Kinetics of Chlorophyll Pigment Leaching From Iraqi Alfalfa

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Abstract

An experimental method of batch leaching was studied to obtained chlorophyll pigment from dehydrated alfalfa using ethanol – hexane mixture as solvent.

The aim of this research is to study the extraction kinetics and the effects of temperature $(30 - 60)°C$, solvent concentration of ethanol (40% to 60%) and particle size $(0.2 - 0.5, 0.5 - 1.0$ and $1.0 - 2.0)mm$ as well as the effect of gathering, drying and transport of alfalfa on the extractability of total chlorophyll from dehydrated Iraqi alfalfa. The maximum extraction value of chlorophyll obtained was found to be 0.31% of alfalfa. The major part of the extractable chlorophyll is water soluble type, which could be used as pigment in toothpastes, soaps, shampoos and medical products.

A batch leaching model was developed and solved numerically. The model parameters were predicted by applying existing experimental correlations and data gathered during this work. The model allows us to predict the rate constant and the saturation capacity with various temperatures. In addition, the activation energy and the frequency factor of the leaching process were also determined and found that the process is an endothermic process with activation energy equal to 30.7 kJ/mol.

The mathematical model applied showed a good coincidence with the experimental results, which allows their application in modeling and optimization of chlorophyll extraction process from Iraqi alfalfa.

Key words: Alfalfa, Chlorophyll, Leaching, Kinetics, Modeling.

حركية استخلاص صبغة الكلوروفيل من نبات الجت العراقي

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الخلاصة:

لقد تم استخدام طريقة مختبريه للاستخلاص الدفعي في دراسة استخلاص صبغة الكلوروفيل من نبات ألجت المجفف بواسطة استخدام مزيج الايثانول و الهكسان كمذيب.

كان هدف البحث هو دراسة حركية عملية الاستخلاص وتأثير درجة الحرارة (30 – 60) °م و تركيز المذيب من الايثانول في الهكسان (40 % – 60 %) و حجم الجسيمات (0,2 – 0,5 و 0,5 – 1,0 و 1,0 – 2,0) ملم بالإضافة الى تأثير تجميع وتجفيف ونقل الجت على استخلاص الكلوروفيل من نبات ألجت العراقي الجاف. كمية الاستخلاص القصوى من الكلوروفيل قد تم ايجادها مساوية لـ 0,31 % من الجت وان الجزء الاكبر منها هو من نوع الكلوروفيل الذائب في الماء الذي من الممكن استخدامه كصبغة طبيعية لمعاجين الاسنان، الصوابين، الشامبوات والمنتجات الطبية .

تم استخدام و حل موديل لعملية الاستخلاص الدفعي بواسطة طريقة رقمية. معاملات الموديل تم حسابها بواسطة استخدام العلاقات العملية والبيانات التي تم استحصالها من العمل الحالي. الموديل يسمح باحتساب ثابت السرعة و سعة التشبع عند درجات حرارة مختلفة. بالإضافة الى ذلك فقد تم حساب معامل التردد و طاقة التنشيط لعملية الاستخلاص وقد وجد ان العملية هي عملية ماصة للحرارة ولها طاقة تنشيط مساوية لـ 30,7 كج/مول.

الموديل الرياضي المستخدم اثبت تطابق جيد مع القيم المختبرية و بذلك فمن الممكن استخدامه في عمليات التمثيل الرياضي و إيجاد الظروف المثلى لعملية استخلاص الكلوروفيل من نبات ألجت العراقي.

الكلمات المفتاحية: نبات الجت، كلوروفيل، استخلاص، حركية، نموذجة .

Nomenclature

Symbol	Meaning	Unit
a	Empirical constant	-
A	Absorbance at subscripted wave length	-
C	Concentration of chlorophyll	g. l^{-1}
C_o	Initial concentration of chlorophyll	g. l^{-1}
C_s	Concentration of chlorophyll at saturation	g. l^{-1}
e	Square root of the mean square relative error	-
E	Activation energy of leaching	J. mol^{-1}
k	Dissolution mass transfer coefficient	s^{-1}
k_o	Arrhenius equation temperature independent constant	s^{-1}
L	Thickness of spectrophotometer cell	m
N	Number of experimental data	-
R	Universal ideal gas constant (8.314)	J. mol^{-1}. K^{-1}
S	Solid phase concentration of chlorophyll	g. g^{-1}
S_o	Initial solid phase concentration of chlorophyll	g. g^{-1}
t	Time of extraction	s
T	Absolute temperature	K
V_s	Volume of solvent	l
W	Weight of dehydrated alfalfa	g
x	Mass percent of chlorophyll extracted	-
ε	Void fraction of milled alfalfa	-
ρ_b	Bulk density of milled alfalfa	g. l^{-1}
ρ_s	Density of solvent	g. l^{-1}

Introduction

The term chlorophyll refers to the green colored material found in many plants. Chlorophyll usually formed in the chloroplasts of plant's cells under the influence of sun light and necessary for the process of photosynthesis [1]. Chlorophyll concentration in green plants is different with respect to plant type and harvest time [2].

