

# DIFFERENTIAL METHOD TO DETERMINE THERMAL DEGRADATION KINETICS OF CHLOROPHYLL IN VIRGIN OLIVE OIL

AHMET LEVENT INANC

Department of Food Engineering, Engineering and Architecture Faculty,  
Kahramanmaraş Sutcu Imam University, 46100 Kahramanmaraş, Turkey  
email: [linanc@ksu.edu.tr](mailto:linanc@ksu.edu.tr)

## ABSTRACT

Differential method is presented to study thermal degradation kinetics of chlorophyll in virgin olive oil. The oil samples, naturally containing 20.0 mg/kg chlorophyll were stored at 150°, 160°, 170°, 180°, 190° and 200°C until the time at which chlorophyll contents had reduced to the certain amounts. The concentration gradually decreased as heating time increased. A half order equation was found as the best model for the present experimental data. Differential method with graphic and substitution methods was compared for the determination of the rate constant and the half-time. The rate constants and half life at 150°C were determined in the range of 0.20-0.22 and 12.14-13.12 for the thermal process of chlorophyll in virgin olive oil, respectively. The reaction rates increased approximately 1.1 times with increment of every 10°C from temperature of 150°C. Conversely, the half lives decreased 0.9 times for increment of every 10°C. The activation energies were approximately 24 J/kg for differential method, and 22 J/kg for graphic and substitution methods.

- Keywords: chlorophyll, kinetics, thermal degradation, virgin olive oil -

## INTRODUCTION

Chlorophylls are responsible for the green color of all vegetables and fruits. Animal tissues can't synthesis chlorophylls, though animal cells can chemically modify them for assimilation. These compounds should be supplied from food (GIUFFRIDA *et al.*, 2007). Chlorophyll and its various derivatives have been used in traditional medicine and for therapeutic purposes for many years and perhaps have the potential role of these pigments in the prevention of human cancers that has drawn more recent attention (FERRUZZI and BLAKESLEE, 2007).

The color of olive oil is principally related to its perceived quality, and therefore to its acceptability. The economic importance of the appearance of the oils is unquestionable. The color of virgin olive oil is due to the natural pigments chlorophylls, and carotenoids (MINGUEZ MOSQUERA *et al.*, 1994).

Olive oil contains originally the chlorophylls a and b. Chlorophyll a, pheophytin a, is typically found in higher amounts than chlorophyll b. The distribution and content of chlorophyll in olive oil are dependent on a number of factors including species, agroclimactic conditions, pre- and postharvest treatment, and type and degree of food processing (MINGUEZ-MOSQUERA *et al.*, 1990, GANDUL-ROJAS *et al.*, 1996, GIUFFRIDA *et al.*, 2007, CRIADO *et al.*, 2008, CERRETANI *et al.*, 2008, GIUFFRIDA *et al.*, 2011).

The grades of oil extracted from the olive fruit are classified as virgin, lampante, refined and olive pomace oil. Virgin oil is produced by the use of mechanical means only, with no chemical treatment or heat. Virgin oil includes both virgin olive oil (VOO) and extra-virgin olive oil (EVOO) products, depending on quality. Therefore, virgin olive oil should be preferably added as the final seasoning in fresh salads, soups, or more elaborated dishes (CARLA *et al.*, 2013) but olive oil like other vegetable oils is used in several cooking processes such as deep-frying, pan-frying, roasting, microwave cooking, etc. (WATERMAN and LOCKWOOD, 2007; BOSKOU, 2009). Each thermal processing type has particular characteristics as depending on process temperature and time. CARLA *et al.* (2013) summarized several works related to olive oil that used as the cooking base, grouped the works by real and simulated cooking method and showed the analytical parameters chosen by the authors to evaluate olive oil performance. For example, in frying process the both methods were tested with several olive oil commercial grades, at temperatures ranging from 170°C to 180°C in real frying, and from 160°C to 190°C in simulated frying, i.e. being the olive oil heated without any food. Some authors also compared the effects of adding fresh oil between frying sessions in the oil performance. In the previous studies it was made on thermal stability of olive oil. The studies on thermal stability of olive

include the thermal decomposition of commercial vegetable oils of some of their thermal properties (DWECK *et al.*, 2004), the thermal degradation study of four unsaturated or saturated esterified C18 fatty acids with glycerol (VECCHIO *et al.*, 2008), stability of olive oil during heating (BERSATEGI *et al.*, 2012), the heat-oxidation stability of binary blends made with palm oil and several extra virgin olive oils (DE LEONARDIS and MACCIOLA, 2012) and effects of the main virgin olive oil antioxidants under mild temperature conditions (MANCIBO-CAMPOS *et al.*, 2014) but, virgin olive oil contains minor constituents together with triglycerides, the thermal effect on chlorophyll stability and degradation in olive oil has not been studied extensively.

