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Degradation Kinetics of Chlorophyll Pigments in Dried Leaves of *Polyscias Fruticosa* (L.) Harms during Storage

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ABSTRACT

Chlorophyll is well-known as the only natural green pigment that owns high biological values with an enormous amount worldwide. In this study, the experiment was designed to a two-level factorial design with three factors, including storage temperature (4 and 28 °C), level of light (with or without light transmission through the package), and level of oxygen (high and low oxygen concentration in package). The chlorophyll content in samples was analyzed every three days. The results showed that the Weibull model could characterize chlorophyll content change in dried *P. fruticose* leaves. Factors including temperature, light, oxygen and their interactions significantly forced the rate of chlorophyll degradation. Storage of dried *P. fruticose* leaves at 4 °C, lack of light and oxygen could get the highest retention of chlorophyll a and b. And in this condition, the half-life was 222 days and 376 days for the degradation of chlorophyll a and b, respectively.

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1. Introduction

Chlorophyll is well-known as the only natural green pigment with an enormous amount worldwide [1]. Especially, chlorophyll was proven to own high biological values such as preventing cancer [2], anti-inflammatory, and antibacterial properties [3], [4], antioxidant activity [2], etc. Therefore, chlorophyll is often used as one of the quality indicators in food processing and preservation studies. While research on chlorophyll retention in final products is numerous, studies on the kinetics of chlorophyll degradation during storage are still limited. Studies on kinetics of chlorophyll degradation were essential to provide vital kinetic parameters that were used to control, design, and optimize the operation to produce the best quality of the product. In the previous limited studies, the kinetics of chlorophyll degradation had been performed in the storage of frozen broccoli [5], thermal treatment of pureed coriander leaves [6], mint leaves puree [7], pasteurization of whole spinach (*Spinacia oleracea*) leaves [8] and microwave drying of Brussels sprouts [9], etc. Most studies reported that chlorophyll degradation could be determined by zero-, first-, and second-order models. Currently, the Weibull distribution was proven that most suitable for the description of chemical [10] or enzymatic reaction kinetics [11], microbial degradation [12], predicting nutrient degradation during drying [13]. Therefore, the Weibull model becomes more convenient in predicting multi-objects at the same time. It is still limited in using the Weibull model to predict the change of chlorophyll during storage.

In food materials containing chlorophyll, *Polyscias fruticosa* (L.) Harms leaves are attractive materials. *Polyscias fruticosa* leaves (PFLs) belong to the *Araliaceae* family [14] that is popularly distributed in moist tropical climates like India, Malaysia, Indonesia, Laos, Cambodia, Vietnam, etc. PFLs are herbs that were figured out therapeutic effects such as

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antipyretic [15], anti-inflammatory [15], [16], anti-tussive [17], etc. Recently, PFLs could be used as herbal tea or food ingredients. However, PFLs have been still underutilized.

Therefore, this study aimed to investigate the kinetics of chlorophyll change storage of dried PFLs. Important factors, including temperature, level of light, and oxygen in the package, were used to design experiments. The results of this study could be used to control the storage operation of PFLs and the suitable storage condition was suggested to enhance the trade value of products.

2. Materials and Methods

2.1. Materials and chemicals

Fresh PFLs originated from a local company in Tay Ninh province, Vietnam was used as materials. To ensure the uniformity of materials, the leaves owned the bright green were sorted by size with 4.2±0.5 cm length and without visual defects. The fresh leaves had the moisture content approximation of 3.76 g/g dry basis (d.b.). Before conducting experiments, these leaves (25 gram per batch) were dried by microwave oven (Electrolux EMM2001W) at the power of 300 W until the moisture content reached about 0.075 g/g d.b. The time for drying in ranging from 9 to 10 min. Then dried PFLs was fractionated in 10 g containers and used as materials.

Acetone (99.8% purity) was purchased from Sigma-Aldrich and distilled water was of analytical grade.

2.2. Experimental design

The design of the experiment used was a full factorial design with three factors, including one numerical factor (storage temperature) and two category factors (level of light and level of oxygen in package). Two-level of each factor were applied. The temperature varied 4 and 28 °C. Dried PFLs were packaged in the transparent plastic and aluminium foil bag that corresponded to two levels of light in a package: presence and absence of light. And the presence and absence of oxy were investigated using non-vacuum and vacuum packing. There are total of 16 runs was preserved during 30 days and each run was determined the chlorophyll content at each 3-day interval. All the experiments were replicated three times, and the average values were used.

