (Tran *et al.*, 2023).

In the market, it is also necessary to investigate appropriate packaging materials and estimate the product's shelf life to maintain quality attributes and minimize external impacts besides the finding the optimal conditions of processing (Robertson, 2017; Singh *et al.*, 2025). Up to now, various new packaging materials have been developed, including plastic films, aluminum foil, biodegradable polymers, and nanocomposites (Deshwal & Panjagari, 2019). Although these packaging types differ, they are all based on specific base materials possessing characteristic properties for preserving different products. For example, packaging made from metal-based materials can prevent moisture absorption and oxidation; therefore, the shelf life of biscuits can be extended (Deshwal & Panjagari, 2019). At the same time, estimating the storage duration and quality deterioration of biscuits under different packaging materials and environmental conditions is highly important. In fact, there are many factors affecting the storage duration of biscuits, which are temperature, equilibrium relative humidity, oxygen partial pressure,

light exposure, packaging permeability, and packaging configuration (Singh *et al.*, 2025).

Therefore, the application of kinetic models as computational tools to predict the quality deterioration of biscuits during storage has become a crucial research line and has demonstrated high reliability (Kumar & Mishra, 2004; Van Boekel, 2008; Dak *et al.*, 2014). Most studies indicate that the product quality degradation during storage follows first-order kinetics (Kumar & Mishra, 2004; Van Boekel, 2008; Dak *et al.*, 2014). However, the product quality deterioration may also follow different kinetic models such as Biphasic kinetics or Weibull kinetics, depending on the types of product, storage conditions, and the specific quality attributes being monitored (Verbeyst *et al.*, 2013; Kumar *et al.*, 2015). So, selecting an appropriate kinetic model can be the crucial to predict shelf life accurately and ensure product stability, as different quality parameters may deteriorate at different rates under the same storage conditions. In order to inherit and further develop the values of previous research, as well as to support the commercialization of biscuits supplemented with powder from *E. asperula* leaves, this study focuses on analyzing changes in moisture content, water activity, hardness, color, and bioactive compound content of the biscuits. In addition, kinetic analysis was applied to evaluate the changes in selected quality parameters of the biscuits under different packaging materials.

2 Materials and methods

2.1 Raw materials

E. asperula leaves were procured from March to July from Cao Thang, Luong Son, Hoa Binh, transported to the laboratory under ambient conditions on the same day, and stored in a cold room at 2-4°C. The raw materials were required to be free of impurities, intact, undamaged, and free from pest infestation. In addition, *E. asperula* leaf powder was prepared according to the method described by Tran *et al.* (2023) and stored in Al/PET/PE packaging until use.

2.2 Sample preparation

The biscuits supplemented with *E. asperula* leaf powder formulation were 44% wheat flour, 22% sugar, 15% butter, 18% egg yolk, 0.5% salt, and 0.5% sodium bicarbonate, with 12% *E. asperula* leaf powder substituted (based on the initial weight of wheat flour) (Tran *et al.*, 2024). Firstly, sugar, butter, and egg yolk was mixed vigorously beaten to form a smooth and homogeneous emulsion. Subsequently, the flour mixture and other dry ingredients were gradually added to the emulsion and mixed until an elastic

and cohesive dough was obtained. The dough was then sheeted and shaped to a fixed dimension (5 mm thickness and 5 cm diameter). The biscuits were baked at 170°C in an oven (ATO-CRL8076, Aqua, China) for 15 minutes, and cooled to room temperature before storage.

2.3 Storage conditions

Five biscuits/bag in Al/PET/PE, PAPER/PET/PE, and PP sealed by vacuum (Boxer 42, Henkelman, Netherlands) sealer, approximately 0.5-2% residual air. Then, samples were stored at room temperature (30±2°C and relative humidity of approximately 90%, which was measured using a digital hygrometer – LR5001, HIOKI, Japan) for 8 weeks. The samples were arranged upright on racks to ensure the same conditions. For each type of packaging, samples were randomly collected for analysis of quality parameters.

2.4 Quality evaluation

Moisture content was determined using a convection drying oven (UNB200, Memmert, Germany) by drying the samples at 105°C to constant weight according to the AOAC method 950.46 (AOAC, 2000). Meanwhile, water activity was determined at 25 °C using a water activity meter (TDL, Aqualab, USA).

The hardness of the biscuits, defined as the maximum penetration force, was determined using a texture analyzer (TA.XTplus, Stable Micro Systems, UK). The biscuits were placed horizontally on a flat platform and centered under a P/5S probe. The measurement conditions were set as follows: pre-test, test, and post-test speeds of 1, 2, and 10 mm/s, respectively, and a penetration distance of 5 mm (Tran *et al.*, 2024).

The color of biscuits supplemented with *E. asperula* leaf powder was determined using a handheld colorimeter (CR-410, Konica Minolta Measuring Instruments, Japan). Color was expressed in the CIELab coordinate system with the following parameters: lightness (L*: 100=white, 0=black), redness (a*: ± red–green), and yellowness (b*: ± yellow–blue). Each sample was measured in triplicate at different positions on the biscuit surface.

Extraction of bioactive compounds from *E. asperula* biscuits was conducted following Nguyen *et al.* (2019) with slight modifications. Briefly, 0.5 g of ground biscuit was homogenized in 15 mL of methanol using an Ultra-Turrax homogenizer (T 18 digital ULTRA-TURRAX®, IKA, Germany) for 45 s at 1000 rpm. The extracts were centrifuged at 3000 rpm for 15 min (DM0412, DLAB, USA). Throughout extraction, samples were kept cold (0-5°C) on ice until analysis.

