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COMPARATIVE KINETICS STUDY OF CHLOROPHYLL AND COLOUR DEGRADATION FOR THREE LEAFY VEGETABLES

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Abstract: The chlorophyll and colour degradation of lettuce, spinach and rocket, harvested at their commercial maturity were estimated at 0, 5, 10 and 20 ^{o}C. A first-order kinetics model based on the time-temperature integral was developed and tested to describe the chlorophyll and colour degradation of the three vegetables. The kinetic model described efficiently the experimental chlorophyll and colour degradation data. The spinach and rocket exhibited similar chlorophyll degradation rate while lettuce had different. The energy of activation for the chlorophyll degradation was in the same order for the rocket and spinach and 21% higher for the lettuce. The spinach exhibited increased sensitivity regarding colour degradation at 20 ^{o}C compared to rocket and lettuce. Plotting the total chlorophyll values with the respective hue angles generated three parallel linear plots, one for each vegetable, the classification of which follows the respective ranking of respiration rate of the three vegetables.

Keywords: kinetics; chlorophyll degradation; hue angle; multiresponse modelling.

1. Introduction

One of the most significant and readily identifiable quality attributes of fresh agricultural products is skin colour which influences consumers' acceptability. Consumers usually purchase fresh agricultural products driven by their visual appearance, while other quality attributes such as texture and aroma affect consumers' choice to re-purchase the same product [1]. Colour is often taken as an indicator of freshness, ripeness, palatability and nutritional value although sometimes this perception is misleading [2]. Food colour is driven by chemical, biochemical, microbial and physical changes taking place during growth, maturation, postharvest handling and processing [3].

Skin colour of fruits and vegetables results from the development of chlorophylls and carotenoids pigments in the chloroplasts and chromoplasts and the phenolic pigments in vacuole [4]. The colour of green vegetables is mainly shaped up by the dominant chlorophyll pigments. Several chlorophylls can be traced in nature; chlorophyll *a* and *b* is found in green plants in an approximate ratio of 3:1 although deviations from this ratio have been reported [5].

The main causal factor of discolouration in stored green vegetables (i.e. broccoli, spinach, lettuce, rocket, etc) is chlorophyll breakdown [6]. Chlorophyll breakdown shifts colour from bright green to a variety of colour shades such as yellow, brown, and orange in senescent tissues [7]. Chlorophyll degradation is considered the first visual symptom of senescence but by the time leaf yellowing appears, the senescence has already progressed [8]. Chlorophyll breakdown in vascular plants is a complex multistep pathway, as chlorophyll biosynthesis is, but can be summed up in two basic groups of reactions; the first one produce greenish derivatives while the second one, colourless compounds by an oxidative ring opening. The previous process is very rapid and despite considerable efforts, detection of intermediate products is very difficult [9].

The chlorophyll degradation in senescent tissues is initiated by external factors such as water stress [10, 11], insufficient daylight [12], temperature variation [13] increased levels of ethylene [14] or other factors or combinations thereof (humidity, certain plant hormones, damage of vegetables) [15].

Temperature affects significantly the shelf-life of freshly harvested vegetables due to its profound role on their metabolism and respiration. Temperature abuse is one of the main known factors to hasten the loss of shelf-life of stored vegetables; high temperatures accelerate yellowing in leafy vegetables.

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Many green vegetables are not consumed raw, the effect of food handling, cooking, and industrial processing on their appearance and acceptance, has been studied employing first-order kinetic models of chlorophyll degradation. The respective temperature dependence of the degradation rate is described by modified Arrhenius equations. Green colour degradation of vegetables is measured by various chromatic indices on the CIEL*a*b* system (Commission International d' Eclairage) which also follow first-order kinetics, where the rate constant is temperature dependent [16, 17, 3].

Leafy vegetables, such as lettuce, spinach, rocket etc. are widely used in Mediterranean diet in various fresh, mixed or garnish salads. Over the last decade has been scientifically proved and promoted that these vegetables constitute important functional food components by contributing vitamins, minerals and biologically active compounds associated with dietary activities [18]. Leafy vegetables are highly perishable, characterised by a short life-time and high transpiration rates due to high surface to mass ratio, fact that contributes to accelerating senescence and colour loss. Therefore, maintaining much of their original colour along with other sensory characteristics, perceived as quality issues by the consumers, consist a major challenge during the storage of leafy vegetables [17].

The objectives of this study are:

- estimate degradation kinetics of total chlorophyll in rocket, spinach, and lettuce employing spectrophotometry;
- estimate green colour degradation kinetics of rocket, spinach, and lettuce, by means of hue angle. A first-order kinetics model was developed to describe the previous kinetics parameters of hue angle and total chlorophyll degradation as function of the storage time-temperature integral;
- correlate the total chlorophyll and hue angle and map the relative sensitivity of total chlorophyll with the respective hue angle degradation.