Alfalfa grows widely in the tropical area of Asia and it is a native Iraqi plant which has a colorful properties. This plant has been extensively used as a popular natural source of chlorophyll pigment for detergents [3].

Chlorophyll is a mixture of green pigments, which is always combined by other pigments, principally those of the carotenoid group [4]. The most important components of chlorophyll are: chlorophyll a ($C_{55}H_{72}O_5N_4Mg$) and chlorophyll b ($C_{55}H_{70}O_6N_4Mg$). Ratio of chlorophyll a to chlorophyll b in most plants usually in the range 2.5: 1.0 [5].

Chlorophyll may be classified traditionally into two types. The first type is soluble in oil which is used as pigment in lubricating oils, paints, waxes, foods, cosmetics, ointments, pharmaceuticals and as antiknock in gasoline. The second type is soluble in water which is used as pigment in toothpastes, soaps, shampoos, candies, mouth washes, deodorants and medical products [1].

The liquid solvent extraction is the most common method for separating chlorophyll components from their natural resources. Several solvents are usually used in chlorophyll extraction process; the first one has the ability to extract water soluble chlorophyll such as pyridine, methanol, ethanol and acetone. The other solvent has the ability to extract oil soluble chlorophyll such as diethyl ether, dioxane and hexane.

Literature data on the extraction of chlorophyll from alfalfa are scare. Therefore, it is important to study this process experimentally and mathematically. Schertz used a mixture of petroleum ether – acetone to extract chlorophyll from stinging nettle [6]. Iriyama et al. used methanol – dioxane mixture as a solvent to extract chlorophyll from spinach [7]. Many attempts have been made to formulate a general expression describing the kinetics of leaching systems. The rate of leaching is the most important parameter when batch leaching systems have to be designed. Consequently, it is of prime importance to establish the time dependence of such systems under various process conditions. Numerous leaching processes have been studied in an attempt to find a suitable explanation for the mechanism and kinetics of the leaching processes [8, 9].

In the investigation reported herein, dehydrated alfalfa was used because it is available at all seasons of the year and has a cheep price. Ethanol – hexane mixture was used as solvent in the chlorophyll extraction experiments due to local availability, moderate cost, low toxicity and environmental safety.

The aim of this work is to identify the influence of the main operating parameters (particle size, solvent concentration and temperature) on the degree of extraction. Further, determination the kinetic function for the extraction process and developing a mathematical model describes the process which could be used in optimization and design purposes.

Experimental Work

A schematic diagram and photographic picture of the experimental apparatus are shown in **Figure (1)** and **Figure (2),** respectively. The extraction was carried out in a batch double – jacketed vessel of a 2 liters volume. The vessel was fitted with an impeller having variable speed mechanical stirrer. The working part of the stirrer is blade – shaped constructed of stainless steel (type, CF – 8M), ensured good mixing of the vessel's content. The temperature of the medium was measured using a thermometer and maintained at a constant level by circulation of thermostatically fluid (water) supplied from thermostatted bath in the double jacketed of the vessel. Samples of the solution could be withdrawn at different times by using a sampling port immersed in the vessel. A high efficiency glass condenser with ethanol at −10 °C working fluid was attached to the extraction vessel to avoid loss of solvent due to evaporation. The low temperature ethanol was supplied from external chiller.
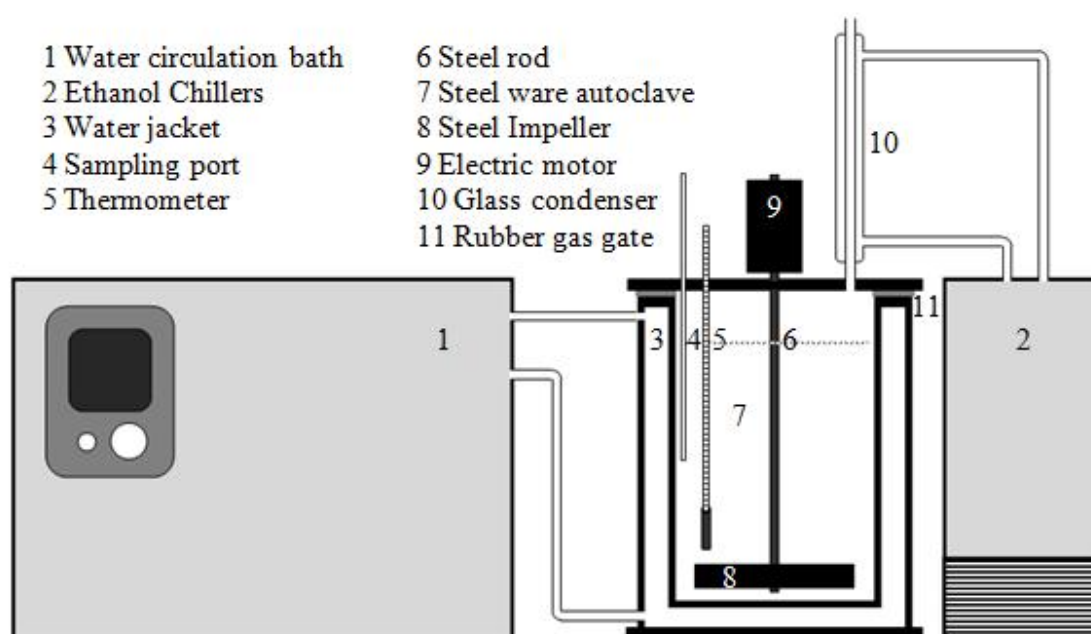

