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Ultrasound assisted extraction of carotenoids from Sargassum Angustifolium algae

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ABSTRACT

An experimental batch extraction method was studied to obtain a carotenoid pigment from dried Sargassum Angustifolium algae using ultrasound as an enhancer and a mixture of ethanol-isopropanol as a solvent. The purpose of this work is to investigate the extraction kinetics as well as the temperature effects (303 to 333) K, solvent concentration (40% ethanol - 60% isopropanol to 60% ethanol - 40% isopropanol) and particle size (250 - 500, 500 - 710 and 710 - 1000) micrometer. Also, the effect of collecting, drying and transporting Sargassum Angustifolium algae on the total carotenoids extraction from dried algae was studied. The ultimate extraction value of the carotenoids was found to be 0.29% from algae. A batch model for extraction was created and numerically solved. The model parameters were calculated using existing empirical correlations and data gathered during this project. The model predicts the mass transport rate constant and saturation capacity at various temperatures. Furthermore, the energy of activation and frequency factor of the extraction process were enumerated and it was found that the process is endothermic with activation energy equals 28.9 kJ/mol. The utilized mathematical model agreed well with the experimental data, allowing it to be used in modeling and improving the carotenoids extracting process from Sargassum Angustifolium algae.

Keywords: Sargassum Angustifolium algae, Carotenoids, Extraction, Kinetics, Modeling

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1. Introduction

Carotenoids (also known as carotenoid extract) are natural yellow, red or orange pigments. Almost carotenoid pigments are carotenoids derived from plants. Carotenoids are observed in the chloroplasts as a part of the biosynthetic system in green vegetation, but they are more numerous, marked, and colorful in fruits, flowers and roots [1]. Those photosynthetic organisms such as cyanobacteria, algae and higher plants, and also some non-photosynthetic organisms like bacteria and fungi have the ability to biosynthesize carotene in nature [2], [3]. Carotenoids play an important role in human health and survival. Also, they can boost the immune system, protect against cancer, and also act as an antioxidant. Therefore, carotenoids are an excellent oxygen scavenger, and are used as food coloring, food additives, pharmaceuticals and cosmetics because they bring health benefits to consumers [4].

The brown alga of Sargassum Angustifolium widely grows in the equatorial zone of Asia and it is a native Iraqi alga with colorful properties. It is abundant in the marine waters of the Shatt al-Arab in all seasons. Therefore, it can be considered as a sustainable source of carotenoid pigments.

Carotenoids are poly isoprene substances composed of eight isoprene units and forty carbon atoms, with the

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formula $C_{40}H_{56}$. There are approximately 600 compounds with in carotenoid group that could be characterized as carotenoids or carotenes, and xanthophyll, which are oxygenated hydrocarbons that have at least one oxygen atom. Carotenoid pigments seem to be hydrophobic compounds that are only soluble in organic solvents due to their hydrocarbon structure [5]. The main hydrocarbon carotenoids are: lycopene (red color), alpha-carotene (copper color), beta-carotene, and lycopene beta-carotene (purple red color) [6].

In general, carotenoids are extracted from algae by conventional solvent extraction using organic solvents [7]. However, these methods are time consuming; often require the use of relatively large amounts of solvent, and expensive and not environmentally friendly. In recent years, there has been an increase in interest of the utilization of unconventional innovative methods based on the permeability of physical membrane or hydrolysis to eclectically or non- eclectically enhance the transfer rate of carotenoids from intra-cellular compartment of microalga species and seaweed. Creative extraction could be accomplished utilizing two distinct types of techniques: Mechanical, including pressurized systems, microwave, electrical fields, ultrasound and super-critical extraction; Non-mechanical, including thermal, chemical, and enzymatic. Some techniques rely on interactions between non-mechanical and mechanical properties [8]. Among these, ultra sound extraction (UAE) is a quick and effective extraction technology. The improvement is attributed to an extraction obtained using ultrasound, which is primarily to the influence of an acoustic cavity generated in solvents by the passing of ultrasound waves [9], [10]. Ultrasound also has mechanical impacts, enabling solvents to penetrate deeper into tissues, so increasing the area of contact between both the liquid and solid phases. Therefore, the solute quickly spread from the solid phase to the liquid phase [11]. As a result, the UAE had been widely used to extract a variety of natural products [12] to [15]. Even so, it was unknown whether the UAE could improve the extraction efficiency of carotenoids from Sargassum Angustifolium algae. Therefore, it is crucial to examine this process both mathematically and experimentally.