2. Materials and methods

2.1 Plant material and sample preparation

Romaine lettuce (*Lactuca sativa* var. *longifolia*), savoy spinach (*Spinacia oleracea*) and rocket (*Eruca sativa* Mill) cultivated in an experimental field of Technological Educational Institute of Kalamata, Greece and harvested at their commercial maturity based on their leaves' number for lettuce and size for spinach and rocket. The vegetables were hand-picked early in the morning and immediately transported to the lab for further processing. From the sorting, non defective lettuce heads of similar size were used and the outer leaves were removed. In spinach and rocket, senescent and defective leaves were also removed and only healthy leaves of the same growth stage were used. Finally, the sorted vegetables were thoroughly rinsed with tap water at 5 ^{o}C and then dried with water-absorbing kitchen paper.

2.2 Storage conditions and sampling measurement

The three vegetables were stored in dark to prevent potential interference of photosynthesis on chlorophyll breakdown. Storage at 0, 5 and 10 °C lasted for 13 days in the case of lettuce and spinach and 10 days in the case of rocket. At 20 ^{o}C lettuce was stored for 10 days, spinach for 6 days and rocket for 7 days. Sampling for measurement of colour and chlorophyll content (mg g_{fw}^{-1}) was carried out on the 0, 3, 8, 10 and 13 day of the experiment for lettuce, on the 0, 3, 6, 10 and 13 for spinach and on the 0, 3, 7 and 10 for rocket. In each sampling day, six lettuce heads and six spinach and rocket samples of five leaves per sample (in total 30 leaves) were tested per storage temperature. The colour measurement was carried out in the case of the spinach and rocket, on 30 leaves, 1-2 cm from the leaf tip; in lettuce, measurements were carried out at the four outer leaves of each tested head (in total 24 leaves), 1-2 cm from the leaf tip. The areas of the leaves used for colour measurements afterwards were cut into thin strips and randomly mixed for chlorophyll extraction. Thus, chlorophyll measurement was carried out at 6 samples per measurement day and storage temperature. All experiments were duplicated.

2.3 Assessment of leaf surface colour and chlorophyll content

The colour of the leaves was measured by a Minolta CR-300 Chromameter (Minolta Corp. Tokyo, Japan) on CIE *L*a*b** chromatic space and expressed as hue angle (h_{ab}). The CIE *L*a*b** chromatic space is widely used for foods, because corresponds to the visible to human eye spectrum. The *L** index is related to the luminosity taking values between black (*L**=0) and white (*L**=100); the *a** coordinate is negative for greenish and positive for reddish colours; the *b** coordinate is positive for yellowish colours and negative for

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bluish colours. The total colour difference (ΔE*), the chroma (C^*_{ab}) and the h_{ab} provide valuable information on colour degradation. The ΔE* is important when the relationship between the visual and the numerical analyses is evaluated while the C^*_{ab} and h_{ab} are the quantitative and qualitative attributes of colour, respectively [19]. In particular, the h_{ab} is the angle in degrees that corresponds to the 3-D colour diagram (i.e., 0^o for red, 90^o for yellow, 180^o for green and 270^o for blue). Depending on the sampling strategy and pursued accuracy, the *L**, *a**, *b**, h_{ab}, C^*_{ab} or a combination thereof, can provide satisfactory colour description. The calculation of the h_{ab} should be performed by compensating for the quadrant, in which the data appear so as the h_{ab} to be positive,

$h_{ab}=\arctan(b^*/a^*)$, when $a^*>0$ and $b^*>0$ (1a)

$h_{ab}=180+\arctan(b^*/a^*)$, when $a^*<0$, $b^*>0$ and $a^*<0$, $b^*<0$ (1b)

$h_{ab}=360+\arctan(b^*/a^*)$, when $a^*>0$, $b^*<0$ (1c)

The chlorophyll assay was performed in accordance to Hiscox and Israelstam (1979) [20] spectrophotometer protocol employing dimethyl sulphoxide (DMSO). About 100 mg of fresh leaf tissue was cut into strips of 2-5 mm wide and placed in a test tube containing 10 ml of DMSO. The test tubes incubated at 60 °C for 60 min. Preliminary tests showed that chlorophyll extraction at 60 °C was carried out within 40-50 min with no loss of chlorophyll in the heated DMSO during the first 60 min; therefore the extractions were ran for 60 min. Upon cooling, 3 mL of the extract was placed in a quartz cuvette and absorbance readings were carried out at 665 nm (A^{665}) and 648 nm (A^{648}) by a U-2000 Hitachi double-beam spectrophotometer (Hitachi High-Technologies Corp., Tokyo, Japan). The spectrophotometer was calibrated at zero absorbance using a blank quartz cuvette bearing pure DMSO. The total chlorophyll was determined as the sum of chlorophyll *a* (Chl_a) and *b* (Chl_b), where $Chl_a=14.85\times A^{665}-5.14\times A^{648}$ and $Chl_b=25.48\times A^{648}-7.36\times A^{665}$ [21].

