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Subcritical Water Extraction of Mannitol from Olive Leaves

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***Abstract*—**Subcritical water extraction was investigated as a novel and alternative technology in the food and pharmaceutical industry for the separation of Mannitol from olive leaves and its results was compared with those of Soxhlet extraction. The effects of temperature, pressure, and flow rate of water and also momentum and mass transfer dimensionless variables such as Reynolds and Peclet Numbers on extraction yield and equilibrium partition coefficient were investigated. The 30-110 bars, 60-150˚C, and flow rates of 0.2-2 mL/min were the water operating conditions. The results revealed that the highest Mannitol yield was obtained at 100˚C and 50 bars. However, extraction of Mannitol was not influenced by the variations of flow rate. The mathematical modeling of experimental measurements was also investigated and the model is capable of predicting the experimental measurements very well. In addition, the results indicated higher extraction yield for the subcritical water extraction in contrast to Soxhlet method.

***Keywords*—**Extraction, Mannitol, Modeling, Olive leaves, Soxhlet extraction, Subcritical water.

I. Introduction

MANNITOL is a naturally occurring polyol or sugar alcohol with six carbons and a relative sweetness of 40-50% compared to sucrose. Mannitol is assumed to have several beneficial effects, as an antioxidant (protection against oxidative damage by oxygen radicals) and as a non-metabolizable sweetener. It has a reduced caloric value (1.6 kcal g-1) compared to sucrose (4 kcal g-1) and is commonly used as an artificial sweetener in "light" foods such as sugar-free chewing gums. Uptake of Mannitol is independent of insulin. Thus, it is also applicable in diabetic food products [1]. In addition to food industry, Mannitol is utilized in pharmaceuticals, chemistry, and as medicine [2]. Mannitol finds its principal use in pharmaceutical applications. It is used as a base in chewable, multilayer, and press-coated tablets of vitamins, antacids, aspirin, and other pharmaceuticals, sometimes in combination with sucrose or lactose. It provides a sweet taste, disintegrates smoothly, and masks the unpleasant taste of drugs such as aspirin. Tablets containing Mannitol retain little moisture because of the low affinity of Mannitol for water, making it an excellent excipient and thus suitable for use with moisture-sensitive actives. Mannitol is available as a powder for wet granulation tableting and in a granular form for direct compression tableting. Also Mannitol is administered intravenously as osmotic diuretics and mannitol hexanitrate is antianginal drug as a cardiovascular agent. Another application of mannitol is in the field of bacteriological media. Blood is protected during freezing, storing, and thawing by adding 15-20% of Mannitol. Mannitol protects freeze-dried bacterial culture during storage, and animal semen is preserved by the addition of small quantities of Mannitol and sorbitol together with other materials [3].

Mannitol is widely distributed in nature, occurring in olive tree, plane tree, manna ash, marine algae and fruit and vegetables (e.g., celery, pumpkin, hedge parsley, onions, mushrooms) [3]. It has been experimentally demonstrated that Mannitol occurs in olive leaves with a seasonal variation in Mannitol content which can reach 30% in the summer and in deciduous species. However, Mannitol content in the evergreen species of olive leaves can reach 8% on September [4], [5].

Mannitol is obtained commercially in large quantities by catalytic hydrogenation from glucose-fructose mixtures. Glucose is completely converted in the chemical hydrogenation process into sorbitol, whereas fructose is converted into Mannitol and sorbitol [2]. The catalytic hydrogenation process has several drawbacks including the need for high-purity raw materials (including hydrogen gas) due to the use of a substrate-unspecific metal catalyst, the required high reaction temperature and pressure, a low Mannitol yield from sugars, the necessary chromatographic purification to discard the metal catalyst from the product, and a difficult separation of Mannitol from its stereoisomer, sorbitol.

Some alternative processes based on the use of enzymes or microbes have been suggested in the literature [1]–[2], [6]–[8]. Conventional Soxhlet solvent extraction of Mannitol from plant raw material (e.g. manna, seaweed or algae) is no longer economically relevant. However, this procedure is used in China for the production of Mannitol [9]. Nowadays, the desire to reduce the use of the organic solvents in food and medicine processing has led to new extraction methods including supercritical fluid extraction and subcritical water extraction (SWE). In 2001, supercritical fluid extraction of

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plane tree leaf as an alternative technology in the production of Mannitol was modeled mathematically by Ghoreishi and Sharifi [10]. They investigated the theoretical feasibility of supercritical extraction of Mannitol from plane tree leaves using carbon dioxide. Their results revealed that due to the polar nature of Mannitol, it is very difficult to solve it in the nonpolar carbon dioxide without an entrainer.