2.3. Analytical method

The chlorophyll content was determined by the method of Kumar et al. (2015) [18] with some modifications. Samples (0.1 g) were grinded and extracted with 10 mL acetone 80% (v/v). The extract was separated from the crushed mass by vacuum filtration through Whatman No. 1 filter paper and washed with 10 mL acetone 80% (v/v). The chlorophyll extract was taken in a 25-mL volumetric flask, then measured at 663, 645 nm using a UV-Vis spectrophotometer (SHIMADZU UV 1800). Chlorophyll a and b (mg/g dry basis) was calculated according to the below Eq. (1) and (2), respectively:

$$\frac{\left(-2,59\times OD_{645}+12,72\times OD_{663}\right)\times V_{dm}}{1000\times m_{ck}} \quad (1)$$

$$\frac{\left(22,9\times OD_{645}-4,67\times OD_{663}\right)\times V_{dm}}{1000\times m_{ck}} \quad (2)$$

2.4. Mathematical model for chlorophyll degradation

Many mathematical theories have been used to model the change of chemical compounds during processing and preservation [19]. Previous studies confirmed these changes mainly follow the zero-, first-, or pseudo-first-order model [19]. Recently, the Weibull model has received a large interest in the kinetic study on degradation. Weibull model was proven to be suitable for predicting the degradation of vitamin C and total polyphenol during drying [13]. Therefore, in this study, the Weibull model was used to characterize chlorophyll degradation during the storage of dried PFLs at different conditions. Weibull model was displayed following equation [13]:

$$\frac{C_t}{C_0} = \exp\left[-\left(\frac{t}{\tau}\right)^{\alpha}\right] \quad (3)$$

Where, C_o and C_t corresponded to chlorophyll (a or b) content at initial and time t (mg/g d.b.); τ can be considered the reaction time constant (day); α is a fitting parameter.

The half-life was also calculated from the estimated coefficient in the Weibull model as shown:

$$t_{1/2} = (\ln 2)^{1/\alpha}\,\tau \quad (4)$$

The statistic parameters including R^2 value, RMSE standard deviation, and χ^2 value was shown to identify the ability in the prediction of the tested model

$$R^2 = 1 - \frac{\sum_{i=1}^{N}\left(MR_{\exp,i} - MR_{pre,i}\right)^2}{\sum_{i=1}^{N}\left(\overline{MR}_{\exp} - MR_{pre,i}\right)^2} \quad (5)$$

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(MR_{\exp,i} - MR_{pre,i}\right)^2} \quad (6)$$

$$\chi^2 = \frac{\sum_{i=1}^{N}\left(MR_{\exp,i} - MR_{pre,i}\right)^2}{N - Z} \quad (7)$$

2.5. Data analysis

All experiments were conducted in triplicate. Microsoft Excel (2019) and MATLAB R2014 software were used to calculate and analyze the experimental data. Analysis of factor effects and interactions between factors were conducted using Design-Expert software 10.1.

3. Results and Discussion

3.1. The change of chlorophyll content during storage of dried PFLs

The photograph of dried PFLs during 30-day storage are shown in Figure 1 and the changes in chlorophyll a and b during the storage of dried PFLs under different conditions are shown in Figure 2.

At 4°C with the lack of light and presence of oxygen in the package (4LP)

At 4°C with the lack of light and oxygen in the package (4LL)

At 4°C with the presence of light and lack of oxygen in the package (4PL)

At 4°C with the presence of light and oxygen in package (4PP)