Dehydrated Sargassum Angustifolium has been used in the reported research herein since it is inexpensive and locally available throughout all seasons. the ethanol – isopropyl alcohol mixture has been used as a solvent in carotenoids extraction experimentations because of it was readily available, reasonably priced, low in toxicity, and environmentally safe.

The objective of this research was to evaluate ultrasound technique in the presence of the ethanol - isopropanol mixture as a pretreatment for carotenoids extraction from Angustifolium algae. The impact of key operational parameters including: particle size, solvent concentration and temperature were investigated. Moreover, identifying the kinetic functions and developing a mathematical model that describes the extraction process was represented. This information is valuable for improvement and design of the carotenoids extraction process.

2. Material and methods

A schematic schema for the experimental apparatus is displayed in Fig. 1. The extraction was carried out in a double jacketed batch vessel of 2 liters capacity. The vessel was outfitted with a variable-speed mechanical mixer. The working part of the mixer is a four blade - form made from carbon steel (ASME SA516, Grade 70), which provides proper mixing of the vessel material. The temperature of the mixture was monitored using a mercury thermometer and kept constant by circulating conditioning water as a thermal fluid delivered from the constant temperature bath in the vessel's jacket. Samples of the extraction solution could be drawn at variable times using a sampling exit route embedded inside the vessel. A highly efficient glass condenser with ethyl alcohol at 263K labor fluid was connected to the extraction vessel to prevent hydrocarbon loss by evaporation. Alcohol was being supplied at a low temperature by an outer cooling system (Henan Lanphan, DLSP, China). The extraction device was supplied with ultrasound equipment (Hielscher, UP 200 S, Germany), probe diameter of 14 mm, with energy density varying from 12 to 600 Wcm^{-2} and amplitude ranged from 12 to 260 µm.

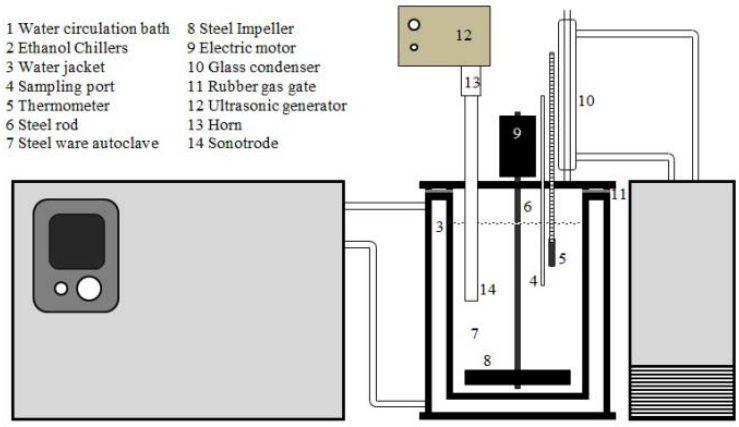

Figure 1. Diagram of the carotenoids extracting apparatus

The fresh Sargassum Angustifolium algae were collected from Shatt al-Arab River in Basra in July. The first stage would have been to remove any foreign objects like dirt and mud before heating at 333 K for overnight in a dim environment. Materials are grinded in the mechanical milling machine (Brabender, SM4, Germany), then sectioned by screening into a number of sieves (Retsch, AS 200, Germany). Three classes (250 - 500, 500 - 710 and 710 - 1000) µm were used in the present research. The vessel was initially filled with 1200 ml of ethanol-isopropanol solvent, which is then conditioned at the required temperature (303, 313, 323, or 333K) using elevated agitation inside the vessel and water circulation inside the jacket. 50 grams of dry Sargassum Angustifolium algae were added to the vessel content to ensure a large surplus of solvent. The extraction mixture was mixed at the desired temperature for the study period of time with ultrasonic frequency of 24 kHz and intensity of 105 W/cm^2. Samples of extraction mix were drawn at variable times and carotenoids mass concentration was measured in the liquor.

