Heat Degradation Kinetics of the Chlorophyll from Spinach and its Correlation with the Reflection Spectra

ELENA CORINA POPESCU*, CLAUDIA LAVINIA BURULEANU
Valahia University of Targoviste, Faculty of Environmental Engineering and Food Sciences, Food Engineering Department, 13 Sinaia Alley, Targoviste, Romania

Chlorophyll, the pigment responsible for the green color specific to the spinach leaves, is sensitive to temperature, pH, activity of enzymes. The heat degradation kinetics of the chlorophyll a and chlorophyll b from the spinach leaves was studied at different temperatures (60, 70, 80 and 90^{0}C) and different times (3, 9, 12, 15 min). The degradation of the chlorophyll a and chlorophyll b followed a first-order reaction, and the rate constant had values between 0.0199-0.0695 min^{-1} for chlorophyll a and 0042 -0.011 min^{-1} for chlorophyll b respectively. The dependence between the chlorophyll degradation and temperature was modeled using the Arrhenius'a equation. The activation energy was by 44.316 Kjmol^{-1} for chlorophyll a and 35.384 Kjmol^{-1} for chlorophyll b respectively. Using the rapid, non-invasive measuring method of the reflection spectra in red band and in NIR region and the calculation of the index $Cl_{red\ edge}$, the chlorophyll quantity from spinach leaves was measured with a relative error E_{rCle} between 1.27 and 8.65%.

Keywords: chlorophyll, spinach, chemical kinetic, spectral reflectance

Spinach (Spinacia oleracea L.) is a plant belonging to the family Chenopodiaceae, commonly used as human food. Spinach can be eaten fresh, as salad, juice, or as creams, smoothies, soups.

Spinach leaves are well known for their diuretic, laxative, anthelmintic, hepatoprotective effects, being rcognized as useful in the case of the bowel inflammation, sore throat, joints pains, asthma, with a protective effect against the oxidative stress [1,2].

Spinach has high nutritional value and it is extremely rich in antioxidants (chlorophyll, flavonoids, carotenes), vitamins from B group, vitamin C, vitamin E, vitamin K, iron, potassium, calcium, magnesium [3-6].

Spinach leaves contain chlorophyll, responsible for the characteristic green color of vegetables and fruits and â-carotene, as major pigments, as well as small quantities of other pigments (xanthophylls, lutein), [5].

The preponderant chlorophylls are chlorophyll *a* and chlorophyll *b*, in the ratio of 3:1. The difference between the two chlorophylls consists in the fact that chlorophyll *a* has a methyl group -CH_3) in the position in which the chlorophyll *b* has an aldehyde group (-CHO). The chlorophyll *a* is blue-green colored, since the chlorophyll *b* is yellow-green colored [5, 7]. The chlorophyll controls and adjusts the level of the carbohydrates and calcium in the human body, has a beneficial role in case of constipation, detoxification, wounds healing, it helps strengthen the body [8].

Chlorophyll is sensitive to temperature, pH, enzymes, weak acids, oxygen and light.

The pH influence on the degradation of chlorophyll from the blanched peas was studied by Nuray et al, 2005. They demonstrated that the chlorophyll degradation is favored by an acid *p*H [7, 9].

The enzymatic degradation of chlorophyll takes place, especially, under the peroxidase influence, and less under the chlorophyllase one. Chlorophyllase is involved both in the chlorophyll oxidation and biosynthesis reactions [10, 11].

The vegetables blanching inactivates such enzymes, responsible for senescence and loss of the greenish color, but it induces structural and chemical changes at the tissue level, changes which initiate the chlorophyll degradation [7].

Chlorophyll is sensitive to the heat treatment [12]. The loss of the green color during the heat treatment is mainly attributed to the transformation of chlorophyll *a*, of green color, in pheophytins, of brown color, due to the replacement of the magnesium ion in the porphyrin ring of the chlorophyll by two ions of hydrogen, reaction which takes place in acid environment. The pheophytins formation during the spinach heating is initiated by the cellular acids releasing and new acids synthesis [13, 11].