1 Water circulation bath
2 Ethanol Chillers
3 Water jacket
4 Sampling port
5 Thermometer
6 Steel rod
7 Steel ware autoclave
8 Steel Impeller
9 Electric motor
10 Glass condenser
11 Rubber gas gate

Fig .(1), Schematic diagram of the chlorophyll extraction experimental apparatus

Fig .(2) Photographic picture of the chlorophyll extraction experimental apparatus

The fresh alfalfa used in the experiments was collected from plants conventionally grown in Baghdad in June. The first step was to remove foreign materials such as sticks and dirt and then it is heated at 60°C overnight in a dark place. The material was milled in a mechanical grinder (Philips, HP 2860) and was then separated by sieving in a set of sieves (Retsh, AS 200) into three classes (0.2 – 0.5, 0.5 – 1.0 and 1.0 – 2.0) mm.

1200 ml of solvent was initially placed in the vessel and then conditioned to the desired temperature (30, 40, 50 or 60°C) by using high-speed agitation while the conditioned water circulated in the jacked. 50g of the sample was added to ensure large excess of solvent was used. The mixture was stirred at this temperature during the study period. Liquid samples were withdrawn at different times and the chlorophyll concentration in the liquid phase was measured.

There are several methods for the determination of total chlorophyll content was used in literature [2, 5, 6, 7, 10]. Spectrophotometeric method was used with Shimadzu (UV – 1700) spectrophotometer [10]. The weight percent of chlorophyll extracted was calculated by using the following formula derived from Lambert Reevt law:

$$x = \frac{345.3\left[A_{670} - 0.5\left(A_{630} + A_{710}\right)\right]}{L} \times \frac{r_s V_s}{W} \times 100 \qquad .(1)$$

where x is mass percent of extracted chlorophyll, A is spectrophotometer absorbent at subscripted wavelength and L is thickness of spectrophotometer cell.

Apparent, packing and bulk densities for solid were determined according to standard methods (ASTM D2854 – 83).

Mathematical Work

In this work, it was assumed that the main mechanism that controls the rate of chlorophyll extraction is mass transfer of chlorophyll from alfalfa to bulk liquid solvent. Furthermore, the leaching experiments have two distinct phases. The first phase continues until all pores achieve effective solvent saturation. During this period, soluble chlorophyll in alfalfa readily dissolves into pore solvent with partial offset by dilution of incoming solvent. The second phase following immediately the first phase upon achievement of the effective solvent solution in the pores and subsequent onset of net solvent outflow from the pores. So, rate of mass transfer of chlorophyll from alfalfa to bulk liquid could be written as:

For the liquid phase:

$$\frac{dC}{dt} = \left(\frac{S}{S_o}\right)^a k\left(C_s - C\right) \qquad C = C_o \;\; \forall \;\; t = 0 \qquad\qquad .(2)$$

For the solid phase:

$$\frac{dS}{dt} = -\frac{e}{r_b}\left(\frac{S}{S_o}\right)^a k\left(C_s - C\right) \qquad `S = S_o \;\; \forall \;\; t = 0 \qquad\qquad .(3)$$

where C is mass concentration of chlorophyll in the solvent, S mass concentration of chlorophyll in the alfalfa and k is dissolution mass transfer coefficient.

The mathematical model equations were solved using the fourth order Runge – Kutta method for the set of discretized equations for 420 stages (each of 1 min). The parameters, k and a were adjusted using the experimental data. These parameters were estimated by minimization of the sum of squares of errors between the experimental and the predictive data using Nelder – Mead simplex method [11].

The magnitude of the overall discrepancy between the experimental data and the values predicted by the model was calculated as the square root of the mean square relative error according to the following formula:

$$e = \sqrt{\frac{1}{N}\sum\left(\frac{Experimental\ value - Calculated\ value}{Experimental\ value}\right)^2} \qquad\qquad .(4)$$

where e is square root of the mean square relative error and N is the number of the experimental data points.

Results and Discussion

In order to determine the initial amount of extractable chlorophyll in alfalfa, a soxhlet extraction experiment has been made with different contact times (12, 18 and 24) hours at 60 °C (higher temperature is not recommended due to the possibly of chlorophyll degradation) and <0.2 mm particle size. 50% ethanol – 50% hexane was used as solvent [12]. The maximum extraction value of chlorophyll obtained was found to be 0.31% of alfalfa. This later result is the average of five experiments with a precision of 4%.