Many procedures for quantitative determination of total carotenoids content were used in the literature [1] to [3]. The Spectrophotometric method was used and was accomplished with the Shimadzu spectrophotometer (UV-1700) [16]. The concentration of total carotenoids extracted was calculated using the following formula dependent on the absorbance at 480 nm and 510 nm:

$$C = \left(7.6 \times A_{480} - 1.49 \times A_{510}\right) \times \frac{V}{m} \quad (1)$$

Where (C) is the concentration of total carotenoids expressed by grams of carotenoids per gram of dry Sargassum Angustifolium algae, (A) is the spectrophotometer absorbance in the subscripted wavelength, (V) is the size of the extracted phase and (m) is the mass dried sample. Bulk, apparent and packing densities of solid materials were calculated according to standard analyzing procedures (ASTM D2854 – 83).

3. Theory and calculations

In the present study, it was suggested that the primary mechanism which controlled the mass transfer rate of carotenoids extraction is the comprehensive transport of carotenoids from algae to bulk solvent. Moreover, extraction experiment has two distinguished phases. One phase carries on until all pores fulfill saturation with an efficient solvent. During this period of time, the soluble carotenoids in Sargassum Angustifolium algae readily dissolve into solvent in pores with partial compensation by mitigating of forthcoming solvent. The other phase is instantaneously following the initial phase when achieving the effective solvent mixture inside pores and subsequently beginning of flow of solvents from the pores. Therefore, the comprehensive transport rate of the Carotenoids from Sargassum Angustifolium algae to the bulk liquid mixture could be represented as follows:

For the liquid phase:

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

For the solid phase:

$$\frac{dS}{dt} = -\frac{\varepsilon}{\rho_b}\left(\frac{S}{S_o}\right)^a k\left(C_s - C\right) \qquad `S = S_o \ \forall \ t = 0 \tag{3}$$

Where, (C) is the carotenoids mass concentration in the liquid phase, (S) is the carotenoids mass concentration of in the solid phase, (k) is the comprehensive mass transport coefficient, (ε) and (ρ_b) are the void portion and bulk density of ground Sargassum Angustifolium algae, respectively. The mathematical model's equations were solved numerically by using the forth-ordered Runge-Kutta technique for the 420 estimated stage set of the discretized finite difference equations (interval of 1 minute). The coefficients (k) and (a) were evaluated utilizing the experimental results. The simplex method of Nelder-Mead [17] was used to adjust these coefficients by minimizing the summation of square of errors between both the experimental and predictive data. The amplitude of the overall mismatch between experimental results and model predictions has been calculated as the squared root of the relative mean error utilizing the formula:

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

Where (e) is the squared root of the relative mean square error and (N) is the experimental results number.

4. Results and discussion

A Soxhlet leaching experiments were performed with variable extraction times (720, 1180 and 1440) minutes at 333 K and < 250 μm particle size in order to identify the total quantity of the extractable carotenoids in the Sargassum Angustifolium algae. High temperatures are not recommended because of possible degradation of carotenoids. 50wt% ethanol – 50wt% isopropanol was utilized as a solvent and an ultrasonic frequency of 24000 Hz and an intensity of 105 W/cm^2 were used as an enhancer. The maximum value for extracting the carotenoids was found to be 0.29 wt% of total mass of Sargassum Angustifolium algae. This subsequent outcome was the mean of six runs with an accuracy of 3%.