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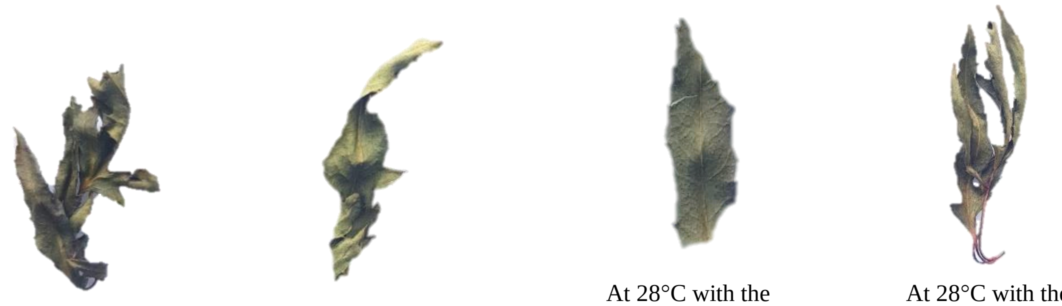

At 28°C with the lack of light and presence of oxygen in the package (28LP)

At 28°C with the lack of light and oxygen in the package (28LL)

At 28°C with the presence of light and lack of oxygen in the package (28PL)

At 28°C with the presence of light and oxygen in the package (28PP)

Figure 1. *The photographs of dried PFLs after 30-day storage at different conditions*