The effect of gathering, drying and transport of alfalfa can affect their initial composition of chlorophyll. So, two experiments were carried out with alfalfa at different aging (New sample, which is dried, crushed and used on the same day; Old sample, which is dried, crushed and used after 7 days) for each particle sizes (0.2 – 0.5, 0.5 – 1.0 and 1.0 – 2.0) mm. As shown in **Figure 3,** old samples show a degradation of chlorophyll of about 9%, 8% and 6% relative to new one, for each particle sizes (0.2 – 0.5, 0.5 – 1.0 and 1.0 – 2.0) mm, respectively. This degradation is linked to the photolability of the alfalfa. It seems that a chemical action occurs between chlorophyll, the air and the light. It is more important when the contact area is increasing, since smaller particles show a higher degradation than larger particles. Therefore, in order to preserve the chlorophyll under optimal conditions, any contact with air and light has to be avoided. In addition, the alfalfa has to be operated immediately after drying and crushing. Tsao and Deng also indicated that the chlorophyll is relatively labile and during isolation, it is necessary to protect it from degradation [3]. However, as a general precaution, it is advantageous to work in dim light and low temperatures to avoid pigment loss.
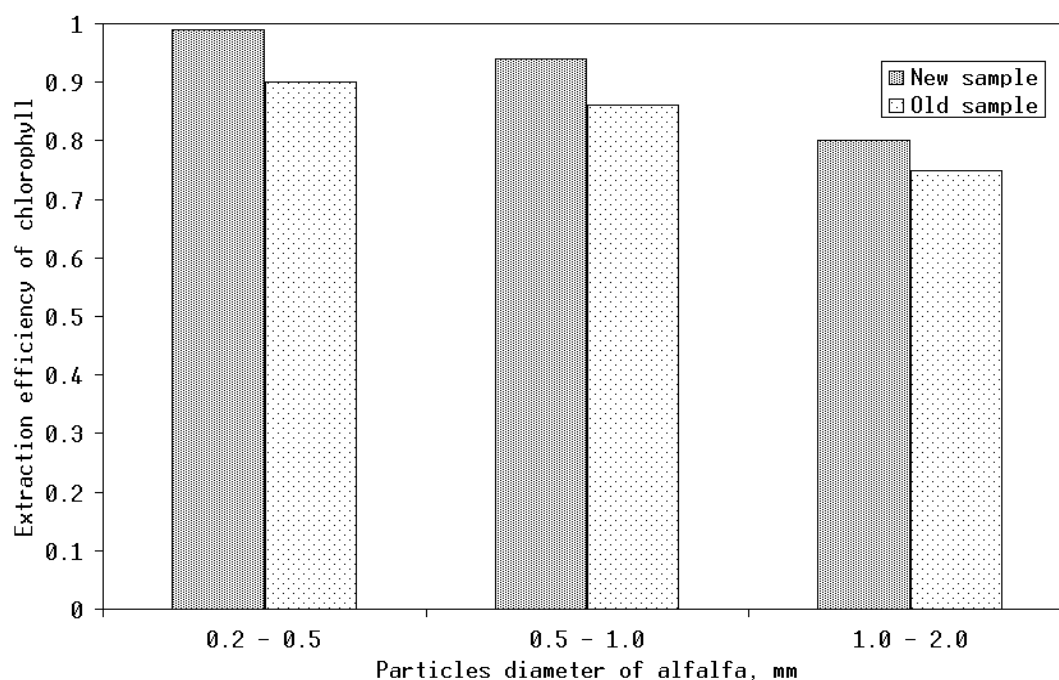

Fig .(3) Effect of alfalfa aging and particle size on the extraction efficiency of chlorophyll (Concentration of ethanol 50%; Temperature 40 °C; Time 420 min)

Figure 4 shows the effect of ethanol concentration as solvent on the concentration of extracted chlorophyll. The final concentration of chlorophyll increased with the percentage of ethanol increasing. Chlorophyll is a polar and either hydrophilic or hydrophobic material depending on it is ability to dissolve in water (water – soluble chlorophyll) or oil (oil – soluble chlorophyll) solvents. Ethanol and water have similar solubility properties because they are both polar and contain hydroxyl group which is hydrophilic, whereas, hexane is non – polar and hydrophobic. Hence, it could be concluded that the major part of chlorophyll found in alfalfa is of the water – soluble that could be used as pigment in toothpastes, soaps, shampoos, candies, mouthwashes, deodorants and medical products.