The effects of collecting, dehydration and transporting of Sargassum Angustifolium algae could impact its primary content of carotenoids. Therefore, a double of experiments were implemented on Sargassum Angustifolium algae at different times of ages (updated specimen, which is dehydrated, milled and used at the same time; outdated sample, which is dehydrated, milled and used after 14 days) for each size of particles (250–500 , 500–710 and 710–1000) micrometers. As shown in Fig. 2, the updated samples show degradation in carotenoids of 10%, 8% and 6% comparative to the updated one, for every size of particles (250–500, 500–710 and 710–1000) μm, respectively. This degradation is related to the Sargassum Angustifolium algae's photo ability. It was concluded that there is a chemical effects takes place among the carotenoids, light and air. Furthermore, the degradation was more pronounced when the contacting area was increased because the particles with smaller sizes show higher values of degradation than particles with larger sizes. Therefore, any contacting with light and air should be obviated to maintain the carotenoids at the preferable conditions. Additionally, the Sargassum Angustifolium algae should be turned on instantaneously after dehydration and milling. Also Pagels et al. showed that the carotenoids are relatively mutable during sequestration and it is indispensable to conserve them from the degradation [18]. Therefore, as a general precaution, it is useful to labor in dark place and low temperature to prevent losing dye.

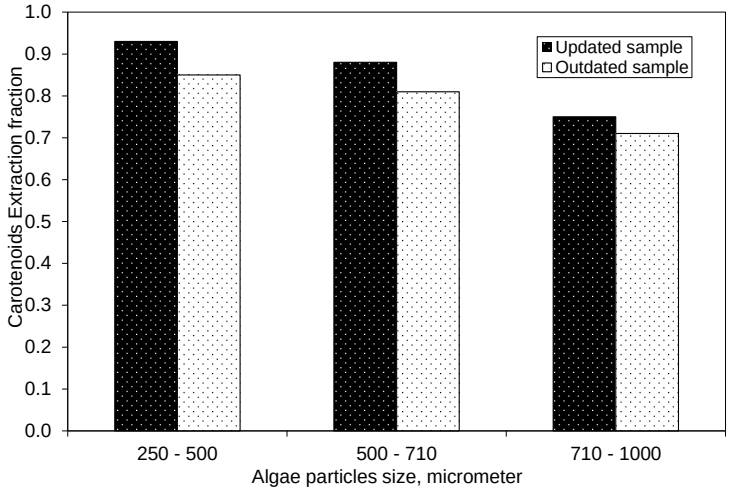

Figure 2. Influence of Sargassum Angustifolium algal age - related changes and size distribution of particles on the extraction fraction of carotenoids
(Ethanol 50% - isopropanol 50%; temp. 313 K; time 7 hours)

The impact of ethyl – isopropyl alcohols content in the solvent on the extracted carotenoids concentration is shown in Fig. 3. The concentration of carotenoids increases with increasing the concentration of ethanol in the solvent. Carotenoids fall into two subcategories, more polar compounds called xanthophylls or oxycarotenoids and nonpolar hydrocarbon carotenes [19]. Ethanol has solubility properties because it is a polar and contains a hydrophilic -OH group, while isopropanol is nonpolar and hydrophobic, although there is a small region of the molecule remains polar, the majority of the isopropyl alcohol molecule is non-polar allowing it to dissolve oils. Thus, it can be concluded that the bulk of the carotenoids present in Sargassum Angustifolium algae are oxycarotenoids, which could be used as a pigment in mouthwashes, hand sanitizers, hair products, sweets, personal care products, antiperspirants, and pharmaceutical products.
The experimental data points of Fig. 3 can be re-drawn to find out the extraction efficiencies that could be defined as the percentage of the amount of extracted carotenoids relative to the ultimate amount of extractable carotenoids at a specified concentricity of the solvent. This representation is shown in Fig. 4. This impersonation permits us to investigate the dynamics of the phenomenon regardless of the saturation status. The lines show that the concentration of ethyl alcohol in the solvent has no considerable effect on the kinetics of the process because all lines have analogous demeanor.