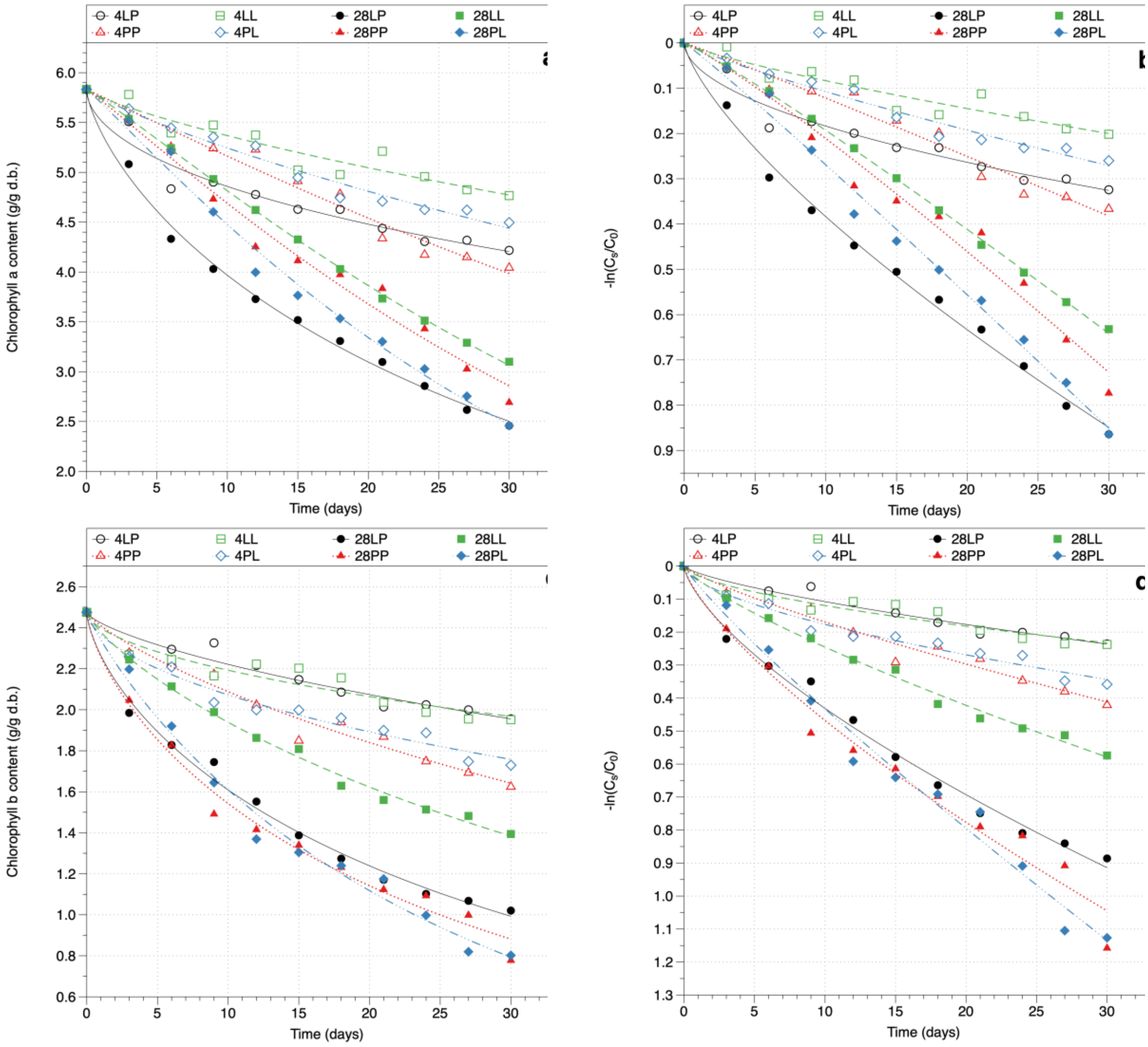

Figure 2. *The change of chlorophyll a and b in dried PFLs during 30-day storage (a and c), and the logarithm of the normalized concentration of chlorophyll-a (b) and chlorophyll b (c) versus time (days)*

Figure 2 showed that both chlorophylls a and b in PFLs were decreased during storage. In which, all dried leaves were stored at 4 °C retained higher chlorophyll a and b than stored at 28 °C in condition with/without contact light and/or oxygen. Chlorophyll degradation during storage may be related to thermal processing, and in this case, it would be the drying process. Under thermal treatment, the structure of chlorophyll could be changed due to the linkage broken between protein and chlorophyll [20]. After drying, free chlorophyll was released and different derivatives of chlorophyll were created, leading to chlorophyll compounds being easy to degrade during storage. The loss of chlorophyll content was confirmed by enzymatic and non-enzymatic reactions [20]. Consequently, the color of the product was changed [20], and lower sensorial value of the product (see Figure 1). Comparison between results as shown in Figure 2, it was indicated the change of color would depend on the level of chlorophyll degradation. Specifically, the color could vary from bright green to dark green even to brown color after 30-day storage of dried leaves.

3.2. Kinetic of chlorophyll degradation during storage of dried PFLs

Results from Table 1 presented that all R^2 values are greater than 0.90. Besides, the RMSE and χ^2 were low ranging from 0.0033 to 0.0256 and 1.20×10^{-5} to 7.23×10^{-4}, respectively. Therefore, the Weibull model could be used to predict the change of chlorophyll (a and b) during the storage of dried PFLs.

Table 1. *Statistical parameters of non-linear regression analysis for chlorophyll degradation during storage of dried PFLs*

Storage Conditions	Chlorophyll a			Chlorophyll b		
	R^2	RMSE	χ^2	R^2	RMSE	χ^2
4LP	0.9554	0.0175	3.37E-04	0.9298	0.0169	3.14E-04
4PP	0.9631	0.0193	4.10E-04	0.9689	0.0183	3.69E-04
4LL	0.9029	0.0186	3.80E-04	0.9040	0.0256	7.23E-04
4PL	0.9729	0.0125	1.71E-04	0.9709	0.0146	2.35E-04
28LP	0.9953	0.0117	1.51E-04	0.9920	0.0158	2.76E-04
28PP	0.9829	0.0215	5.09E-04	0.9867	0.0222	5.43E-04
28LL	0.9995	0.0033	1.20E-05	0.9944	0.0101	1.12E-04
28PL	0.9903	0.0184	3.73E-04	0.9877	0.0235	6.09E-04

Note:
4LP: At 4°C with the lack of light and presence of oxygen in the package
4PP: At 4°C with the presence of light and oxygen in the package
4LL: At 4°C with the lack of light and oxygen in the package
4PL: At 4°C with the presence of light and lack of oxygen in the package
28LP: At 28°C with the lack of light and presence of oxygen in the package
28PP: At 28°C with the presence of light and oxygen in the package
28LL: At 28°C with the lack of light and oxygen in the package
28PL: At 28°C with the presence of light and lack of oxygen in the package

From the Weibull model, kinetic parameters of chlorophyll degradation during storage at different conditions were estimated and summarized in Table 2. In addition, the half-life values ($t_{1/2}$, days) calculated from Eq. (4) are also presented in Table 2.

The relationship between half-life value and investigated factors was determined by linear regression method using Design-Expert software 10.1. Pareto chart presented the level of effects of each factor and interactions of factors in Figure 3.