Glowacz et al, 2013 studied the behavior of the chlorophyll *a* and *b* when the spinach is introduced in hot water, at 45^{0}C for 60 seconds and they found out that these parameters do not produce changes in the chlorophyll structure [14].

Wang et al, 2013 replaced the traditional pasteurization with the application of a high hydrostatic pressure (HHP), for minimizing the chlorophyll degradation and loss of color in the spinach puree [15]. Bunea A. et al, 2008 concluded that the chlorophyll stability depends on its location and distribution in the plants tissues, that is why it is important to study the chlorophyll behavior from different plants matrix [9,12].

Food constituents degradation kinetics is a method recommended by many scientists for studying of the food quality change during the technological processes [16,17]. Many researchers studied the degradation of the chlorophyll from vegetables and demonstrated that it follows a first-order kinetic model [7, 13, 16, 18, 19]. However, there are not enough studies regarding the degradation of the chlorophyll from the spinach leaves. Taking into account that the green color is an important sensory characteristic for establishing the quality and acceptability of the vegetsbles by the consumer, it is important to minimize its loss during the heat treatment.

Traditionally, in order to determine the chlorophyll quantity, its extraction from the spinach leaves is required using different organic solvents (acetone, ethyl alcohol, etc) followed by the spectrophotometric analysis. The

* email: corinapopescu2003@yahoo.com; Phone 0722844864

pigments quantity from solutions is calculated using Lichtenthaler's equations [20].

Recently, different alternatives for the pigments analyze have been developed (chlorophyll, carotenoids, anthocyanins) analysis using non-invasive optical methods have been developed. These non-invasive methods are cheap and rapid and consist in the analysis of the leaves reflection spectra.

The measuring of the reflection spectra is widely applied for the noninvasive estimation of the chlorophyll carotenoids, and anthocyanins contents [21- 23].Gitelson et al, studying the reflection spectra of many types of plants concluded that the spectral bands between 450±20nm, 550±20nm, 715±20 nm and the NIR bands over 750 nm respectively have been proved to be correlated with the chlorophyll quantity from leaves.

Lin C. et al have demonstrated that the reflection from the visible infrared region increases as the water quantity from leaves drops [24]. Water scarcity causes the chlorophyll degradation, the Mg^{2+} ion is removed and the chlorophyll turns into phaeophytins. The spectral characteristics of the red edge and of the green apex are directly or indirectly correlated with the chlorophyll content of the leaves.

In the green regionof the spectrum (λ =540-550 nm) adsorb both the anthocyanin pigments and the chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*). In the red region of the spectrum adsorb only the Chl *a* and Chl *b* [25].

The estimated amount of chlorophyll has been calculated using the ratio (index) proposed by Gitelson et. al in 2009 [26]. This index is based on the reflectance (ρ), in few spectral bands (red region and NIR).

The study goals were :

I. Determining the kinetic parameters for chlorophyll *a* and *b* from the spinach leaves blanched at the temperature of 60, 70 , 80 or 90^{0}C, during 3, 9, 12 or 15 min. The chlorophyll extraction from spinach has been done using ethanol.

II. The analysis of the reflection spectrum of the fresh and blanched spinach leaves, in the spectral interval between 400 and 850 nm. The following spectral bands specific to the chlorophyll have been studied: λ=430-448nm, λ green = 540 – 560 nm, λ red = 660 – 680 nm, λ red edge =690 – 710 nm, λ NIR = 760 – 800 nm.

The reflection spectra have been carried out using the UV-Vis-NIR V-600, JASCO. spectrophotometer. This spectrophotometer is equipped with an integrating sphere and support for solid products which make possible the measuring of the spinach leaves reflectance. The spectral resolution is 1 nm.