Subcritical water extraction is based on the unique solvent properties of water, namely its disproportionately high boiling point for its mass, a high dielectric constant and high polarity [11]. As the temperature rises, there is a marked and systematic decrease in permittivity, an increase in the diffusion rate and a decrease in the viscosity and surface tension. In consequence, more polar target materials with high solubility in water at ambient conditions are extracted most efficiently at lower temperatures, whereas moderately polar and non-polar targets require a less polar medium induced by elevated temperature [12]. The most important advantages of SWE over traditional extraction techniques are shorter extraction time, higher quality of the extract, lower costs of the extracting agent, and an environmentally compatible technique [13]. Numerous applications of this technique for extraction of essential oils from various plant materials have been published [14]–[16]. In addition to essential oils, the technique has also been utilized for the extraction of sweet components from Siraitia grosvenorii [17], lactones from kava roots [18] or from Ginkgo biloba [19], and biophenols from olive leaves [20].

In order to improve the total process yield of Mannitol it would be advantageous to develop a process with Mannitol as the main product and with no sorbitol formation. Therefore, the major aim of this research was to investigate the experimental extraction of Mannitol from olive leaves with subcritical water as an alternative procedure for the production of this sugar alcohol. The experimental optimization of operating conditions of subcritical water as a function of Mannitol yield and equilibrium partition coefficient was accomplished. In order to simulate the experimental results of this study, a mathematical model was also developed and its authenticity was validated versus the obtained experimental data. For comparison purposes, the conventional method of Soxhlet solvent extraction of Mannitol from olive leaves using ethanol was carried out.

II. Experimental

A. Materials

Olive leaves obtained from evergreen trees located in Isfahan University of Technology campus, Iran on September 2006 were passed through a sieve with mesh size of 18-35 (1-0.5 mm) and then dried to a constant weight. They were kept within sealed bag in a cold and dry place until they were used. Since extraction kinetics in this study was controlled by the kernel particle size, an important sieving step was carried out to achieve reproducible extraction yield. Water (Double distilled, de-gassed and purified through a Milli-Q de-ionizing unit (Millipore, Bedford, MA, USA)) and ethanol (99.6%, Merck Co.) were utilized as the extraction solvents. Pure Mannitol (99+ %, Aldrich, 24018-4) was used in the HPLC analysis to obtain standard spectrum.

B. Subcritical Water Extraction: Apparatus and Procedure

The subcritical water extractions were carried out using a bench-scale apparatus shown in Fig. 1. This system operates at maximum temperature and pressure of 200°C and 350 bars, respectively. Deionized water filled into a glass reservoir (1) was first purged for 2 h to remove dissolved O_2. Teflon filter (2) which is placed at the entrance of the pump purified de-ionized water, thus, solid particles were not allowed to enter the pump. A water feed pump (Reciprocating pump, Jasco Co., PU-2080, Japan, flow rate= 0.1-9.9 mL/min, maximum pressure= 50 MPa) (3) was used to deliver water through system with various flow rates. Deionized water was heated before entering the extractor using a coil preheater (6) that was placed in an oven (Fan Azma Gostar Co., Iran) (9). The system pressure was controlled by a back pressure regulator (TESCOM Co., 26-1762-24, USA, Maximum adjustable pressure= 408 bars) (11).

The extraction column (height=12.5 cm, inner diameter (ID) = 0.9 cm, and outer diameter (OD) = 1.3 cm) (7) fitted with cotton wool (8) at the effluent was manually charged with 2.5 g of shredded olive leaves and crushed glass (broken Pyrex laboratory glassware passed through sieve with mesh size of 18-35 (1-0.5 mm)) in the percent ratio of 40:60 (w/w), respectively [22].

The experimental extraction procedure consisted of static extraction, followed by dynamic extraction in which the deionized water was passed through the packed bed at different flow rates with various pressures and temperatures. The static extraction time was optimized via different experimental runs at 5 min in which the maximum yield was obtained. The extracted material in all experiments except the one carried out for model validation was collected at the packed bed effluent in 15 mL water container (8). Then, they were kept in a refrigerator until analyzed by a high performance liquid chromatography apparatus (HPLC).