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Table 2. *The kinetic parameters of chlorophyll degradation during storage of dried PFLs*

Storage Conditions	Chlorophyll a			Chlorophyll b		
	τ (days)	α	$t_{1/2}$ (days)	τ (ngày)	α	$t_{1/2}$ (days)
4LP	247.33	0.530	124	230.48	0.709	137
4PP	76.16	1.036	53	91.00	0.803	58
4LL	221.68	0.803	140	375.64	0.578	199
4PL	136.35	0.858	89	181.11	0.597	98
28LP	37.87	0.716	23	34.24	0.684	20
28PP	41.02	1.078	29	28.68	0.711	17
28LL	44.74	1.102	32	59.67	0.789	38
28PL	34.54	1.072	25	25.92	0.885	17

Note:

4LP: At 4°C with the lack of light and presence of oxygen in the package

4PP: At 4°C with the presence of light and oxygen in the package

4LL: At 4°C with the lack of light and oxygen in the package

4PL: At 4°C with the presence of light and lack of oxygen in the package

28LP: At 28°C with the lack of light and presence of oxygen in the package

28PP: At 28°C with the presence of light and oxygen in the package

28LL: At 28°C with the lack of light and oxygen in the package

28PL: At 28°C with the presence of light and lack of oxygen in the package

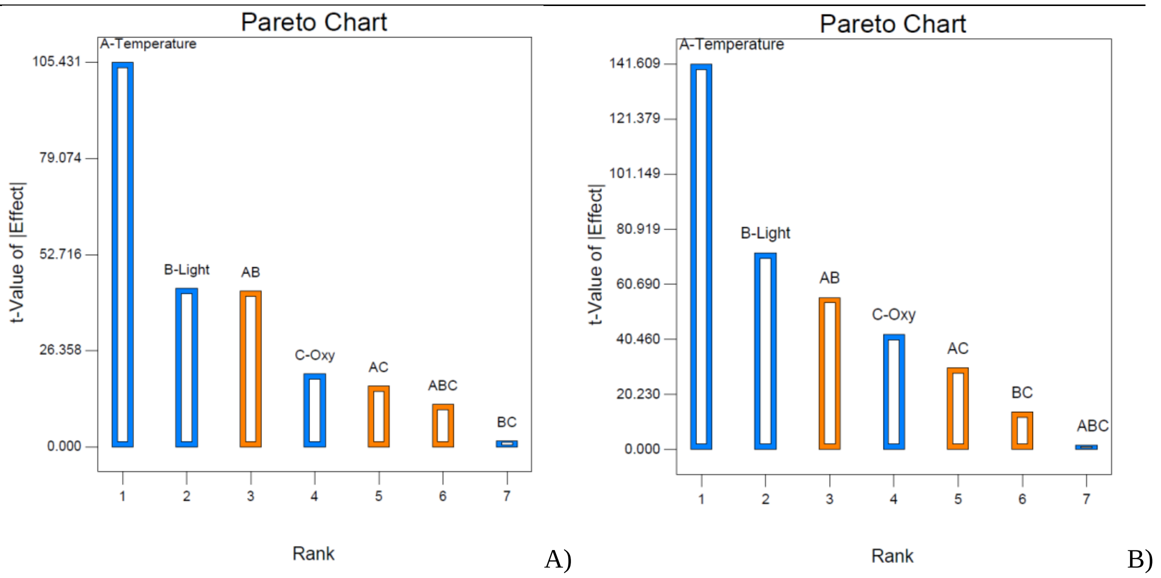


Figure 3. *Pareto chart presented effects of storage conditions on degradation of chlorophyll a (A) and b (B) in the dried PFLs*