III. The chlorophyll content estimation using a rapid, non-invasive method, based on the spinach leaf reflection.

Material and methods

The fresh spinach (*Spinacia oleracea L.*), Viroflay variety, grown in greenhouse, has been acquired in Apryl from the local market.

Heat treatment

The washed spinach leaves were blanched in a thermoregulated water bath at the temperature of 60, 70, 80 and 90^{0}C respectively. The blanching time was of 3, 9, 12 and 15 min, for each temperature.

The spinach has been analyzed from the point of view of its chlorophyll content (chlorophyll *a*, *b* and total chlorophyll), using the classical method (extraction with solvents) and also the rapid, non-invasive method, using the reflection spectra.

Chlorophylls extraction

The chlorophyll content was determined in compliance with the following method; 0.5 g spinach leaves chopped were extracted in 25 mL 95% (v/v) ethanol. The plant material covered by ethanol was boiled on a sand bath until the spinach complete discolouring. The extracts were rapidly cooled, in the absence of light and brought to a volume of 25 cm^3 using ethanol 95 %. The pigment extracts were centrifuged for 3–5 min in glass tubes to make the extract fully transparent. The extracted solution was analyzed spectrophotometrically.

Spectrophotometric determination of the chlorophylls from spinach

The extracts adsorption was measured using an UV-Vis-NIR V-600, JASCO spectrophotometer at the wavelength of 665 nm for the chlorophyll *a* and 649 nm for the chlorophyll *b*. The pigments concentration was calculated using the Lichtenthaler's equation, 1987 [20]:

$$Chla=\frac{13,95\times A665-6,88\times A649}{d\times W\times 1000}\times V\times D(mg/g) \quad (1)$$

$$Chlb=\frac{24,96\times A649-7,32\times A665}{d\times W\times 1000}\times V\times D(mg/g) \quad (2)$$

$$Total..Chl=\frac{6,63\times A665+18,08\times A649}{d\times W\times 1000}\times V\times D(mg/g) \quad (3)$$

where:

A - absorbance at a certain wavelength;
V - volum of total extract (mL);
W = fresh weight (g), D - coefficient of dilution;
FW = fresh weight (g);
d = thickness of cuvette (mm);
1000 = conversion factor μg - mg.

Calculation of kinetic parameters

Calculation of kinetic constant (k)

Studies showed that the chlorophyll degradation follows a first-order kinetic model [13, 16, 19].

$$\ln\frac{C}{C_0}=-kt \quad (4)$$

where:

C is the concentration of the chlorophyll *a, b*, at any time t, C_0 is the initial chlorophyll content;
k is the first-order rate constant(min^{-1});
t: time (min).

Calculation of half-life values ($t_{1/2}$)

Half-life value ($t_{1/2}$), the time needed for 50 % degradation for chlorophyll *a* and chlorophyll *b*, was calculated following the equation:

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

where k is the rate constant (min $^{-1}$).

Calculation of activation energy (E_a)

The dependence of the chlorophyll degradation and colorur loss on temperature was determined by the Arrhenius equation:

$$k=k_0\times e^{-Ea/RT} \quad (6)$$

where:

k is the rate constant (min $^{-1}$);
k_0 is the pre-exponential factor, Ea is the activation energy (kJ mol^{-1});
R is the universal gas constant (8.314J mol^{-1} K^{-1});
T is the temperature in K.

Plotting lnK depending on 1/T it is got a straight-line (y=a-bx, where x=1/T, y= lnk, a=lnA, and b= -E_a/R), from its slope which the activation energy can be calculated.

Spectral characteristics of the spinach leaves and their relationship with the total chlorophyll content

The reflection spectra of the fresh and blanched spinach leaves were measured in the spectral interval between 400-840 nm, with a spectral resolution of 1 nm, using an UV-Vis-NIR V-600, JASCO spectrophotometer, equipped with an integration sphere and using Spectralon as standard. For this determination, disks with a diameter of 1.6 cm were cut from the spinach leaves.