The data of **Figure 4** could be redrawn to find the efficiency of extraction, which is defined as the percent of chlorophyll extracted to the maximum amount of extractable chlorophyll at the specified concentration of solvent, as shown in **Figure 5**. This representation allows us to study the dynamic of the phenomena independently of the equilibrium conditions. The curves show that the percentage of ethanol has no significant influence on the kinetic of extraction since all curves have similar behavior.
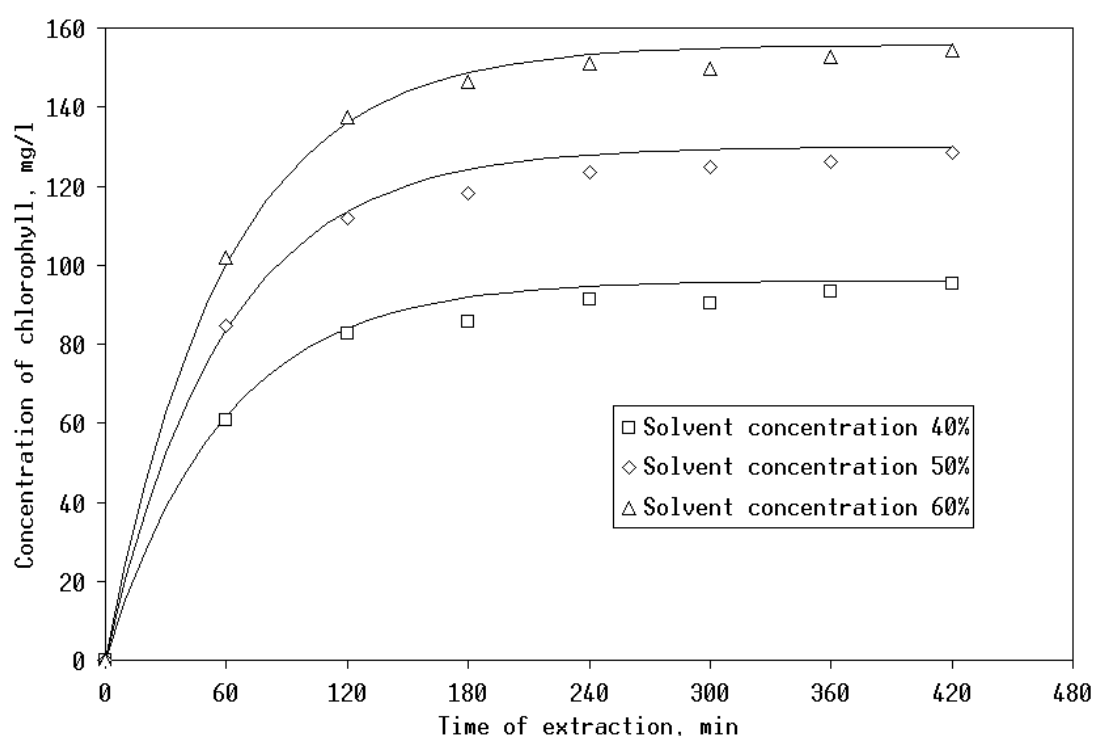

Fig .(4), Effect of solvent concentration and time on the extractable chlorophyll concentration (Temperature 40 °C; particle size 0.2 – 0.5 mm)
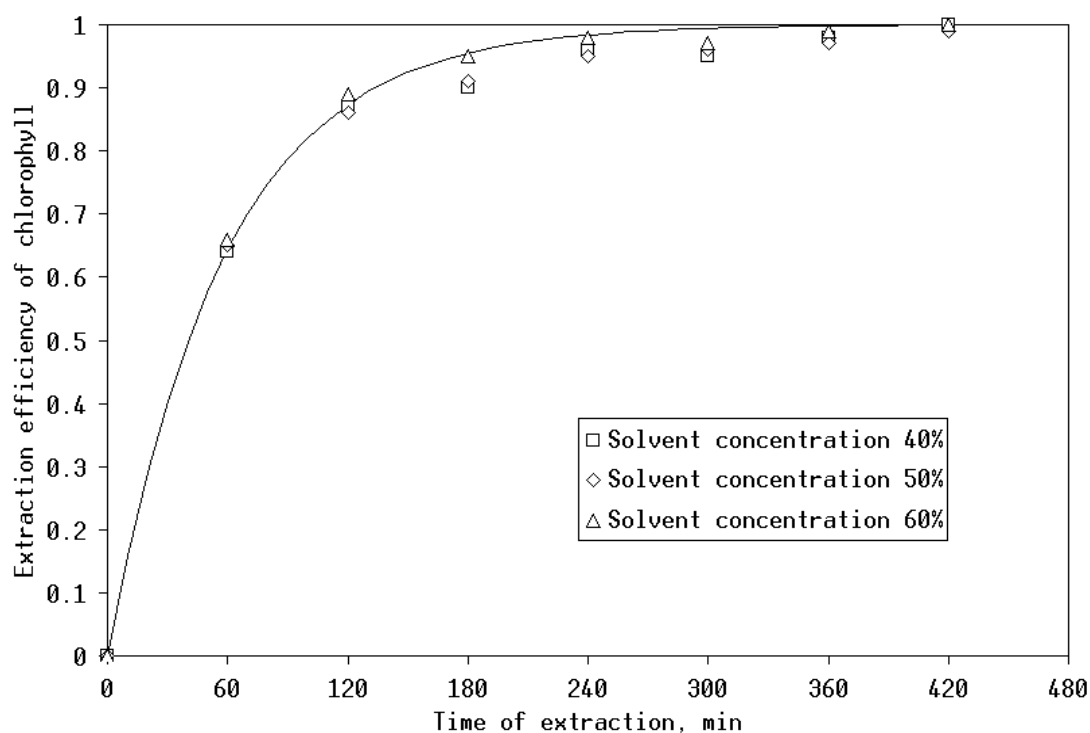
Fig .(5) Effect of solvent concentration on the extractable chlorophyll extraction efficiency (Temperature 40 °C; particle size 0.2 – 0.5 mm)