2.4 Colour kinetics analysis

Most of the cited studies, related to colour and chlorophyll degradation kinetics in foods, are zero- or first-order. The rate of change of a quality factor A can be expressed as:

$$-\frac{dA}{dt}=k_r\exp\left[-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_r}\right)\right][A]^n \quad (2)$$

where n is the reaction order, k_r is the frequency factor, E_a is the activation energy (J mol^{-1}), R is the gas constant (J $mol^{-1}K^{-1}$), T is the absolute temperature (K) and T_r is the reference temperature (K). Dolan (2003) [22] presented three methods of T_r estimation for isothermal and non-isothermal conditions. From the two available methods for parameter estimation of Eq. 2 the *integral method* is preferred compared to the *differential method* as less sensitive to experimental errors. Following integration of Eq. 2,

$$-\int_{A_o}^{A}[A]^{-n}dA=k_r\int_0^t\exp\left[-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_r}\right)\right]dt \quad (3)$$

where t is the storage time (h). Upon integrations in Eq. 3, Eqs 4a and 4b are obtained for zero- and first-order reactions which can be used for parameter estimation employing a nonlinear optimisation procedure,

$$n=0,\ A-A_o=\pm k_r t\exp\left[-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_r}\right)\right] \quad (4a)$$

$$n=1,\ \ln(A)-\ln(A_o)=\pm k_r t\exp\left[-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_r}\right)\right] \quad (4b)$$

where (+) and (-) indicate formation and degradation of a quality parameter A that is the C_t/C_o ratio and the h_{ab} for chlorophyll and colour degradation respectively in our study. Xanthopoulos et al. (2014) [23] discussed that the reaction order in each reactant may change during the various phases of degradation and furthermore the same process could contain consecutive or parallel reactions such as in the case of chlorophyll degradation in plant tissues as Marquez & Sinnecker (2007b) [17] discussed. In our study, the reaction order was chosen testing Eq. 4a and 4b employing nonlinear regression and then the accuracy was improved introducing an exponent *m'* in the time term.

$$n=0,\ A=A_o\pm k_r t^{m'}\exp\left[-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_r}\right)\right] \quad (5a)$$

$$n=1,\ \ln(A)=\ln(A_o)\pm k_r t^{m'}\exp\left[-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_r}\right)\right] \quad (5b)$$

2.5 Experimental design and statistical analysis

The experiment was performed according to a full factorial design (storage temperature×vegetable type×storage time) and subjected to an analysis of variance (ANOVA) using the Statgraphics Centurion XVI (Statpoint Technologies, Inc, VA,

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USA) statistical software. Mean values were subjected to Fisher's Least Significant Difference (LSD) test at $P \leq 0.05$. This statistical test is liberal with respect to the comparison wise error rate, but is powerful for detecting true differences [24, 25]. The data points in the figures and tables are the mean values from the two experimental series since no significant differences between these series were detected. The root mean square error (RMSE) was used as a measure of the fitting quality since the errors (X_{exp}-X_{pred}) are squared before they are averaged and thus RMSE provides a relative high weight to large errors. The RMSE is valid for unbiased errors following a normal distribution.

$$RMSE=\left[N^{-1}\sum_{t=0}^{t_f}\left(X_{exp}-X_{pred}\right)^2\right]^{1/2} \quad (6)$$

where t_f the total storage time, N the number of measurements, X_{exp} an experimental value and X_{pred} the respective predicted value.

3. Results and Discussion

Estimation of the chlorophyll *a* and *b* in the lettuce, rocket and spinach showed that the mean value of Ch_a/Ch_b equals to 5.5±1.0, 2.3±0.6 and 5.5±1.0 respectively. Bohn et al. (2004) [26] reported values of Ch_a/Ch_b for lettuce, rocket and spinach as 4.3, 3.5 and 3.0 respectively with no information regarding the tested vegetables and storage conditions. The Ch_a/Ch_b varies according to produce, growing conditions, agronomic practices, growth stage [27] or combinations of factors (humidity, leaf damage etc.). During storage, the Ch_a/Ch_b varies also due to faster degradation of Ch_a compare to Ch_b [28].

Chlorophyll and green colour degradation studies in stored fresh products are scarce compared to heat-treated products mainly in puree form [26, 9]. Pulping samples, simplifies chlorophyll and colour assessment due to homogenisation while in the case of intact products (i.e. leafy vegetables) sampling is laborious due to multiple sampling points required to cope with the high variability of the assessed chlorophylls and colour indices. The ANOVA indicated the significance of storage time, storage temperature and vegetable type ($P \leq 0.05$) on chlorophyll degradation (cf. Table 1). The F-ratio of storage temperature (F-ratio=73.40) is close to the F-ratio of storage time (F-ratio=79.87), indicating that both variables affect to the same extent chlorophyll degradation which is consistent with the literature, that enzymatic reaction of chlorophyll degradation is driven by the integral of storage temperature and time.