The reflection spectra variation was analyzed in the spectral bands: λ=430-448nm, λ green = 540 – 560 nm, λ red = 660 – 680 nm, λ red edge =690 – 710 nm, λ NIR = 760 – 800 nm [21].

A molecular weight of 907 (g·mol^{-1}) for Chlorophyll was recognized.

The total quantity of chlorophyll was estimated using the reflection index proposed by Gitelson et al in 2009 [26].

Chl content was estimated using the red edge chlorophyll index, $Cl_{red\ edge}$:

$$Chl_{red\text{-}edge} = \rho_{NIR} / \rho_{red\text{-}edge} - 1 \quad (7)$$

where:

ρ_λ- reflectance in the spectral band;

λ red edge = 690 – 710 (nm);

λ NIR = 760 – 800 (nm).

The relationship between the chlorophyll reflectance index ($Cl_{red\ edge}$) and the chlorophyll content (in nmol·cm^{-2}) is:

$$Y = 0.0737x + 0.0319 \quad (8)$$

where:

Y is index $Cl_{red\ edge}$,

x is Chlorophyll content (nmol·cm^{-2}).

Relative error calculation

The relative error represents the deviation of the estimated value compared to the measured value. The relative value was calculated using the formula:

$$E_{rCle} = \frac{\Delta Cl}{Cl_e} = \frac{Cl - Cl_e}{Cl_e}(\%) \quad (9)$$

$$E_{rCle} = \frac{\Delta Cl}{Cl_e} \cdot 100(\%) \quad (10)$$

where:

Cl – the measured chlorophyll content (mg/g);

Cl_e - the estimated chlorophyll content (mg/g).

Statistical analyses

Kinetic data were analyzed by regression analysis using MS Excel.

Results and discussions

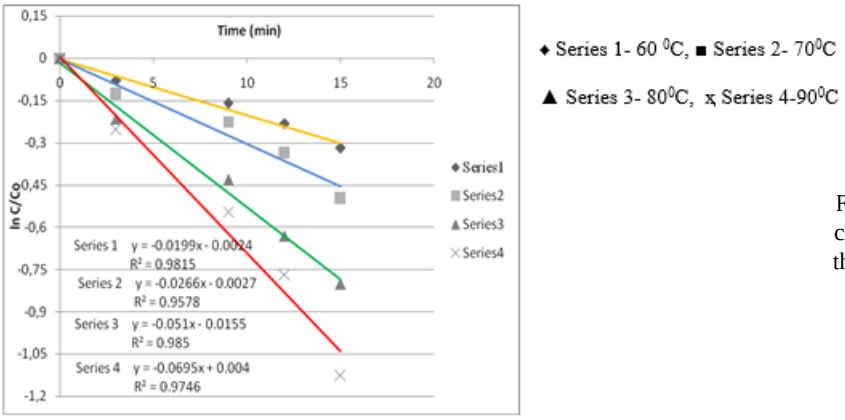
Kinetics of Thermal Degradation of Chlorophyll din spanach

The heat degradation of the chlorophyll *a* and *b* from spinach was studied at the temperatures : 60, 70, 80 and 90^{0}C. The heat treatment duration was of 3, 9, 12 and 15 minutes respectively.

The graphical representation of the ln C/C_0 variation depending on time (min), for chlorophyll *a*, for the four analyzed temperatures is shown in figure 1, and for chlorophyll *b* in figure 2.

Analyzing the regression equations got in the studied temperature interval, it can be observed a linear variation, this way being obvious that the chlorophyll degradation follows a first-order kinetic model.