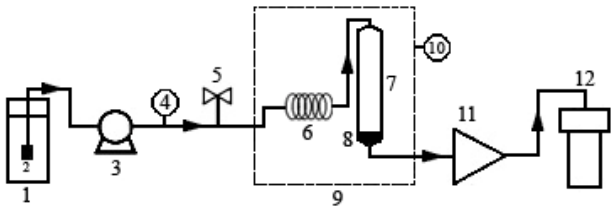

Fig. 1 The experimental set-up for the subcritical extraction system: (1) solvent reservoir, (2) teflon filter, (3) HPLC pump, (4) pressure gauge, (5) valve, (6) coil preheater, (7) extraction column, (8) cotton wool filter, (9) oven, (10) temperature controller, (11) back pressure regulator, (12) container

C. HPLC Separation-Detection

Pure standard Mannitol and Mannitol contained in the extracted samples were analyzed and quantified by HPLC apparatus (Jasco Co., Japan) equipped with a refractive index detector using an anion-exchange column (Aminex HPX-87H, Bio RAD Co., USA) at 60 °C, with 5 mM H_2SO_4 as the elution fluid and a flow rate of 0.5 mL/min [23].

D. Mannitol Determination

In the yield calculation, the initial amount of Mannitol in the olive leaves was determined using the following procedure [4]. Aliquots of the powdered leaves (300 mg) were extracted twice in 10 ml 80 % ethanol (v/v) and once in 10 ml H_2O overnight at room temperature. Combined extracts were partitioned against 10 ml of $CHCl_3$, and a 10 ml aliquot of the aqueous phase was passed through an ion exchange column (Sephadex QAE-A-25; Sigma, Steinheim, Germany) equilibrated with ammonium buffer, pH 9.5, and brought to neutrality with water [24], [25]. An aliquot (10 ml) of the eluate was dried at 60°C using a rotary vacuum evaporator (Rotovapor; Buchi, Milan, Italy). The dry residue was dissolved in 5 ml 99% pyridine, to which 250 μl hexamethyldisilazane and 100 μl trimethylchlorosilane were added for derivatization. Analyses were carried out using a temperature program Hewlett-Packard 6890 gas chromatograph with an HP 5973 mass spectrometry detector, on an HP 5MS column (30 m × 250 μm × 0.25 μm coating thickness). Peaks were identified by comparison with standards and with the NIST database. For quantitative analysis, arabinose was added to the samples before derivatization as an internal standard, since it was never found in the plant extract. In preliminary experiments, the accuracy of the method was tested by adding Mannitol as an internal standard to replicate samples before extraction; recovery was 98%. Mean values including standard deviations were calculated and the significance ($P < 0.05$) of differences between sets of data was tested using a Student's *t*-test. Pearson's correlation coefficient was calculated to measure the degree of correlation between data and the significance ($P < 0.05$) was tested using a one-tailed test. Using this method, the amount of Mannitol in the initial olive leaves sample was determined to be 8 % (w/w).

E. Soxhlet Extraction

Traditional Soxhlet extraction was carried out in standard apparatus using 2 g of shredded olive leaves by standard method [16] for 8 h (2 cycles per h) with 280 mL of ethanol.

III. Model Description

The variety of physicochemical models had been used to get a complete description for the supercritical carbon dioxide extraction process [10], [26]–[28]. These models were proposed and developed to simulate mobility of Mannitol in plane tree leaves-supercritical carbon dioxide and hydrocarbons in soil-water and soil-supercritical carbon dioxide systems. In this work, modeling of a packed extraction column filled with olive leaves-glass beads and subcritical water was studied in order to apply a validated model to optimize the operating conditions without any further experiments.

This model was comprised of two mass transfer mechanisms: (1) Convective flow transport between particles and bulk phase (subcritical water) with an external mass transfer coefficient (k_f), and (2) Linear equilibrium isotherm on the solid matrix surface with equilibrium coefficient (h). The equilibrium coefficient for the case of subcritical water extraction was composed of two mass transfer steps, i.e. internal diffusion and the adsorption/desorption equilibrium.

In order to develop an applicable mathematical model of transport of Mannitol in olive leaves-subcritical water system, some hypotheses, simplifying assumptions, and physical criteria were used as follows to simulate the extraction process: (1) The process was a one-dimensional, unsteady state, axial flow system; (2) Axial and radial dispersions were neglected due to column geometry; (3) The system was isothermal; (4) The solute was considered completely soluble in the subcritical water; (5) The system was considered as a "fixed bed" which included two phases: (a) Solid (stationary) phase contained shredded olive leaves, and (b) Fluid (mobile) phase contained subcritical water; (6) A linear relationship between solute concentrations in stationary and mobile phases was assumed; (7) Solvent flow rate, density and viscosity were considered constant during the process; (8) Pressure and temperature gradients were neglected. As a result of the last two assumptions superficial velocity could be considered constant along the fixed bed.