The chlorophyll degradation was expressed as Chl_t/Chl_{t_o}, where Chl_{t_o} is the initial total chlorophyll. The ratio of Chl_t/Chl_{t_o} expresses the remaining percentage of the initial total chlorophyll Chl_{t_o}. At the end of the storage, the Chl_{t_o} reduced by 50% in all the tested vegetables stored at 10 ^oC and 20 ^oC. The chlorophyll ratio Chl_t/Chl_{t_o} follows a first-order reaction kinetics, exhibiting good agreement between the fitted model (Eq. 5b) and the experimental data as it is displayed in Fig. 1a-c, with R^2_{adj} >90%, SEE≤0.09 and RMSE<5.9% (cf. Table 2a). The tested zero-order kinetics model exhibited less accuracy between the fitted model (Eq. 5a) and the experimental data with RMSE ranged between 4.3 and 6.1% (cf. Table 2b). The previous findings agree with Marquez and Sinnecker (2007a) [9] review, that in most of the studies, chlorophyll degradation follows a first-order reaction kinetics. The T_r value used in Eqs 5a and 5b was 281.15 K, calculated from the equation $T^{r^1}=m^{-1}\sum_{m} T^{r^1}$ for the four tested isothermal conditions (0, 5, 10 and 20 ^oC). Spinach (cf. Fig. 1b) and rocket (cf. Fig. 1c) exhibited similar chlorophyll degradation rate against lettuce (cf. Fig. 1a) degradation rate which followed different trend.

Many factors have been indentified to affect chlorophyll degradation such as water stress (due to high water vapour deficit), sun light, storage temperature, excessive ethylene presence and combinations of these factors [13, 10, 11, 14, 12, 7, 29, 15]. Among the previous factors, temperature is considered the most significant, affecting postharvest quality of leafy vegetables. The reduction of the green colour intensity in the green leafy vegetables is associated with the senescence of the plant tissues, the nutritional deprivation and consequently the degradation of the overall quality [30]. The deterioration rate of the harvested produce is well-known that is proportional to their respiration rate [31]. Reported respiration rates (RR) of lettuce (Romaine, whole leaf) is moderate as 24-35 mg_{CO_2} kg^{-1} h^{-1} and very high for spinach (leaf) 35-58 mg_{CO_2} $kg^{-1}h^{-1}$ and rocket salad 47-95 mg_{CO_2} $kg^{-1}h^{-1}$ [32, 33, 34]. The previous classification can explain the shape of the chlorophyll and hue angle degradation rate with storage time (cf. Fig. 1 and 2). The higher the respiration rate of a produce, the faster the degradation rate and the shorter its

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storability which explains the shorter by 3 days (10 days) storage life of the rocket compared to 13 days of lettuce and spinach. Differences of the calculated activation energy E_a (cf. Table 2) were detected, where rocket and spinach had similar E_a values 47.6-48.5 kJ mol^{-1} while lettuce E_a was 21% higher, 57.9 kJ mol^{-1}.

Degradation of chlorophyll in green plant organs is expressed mainly as green colour degradation. Although many different ways of colour assessment have been proposed [3], h_{ab} as a qualitative colour attribute [19] provides a direct assessment of colour variation. In our study, the green colour degradation was described based on the h_{ab} following a first-order reaction kinetics. Experimental and predicted (cf. Eq. 5b) h_{ab} data of the tested vegetables are presented in Fig. 2a-c. In all the tested vegetables and storage temperatures, the initial h_{ab} did not reduce more than 16-20% which shows that chlorophyll degradation had been advanced before the respective colour degradation become detectable.

The calculated parameters of Eq. 5b derived from the nonlinear regression of the experimental h_{ab} values are tabulated in Table 3a. The calculated statistical parameters from the previous regression (cf. Table 3) revealed good agreement between the experimental and the predicted h_{ab} with R^2_{adj} >95%, SEE≤0.01 and RMSE<1.7%. The respective zero-order model Eq. 5a gave marginally less accurate h_{ab} prediction with RMSE ranged between 1.0 and 1.4%. The response surfaces (cf. Fig. 3a-c) of h_{ab} with storage time and temperatures depict the effect of time-temperature integral on h_{ab} degradation. As can be seen in these plots the spinach (cf. Fig. 3b) exhibited increased sensitivity at 20 ^oC compared to rocket (cf. Fig. 3c) and lettuce (cf. Fig. 3a).

ANOVA was carried out considering the Chl_t as response and h_{ab} and vegetable type as main factors. Based on this selection, if h_{ab} is known, then Chl_t degradation can be evaluated with no need of laborious chemical analyses. Regression of h_{ab} with Chl_t (mg g_{fw}^{-1}) resulted to R^2_{adj}=0.918 and SEE=0.876. The calculated P-values for the slopes (P-value=0.135>0.05) and intercepts (P-value≤0.001) showed no significant difference among the slopes for the three tested vegetables. Based on this analysis the resulted model was,

$$Chl_t=-31.285+0.351\times h_{ab}+3.636\times Rocket +2.013\times Spinach \quad (7)$$

The *Rocket* and *Spinach* terms are indicator variables which take the value 1 if true and 0 if false. According to Eq. (7) three parallel plots are derived (cf. Fig. 4a) one for each tested vegetable. For lettuce, the model becomes $Chl_t=-31.285+0.351\times h_{ab}$, for rocket the model becomes $Chl_t=-27.649+0.351\times h_{ab}$ and for spinach the model becomes $Chl_t=-29.272+0.351\times h_{ab}$.