The TPC in the extracts was measured using the Folin-Ciocalteu technique described by Nguyen *et*

al. (2019) with some modifications. Specifically, 1 mL of the extract was thoroughly mixed with 1 mL of water, then 0.5 mL of 10% (v/v) Folin-Ciocalteu reagent was added, and the mixture was left at room temperature for 6 minutes. Subsequently, 1.5 mL of 20% (w/v) sodium carbonate solution was added to the mixture, which was then incubated for 2 hours. The absorbance of the mixture was measured using a spectrophotometer (UV-1800, Shimadzu, Japan) at 765 nm. The result was calculated and expressed in gallic acid equivalent per gram of dry weight of sample (mg GAE/g) according to eq(1).

The TFC in extracts was measured using an aluminum chloride colorimetric method of Pham *et al.* (2020) with some modifications. 500 µL of extract or standard solution of various concentrations was mixed with 1.5 mL of methanol, 100 µL of 10% AlCl₃, 100 µL of 1 M CH₃COOK, and 2.8 mL of distilled water. This mixture was incubated at room temperature in the dark for 45 min before absorbance was measured against the blank at 415 nm using a spectrophotometer (UV-1800, Shimadzu, Japan). The result was calculated and expressed in quercetin equivalent per gram of dry weight of sample (mg QE/g) according to eq(1).

$$P = C \times \frac{V \times k}{M'} \quad (1)$$

Where, P is the TPC (GAE mg/g of sample) or TFC (QE mg/g of sample), C is the concentration of sample established from the calibration curve (mg/mL), V is the volume of extract (mL), M' is the weight of extract (g), and k is dilution coefficient (if any).

Microbiological analysis was performed by determining the total aerobic mesophilic count and total yeast/mold count using the plate count method on Plate Count Agar (PCA, Himedia, India) and Yeast Glucose Chloramphenicol (YGC, Merck, Germany) media, respectively. The procedures followed the Vietnamese standard TCVN 4884-1:2015 (Directorate for Standards, Metrology and Quality [DSMQ], 2015).

2.5 Modelling of quality changes

Kinetic models can be used to describe the reaction rate as a function of storage time and to predict quality changes in food products during processing and storage (Van Boekel, 2008; Kumar & Mishra, 2004). The degradation of quality attributes (such as polyphenols, vitamins, and antioxidants) is commonly described by a first-order kinetic model (Ramaswamy & Marcotte, 2006) or a Weibull model (Peleg, 2006), depending on the nonlinear behavior of the experimental data. Some studies have reported that food quality may involve two simultaneous degradation mechanisms, which can be appropriately represented by a Biphasic model (Ling & Lund, 1978; Verbeyst *et al.*, 2013). Accordingly, this study

examined the suitability of several common kinetic models (Table 1) in describing the degradation behavior of biscuits supplemented with *E. asperula* leaf powder during storage.

Table 1. Predictive kinetic modeling of food quality during storage.

No.	Model name	Equation
1	First-order	$\frac{Q_t}{Q_o} = \exp(-kt)$ (2)
2	Biphasic	$\frac{Q_t}{Q_o} = \alpha \exp(-k_1 t) + (1 - \alpha) \exp(-k_2 t)$ (3)
3	Weibull	$\frac{Q_t}{Q_o} = \exp\left(-\frac{t}{k}\right)^n$ (4)

Where Q_o and Q_t denote the quality parameters (L^* , a^* , TPC, and TFC) measured at the initial time and at different storage periods (t , weeks). The term k represents the reaction rate constant (week^{-1}), while k_1 and k_2 correspond to the rate constants of the first and second stages, respectively. The parameter α indicates the fraction of the fast phase, and n describes the shape of the degradation curve.

In the first-order model, the half-life ($t_{1/2}$) is the time required to degrade the initial quality by 50% at a specified temperature (eq 5). While, the decimal reduction time (D) is the time required to degrade the initial quality by 90% at a specified temperature (eq 6):

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

$$D = \frac{\ln(10)}{k} \quad (6)$$

2.6 Statistical analysis

Nonlinear regression analysis was performed using Statgraphics Centurion software (version 19.1, Statgraphics Technologies, Inc., Virginia) to estimate the model coefficients. In the regression analysis, the best-fitting model describing storage behavior across different packaging conditions was identified using statistical indicators such as the correlation coefficient (R^2), chi-square (χ^2), and root mean square error (RMSE). A higher R^2 combined with lower χ^2 and RMSE values indicates a better agreement between the experimental observations and the predicted data of the mathematical model. Statgraphics Centurion software was also used to test the differences between treatments using analysis of variance (ANOVA) and the least significant difference (LSD) test at a 5% significance level.

3 Results and discussion

3.1 Changes in moisture content, water activity, and hardness of *E. asperula* leaf powder-supplemented biscuits during storage

The results in Figure 1a show that the moisture content of the biscuits increased linearly over 8 weeks of storage. Specifically, the biscuit moisture content gradually increased from 6.44% to 10.89% after 8 weeks, compared with the initial value of 3.70%. Similarly, Silav-Tuzlu and Tacer-Caba (2021) reported an increase in the moisture content of buckwheat-chia biscuits from 5.4% to 9.1% after 45 days of storage. Biscuits are characterized by low moisture and low water activity, which makes them highly hygroscopic; thus, they readily absorb moisture from the surrounding environment during storage (Silav-Tuzlu & Tacer-Caba, 2021). The present study also indicated that aluminum-laminated packaging (Al/PET/PE) most effectively preserved the quality of *E. asperula* leaf powder-supplemented biscuits, followed by polypropylene (PP) and paper-laminated (PAPER/PET/PE) packaging. After eight weeks, the moisture content of biscuits stored in Al/PET/PE packaging increased 1.66-fold compared with the initial value, which was lower than that stored in PP (2.46-fold) and paper-laminated (2.81-fold) packaging. In a related study, Jan and Singh (2017) found that the moisture content of millet-based biscuits increased from approximately 3.4% to 4.8% when stored in LDPE, while it remained almost unchanged over 120 days in MET-PPE packaging. These findings suggest that the rate of moisture uptake in biscuits strongly depends on the type of packaging material and its barrier properties (Robertson, 2017; Singh et al., 2025). Similarly, a_w increased during storage (Figure 1b). However, the fluctuation was not consistent: during the first two weeks, a_w slightly decreased, then gradually increased and became unstable from week 3 onward. A comparable trend was observed by Jan and Singh (2017), who reported that the water activity of biscuits increased from 0.58 to 0.60 after 120 days of storage.