The effect of particle diameter (0.2 – 0.5, 0.5 – 1.0 and 1.0 – 2.0) mm on extraction efficiency at different temperatures (30, 40, 50 and 60 °C) were calculated and shown in **Figures (6, 7 and 8)** . It is clear that efficiency increases with the decrease of the size of the solid particles and the equilibrium concentration (maximum extraction degree) was achieved in shorter extraction time with the decreasing of the solid particle size. Indeed, increasing the contact area enhances the solute mass transfer. It is a classical result since the pore diffusion path increases with the particle size increasing. But in practice, it is not recommended to use particle sizes smaller than 4mm, since higher grinding could result in high losses of raw material and environmental pollution (due to wind effect) and high degradation of chlorophyll (due to light and air effects). Therefore, the size classes obtained depend on the diameter for the stem and on the width for the leaves, (the mean percentage of leaves to stems is about 75%). Therefore, the effect of the size cannot be very important in the industrial applications, since in the case of the leaves, which contain the main amount of solute, the relevant dimension for the diffusion is the thickness.
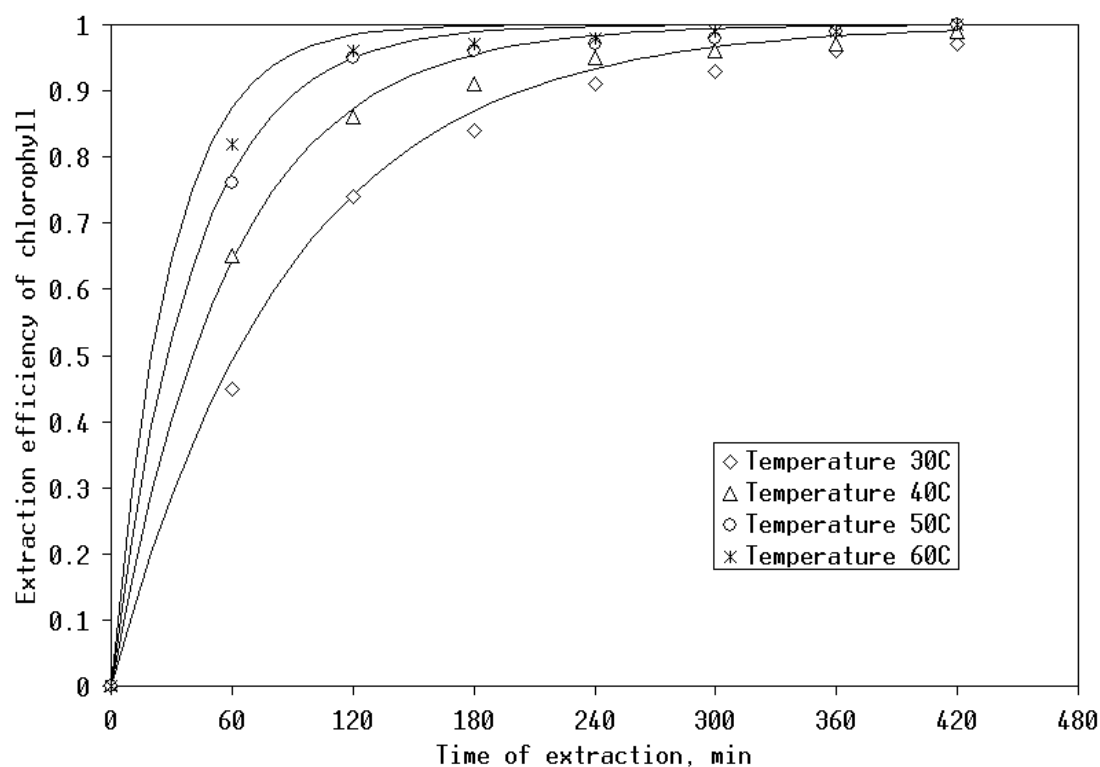
Fig(6) Effect of temperature and time on the extraction efficiency of chlorophyll (Concentration of ethanol 50%; particle size 0.2 – 0.5 mm)
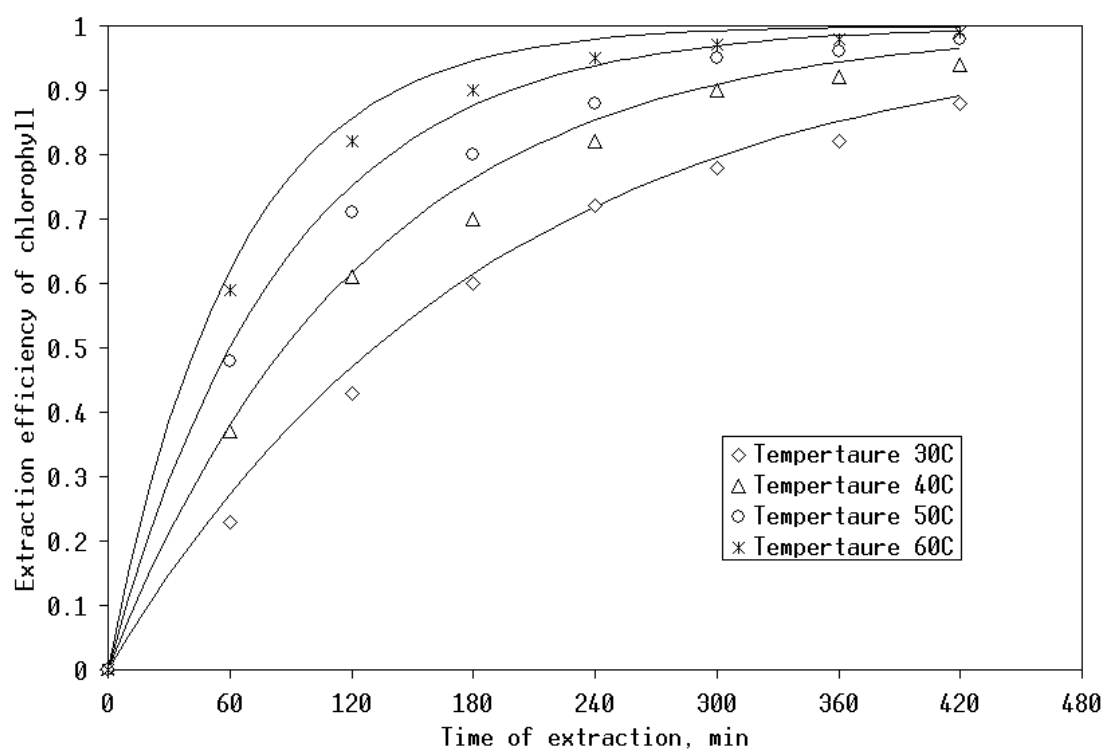
Fig(7) Effect of temperature and time on the extraction efficiency of chlorophyll (Concentration of ethanol 50%; particle size 0.5 – 1.0 mm)