In this extraction process, the Mannitol was transported by bulk flow and the mass balances of the solute in the mobile and solid phases may be written:

$$\varepsilon\frac{\partial C}{\partial t} = -u_z\frac{\partial C}{\partial z} - (k_f a)(C - c^*) \quad (1)$$

$$(1-\varepsilon)\frac{\partial c_s}{\partial t} = (k_f a)(C - c^*) \quad (2)$$

with equilibrium equation:

$$c_s = hc^* \quad 0 \le z \le L,\ 0 \le t \le T \quad (3)$$

and finally, with boundary and initial conditions:

$$z = 0 \rightarrow C = 0 \quad (4)$$

$$t = 0 \rightarrow C = 0 \quad (5)$$

$$t = 0 \rightarrow c_s = c_{s0} \quad (6)$$

where a is specific solid surface (1/cm), C is the concentration of Mannitol in the fluid phase (mol/cm^3 water), c_s is the concentration of Mannitol in the solid phase (mol/cm^3 solid), c_{s0} is the initial concentration of Mannitol in the solid phase (mol/cm^3 solid), c^* is the equilibrium concentration of Mannitol in the fluid phase (mol/cm^3 water), h is the equilibrium coefficient (cm^3 water/ cm^3 solid), k_f is the external mass transfer coefficient (cm/s), L is the bed length (cm), t is time (min), u_z is the superficial velocity (cm/s), z is

axial coordinate (cm), and ε is the bed void fraction.

These Equations could be solved analytically for the effluent concentration $C(z,t)$ and solid concentration $c_s(z,t)$. In this case, since the extraction curve as a dimensionless variable was defined to be the cumulative extracted amount of Mannitol per total amount of Mannitol in olive leaves, thus, it was essential to integrate the effluent flow rate as a function of time as follows:

$$\text{Extraction Yield} = \frac{m_M}{m_{M\infty}} = \frac{\int_0^t Cdt}{\int_0^\infty Cdt} \tag{7}$$

where m_M is the extracted Mannitol weight (g), and $m_{M\infty}$ is the maximum extractable Mannitol weight (g).

IV. Analytical Solution in the Laplace Domain

The model composed of (1) to (3) with two adjustable parameters was solved by Laplace Transform Technique. The final results were obtained in (8) and (9):

$$C(z,t) = \begin{cases} K_2\left[1+\exp(-K_1 t)\right], & 0 \le t \le \dfrac{\varepsilon z}{u_z} \\ K_2\left[1+e^{-K_1 t} - e^{-Az}\varphi_0\left(z, t-\dfrac{\varepsilon z}{u_z}\right) + e^{-Az}\varphi_{K_1}\left(z, t-\dfrac{\varepsilon z}{u_z}\right)\right], & t \ge \dfrac{\varepsilon z}{u_z} \end{cases} \tag{8}$$

$$c_s(z,t) = \begin{cases} h\times\left(K_2 + K_3 e^{-K_1 t}\right), & 0 \le t \le \dfrac{\varepsilon z}{u_z} \\ h\times\left[K_2 + K_3 e^{-K_1 t} - K_2 e^{-Az}\varphi_0\left(z, t-\dfrac{\varepsilon z}{u_z}\right) - K_3\varphi_{K_1}\left(z, t-\dfrac{\varepsilon z}{u_z}\right)\right] + c_{s0}e^{-Az}\varphi_{K_4}\left(z, t-\dfrac{\varepsilon z}{u_z}\right), & t \ge \dfrac{\varepsilon z}{u_z} \end{cases} \tag{9}$$

where

$$\varphi_\eta(z,t) = e^{-\eta t} + \sqrt{\frac{\gamma\delta z}{\lambda}}\int_0^t \frac{e^{-\eta(t-s)-\delta s}}{\sqrt{s}} I_1\left(2\sqrt{\gamma\delta z s}\right) ds \tag{10}$$

$$\lambda = u_z/\varepsilon \tag{11}$$

$$\gamma = k_f a/\varepsilon \tag{12}$$

$$\delta = (k_f a)/(h(1-\varepsilon)) \tag{13}$$

$$A = (k_f a)/u_z \tag{14}$$

$$K_1 = k_f a((1/\varepsilon)+(1/(h(1-\varepsilon)))) \tag{15}$$

$$K_2 = c_{s0}/(h+(\varepsilon/(1-\varepsilon))) \tag{16}$$

$$K_3 = c_{s0}/(h(1+h(1-\varepsilon)/\varepsilon)) \tag{17}$$

$$K_4 = k_f a/(h(1-\varepsilon)) \tag{18}$$

Where I_1 is the modified Bessel function of the first kind, φ_0, φ_{K_1} and φ_{K_4} were defined by (10) with $\eta = 0$, $\eta = K_1$, and $\eta = K_4$. The integral term in (10) was solved numerically by Simpson's method. Since the experimental extraction yields were based on the amount of Mannitol in the effluent of the extraction column, therefore, the axial coordinate (z) in the model was considered constant and equal to bed length (L). As a consequence, concentration profile and eventually, extraction yield in the model was only based on a single independent variable (time). The Simplex Optimization Method was applied in order to fit adjustable parameters using experimental measurements. The obtained values for the parameters from Simplex method were strongly dependent on selected initial data and subsequently the computer program converged output data was not always meaningful. Thus, physical evaluation and interpretation of Simplex method's final results was necessary.