Moisture content and a_w are two physical parameters that are directly proportional to each other; however, they are inversely related to product hardness. The results showed that the hardness of the biscuits significantly decreased after 8 weeks of storage; hardness declined from 43.36 ± 0.07 N to 19.87 ± 1.16 N for paper-based packaging, 22.11 ± 1.03 N for PP, and 26.02 ± 0.43 N (Figure 1c). The most pronounced reduction was observed in paper-based packaging (53.35%), followed by PP packaging (47.96%), while aluminum-based packaging exhibited

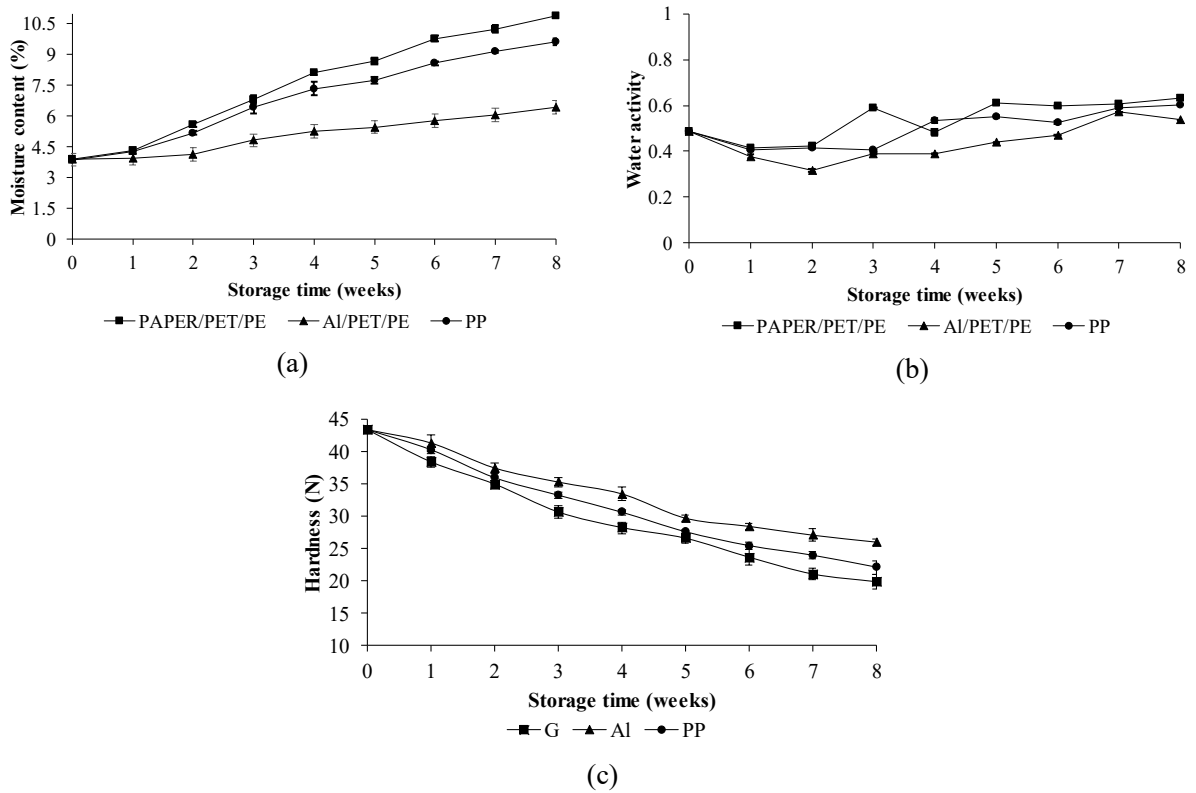


Figure 1. Changes in moisture content (a), water activity (b), and hardness (c) of *E. asperula* leaf powder-supplemented biscuits during storage in different types of packaging. Data are presented as mean $\pm$ standard deviation, $n = 3$.

Table 2. Changes in L^* and a^* values of *E. asperula* leaf powder-supplemented biscuits stored in different types of packaging over time.

	Time (weeks)	PAPER/PET/PE	Al/PET/PE	PP
L^*	0			46.68 $\pm$ 0.38 ^a
	1	45.76 $\pm$ 0.21 ^b	46.36 $\pm$ 0.31 ^{ab}	46.19 $\pm$ 0.16 ^b
	2	45.38 $\pm$ 0.12 ^c	46.16 $\pm$ 0.34 ^b	45.84 $\pm$ 0.37 ^b
	3	44.46 $\pm$ 0.03 ^d	45.53 $\pm$ 0.16 ^c	44.64 $\pm$ 0.10 ^c
	4	43.88 $\pm$ 0.07 ^e	44.48 $\pm$ 0.24 ^d	44.14 $\pm$ 0.42 ^d
	5	43.53 $\pm$ 0.05 ^f	44.18 $\pm$ 0.21 ^d	43.85 $\pm$ 0.13 ^d
	6	43.01 $\pm$ 0.04 ^g	43.80 $\pm$ 0.17 ^e	43.23 $\pm$ 0.19 ^e
	7	42.82 $\pm$ 0.03 ^g	43.60 $\pm$ 0.16 ^{ef}	42.93 $\pm$ 0.09 ^{ef}
	8	42.50 $\pm$ 0.14 ^h	43.33 $\pm$ 0.13 ^g	42.79 $\pm$ 0.17 ^f
a^*	0			-6.22 $\pm$ 0.19 ⁱ
	1	-5.41 $\pm$ 0.02 ^h	-6.05 $\pm$ 0.10 ^f	-5.63 $\pm$ 0.09 ^h
	2	-4.68 $\pm$ 0.07 ^g	-5.66 $\pm$ 0.14 ^f	-4.82 $\pm$ 0.09 ^g
	3	-3.77 $\pm$ 0.04 ^f	-5.05 $\pm$ 0.24 ^e	-4.18 $\pm$ 0.09 ^f
	4	-3.19 $\pm$ 0.07 ^e	-4.46 $\pm$ 0.10 ^d	-3.38 $\pm$ 0.28 ^e
	5	-2.81 $\pm$ 0.13 ^d	-3.91 $\pm$ 0.13 ^c	-2.88 $\pm$ 0.15 ^d
	6	-2.27 $\pm$ 0.08 ^c	-3.6 $\pm$ 0.19 ^b	-2.42 $\pm$ 0.16 ^c
	7	-1.93 $\pm$ 0.11 ^b	-3.50 $\pm$ 0.11 ^a	-2.12 $\pm$ 0.21 ^b
	8	-1.48 $\pm$ 0.12 ^a	-3.38 $\pm$ 0.07 ^a	-1.81 $\pm$ 0.11 ^a