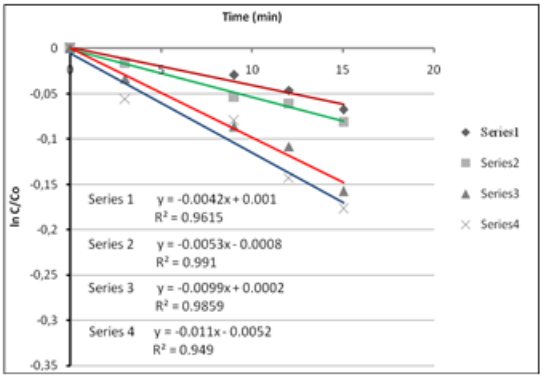
The correlation coefficient R^2 varied between 0.9578 and 0,985 for chlorophyll *a* and 0.949 and 0.991 for chlorophyll *b* respectively. The content of chlorophyll *a* and chlorophyll *b* showed similar trends at different temperatures.



♦ Series 1- 60 ^{0}C, ■ Series 2- 70^{0}C

▲ Series 3- 80^{0}C, x Series 4-90^{0}C

Fig. 1 First order thermal degradation of chlorophyll *a* (ln C/C0) in spinach during thermal process at various temperatures



♦ ♦ Series 1- 60 ^{0}C, ■ Series 2- 70^{0}C

▲ Series 3- 80^{0}C, x Series 4-90^{0}C

Fig. 2. First order thermal degradation of chlorophyll *b* (ln C/C0) in spinach during thermal process at various temperatures

From the slope of the straight-line got, the rate constant (K) of chlorophyll degradation depending on the heat treatment is calculated. The reaction rate constants of the chlorophyll *a* and chlorophyll *b* and the values of the half-life values ($t_{1/2}$), calculated according to the equation 5 are shown in table 1.

The results obtained prove similar degradation trends of the chlorophyll *a* and chlorophyll *b* under identical conditions of temperature and blanching time. The rate constants increase with the temperature, and the chlorophyll *a* is degraded more rapidly than chlorophyll *b*.

The results are in compliance with the reported data [7, 13, 18, 19, 27].

Table 1

RATE CONSTANTA (k) AND HALF-LIFE ($t_{1/2}$) OF CHLOROPHYLL DEGRADATION IN SPINACH

Temp (°C)	Chlorophyll *a*		Chlorophyll *b*	
	K (min^{-1})	$t_{1/2}$ (min)	K (min^{-1})	$t_{1/2}$ (min)
60	0.0199	34.82	0.0042	165.02
70	0.0266	26.05	0.0053	130.77
80	0.0513	13.51	0.0099	70.01
90	0.0695	9.97	0.0110	63

At the temperature of 60°C, it was got a half-life period $t_{1/2}$ of the chlorophyll *a* by 4.74 times lower than the half-life period of the chlorophyll *b*. At the temperature of 70°C, the half-life period of the chlorophyll *a* was 5,01 times lower than the half-life period of the chlorophyll *b*, at the temperature of 80°C 5.18 times lower, and at the temperature of 90°C 6.31 times lower respectively.

Analyzing these data, we can conclude that at the same time with the temperature increasing, the half-life value ratio between the chlorophyll *a* and chlorophyll *b* increases too. Then, the chlorophyll *a* is more sensitive at the temperature increasing compared to the chlorophyll *b*. The got data are in compliance with the results of other researchers who reported a degradation of the chlorophyll *a* from 2 up to 10 times more rapid than that one of the chlorophyll *b* [7, 11, 19].

Activation energy calculation

The variation of the natural logarithm of the rate constant (lnK) depending on the mutual of the absolute temperature (1/T) in K, for chlorophyll *a* and chlorophyll *b* is shown in figure 3.

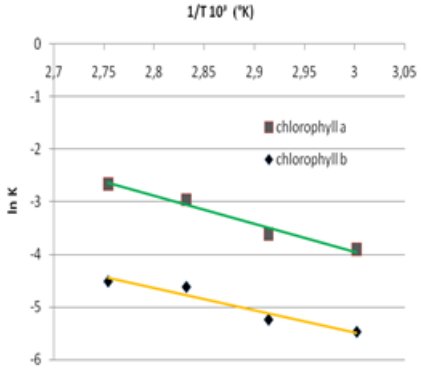


Fig. 3 Arrhenius plots of chlorophyll degradation in spinach

Multiplying the regression line slope by 8,314 the activation energy is got in kJ mol^{-1}. The value of the activation energy for chlorophyll *a* and chlorophyll *b*, as well as the correlation coefficients R^2) are shown in table 2.