The efficiency of the proposed model (cf. Eq. 7) was also tested based on the residual error graph, Fig. 4b. This graph is used for visual examination of the homoscedasticity hypothesis where the variance (σ^2) of the residuals is constant over the entire range of the residuals; residuals must exhibit a non-systematic pattern. Based on Fig. 4b can be concluded that the homoscedasticity hypothesis is fulfilled since the plotted studentised residuals of the predicted Chl_t (mg g_{fw}^{-1}) show no systematic patterns and are allocated around zero in a narrow zone of -3.0 and 3.0. The variation between the Chl_t and the h_{ab} of the three vegetables exhibited a systematic trend. The RR classification of these vegetables ($RR_{rocket}>RR_{spinach}>RR_{lettuce}$) agrees with the apposition of the three linear plots seen in Fig. 4a where rocket lies higher than spinach and lettuce in the row.

Future research will focus on the existence of similar correlations between the Chl_t and the h_{ab} for different leafy vegetables and varieties of the same vegetables, harvested on their commercial maturity stage and stored in similar storage conditions, aiming to map the relative sensitivity of the Chl_t with the respective h_{ab} variation.

4. Conclusions

The high explained parts (R^2_{adj}) and the low resulted errors (SEE and RMSE) obtained from the integral analysis confirm that the proposed first-order kinetics model can efficiently describe the Chl_t and h_{ab} degradation during the storage of the three tested vegetables. Spinach and rocket exhibited similar chlorophyll and hue angle degradation rate while lettuce followed different trend which agrees with the respiration rate ranking of the three vegetables. This trend was accompanied from similar E_a for rocket and spinach and significant different for lettuce.

Statistical analysis of Chl_t as response and the h_{ab} and vegetable type as main factors, resulted to three linear equations of the same slope. The apposition of the three linear plots follows the inverse ranking of the E_a calculated from the nonlinear regression of chlorophyll degradation. Also, the apposition of the

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three linear plots agrees with the RR ranking of the three vegetables and the associated senescence process as this is induced by the high respiration rates.

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Table 1. ANOVA of the factors affecting the Chl_t during the storage of the tested vegetables.

Source	*df*	F-ratio[a]	P-value
Main effects			
A: Storage temperature	3	73.40	≤0.001*
B: Storage time	6	79.87	≤0.001*
C: Repetition	1	0.10	0.75
D: Vegetable type	2	46.56	≤0.001*
Residual	1043		
Total	1055		

[a]All F-ratios are based on the residual mean square error.
*Significant at P≤0.05

Table 2a. Estimated parameters of the $\ln(Chl_t/Chl_o)$ (cf. Eq. 5b).

Produce	k_r	E_a (J mol^{-1})	T_r (K)	n	R^2_{adj} (%)	SEE	RMSE (%)
Lettuce	0.004	57,954	281.15	1.89	90.02	0.09	5.9
Rocket	0.062	47,581	281.15	0.82	98.12	0.03	2.8
Spinach	0.052	48,544	281.15	0.95	98.11	0.03	2.1

Table 2b. Estimated parameters of the Chl_t/Chl_o (cf. Eq. 5a).

Produce	k_r	E_a (J mol^{-1})	T_r (K)	n	R^2_{adj} (%)	SEE	RMSE (%)
Lettuce	0.006	52,295	281.15	1.64	80.96	0.09	6.1
Rocket	0.047	36,136	281.15	0.92	83.31	0.07	4.8
Spinach	0.089	48,494	281.15	0.60	97.84	0.03	4.3

Table 3a. Estimated parameters of the ln(Hue) (cf. Eq. 5b).

Produce	k_r	E_a (J mol^{-1})	T_r (K)	n	R^2_{adj} (%)	SEE	RMSE (%)
Lettuce	0.002	48,302	281.15	1.52	95.75	0.01	1.0
Rocket	0.018	62,122	281.15	0.67	95.55	0.01	1.5
Spinach	0.021	65,575	281.15	0.65	95.14	0.01	1.1

Table 3b. Estimated parameters of the Hue (cf. Eq. 5a).