Data are presented as mean $\pm$ standard deviation, $n = 3$; different letters within the same column (a, b, c, ...) indicate statistically significant differences among treatments at $p < 0.05$.

the best retention, with a decrease of 38.76% compared to the initial week (43.36 ± 0.07 N). Similar results were reported according to Jan and Singh (2017), which were a significant decrease in biscuit hardness during storage in both packaging types (LDPE and MET-PPE), with a greater reduction in LDPE due to its higher moisture absorption capacity.

3.2 Changes in color (L^* and a^*) of *E. asperula* leaf powder-supplemented biscuits during storage

L^* and a^* values are crucial parameters determining the lightness and greenish hue of *E. asperula* leaf powder-supplemented biscuits. Table 1 shows that the lightness (L^*) of the biscuits decreased across all three packaging types. Similarly, the brightness of the biscuits decreased from 36.60 ± 2.60 to 24.50 ± 0.30 when stored in LDPE and to 34.78 ± 0.50 in MET-PPE (Jan & Singh, 2017). In this study, the L^* value of *E. asperula* biscuits was 46.68 ± 0.38 . After 8 weeks, the L^* of biscuits stored in Al/PET/PE (43.33 ± 0.13) maintained the brightness better than the PAPER/PET/PE and PP packaging (42.50 ± 0.14 and 42.79 ± 0.17 , respectively). In addition, the a^* value representing the characteristic blue pigment of the biscuits showed an increase, indicating a decrease in blue color intensity (Table 1). PAPER/PET/PE and PP packaging showed a significant decrease in a^* values during the first few weeks of storage, and

after 8 weeks, the a^* values were -1.48 ± 0.12 and -1.81 ± 0.11 , respectively, compared to -6.22 ± 0.19 at week 0. This change is attributed to biochemical reactions occurring within the product during storage, primarily oxidative browning and storage-induced non-enzymatic reactions, which decrease brightness and alter the color. As a result, the biscuits gradually turned brown due to the formation of reaction products (Rai & Chauhan, 2008), thereby reducing L^* and increasing a^* values.

3.3 Changes in TPC and TFC of *E. asperula* leaf powder-supplemented biscuits during storage

The results in Table 3 show that both TPC and TFC decreased significantly over the storage period. Specifically, TPC and TFC decreased by 2.46-3.47 times and 4.56-7.22 times, respectively - a trend also reported in several previous studies. TPC of burger meat decreased significantly after 14 days of storage (from 45.34-75.03 mg gallic acid/100 g meat to 24.37-49.47 mg gallic acid/100 g meat) (Akcan *et al.*, 2023). Pasqualone *et al.* (2014) observed a similar loss of phenolic compounds in grape pomace-enriched biscuits during storage. Furthermore, different packaging materials exhibited varying effects on the stability of bioactive compounds in the biscuits.

Table 3. Changes in TPC and TFC of *E. asperula* leaf powder-supplemented biscuits stored in different types of packaging over time.

	Time (weeks)	PAPER/PET/PE	Al/PET/PE	PP
TPC (mg GAE/g)	0			2.88 ± 0.27^a
	1	2.51 ± 0.04^a	2.40 ± 0.01^b	2.31 ± 0.01^b
	2	2.01 ± 0.01^b	2.17 ± 0.05^{bc}	2.13 ± 0.15^{bc}
	3	1.54 ± 0.07^c	2.04 ± 0.06^c	1.98 ± 0.12^c
	4	1.42 ± 0.06^{cd}	1.65 ± 0.07^d	1.57 ± 0.04^d
	5	1.25 ± 0.01^{cd}	1.56 ± 0.02^{de}	1.55 ± 0.06^d
	6	1.13 ± 0.01^{de}	1.44 ± 0.05^{def}	1.42 ± 0.02^{de}
	7	0.95 ± 0.03^{ef}	1.29 ± 0.05^{ef}	1.23 ± 0.04^{ef}
	8	0.83 ± 0.03^f	1.17 ± 0.02^f	1.08 ± 0.01^f
TFC (mg QE/g)	0			2.6 ± 0.10^a
	1	2.04 ± 0.04^b	2.12 ± 0.15^b	2.06 ± 0.06^b
	2	1.60 ± 0.06^c	1.88 ± 0.06^c	1.79 ± 0.01^c
	3	1.45 ± 0.04^c	1.67 ± 0.03^d	1.34 ± 0.08^d
	4	0.87 ± 0.07^d	0.88 ± 0.05^e	0.89 ± 0.03^e
	5	0.63 ± 0.08^e	0.75 ± 0.03^{ef}	0.78 ± 0.04^{ef}
	6	0.53 ± 0.06^{ef}	0.68 ± 0.03^{ef}	0.68 ± 0.02^{fg}
	7	0.49 ± 0.10^{ef}	0.63 ± 0.14^f	0.58 ± 0.04^{gh}
	8	0.36 ± 0.09^f	0.57 ± 0.04^f	0.45 ± 0.03^h

Data are presented as mean $\pm$ standard deviation, $n = 3$; different letters within the same column (a, b, c, ...) indicate statistically significant differences among treatments at $p < 0.05$.