The results from Figure 3 could divide into three sections depending on the level of effect on the half-life of chlorophyll (a and b). The first group had a high level of effect that could include following order temperature (denoted A) > level of light (denoted B) > interaction of temperature and level of light (denoted AB). The second group had a medium level of effect were the level of oxygen (denoted C) and interaction of temperature and level of oxygen (denoted AC). Finally, the last group had a low level of effect including the interaction of level of light and level of oxygen (denoted BC) and interaction between temperature, level of light, and level of oxygen (denoted ABC). Based on these results, using linear regression of the experimental data, the half-life for chlorophyll a and b, $t_{1/2}$ (days) correlated with investigated factors was shown corresponding to the following equations:

$$t_{1/2}\left(Chla\right) = 64.41 - 37.28A - 15.37B - 7.105C + 15.11AB + 5.93AC \quad (7)$$

$$t_{1/2}\left(Chlb\right) = 73.02 - 50.07A - 25.55B - 14.95C + 19.72AB + 10.59AC + 4.86BC \quad (8)$$

where A, B, and C are the encoded temperature, levels of light and oxygen, respectively, such that the low, and high levels of temperature, or levels of light or oxygen, correspond to −1, and 1, respectively. The standard error of Equation (7) and (8) corresponded to 8.30 and 1.41 days and the adjusted R^2 value for the fit was 0.969 and 0.999, respectively. Very high R2 values (closely 1) indicated the strong correlation between observed values and predicted values. Effects of each factor and these interactions on the half-life of chlorophyll a and b during storage of dried PFLs was presented in Figure 4 and 5, respectively.

Based on results shown in Eq. (7), (8), and Figure 4, 5, it was indicated that both the half-life of chlorophyll a and b decreased when temperature or level of light or level of oxygen increased. Coefficients from Eq. (7), (8) also showed that temperature was the factor that had the largest effect on the degradation of chlorophyll, following the level of light, and finally the level of oxygen in packages. For effects of factor interactions, results showed that at the same storage temperature, an increment of light or oxygen in the package could lower the half-life of chlorophyll a and b. In other words, the larger the rate of chlorophyll degradation, the more level of light or oxygen in the package. However, storage of dried leaves at low temperature (4 °C), this increase of chlorophyll degradation rate was larger than at high temperature. And, the results were found to be similar interactions between the level of light and the level of oxygen. But, when the light resistance package was used, the increase of chlorophyll degradation rate was insignificant larger than light transmission package.

The higher chlorophyll retention at low-temperature storage had also been reported in the previous study [21], which led to sensorial values being higher than at high-temperature storage. In this study, chlorophyll a and b in dried PFLs was degraded both lack and presence of light in the package. In previous study, chlorophyll degradation in lack of light condition was reported due to free radical mechanism with slow rate [22] and mainly produced phytoene, 3,7,11,15-tetramethyl-2,3-epoxyhexadecan-1-ol, and 3,7,11,15-tetramethylhexa decane-1,2,3-triol [23], [24]. And, the rate of chlorophyll was faster at a higher level of light [22]. For the impact of oxygen on chlorophyll degradation, many previous publications have been reported [23], [24]. Double bonds in porphyrin ring and isoprenoid phytin branched-chain were easy to react with singlet oxygen and produce hydroperoxides along with transit double bonds [25]. And then, hydroperoxides would break oxygen-oxygen bonding to release free radicals [25]. The oxidation reaction by singlet oxygen was reported that it was the main oxidative mechanism of phytol in chlorophyll b in the presence of light [23]. And our results supported the finding that the presence of light was more crucial on chlorophyll degradation than singlet oxygen in the package.