Produce	k_r	E_a (J mol^{-1})	T_r (K)	n	R^2_{adj} (%)	SEE	RMSE (%)
Lettuce	0.311	46,477	281.15	1.45	95.51	1.25	1.0
Rocket	1.690	67,102	281.15	0.73	91.65	1.93	1.4
Spinach	2.036	67,166	281.15	0.74	95.63	1.49	1.2

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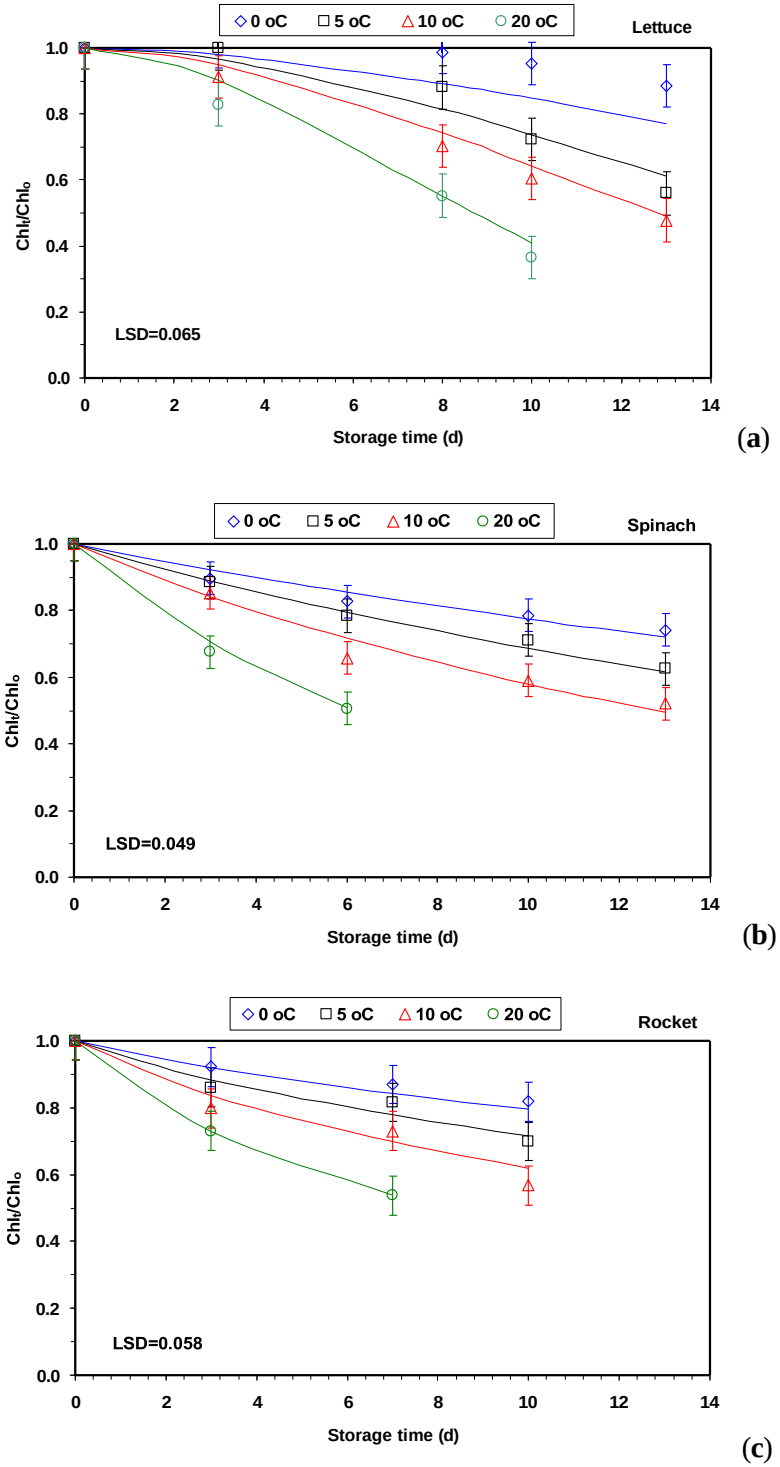

Fig. 1. First-order kinetics of Chl_t degradation during storage of lettuce (**a**), spinach (**b**) and rocket (**c**) at 0, 5, 10 and 20 ^{o}C. Experimental (points) and predicted (lines) data are shown. Data points are the means of 12 replicates±LSD. Error bars are the LSD intervals (P≤0.05).