Table 4. Total aerobic mesophilic bacterial count (AMBC) and total yeast and mold count (YMC) of biscuits stored in different types of packaging over time.

Packaging	Parameter	Time (weeks)								
		0	1	2	3	4	5	6	7	8
PAPER/PET/PE	AMBC (CFU/g)	X<10	X<10	X<10	X<10	X<10	X<10	X<10	X<10	X<10
	YMC (CFU/g)	X<10	X<10	X<10	X<10	X<10	X<10	X<10	X<10	X<10
Al/PET/PE	AMBC (CFU/g)	X<10	X<10	X<10	X<10	X<10	X<10	X<10	X<10	X<10
	YMC (CFU/g)	X<10	X<10	X<10	X<10	X<10	X<10	X<10	X<10	X<10
PP	AMBC (CFU/g)	X<10	X<10	X<10	X<10	X<10	X<10	X<10	X<10	X<10
	YMC (CFU/g)	X<10	X<10	X<10	X<10	X<10	X<10	X<10	X<10	X<10

Data are presented as mean $\pm$ standard deviation, $n = 3$.

The aluminum-laminated packaging most effectively maintained the bioactive components, with final values of 1.17 ± 0.02 mg GAE/g (TPC) and 0.57 ± 0.04 mg QE/g (TFC). This was followed by PP packaging (1.08 ± 0.01 mg GAE/g and 0.45 ± 0.03 mg QE/g), while paper-laminated packaging showed the fastest degradation (0.83 ± 0.03 mg GAE/g and 0.36 ± 0.09 mg QE/g) after 8 weeks. Paper and PP films have lower gas-barrier properties, whereas aluminum film provides a high barrier to gas transmission. Consequently, gas permeation-particularly oxygen-occurs more readily through paper and PP packaging than through aluminum (Deshwal & Panjagari, 2019). The varying gas permeability and diffusion rates among packaging materials directly influence the internal quality of stored products, as oxygen exposure accelerates the degradation of phenolic compounds present in the flour (Pasqualone et al., 2014).

3.4 Total aerobic mesophilic bacterial count and total yeast and mold count of biscuits supplemented with *E. asperula* leaf powder over time

The results of the effect of different packaging types on the microbiological quality of *E. asperula* leaf powder-supplemented biscuits are presented in Table 4. The findings indicate that the total yeast and mold count, as well as the total aerobic mesophilic bacteria in the biscuits, monitored over 8 weeks under the same storage conditions in three types of packaging, were below the detection limit (<10 CFU/g) (Table 4). Throughout the storage period, no microbial growth was detected in the biscuit samples. These results are consistent with the Vietnamese Standard TCVN 12940:2020 for *E. asperula* leaf powder-supplemented biscuits, which specifies that the total aerobic plate count should be in the range of 5×10^5 – 5×10^6 CFU/g, and the total yeast and mold count should be less than 150 spores/g (DSMQ, 2020). The results showed that after 8 weeks of storage, the biscuits maintained good quality at a safe level, without being contaminated by microorganisms. Overall, the microbiological quality indicators of biscuits in all

three packaging types showed insignificant differences ($p > 0.05$). Accordingly, after 8 weeks of storage, the microbial growth in the biscuits was observed to be very low ($X < 10$ at PAPER/PET/PE, Al/PET/PE, and PP) (Table 4). However, some studies have noted an increase in the number of microorganisms during the storage of nutrient-rich products such as mango soy yogurt powder, extruded products from sprouted soybeans, and biscuits (Kumar & Mishra, 2004; Jan et al., 2017). Jan et al. (2017) showed microbial counts in extruded products and biscuits significantly increase from 1.7×10^3 to 7.8×10^4 CFU/g and from 2.0×10^3 to 9.8×10^4 CFU/g, respectively, when stored in LDPE after 120 days. Besides, Kumar and Mishra (2004) noted that multi-layered aluminum packaging inhibited the growth of microorganisms in nutrient-rich products such as mango-soy yogurt powder. Similarly, biscuits preserved in PE/AL/PP multilayer film showed slower microbial growth after 120 days, in which the total colony count increased from 1.7×10^3 to 4.5×10^4 CFU/g for extruded products and from 2.0×10^3 to 6.84×10^4 CFU/g for biscuits (Jan et al., 2017). This shows that packaging materials play a crucial role in maintaining the stability of microbial growth during storage, mainly due to differences in gas and water vapor permeability. Specifically, PE packaging has higher oxygen and moisture permeability than aluminum, thus accelerating the absorption of moisture and gases necessary for microbial growth (Robertson, 2017).