113
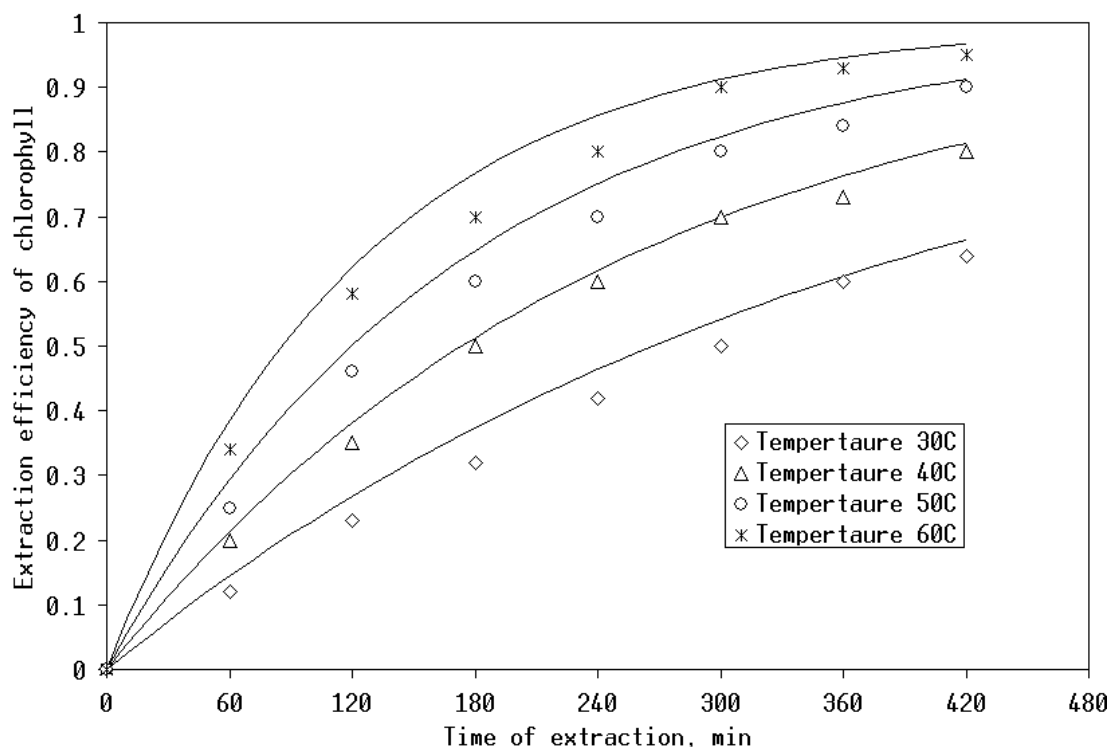
Fig(8) Effect of temperature and time on the extraction efficiency of chlorophyll (Concentration of ethanol 50%; particle size 1.0 – 2.0 mm)

The figures show that the concentration increased with the temperature increasing. This must be due to the thermodynamic effect of temperature on solubilization inside of the solid. The solubility determined the number of interactions between solvent and solute molecules. If enough interactions occurred, the cohesive forces between individual solute molecules were broken down rapidly and solubilization would occur. Therefore, solubilization of solute in solvent will be a function of the molecular weight of the solute and the level of interaction between solvent and solute molecules. This phenomenon could be explained also in viewing the fact that the chlorophyll viscosity decreases as the temperature increases and the chlorophyll was easier eluted from the plant by the solvent.