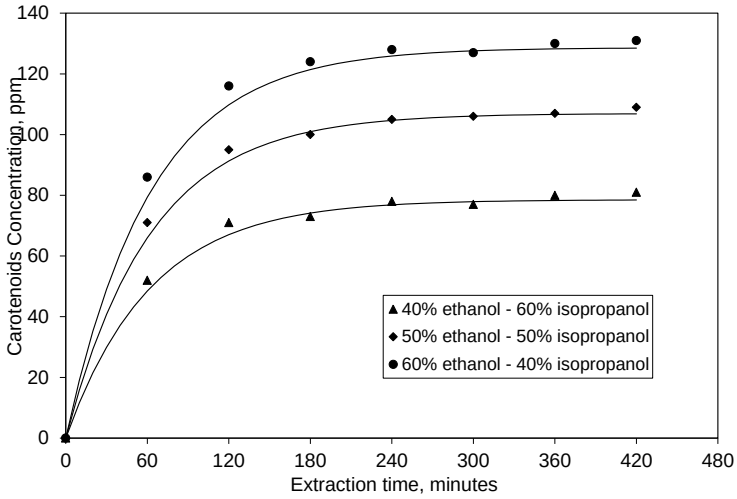

Figure 3. Kinetics of carotenoids concentration at different concentrations of solvent
(Temp. 313 K; Size of particles 250-500 μm)

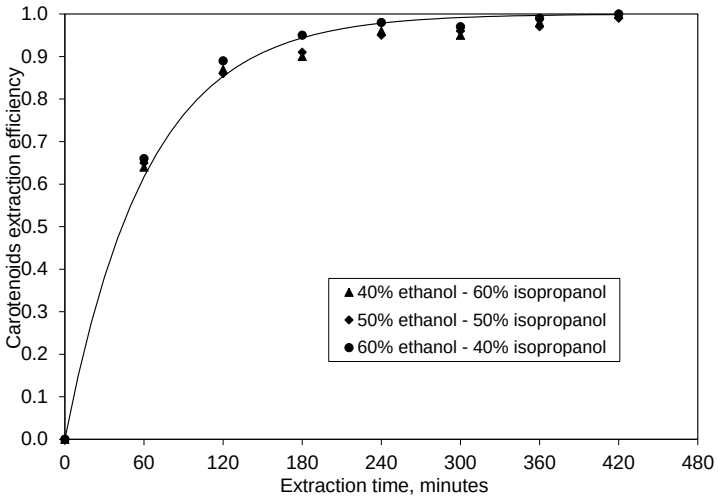

Figure 4. Kinetics of carotenoids extraction efficiency at different concentrations of solvent (Temp. 313 K; Size of particles 250-500 μm)

The effect of particle size (250 - 500, 500 - 710 and 710 - 1000) μm on the extraction efficiency at different temperatures (303, 313, 323 and 333 K) was examined and presented in Fig. 5, 6 and 7, respectively. It is clear that the carotenoids extraction efficiencies increase as the particle size of solids is reduced and the maximum concentration (equilibrium concentration) was obtained through a smaller time of extraction with reducing the size of solid particle. In fact, the transport of the dissolved mass is improved by increasing the area of contact and interfacial interaction. This is a classical outcome because the path of diffusion through pores increases with increasing the size of particles. However, in practice, it is not preferred to be using sizes of particles lesser than 1000 μm because elevated milling can result in a significant raw material losses, pollution of environment because of wind actions and significant carotenoids degradation because of air and light effects. Though, the obtained classes of size lean on the shape and width of the leaves of the Sargassum Angustifolium algae. Since, the particle size effect is not extremely paramount in industrial application because the leaves that contain the most quantity of carotenoids, the pertinent dimension of diffusive transport are the thickness of leaves.