3.5 Kinetics of quality changes in biscuits supplemented with *E. asperula* leaf powder during storage

The suitability of the kinetic models and the kinetic parameters describing the degradation of TPC, TFC, L^* , and a^* was evaluated in this study (Table 5). Accordingly, all three selected models (First-order, Biphasic, and Weibull) demonstrated a high degree of goodness-of-fit, with high average correlation coefficients (R^2) and low chi-square and RMSE values ($R^2 > 0.95$, $\chi^2 < 4.10 \times 10^{-3}$, and $RMSE < 0.05$). The results indicated that the Biphasic model provided

the best fit for describing the degradation of TPC and L* ($R^2=98.87$; $\chi^2=1.00 \times 10^{-3}$; RMSE=0.0262 and $R^2=97.80$; $\chi^2=5.18 \times 10^{-5}$; RMSE=0.0063, respectively). Meanwhile, TPC in germinated rice during drying followed a zero-order model (R^2 ranged from 0.9833-0.9925) (Loan *et al.*, 2025). In contrast, the First-order model showed better suitability for characterizing the degradation of TFC and a* ($R^2=97.65$; $\chi^2=2.30 \times 10^{-3}$; RMSE=0.0433 and $R^2=98.24$; $\chi^2=8.30 \times 10^{-4}$; RMSE=0.0263, respectively). The Biphasic model is known to be commonly used to describe two-stage degradation behavior of compounds (or enzymes) that exhibit different decomposition rates, in which the more labile fraction is rapidly degraded while the remaining, more stable fraction breaks down more slowly (Ling & Lund, 1978; Verbeyst *et al.*, 2013). In contrast, the First-order model describes linear degradation as a function of time at a given temperature (Van Boekel, 2008). This indicates a similarity in the theoretical basis of the two models. TPC is known as the total content of phenolic compounds in *E. asperula* leaves, including flavonoids, phenolic acids,

and tannins (Tran *et al.*, 2023). Different compounds in terms of structure and thermal stability present in a product lead to heterogeneous degradation via various mechanisms and rates (Vamos-Vigyázó, 1981). This results in a non-linear degradation curve of TPC, with a rapid initial decline followed by a slower decline later on (Figure 2a). In contrast, TFCs have relatively homogeneous chemical characteristics, mainly consisting of flavone or flavonol glycosides or aglycones, so they degrade linearly and follow first-order kinetics (Ramaswamy & Marcotte, 2006). Regarding color, brightness (L*) is simultaneously affected by many factors, among which the oxidation of polyphenols, chlorophyll, or other easily degradable pigments occurs abruptly in the early stages, causing the formation of brown compounds, which is the main reason for the gradual decrease in the L* value of biscuits during storage (Verbeyst *et al.*, 2013). In contrast, changes in the a* value are largely governed by the degradation of dominant red pigments, a process that generally follows first-order kinetics (Rathinasabapathi *et al.*, 2024).

Table 5. Kinetic parameters of the degradation of TPC, TFC, L*, and a* in biscuits supplemented with *E. asperula* leaf powder stored in different types of packaging.

Models	Packaging	Model constants	R^2	χ^2	RMSE
TPC	First-order	PAPER/PET/PE k=0.1679	98.3418	0.00113	0.0317
		Al/PET/PE k=0.1212	97.6865	97.40 0.00100	1.29 x10 ⁻³ 0.0299
		PP k=0.1288	96.1596	0.00173	0.0392
	Biphasic	PAPER/PET/PE $\alpha=0.7828$; $k_1=0.2418$; $k_2=0.0225$	99.0265	0.00118	0.0281
		Al/PET/PE $\alpha=0.6544$; $k_1=0.0659$; $k_2=0.3180$	98.9515	98.87 0.00081	1.00 x10 ⁻³ 0.0233
		PP $\alpha=0.8784$; $k_1=0.1011$; $k_2=1.6011$	98.6306	0.00101	0.0271
TFC	Weibull	PAPER/PET/PE k=0.6423; n=0.1078	98.3419	0.00148	0.0339
		Al/PET/PE k=1.7575; n=0.2128	97.6866	97.40 0.00132	1.69 x10 ⁻³ 0.0319
		PP k=1.7033; n=0.2192	96.1596	0.00226	0.0419
	First-order	PAPER/PET/PE k=0.2484	98.5023	0.00155	0.0371
		Al/PET/PE k=0.2084	95.5838	97.65 0.00429	2.30 x10 ⁻³ 0.0618
		PP k=0.2274	98.8639	0.00107	0.0309
L*	Biphasic	PAPER/PET/PE $\alpha=0.6431$; $k_1=0.2491$; $k_2=0.2474$	98.5023	0.00276	0.0429
		Al/PET/PE $\alpha=0.5628$; $k_1=0.2093$; $k_2=0.2074$	95.5838	97.65 0.00764	4.10 x10 ⁻³ 0.0714
		PP $\alpha=0.7784$; $k_1=0.2284$; $k_2=0.2236$	98.8639	0.00190	0.0357
	Weibull	PAPER/PET/PE k=0.2127; n=0.0529	98.5023	0.00202	0.0397
		Al/PET/PE k=1.0785; n=0.2248	95.5838	97.65 0.00561	3.01 x10 ⁻³ 0.0661
		PP k=1.7547; n=0.3989	98.8639	0.00140	0.0330
a*	First-order	PAPER/PET/PE k= 0.0131	95.3440	5.05x10 ⁻⁵	0.0067
		Al/PET/PE k= 0.0099	96.1680	96.09 3.30x10 ⁻⁵	3.97 x10 ⁻⁵ 0.0054
		PP k= 0.0121	96.7553	3.55x10 ⁻⁵	0.0056
	Biphasic	PAPER/PET/PE $\alpha=1.1002$; $k_1=0.0142$; $k_2=0.0251$	95.2800	9.10x10 ⁻⁵	0.0078
		Al/PET/PE $\alpha=0.0242$; $k_1=0.0082$; $k_2=0.0099$	96.1680	96.43 5.87x10 ⁻⁵	6.39 x10 ⁻⁵ 0.0063
		PP $\alpha=0.0845$; $k_1=0.1276$; $k_2=0.0046$	97.8469	4.19x10 ⁻⁵	0.0053
a*	Weibull	PAPER/PET/PE k=4.8278; n=0.0634	95.3439	6.59x10 ⁻⁵	0.0072
		Al/PET/PE k=1.1451; n=0.0114	96.1679	96.09 4.31x10 ⁻⁵	5.18 x10 ⁻⁵ 0.0058
		PP k= 3.3360; n=0.0403	96.7553	4.64x10 ⁻⁵	0.0060
	First-order	PAPER/PET/PE k= 0.1651	99.5004	0.00038	0.0185
		Al/PET/PE k= 0.0809	96.1731	98.24 0.00142	8.30 x10 ⁻⁴ 0.0356
		PP k= 0.1489	99.0530	0.00069	0.0248
a*	Biphasic	PAPER/PET/PE $\alpha=0.6775$; $k_1=0.1653$; $k_2=0.1649$	99.5004	0.00068	0.0213
		Al/PET/PE $\alpha=0.0078$; $k_1=0.0795$; $k_2=0.0810$	96.1732	98.24 0.00253	1.48 x10 ⁻³ 0.0411
		PP $\alpha=0.6139$; $k_1=0.1492$; $k_2=0.1489$	99.0534	0.00123	0.0286
	Weibull	PAPER/PET/PE k=0.0807; n=0.0133	99.5004	0.00050	0.0197
		Al/PET/PE k=0.2878; n=0.0233	96.1732	98.24 0.00186	1.09 x10 ⁻³ 0.0380
		PP k=0.1467; n=0.0219	99.0534	0.00090	0.0265