Analyzing the results shown in the table 2, it can be observed that the activation energy of the chlorophyll *a* is ,252 times higher than the activation energy of the chlorophyll *b*. A higher activation energy for chlorophyll *a* indicates that a low temperature variation is sufficient for its more rapid degradation compared to chlorophyll *b*. Generally, chlorophyll *a* from the spinach leaves is less thermally stable than chlorophyll *b*.

A broad range of activation energies has been reported : between 47,78 – 105 kJ mol^{-1} for chlorophyll *a* and between 26,77 -94 kJ mol^{-1} for chlorophyll *b* from green peas, spinach, coriander [9, 13, 28]. The values obtained are included in these intervals.

Characterization of the reflection spectra for te fresh and blanched spinach leaves

Figure 4 presents the reflection spectra for the fresh and blanched spinach leaves at the temperature of 60, 70, 80 and 90°C, for 12 min. This thermostatation duration was chosen because at lower values of the heat treatment the degraded chlorophyll quantity is lower, and the reflection spectra have close values. Analyzing the reflection spectra of the spinach leaves we note many features.

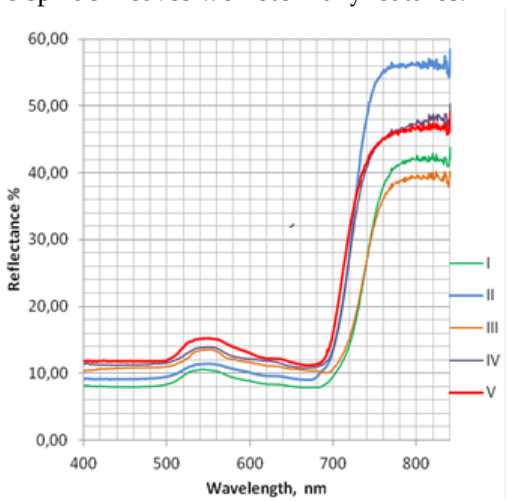


Fig.4 Reflectance spectra of the fresh and blanched spinach leaves at different temperatures . I- fresh spinach leaves, II – spinach leaves blanched at 60°C, III – spinach leaves blanched at 70°C IV- spinach leaves blanched at 80°C V- spinach leaves blanched at 90°C. For all the variants the blanching duration was of 12 min.

The first feature is given by the variation of the spinach leaves reflectance depending on the chlorophyll quantity. The spinach leaves having a lower chlorophyll concentration have a higher reflection degree in the blue band and green band spectra. Similar results got also by Gitelson A. et al, in 2009, Lin C. et al, in 2015 [24, 26].

The reflectance in blue band, λ=430-448nm, presents low values, almost constant for the same type of sample.

A distinctive feature of the reflectance is the presence of a pick in the green band for the wave length between 550-555 nm.The samples reflectance reached minimum values around the wave length of 675 nm, in the yellow band.

Table 2

THE ACTIVATION ENERGY E_a AND THE CORRELATION COEFFICIENT R^2 FOR CHLOROPHYLL *a* AND *b*

	Kinetic equation	E_a (kJ mol^{-1})	R^2
Clorophyll *a*	y= -5.3303x +12.032	44.316	0.9721
Clorophyll *b*	y= - 4.2560 + 7.2811	35.384	0.9334

Another feature is the sudden reflectance of the samples beyond 690 nm, in the so-called red edge range (the so-called red edge range). The reflection increasing in the range 690-760 nm would be caused by an increase of the spinach leaves density, according to the studies of Gitelson A. et al 2003 and Gitelson et al, 2009 [26, 29].