V. Data Analysis

In order to quantify the experimental data deviation from model predicted data, the absolute average deviation (AAD) is defined:

$$AAD = \frac{1}{n}\sum_{i=1}^{n}\left|E_{exp} - E_{model}\right| \times 100 \tag{19}$$

where n is the number of data.

If AAD≤5, data are highly consistent, whereas AAD<10, data are probably consistent, and for AAD≥10, data are probably not consistent.

VI. Parameters

In this study, two important parameters, the equilibrium partition coefficient and the extraction yield, were used for optimization of operating conditions. At equilibrium, the partition of Mannitol between the solid phase and the mobile phase was determined by adsorption isotherm called equilibrium partition coefficient, K_d, as follows:

$$K_d = \frac{\dfrac{\text{Mass of Mannitol in olive leaves}}{\text{Mass of olive leaves}}}{\dfrac{\text{Mass of Mannitol in fluid phase}}{\text{Volume of fluid phase}}} \tag{20}$$

This is similar to the following:

$$K_d = \frac{\text{Volume of fluid phase}}{\text{Mass of olive leaves}} \tag{21}$$

Two mobile and stationary phases were considered in the modeling of the SWE. Considering the fact that the solute in the solid matrix (Mannitol in olive leaves) is extracted by water, thus it can be assumed that the mass of Mannitol in the mobile phase (subcritical water) would finally be the same as the mass of Mannitol in the stationary phase (olive leaves). Multiplying the equilibrium partition coefficient (21) by the density of subcritical fluid at the system operating conditions

results in units of (g of water)/(g of olive leaves). Therefore, equilibrium partition coefficient determines the amount of subcritical water needed to extract Mannitol from 1 g of olive leaves.

$$K_d = \frac{Mass\ of\ subcritical\ water}{Mass\ of\ olive\ leaves} \quad (22)$$

It is imperative to realize that the K_d defined in this study is not the thermodynamic partition coefficient. The thermodynamic partition coefficient is based on the ratio of mole fraction of Mannitol between two phases, namely, the mobile phase (subcritical water) and the stationary phase (olive leaves). Without a definitive molecular weight for olive leaves, the thermodynamic partition coefficient for the subcritical extraction of Mannitol will remain undefined.

In this study, extraction yield was defined as follows:

$$\text{Extraction Yield} = \frac{\text{Mass of Extracted Mannitol}}{\text{Total Mass of Mannitol in olive leaves}} \times 100 \quad (23)$$

The mass transfer coefficient was estimated using the empirical correlation reported by Wakao for conventional processes [28]:

$$N_{Sh} = 2 + 1.1 N_{Sc}^{1/3} N_{\mathrm{Re}}^{0.6} \quad (24)$$

where N_{Sh} is Sherwood number ($(2R_p k_f)/D_p$), N_{Re} is Reynolds number ($(2R_p v\rho_f)/\mu_f$), N_{Sc} is Schmidt number ($\mu_f/(\rho_f D_m)$), D_m is the diffusion coefficient of the organic in the subcritical water (cm^2/s), D_p is the effective diffusivity in shredded olive leaves' pores (cm^2/s), R_p is the particle radius (cm), v is the interstitial fluid velocity (cm/s), ρ_f is the fluid density (g/cm^3) and μ_f is the fluid viscosity (g/cm.s).

The effective pore diffusivity in the particle was given by [29]:

$$D_p = \frac{\varepsilon_p}{\tau_p} D_m \quad (25)$$

where τ_p is the tortuosity factor [30]. The tortuosity factor of solids is usually in the range of 2–8 and the value of constant 3 was used for Mannitol in this study. The diffusion coefficient of Mannitol in subcritical water was obtained using Rohr's experimental data [31].

The particle porosity, ε_p, was defined as:

$$\varepsilon_p = \frac{V_{pores}}{V(1-\varepsilon)} \quad (26)$$

Thus, the effective pore diffusivity was evaluated from (25) using the values of molecular diffusivity, particle porosity, and tortuosity factor.

The axial dispersion coefficients (D_L) for water and carbon dioxide in packed beds (cm^2/s) have been measured by Bear [32], Ghoreishi [33], and Nauman [34]. In this research, the available data from Nauman's study was used for the axial dispersion coefficient. Also the experimental data of Rohr's study was utilized for the densities and viscosities of subcritical water. The bed porosity of the packed column was experimentally measured to be 0.4.