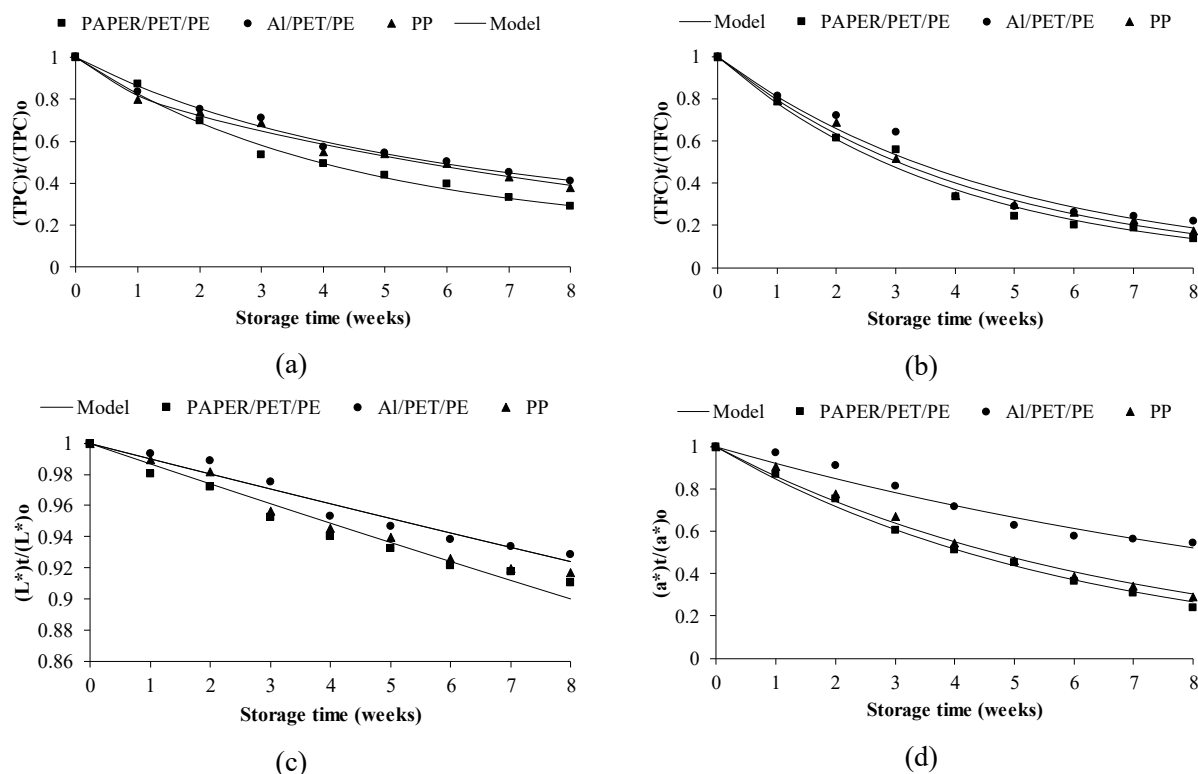


Figure 2. The degradation kinetics of TPC (a), TFC (b), L* (c), and a* (d) in biscuits supplemented with *E. asperula* leaf powder during storage in different types of packaging.

Similarly, the degradation kinetics of L* in tomato puree or the browning index in garlic have been shown to follow biphasic model (Barreiro *et al.*, 1997; Fante & Noreña, 2012). Goñi and Salvadori (2011) also demonstrated that changes in the redness of beef during roasting were well described by a biphasic model. Similarly, Verbeyst *et al.* (2013) noted that the degradation of vitamin C in strawberries and raspberries upon high heat and pressure treatment followed biphasic model. In contrast, the degradation of anthocyanins in blackcurrant juice followed a first-order model (Harbourne *et al.*, 2008). Kumar and Mishra (2004) reported that color degradation of mango-soy yogurt powder during storage followed first-order kinetics. Similarly, Rathinasabapathi *et al.* (2024) demonstrated that color changes in herb-coated pear slices during storage were well described by first-order kinetic models.