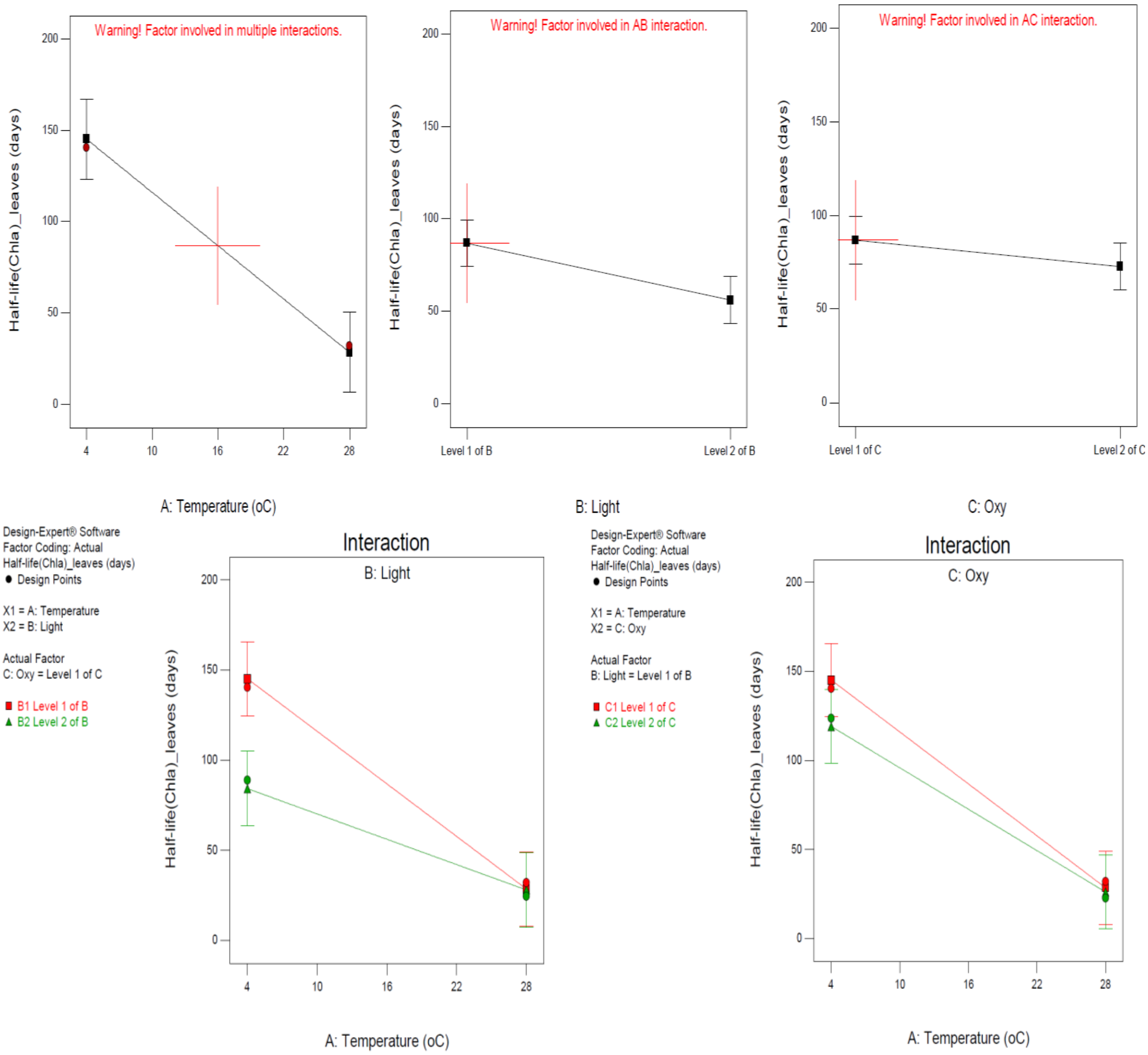
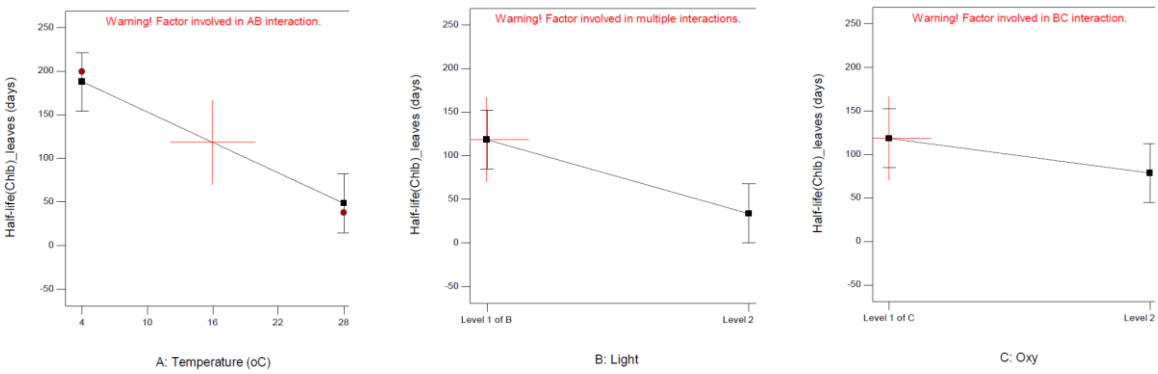