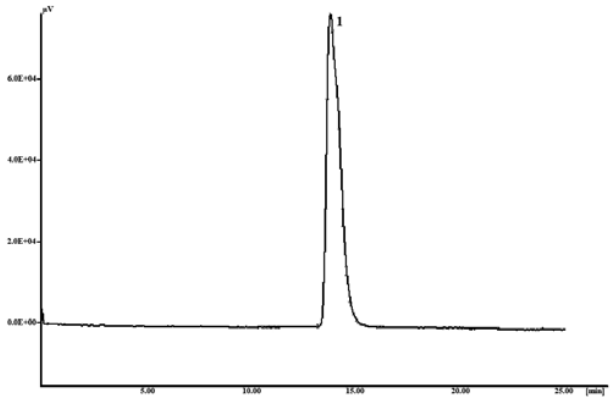

Fig. 2 HPLC spectrum of the pure Mannitol

VII. Results and Discussion

A. HPLC Quantification of Olive Leaves

The olive leaves samples prior to extraction were dried at 70°C for a period of 30 min and weighed and this procedure repeated until the weight of samples became constant which assured of no water content.

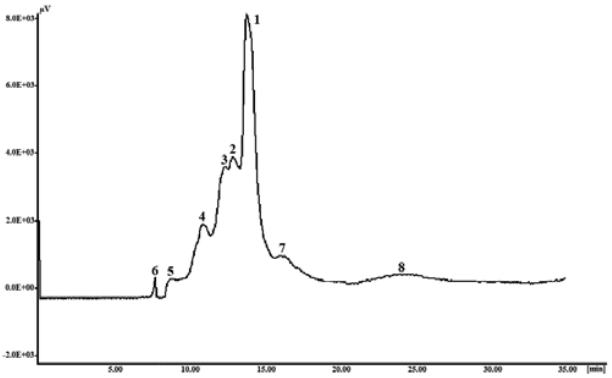

Fig. 3 HPLC spectrum of the extracted sample

The pure Mannitol spectrum is shown in Fig. 2. The data shows that the Mannitol (1) is detected with a high intensity peak at 14.3 min. Similar HPLC spectrum for extracted sample is shown in Fig. 3. A high intensity peak (1) is also detected at 14.3 min which is the indication of Mannitol content of the extract and other constituents are shown with peaks 2, 3, 4, 5, 6, 7, and 8 that may be the indication of different structures of sugar compounds.

By calculating the area under the peak detected at 14.3 min the amount of Mannitol quantified. In order to obtain a calibration curve, the different concentration of pure Mannitol was injected into HPLC and the area under the Mannitol peak was measured and the results are sketched in Fig. 4. A linear calibration was fitted into the data. For quantification of Mannitol content in the extracted samples, the obtained area

under the peak was compared with the linear calibration of Fig. 4. Besides Mannitol (1), which was always one of the soluble carbohydrates in leaf extracts, gas chromatography analyses revealed the occurrence of mannose, glucose, myo-inositol, sucrose and other unidentified soluble carbohydrates in low concentrations. The extraction yield was calculated using (21), Fig. 4, and the data obtained in regard to the area under the Mannitol peak.

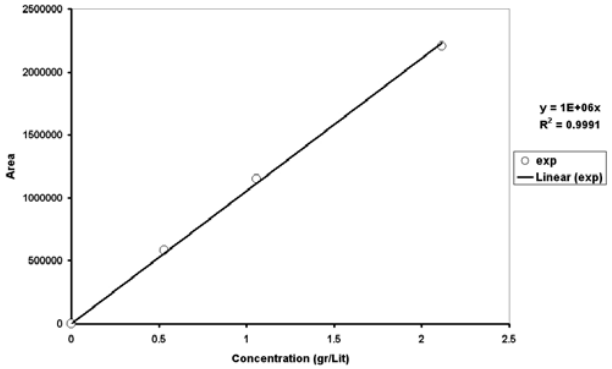

Fig. 4 HPLC calibration curve

B. Soxhlet Extraction

Mannitol was extracted from shredded olive leaves in Soxhlet extractor with ethanol. The results indicated that the extraction yield of Mannitol in 8 hr was 57.34% (w/w) and also the equilibrium partition coefficient for the same period was 194.4 g of ethanol/g of olive leaves.