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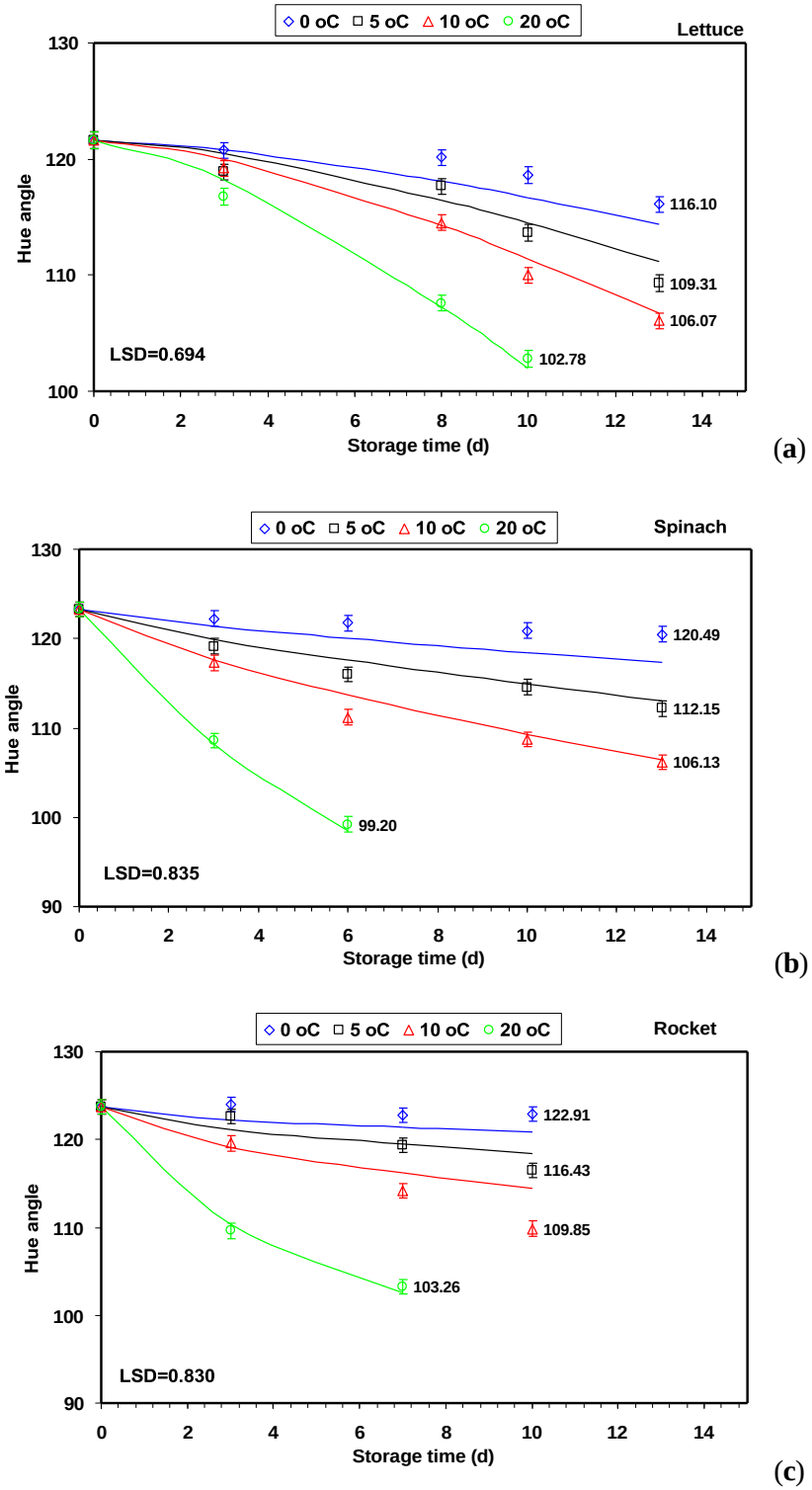

Fig. 2. First-order kinetics of h_{ab} degradation during storage of lettuce (a), spinach (b) and rocket (c) at 0, 5, 10 and 20 ^{o}C. Experimental (points) and predicted (lines) data are shown. Data points are the means of 48 replicates±LSD for lettuce and 60 replicates±LSD for spinach and rocket. Error bars are the LSD intervals (P≤0.05).

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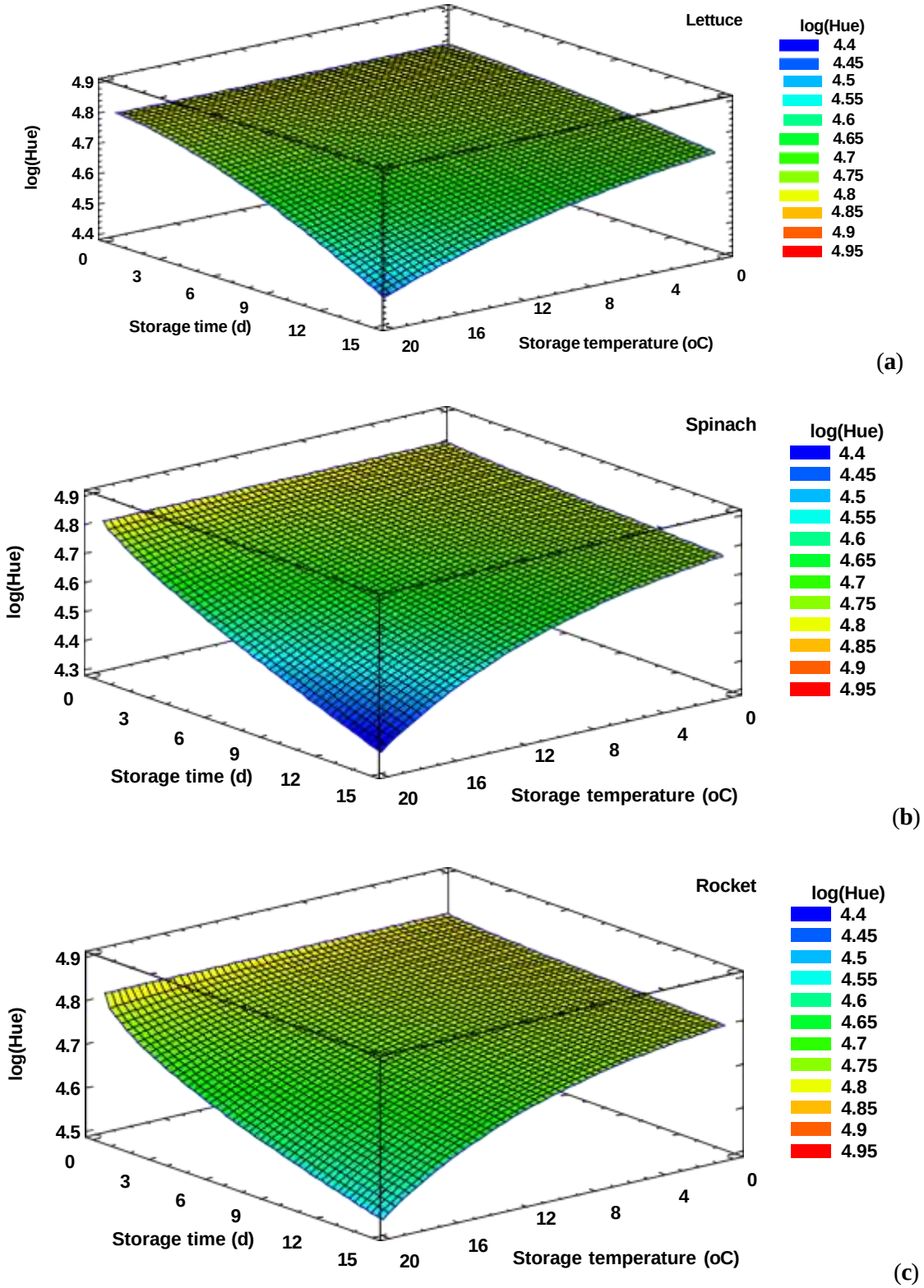