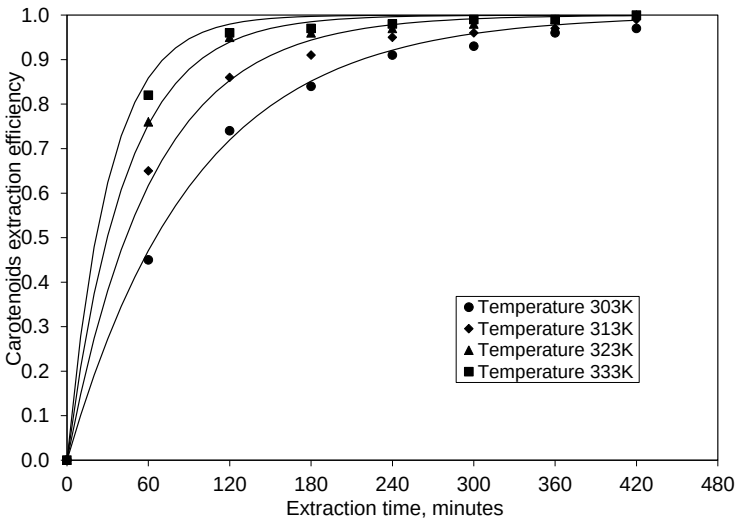

Figure 5. Kinetics of carotenoids extraction efficiency at different temperatures (50% ethanol - 50% isopropanol; Size of particles 250-500 μm)

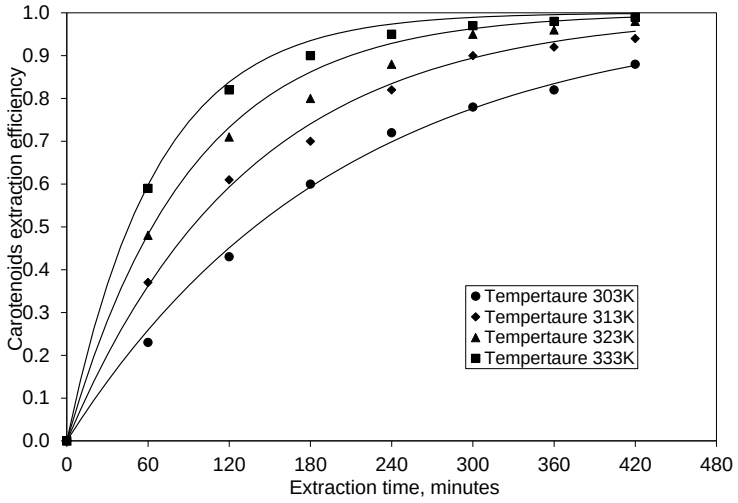

Figure 6. Kinetics of carotenoids extraction efficiency at different temperatures (50% ethanol - 50% isopropanol; Size of particles 500-710 µm)

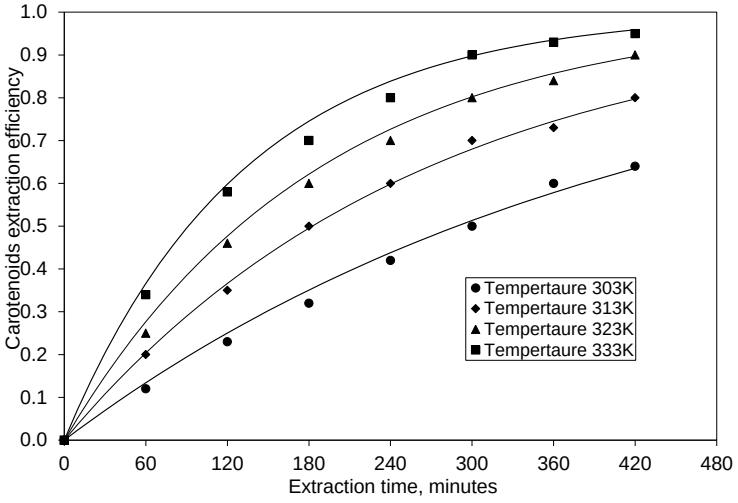

Figure 7. Kinetics of carotenoids extraction efficiency at different temperatures (50% ethanol - 50% isopropanol; Size of particles 710-1000 µm)