Kinetic modelling recently gaining increasing interest in food science gives the possibility of controlling changes in foods such as to control food quality during processing and shelf life (NIAMNUY *et al.*, 2012; GOULA, 2013; GRAUWET *et al.*, 2014; REMINI *et al.*, 2015). Microbiological changes which are called as predictive microbiology have been worked up to recent years but it can also be applied for biological, chemical and physical changes. The rate of a reaction and its temperature dependence, the occurrence of such a reaction can be predicted and controlled under specified conditions. The difficulties in kinetic modelling are choosing the right model for a reaction. For example; one of the difficulties is that too few data points are available to decide for the correct order. In general, researchers in food science have limited themselves often to simple reaction kinetics. i.e. it is trying to fit a zero-, first- or second order model to their data (VAN BOEKEL, 1996; VAN BOEKEL, 1999).

The present study focused on the determination of rate order and characterization of the Arrhenius parameters governing the thermal degradation reactions of chlorophyll in virgin olive oil by using the differential method and was to compare it with other two rate order determination methods.

## 2. MATERIAL AND METHODS

### 2.1. Materials

Olive oil from olive fruits harvested in 2012 - 2013 season were obtained from a local olive oil plant (Demirkol Ltd., Kahramanmaras, Turkey). Working principle of the plant is that olives are stored in the hopper of olive elevator and transported to washing machine. First leaves of olives are removed by leaf remover. Then olives are washed without giving harms to its pulp in the olive washing unit. Olives are transported to crusher by crusher elevator. Olives are crushed and become semi paste in crusher. Semi paste olives are mixed to obtain oil in malaxers. Crushed olive is fed into the decanter without water through a

pulp pump. Input product comes out of decanter as oil and pomace with black water. The characteristic of the olive oil are as follows: free acidity, 0.49 % oleic acid; peroxide value, 5.22 meq O<sub>2</sub>/kg; K<sub>232</sub> and K<sub>270</sub> extinction coefficients, 1.89 and 0.15; respectively, according to the analytical methods described in European Regulation EEC 2568/91 (EEC, 1991) and chlorophyll content, 20.0 mg/kg (POKORNY *et al.*, 1998). The oil samples (25 ml each) were transferred into 50 mL glass bottles. The bottles were sealed with teflon-coated rubber seals and aluminum caps and stored at 150°, 160°, 170°, 180°, 190° and 200°C under dark condition in a forced air oven. Chlorophyll content was measured with 2-h intervals from initial time until the time at which chlorophyll contents had reduced to 1 mg/kg All samples were prepared in duplicate.

## 2.2. Determination of chlorophyll content in olive oil

The chlorophyll content of olive oil was analyzed using the method described by POKORNY *et al.* (1998). The sample was measured at 630 nm, 670 nm and 710 nm in a 10 mm spectrophotometer cell against air, instead of a reference cell. The method is suitable for the determination of quantities of chlorophyll pigments higher than 1 mg/kg. The following equation was used for determining the chlorophyll content

$$[C] = 345.3(A_{670} - 0.5A_{630} - 0.5A_{710})/L$$

Where: [C] = content of chlorophyll pigments in mg of *pheophytin a* in 1 kg of oil,  
A = absorbance at the respective wavelength (nm),  
L = thickness of the spectrophotometer cell (mm).

## 2.3. Kinetic theory

Differential method was used for determination of the degradation rate order and the rate constant of chlorophylls in olive oil.

It was expressed the concentration at any temperature as a function of time in a power series, with constants a, b, c by deriving from the experimental concentration-time data

$$[C] = at^2 + bt + c$$

Where concentration ([C]) and time (t) were expressed in mg/kg and in hour.

Rate of reaction in *mg/kg/h* (*v*) was estimated from the following equation;

$$v = - \frac{d[C]}{dt}$$

The most simple general rate equation was used for a single reactant at concentration [C]:

$$v = k_n [C]^n$$

Where *n* = rate order, *k<sub>n</sub>* = rate constant at order *n*.