Figure 4. *Effects of investigated factors and their interactions on the half-life of chlorophyll during storage of dried PFLs*



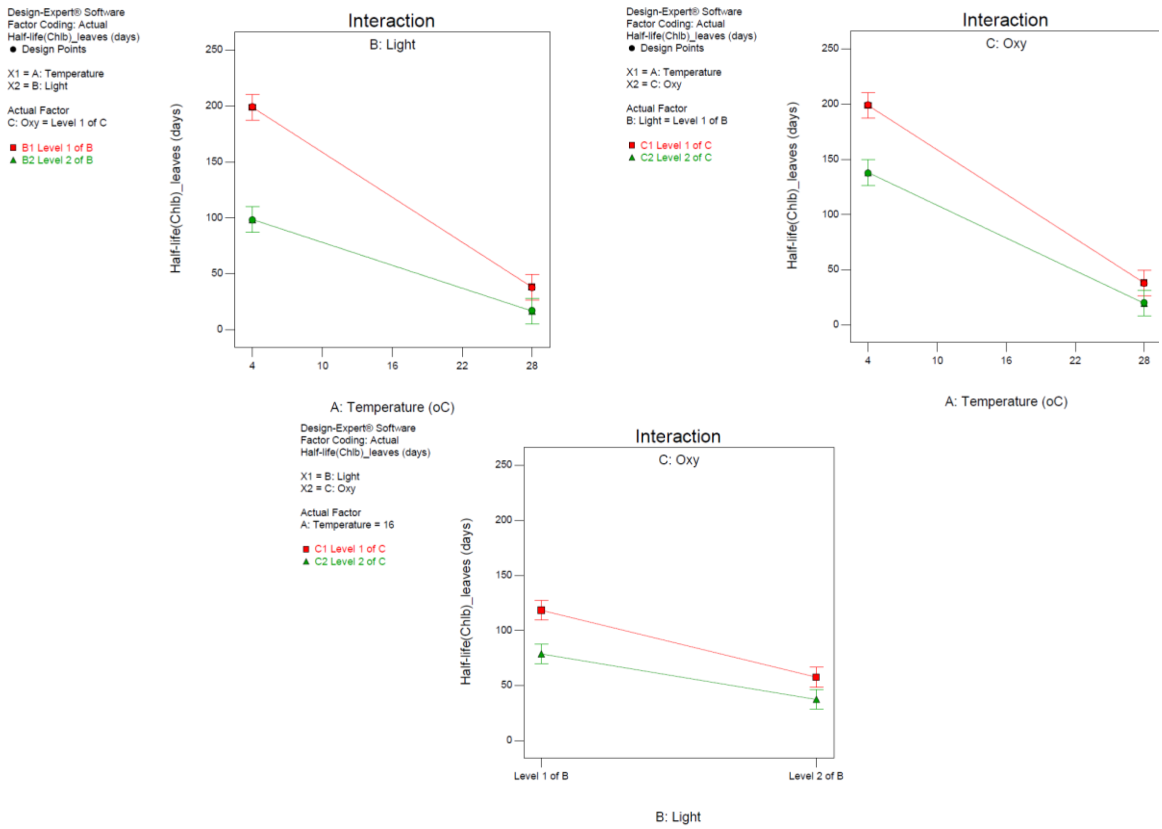


Figure 5. *Effects of investigated factors and their interactions on the half-life of chlorophyll b during storage of dried PFLs*

4. Conclusions

The change of chlorophyll a and b in the dried PFLs at different storage conditions were characterized. The results showed that chlorophyll a and b in the leaf was degraded during storage for all investigated conditions. Investigated factors (temperature, the level of light, and oxygen), and these interactions had significant impacts on the half-life of chlorophyll a and b. Storage of the dried PFLs at low temperature (4°C), lack of light and oxygen in the package would retain the highest content of chlorophyll a and b. And, in this storage condition, the highest half-life values were 222 days and 376 days for degradation of chlorophyll a and b, respectively. Therefore, the application of antioxidants, active packaging, etc. could minimize the degradation of chlorophyll compounds in green leaves.

Acknowledgments

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