The kinetic results describing TPC degradation in biscuits fortified with *E. asperula* leaf powder were best fitted by the Biphasic model, indicating that the fast phase was dominant across all three packaging types ($\alpha > 0.65$) (Table 5). Among them, PP packaging exhibited the highest proportion of the fast phase ($\alpha = 0.8784$), reflecting that most polyphenols underwent rapid degradation during the initial stage. In contrast, Al/PET/PE showed a lower α value (0.6544), suggesting a smaller extent of initial degradation and greater stability in the early storage period. At

the same time, the fast-phase reaction rate constant (k_1) of PAPER/PET/PE reached the highest value (0.2418) among the others, indicating a pronounced initial degradation rate of TPC. In contrast, Al/PET/PE obtained the lowest k_1 value (0.0659), thereby better maintaining TPC. In the slow phase, TPC degradation in Al/PET/PE ($k_2 = 0.3180$) occurred at a more considerable rate compared to PAPER/PET/PE (0.0225). Meanwhile, PP recorded a rapid slow-phase degradation process, reducing the long-term stability of polyphenols ($k_2 = 1.6011$). These trends are consistent with the experimental curves showing two distinct stages of TPC degradation (Figure 2a). The significant decrease in the initial phase may be related to the oxidation of free polyphenols. In contrast, gradual degradation of bound forms or secondary oxidative reactions was mainly performed in the slow phase (Vamos-Vigyázó, 1981; Ling *et al.*, 2015). Paper-based and PP packaging possess lower barrier properties than aluminum-based packaging, particularly in terms of gas permeability (Deshwal & Panjagari, 2019). However, gases (especially oxygen) are significant factors influencing the transformation of phenolic compounds or chlorophyll present in the product (Pasqualone *et al.*, 2014). Al/PET/PE package performed the best TPC preservation capacity due to its lower initial degradation rate and greater stability throughout storage, whereas PP showed a stronger degradation trend in both the fast and slow phases

Table 6. Estimated degradation parameters of biscuits enriched with *E. asperula* leaves stored in different packaging materials.

	Packaging	TPC (mg GAE/g)	TFC (mg QE/g)	L*	a*
$t_{1/2}$ (weeks)	PAPER/PET/PE	-	2.79	-	4.2
	Al/PET/PE	-	3.33	-	8.57
	PP	-	3.05	-	4.66
D (weeks)	PAPER/PET/PE	-	9.27	-	13.95
	Al/PET/PE	-	11.05	-	28.46
	PP	-	10.13	-	15.46

(-): Not determined

The degradation kinetics of L^* based on a two-phase model showed that the L^* of biscuits in PAPER/PET/PE degraded rapidly in the early stages of storage ($\alpha=0.9032$ and $k_1=0.1833$). In contrast, Al/PET/PE showed a slow and stable decrease in L^* ($\alpha=0.0242$, $k_1=0.0082$ and $k_2=0.0099$). Furthermore, the k_2 value of Al/PET/PE is approximately equal to k_1 , confirming that the degradation process is slow and stable. Meanwhile, the L^* of biscuits in PP also showed a more stable decline compared to PAPER/PET/PE, however, it was less stable than Al/PET/PE ($\alpha=0.0845$, $k_1=0.1276$) (Table 5). The study results showed that the brightness (L^*) of biscuits supplemented with *E. asperula* leaf powder was most stable when stored in Al/PET/PE packaging, followed by PP, while PAPER/PET/PE packaging yielded the least stable results. Packaging materials significantly influence the oxidation of polyphenols, chlorophyll, and other biodegradable pigments, leading to the formation of darkening compounds. Therefore, the L^* value of biscuits over storage time was decreased (Verbeyst *et al.*, 2013; Pasqualone *et al.*, 2014). In addition, the degradation of TFC and a^* in *E. asperula* biscuits stored in Al/PET/PE packaging was slower than in PAPER/PET/PE and PP packaging. TFC and a^* recorded the lowest k values when stored in aluminum (Al/PET/PE) packaging (0.2084 and 0.0809, respectively) (Table 4). Al/PET/PE packaging had superior barrier properties, effectively limiting oxidation and flavonoid degradation, while stabilizing chlorophyll and polyphenol pigments. These changes were mainly observed in the a^* value (Ramaswamy & Marcotte, 2006; Patras *et al.*, 2010; Deshwal & Panjagari, 2019). Conversely, PAPER/PET/PE and PP both showed poor barrier properties, accelerated oxidation, and led to faster degradation of TFC and a^* (Deshwal & Panjagari, 2019). In addition, the first-order model allowed estimation of the 50% ($t_{1/2}$) and 90% (D) quality degradation of biscuits in the three types of packaging. The kinetic results showed that biscuits supplemented with *E. asperula* leaf powder packaged in Al/PET/PE packaging maintained the TFC and a^* values effectively ($t_{1/2}=3.33$; $D=11.05$ weeks and $t_{1/2}=8.58$; $D=28.46$ weeks, respectively)

(Table 6). Similarly, Dak *et al.* (2014) found that the shelf life of dried pomegranate seeds stored in aluminum (ALP) packaging reached 187 days, almost double that of HDPP packaging (96 days). In general, Al/PET/PE packaging is suitable for preserving biscuits supplemented with *E. asperula* leaf powder to maintain the biological activity and sensory characteristics of the biscuits, thereby extending their shelf life.

Conclusions

Changes in the quality parameters of biscuits supplemented with *E. asperula* leaf powder were significantly influenced by the type of storage packaging. Parameters such as moisture content, a_w , hardness, color, TPC, TFC, total microbial count, and total yeast and mold count showed significant changes during storage. Accordingly, the results indicated that moisture content and a_w increased significantly, while the other parameters tended to decrease. Meanwhile, total aerobic microorganisms and total yeast and mold counts remained below the detection limit throughout storage. Kinetic results indicated that the degradation of TPC and L^* followed the Biphasic model, whereas TFC and a^* followed first-order kinetics. In addition, the estimated times for 50% and 90% reduction of TFC and a^* in biscuits packaged in Al/PET/PE were better maintained compared to the other two packaging types. Thus, Al/PET/PE demonstrated superior protective effectiveness, maintaining biscuit quality more effectively throughout storage compared to PAPER/PET/PE and PP. However, the study was limited in evaluating quality changes of *E. asperula* biscuits under different storage temperatures to predict a more specific shelf life of biscuits supplemented with *E. asperula* leaf powder.

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