By taking the logarithm of the above equation to base e it follows that:

$$\ln v = n \ln [C] + \ln k_n$$

Rate order and rate constants at different temperatures were determined by plotting graph

$$\ln v \text{ versus } \ln [C]$$

The half-life value (*t*<sub>1/2</sub>) of chlorophyll degradation was calculated using the equation given below after founding rate order and rate constants:

$$t_{1/2} = 0.597 [C_0]^{1/2} / k_{1/2}; [C_0] = \text{initial concentration of chlorophyll}$$

*Lnk*<sub>1/2</sub> was plotted versus 1/T to determine Arrhenius parameters (*A* and *E<sub>a</sub>*) by taking the logarithm of Arrhenius equation; *k*<sub>1/2</sub> = *A*exp(-*E<sub>a</sub>*/RT) to base e; where *E<sub>a</sub>* is the activation energy (J/kg), *A* is the pre-exponential factor or Arrhenius constant, *R*\* is the specific gas constant for pheophytin a (9.543 J/kg K), and *T* is the absolute temperature (K).

Differential method using for determination of the rate constant was compared with substitution and graphic methods.

In substitution method the *k* value at a temperature was calculated by substituting initial concentration, concentration at any time and time values into the following half order rate equation:

$$k_{1/2} = 2/t \times ([C_0]^{1/2} - [C]^{1/2}) \text{ for } n = 1/2$$

In graphic method the above equation was rearranged as  $[C]^{1/2} = [C_0]^{1/2} - (k_{1/2}/2) \times t$  and  $[C]^{1/2}$  was plotted versus *t* to determine *k*<sub>1/2</sub> value (the plot not shown).

## 3. RESULTS AND DISCUSSION

Virgin olive oil is a food matrix contains triglyceride having a high percentage of monounsaturated fatty acids and also other minor constituents such as the phenols, chlorophyll and carotenoids fundamental in contributing to specific characteristics of virgin olive oil. Therefore the kinetic study and characterization of the Arrhenius parameters related with the thermal degradation reactions of chlorophyll in VOO were performed in an oil matrix system to establish mathematical models enabling the prediction of the degradation of this pigment during VOO thermal processing.

Changes with respect to the time in chlorophyll concentration in oil matrix during thermal

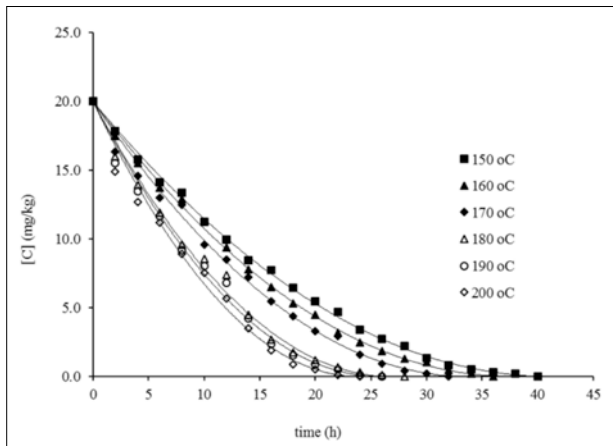


Fig 1 - Changes with respect to the time in chlorophyll concentration in oil matrix during thermal processing.

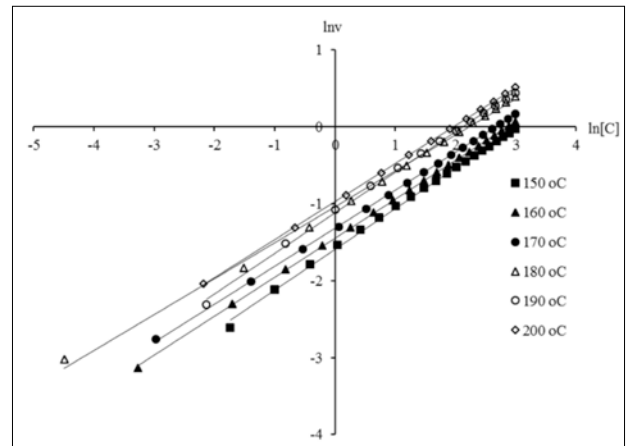


Fig 2 - ln v - ln[C] plot to estimate the rate order and rate constants at different temperatures.