The curves indicate that the carotenoids concentration increases with increasing the temperature of the extraction. This is should be due to the thermo dynamical effects of the temperature on the solubility of carotenoids within the solid. Solubility identifies the quantity of interaction between the molecule of the solute and the solvent. If sufficient interaction occurs, the coherent force between the molecules of solute is quickly disintegrated and dissolution occurs. So, the solubility of the solute molecules in the solvents should be related to the molecular mass of the solute molecules and the grade of interactions among the solute and solvent molecule. These phenomena can also be clarified by looking to the reality that the viscosity of the carotenoids decrease with increasing the temperature and the carotenoids were easily removed from the Sargassum Angustifolium algae by the solvent.

Since the extraction of solute molecules from Sargassum Angustifolium algal matrix is essentially a de-sorption processes. The kinetics coefficient increasing of the de-sorption process will result in an increases of the rate and yield of the extraction. That is because an increasing of the vapor pressures of the dissolved solute and as a consequence an increase in the de-sorption kinetics coefficients with temperature. Mathematically, this dependence could be shown by Arrhenius equation:

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

The scheme of (ln (*k*)) verses (1/*T*) permits the calculation of (k_o) and (*E*). The alterations in different particle sizes (250-500, 500-710 and 710-1000) μm are shown in Fig. 8. There were linear relationships between the extraction rate coefficients and the absolute reciprocal temperatures with a determination coefficient of about 0.96. Table 1 presents the (k_o) and (*E*) magnitudes. It can be realized that the values of (k_o) decrease with increasing particle sizes, which indicates that the extracting rate increased with decreasing particle sizes. The values of energy of activation were found almost constant at about 28.9 kJ/mole for any particle size. This is point out that the extraction of carotenoids requests this amount of energy to complete the process. In addition, they are denoted that the carotenoids extraction is an endothermic process.

5. Conclusions

• The total carotenoids in the Sargassum Angustifolium algal extract have a maximum concentration of 0.29 percent.
• The breakdown of carotenoids is affected by the light, air, and age.
• The kinetics of extraction are unaffected by the ratio of ethanol to isopropanol with in solvents, but the maximum concentration rises as the fraction of ethanol rises.
• The extraction rate increases as the temperature increases or size of particles decreases.
• The energy of activation for carotenoids extraction was estimated and found to be equals to 28.8 kJ/ mole.
• The suggested model might be used to optimize the process of liquid-solid extraction of total carotenoids from Sargassum Angustifolium algae because the estimated experimental and model data were in good agreement.

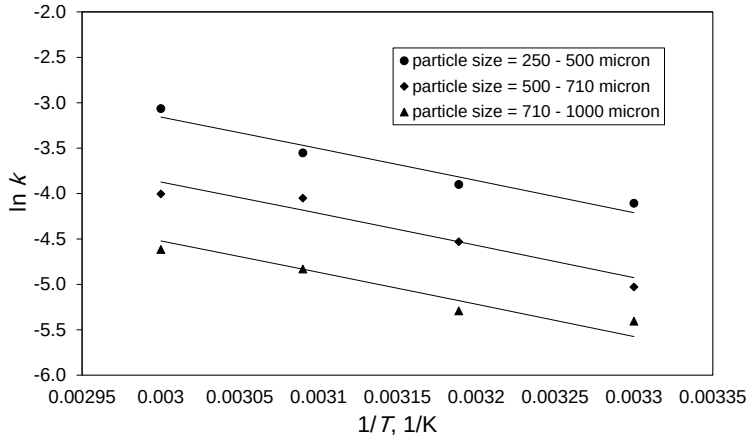

Figure 8. Arrhenius equation plot at different size of particles of carotenoids extraction

Table 1, Arrhenius equation constants values for carotenoids extraction

Size of particles, [μm]	k_o, [1/min]	*E*, [kJ/mol]
250 – 500	2226	28.9
500 – 710	1052	28.9
710 – 1000	506	28.8

Declaration of competing interest

The authors declare that they have no any known financial or non-financial competing interests in any material discussed in this paper.

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