Another important feature which could be noted is the change of the reflection in the visible range compared to that one from the red range, at wave lengths starting with 750 nm. The spinach leaves with a small content of chlorophyll have a higher reflection degree in the visible range, and a lower reflection degree in the infrared range.

The results are in compliance with the remarks made by Lin C. et al, in 2015 [24].

For wave lengths higher than 760 nm, in the so-called region NIR, the reflectance presents variations much lower depending on the wave length.

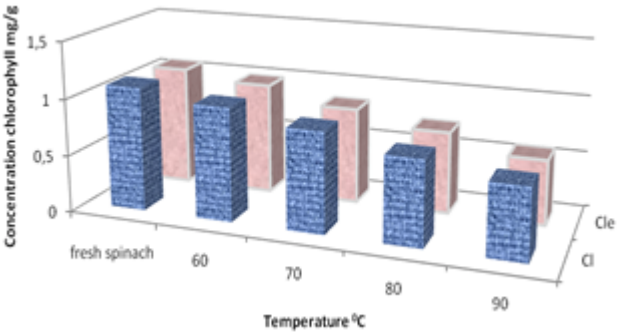
Generally, the reflection increases with the decreasing of the chlorophyll quantity [25].

Estimation of the chlorophyll content from the spinach leaves based on the index proposed by Gitelson et al, 2009

The index $Cl_{red\ edge}$ was calculated using eq. 7 taking into account the reflectance values in red edge and in NIR region.

The total chlorophyll quantity was calculated using equation 3 and the chlorophyll quantity estimated using the regression equation 8 are presented in figure 5.

The following values were got for the relative error : for the fresh spinach E_{rCle} was 2.48%, for the blanched one at



Cl - Chlorophyll content [mg/g]
Cl_e- Chlorophyll content estimation [mg/g]
Fig. 5.The chlorophyll quantity measured and the chlorophyll quantity estimated using the index $Cl_{red\ edge}$

60°C E_{rCle} was 1.27%, at 70°C E_{rCle} value was 4.23°C, at 80°C E_{rCle} was 3.23, and at 90°C E_{rCle} was 8.65.

Conclusions

In this study it was demonstrated that the heat degradation of the spinach chlorophyll follows a first-order kinetic model. The kinetic parameters of the chlorophyll *a* and *b* from the spinach leaves blanched at the temperature of 60, 70, 80, and 90°C respectively, for 3, 9, 12 and 15 minutes were determined.

The rate constants had values between 0.0199 - 0.0695 min^{-1} for the chlorophyll *a* and 0.0042 -0.011 min^{-1} for the chlorophyll *b*.

The activation energy was 44,316 $Kjmol^{-1}$ for the chlorophyll *a* and 35,384 $Kjmol^{-1}$ for the chlorophyll *b*, what indicates a thermal stability lower for the chlorophyll *a* compared to the chlorophyll *b*.

The spinach leaves blanched at different temperatures influenced the spectral reflection in the band between 400-850 nm.The chlorophyll concentration influenced the optical properties of the spinach leaves. The reflectance spectrum presented a negative correlation of the chlorophyll concentration with the reflection in the visible range, while the reflection in infrared is was positively correlated with the chlorophyll from the spinach leaves. These results are in compliance with the results shown by Lin C. et al, 2015 [24].

The reflection index, $Cl_{red\ edge}$, was found to be efficient in the non-destructive estimation of the chlorophyll content in the spinach leaves. The estimation made on the strength of this index registered E_{rCle} values ranging between 1.27 and 8.65%.

This study is a first attempt to correlate the chlorophyll content with the spectral reflection in the visible and NIR range.