Table 1 - Best fit equations for the concentration-time data.

T (oC)	[C]=at <sup>2</sup> -bt+c			R <sup>2</sup>
	a	b	c	
150	0.012	0.970	20.0	0.999
160	0.014	1.066		0.998
170	0.018	1.183		0.993
180	0.028	1.489		0.993
190	0.030	1.558		0.991
200	0.035	1.678		0.987

[C]: Chlorophyll concentration; t: time; a, b and c: function coefficients.

Table 2 - Best fit equations for lnk - ln[C] data.

T (oC)	lnv=n*ln[C]+lnk <sub>n</sub>		R <sup>2</sup>
	n	lnk <sub>n</sub>	
150	0.53	-1.59	0.998
160	0.51	-1.45	0.999
170	0.49	-1.32	0.999
180	0.47	-1.04	0.997
190	0.53	-1.11	0.998
200	0.50	-0.97	0.999

[C]: Chlorophyll concentration; v: reaction rate; k: reaction constant; n: reaction order.

processing, expressed in mg/kg, were shown in Fig. 1. The chlorophyll concentrations gradually decreased while heating times increased. The experimental data was transferred to Sigmaplot (version 12.0) program and trial and error method was applied to find the best fit curve equation on the data. The chlorophyll concentration at any temperature was expressed as a function of time. The best fit mathematical equations for the changes in the experimental data with the reaction time were selected to verify the rates of reaction at any temperature. The equations and their constants are shown in Table 1. The initial concentration of chlorophyll was arbitrarily set at 20.0 units. The reaction mechanism for chlorophyll degradation kinetics was assumed as a simple reaction type;

Pheophytin a → colorless products

where  $k_n$  = rate constant for n order

The rates of reaction were obtained by taking derivatives of the concentrations with respect to time. So ln v versus ln[C] was plotted to estimate the rate order and rate constants at different temperatures (Fig. 2).

Table 2 shows the best fit equations for lnk - ln[C] data. After estimating rate order as half order reaction it was calculated coefficients of the best equations for it (Table 3). An assump-

tion had been made for order of reaction of thermal chlorophyll degradation in a lot of previous studies on the processes of different food matrices such as fermentation of pickles coleslaw and olives (MINGUEZ-MOSQUERA *et al.*, 1992, MINGUEZ-MOSQUERA *et al.*, 1994; HEATON *et al.*, 1996) or thermal processing of spinach (CANJURA *et al.*, 1991; YONGXI *et al.*, 2000) and also such as the visual green color a degradation (STEET and TONG, 1996; WEEMEAS *et al.*, 1999; AHMED *et al.*, 2002; THRON *et al.*, 2001; AHMED *et al.*, 2004; APARICIO-RUIZ *et al.*, 2011, AHMED *et al.*, 2013; MERCALI *et al.*, 2014; DONG *et al.*, 2014), and kinetics studies had been gone on assuming an order of 1. But VAN BOEKEL (2009) reported that the best model for the decomposition of chlorophyll is not only first-order equa-

Table 3 - Best fit equations for half-order rate.

T (oC)	[C] <sup>1/2</sup> =[C <sub>0</sub> ] <sup>1/2</sup> - k <sub>1/2</sub> /2* <sup>1/2</sup> t		R <sup>2</sup>
	k <sub>1/2</sub>	[C <sub>0</sub> ] <sup>1/2</sup>	
150	0.21	4.47	0.998
160	0.24		
170	0.26		
180	0.33		
190	0.34		
200	0.38		

tion, but also could be half-order equation; for example, applying nonlinear regression to the data of SCHWARTZ and VON ELBE (1983), the best order  $n$  is  $0.5 \pm 0.5$  for chlorophyll a and  $0.6 \pm 0.4$  for chlorophyll b ( $\pm 95\%$  confidence interval). Thus, the present data similar to the data of VAN BOEKEL (2009). It was compared the differential method with the other two methods; substitution and graphic method. The results obtained from the three different methods are shown in Table 4. It was found that the rate constants and half-lives at each temperature determined by three methods were close together. The reaction rates increased approximately 1.1 times with increment of every  $10^\circ\text{C}$  from temperature of  $150^\circ\text{C}$ . But, in general the reaction rate doubles for each  $10^\circ\text{C}$  increase in temperature (APARICIO-RUIZ *et al.*, 2010). However, CLARK (2009) reports that this approximation (about the rate of a reaction doubling for a 10 degree rise in temperature) only works for reactions with activation energies of about 50 kJ/mol fairly close to room temperature, and the rate constant goes on increasing as the temperature rise up, but the rate of increase falls off quite rapidly at higher temperatures. The half-life of a reaction is defined as the time at which the concentration of component A is at half its initial value. It provides a highly detailed description of how fast a reaction is occurring. In the present work, the half-life decreased 0.9 times for each  $10^\circ\text{C}$  increase in temperature.