Fig. 3. Response surface of the estimated h_{ab} (cf. Eq. 5b) during storage of lettuce (a), spinach (b) and rocket (c) at 0, 5, 10 and 20 ^{o}C.

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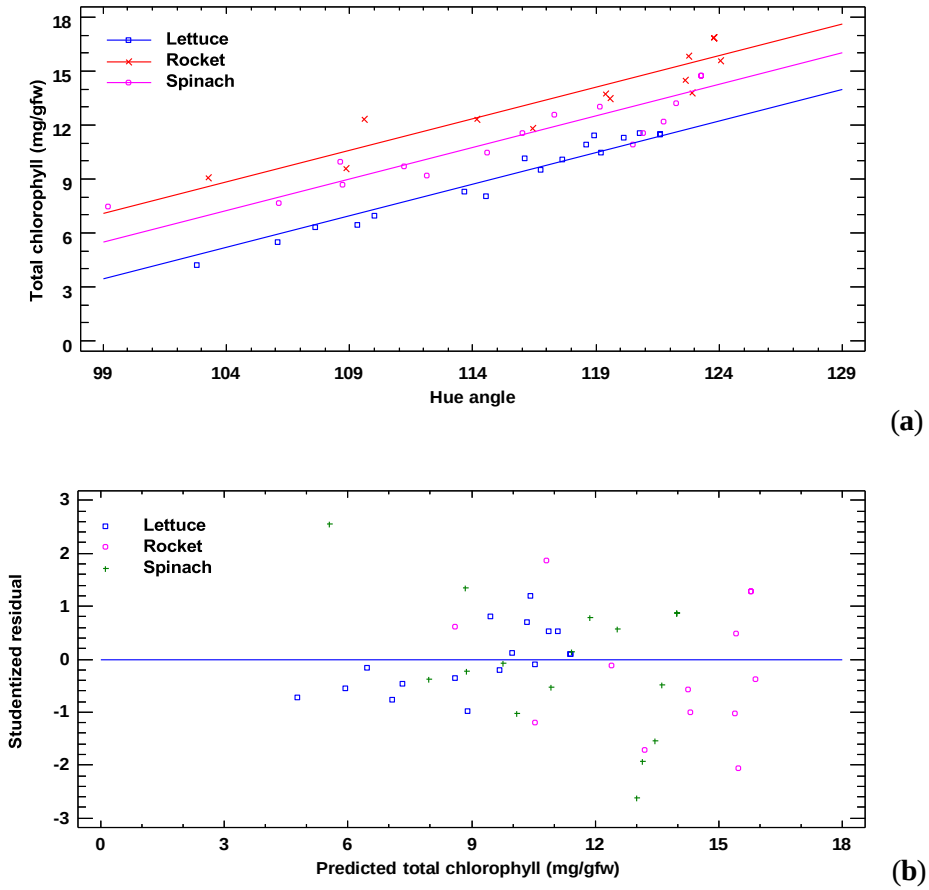

Fig. 4. (a) Hue angle versus total chlorophyll of lettuce, rocket and spinach stored at 0, 5, 10 and 20 ^{o}C; (b) Residual error plot of the predicted Chl_t.