References

1.SUBHASH G. P., VIRBHADRAPPA S. R., VASANT O. K., International Journal of Research in Ayurveda & Pharmacy, **1 (1)**, 2010, p 78.
2.WANG Y., CHANG C.F., CHOU J., CHEN H.L., DENG X., HARVEY B. K., CADET J. L., BICKFORD P. C. Exp Neurol, **193,** 2005, p. 7.
3. LESTER G. E., MAKUS D. J., HODGES D. M., JIFON J. L., J AGR FOOD CHEM, 61(29), 2013, p. 7019.
4.LOMNITSKI L., BERGMAN M., NYSKA A., VARDA B.S, GROSSMAN S., NUTR CANCER, **46(2),** 2003, p 222.
5. LISIEWSKA Z., KMIECIK W., GEBCZYNSKI P., SOBCZYÑSKA L., FOOD CHEM, **126 (2)**, 2011, p.460.
6.ASLAM1 M., SULTANA B., ANWAR F., MUNIR H., TURK J AGRIC FOR, **40,** 2016, p. 136.
7.NURAY K., FERYAL K HANDE S. B., FOOD CHEM **100,** 2006, p. 609.
8.ULBRICHT C., BRAMWELL R., CATAPANG M., GIESE N., ET AL., Journal of Dietary Supplements, **11**, 2014, p. 198.
9.RYAN-STONEHAM T., Tong C.H., Journal of Food Science, **65(8),** 2000, p. 1296.
10.YAMAUCHI N., WATAD A. J. J AM SOC HORTIC SCI, **116(1),** 1991, p. 58.
11.MATILE P., HORTENSTEINER S., ANN REV PLANT PHYSIO., **50**, 1999, p. 67.
12.BUNEA A., ANDJELKOVICB M., SOCACIUA C., BOBISA O., NEACSUA M., VERHÉC R., CAMPB J.V., FOOD CHEM, **108(2)**, 2008, p. 649.
13.ERGE H. S., KARADENTZ F., NURAY K., SOYER Y., GIDA **33 (5)**, 2008, p. 225.
14.GLOWACZ M., MOGRENA L. M.,, READE J. P.H., COBB A. H., MONAGHAN J.M., POSTHARVEST BIOL TEC, **81**, 2013, p. 23.
15.RONGRONG W., XU Q.,, YAO J., ZHANG Y., LIAO X., HU X., WU J., INNOV FOOD SCI EMERG, **17**, 2013, p 63.
16. LABUZA, T.P., J CHEM EDUC**, 61**, 1984, p.348.
17. PETROU A., ROULIA M., TAMPOURIS K., CHEM EDUC RES PRACT**,** **3**, No.1, 2002, p. 87.
18. VONGSAWASDI P., NOPHARATANA M., SASAENG K., TANTEK P., WONGPHAISITPISAN S., As. J. Food Ag-Ind., **3(01)**, 2010, p.441.
19. RUDRA G. S., SARKAR B.C., SHIVHARE U.S., FOOD BIOPROCESS TECH, **1**, 2008, p. 91.
20.LICHTENTHALER H.K., Methods Enzymol, **148**, 1987, p. 350.
21.KONG J., DONG Y., XU L., BAI X., BOT STUD, **55:9**. 2014, p. 1.
22.RICHARDSON A.D., DUIGAN S.P., BERLYN G.P., NEW PHYTOL, **153**, 2002,p. 185.
23.XUE L., YANG L., ISPRS J PHOTOGRAMM**, 64,** 2009, p. 97.
24.CASTRO K.L., SANCHEZ-AZOFEIFA G.A., Sensors- Basel, **8(1),** 2008, p 51.
25.GITELSON A.A., CHIVKUNOVA O.B., MERZLYAK M.N., AM J BOT, **96(10),** 2009, p. 1861.
26. NISHAA P., SINGHALA R. S., PANDITB. A.B., J FOOD ENG **67 (4),** 2005, p. 407.
27. SCHWARTZ S. J., VON ELBE J. H., J FOOD SCI, **48(4),** 1983, p. 1303
28. LIN C., POPESCU S., HUANG S., CHANG P., WEN H.L., Biogeosciences, **12**, 2015, p. 49.
29. GITELSON A. A., J. Plant Physiol. **160,** 2003, p. 271.

Manuscript received: 10.01.2017