The activation parameters were determined for the thermal process of chlorophyll in virgin olive oil in the range between  $150^\circ\text{C}$  and

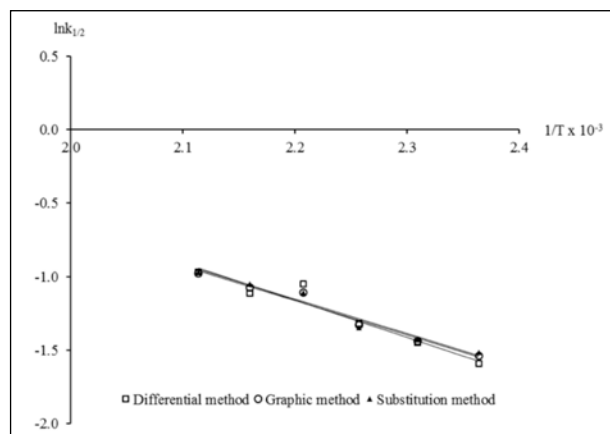


Fig 3 -  $\ln k_{1/2}$  versus  $1/T$  plot for the thermal process of chlorophyll in virgin olive oil.

$200^\circ\text{C}$ . The resulting logarithmic plot is shown in Fig. 3. The estimated values used in the Arrhenius Equation for chlorophyll degradation reaction during heating by using three methods is shown in Table 5. The  $E_a$  determined by graphic method ( $22.43 \text{ J/kg}$ ) was the same value found in substitution method whilst the value in differential method was  $24.05 \text{ J/kg}$ . Average activation energies for chlorophyll with respect to first order reaction were reported to be in range of 14.8 and  $15.3 \text{ kcal/mol}$  in the different temperatures and pH range (RYAN-STONEHAM and TONG, 2000; KOCA *et al.*, 2006). If a compound has low activation energies it is highly sensitive to temperature (JAISWAL *et al.*, 2012)

#### 4. CONCLUSIONS

Thermal processing played an important role for degradation of chlorophyll in virgin olive oil during heating in high temperature range. The kinetics of degradations of chlorophyll in oily food matrices was studied by using differential method. The rate order of chlorophyll degradation reaction was determined as half order reaction that are not yet reported in literature from the experimental results. The degradation reaction of chlorophyll in many of previous studies have been fitted first-order kinetic model by assumption. Use of half-order reaction model for chlorophyll degradation should be encouraged by further studies.

Table 4 - Comparison of methods used for determination of the rate constant and the half-life.

T (oC)	rate constant (k1/2)			half-life (t1/2)		
	D	G	S	D	G	S
150	0.20	0.21	0.22	13.12	12.44	12.14
160	0.24	0.24	0.24	11.36	11.22	11.12
170	0.27	0.26	0.26	9.96	10.09	10.27
180	0.35	0.33	0.33	7.55	8.12	8.09
190	0.33	0.34	0.35	8.12	7.79	7.63
200	0.38	0.38	0.38	7.04	7.11	7.03

D: differential method. G: graphic method. S: substitution method.

Table 5 - Arrhenius constant, and activation energy for chlorophyll.

Method	k=Aexp(-Ea/R*T)			Best fit equations for lnk1/2 vs 1/T data	
	A	Ea (J/kg)	R*(J/kg.K)	Equation	R <sup>2</sup>
D	79.84	24.05	9.543	$\ln k_{1/2} = -2.52 \times 10^3(1/T) + 4.38$	0.93
G	55.70	22.43		$\ln k_{1/2} = -2.35 \times 10^3(1/T) + 4.02$	0.97
S	55.15	22.43		$\ln k_{1/2} = -2.35 \times 10^3(1/T) + 4.01$	0.97

D: differential method. G: graphic method. S: substitution method.

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