However, since extraction of solute from plant matrices is basically a desorption process, increasing the desorption kinetic constant would lead to increase in the extraction rate and extract yield. This is due to the increase in solute vapor pressure and as a result the increase of desorption kinetic constant with temperature according to Arrhenius dependence:

$$k = k_o \, exp\left(-\frac{E}{RT}\right) \tag{5}$$

The plot of *ln k* against *1/T* allows the calculation of k_o and *E*. The variations in different particle sizes (0.2 – 0.5, 0.5 – 1.0 and 1.0 – 2.0) mm are shown in **Figure 8**. There were linear

114

relationships between the leaching rate constant and the reciprocal absolute temperature with a coefficient of determination of about 0.94. Table 1 shows the values of k_o and E. From the table, it could be recognized that the values of k_o drops down as the particles sizes increases, which indicates that the rate of leaching increased as the particles sizes decreased. While the values of activation energy is always constant for all particle sizes at about 30.7 kJ/mol, which indicates that leaching of chlorophyll requires this value of energy for completion. In addition, it is indicated that the leaching is an endothermic process.

Table 1, Frequency factor and activation energy values for chlorophyll extraction

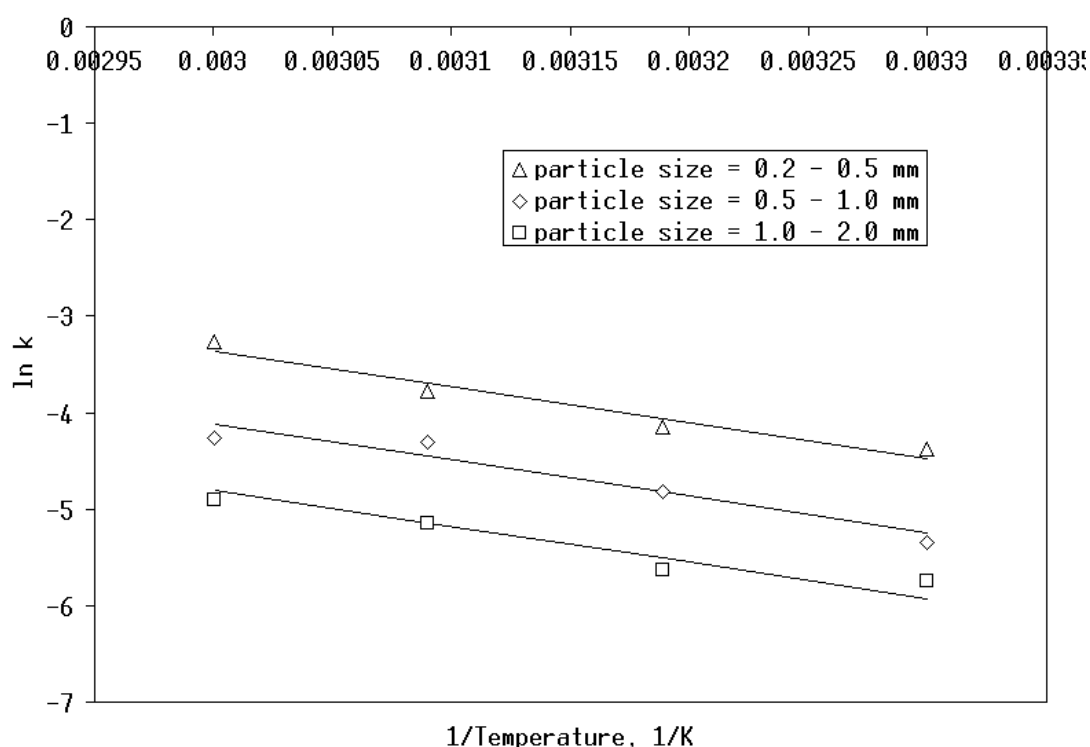| Particle size, mm	k_o, min^{-1}	E, kJ mol^{-1}
0.2 – 0.5	2368	30.7
0.5 – 1.0	1119	30.8
1.0 – 2.0	539	30.6

Fig .(9) Effect of reciprocal temperature and particle size on the rate constant of extraction of chlorophyll

Conclusions

- The maximum concentration of total chlorophyll in the extract of alfalfa is 0.31% for 50% ethanol in hexane.
- Age, air and light have a significant effect on chlorophyll degradation.
- The percentage of ethanol in the solvent has no effect on the kinetic of extraction but the final concentration increases with the percent of ethanol increasing.
- The decreasing of the particle sizes or increasing in temperature increases the rate of extraction.
- The activation energy of leaching was evaluated to be about 30.7 kJ/mol for water-soluble compounds.
- The application of the proposed model for optimization of the process of solid – liquid extraction of total chlorophyll from alfalfa was allowed since a good agreement between the experimental and model calculated data was showed.

Acknowledgements

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