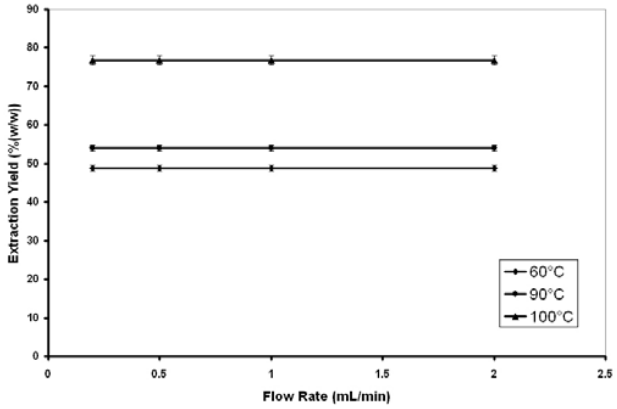

Fig. 5 Effect of water flow rate on the extraction yield at constant pressure of 50 bars and different temperatures of 60, 90, and 100°C

C. Effect of Subcritical Water Flow Rate on Yield and Equilibrium Partition Coefficient

The effect of various flow rates on the extraction yield at constant pressure of 50 bars and different temperature of 60, 90, and 100°C is shown in Fig. 5. The results demonstrate that the extraction yield does not depend on water flow rate, but increases with increasing temperature. In this regard, investigation of two important and effective parameters, mass transfer coefficient and residence time, can clarify the observed phenomenon. Increasing water flow rate at 60°C enhances the mass transfer coefficient as shown in Fig. 6. This behavior is well expected because higher flow rate directly decreases the mass transfer resistance via reduction of water film thickness around the solid particles (shredded olive leaves). By considering this single parameter, one can expect extraction yield enhancement as a function of flow rate, but it is vital to investigate simultaneously the opposite effect of residence time on extraction yield. In other words, higher flow rate decreases the residence time and, consequently, lower yield can be predicted. Therefore, the simultaneous counter effects of these two parameters cancel each other and subsequently in the selected operating flow rates of this study no significant changes on extraction yield are observed.

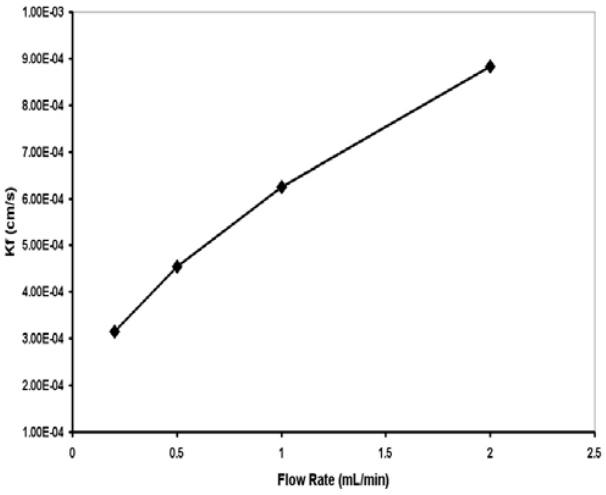

Fig. 6 Effect of water flow rate on mass transfer coefficient

At constant pressure of 50 bars, and different temperatures of 60, 90 and 100°C, the equilibrium partition coefficient was measured at four different flow rates of 0.2, 0.5, 1.0 and 2.0 mL/min and the results in Fig. 7 show that the equilibrium partition coefficient for water-Mannitol-olive leaves system is independent of the flow rate, but decreases with increasing temperature. The obtained result is well expected because flow rate of water does not affect Kd in any way according to the definition of equilibrium partition coefficient (Equation (22)). In other words, the involved parameters in (22), mass of water and mass of olive leaves, are not function of solvent flow rate.

D. Effect of Subcritical Water Temperature on Yield and Equilibrium Partition Coefficient

The effect of different operating temperatures on the extraction yield at constant pressure, flow rate, and extraction time of 50 bars, 0.2 mL/min, and 37.5 min, respectively, is shown in Fig. 8. The results indicate that increasing operating temperature from 60 to 100°C increases the extraction yield

from 48.75 to 76.75% (w/w). However, higher operating temperature from 100 to 150°C resulted in reduction of extraction yield from 76.75 to 64.68% (w/w). Three distinguishable regions with various trends of yields are observed in this Figure. The first region has a mild increasing slope, the second one with a sharp enhancing slope, and finally the third region with a mild decreasing slope in the temperature ranges of 60-90, 90-100, and 100-150°C, respectively. Four different parameters are effective in these observed trends: mass transfer coefficient, Mannitol solubility, Mannitol degradation, and hydrogen-bond breakage.

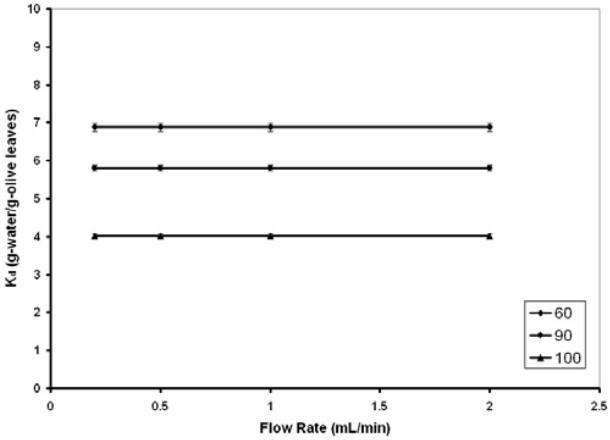

Fig. 7 Equilibrium partition coefficient as a function of water flow rate at constant pressure of 50 bars and different temperature of 60, 90, and 100°C

Fig. 9 shows the effects of different temperatures on mass transfer coefficient by using the data generated via (24). It is clear that an increase in temperature enhances the mass transfer coefficient. Higher mass transfer coefficient results in higher rate of Mannitol transfer from solid matrix to bulk fluid. This seems to be the only parameter affecting extraction yield in the first region. In the second region, in addition to mass transfer coefficient, it is obvious that higher solubility of Mannitol in subcritical water [9], [35], and [36] results in higher rate of Mannitol transfer and thus higher extraction yield.

On the other hand, in the third region, the Mannitol extraction yield decreased and it was observed that at higher temperature range of 120-150˚C an extract with burning smell was produced. Therefore, the lower obtained yield in this region may be the result of degradation of some of constituents such as Mannitol due to burning. Another likely explanation of the behavior of the extraction yield in the third region relies on breakage of water hydrogen bonds [11]. The lower hydrogen-bond concentration in the subcritical water reduces the capability of solubilizing the polar compounds such as Mannitol.

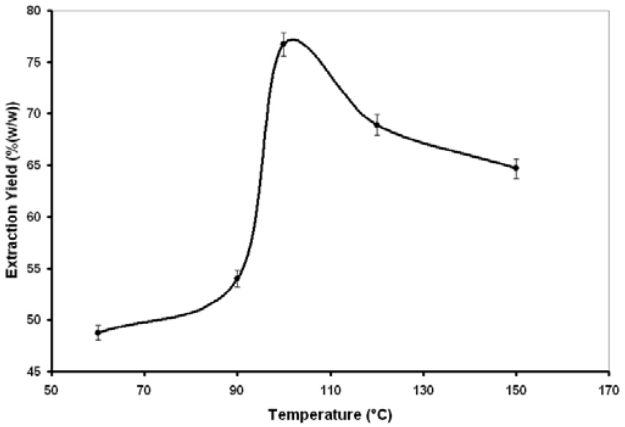

Fig. 8 Extraction yield of Mannitol as a function of temperature at constant pressure of 50 bars, flow rate of 0.2 mL/min, and extraction time of 37.5 min

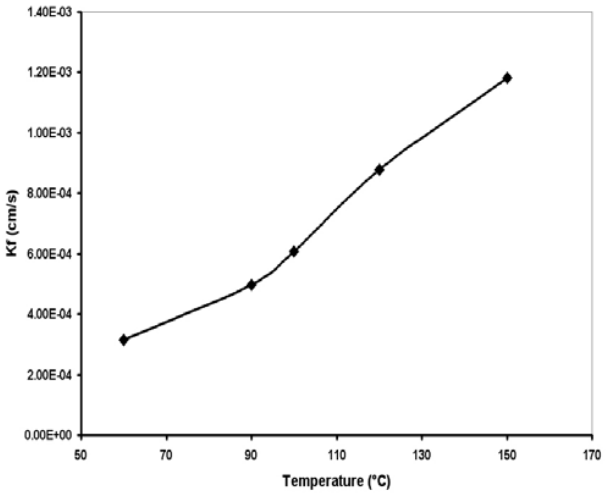

Fig. 9 Mass transfer coefficient as a function of temperature

The variation of equilibrium partition coefficient as a function of temperature at constant pressure, flow rate, and extraction time of 50 bars, 0.2 mL/min, and 37.5 min, respectively, is given in Fig. 10. Consistent with the physicochemical phenomena of the results obtained in Fig. 8, three distinguishable regions with various trends of equilibrium partition coefficient are also observed in Fig. 10. The first region has a mild decreasing slope, the second one with a sharp reducing slope, and finally the third region with a mild increasing slope in the temperature ranges of 60-90, 90-100, and 100-150°C, respectively. The same aforementioned four different parameters of mass transfer coefficient, Mannitol solubility, Mannitol degradation, and hydrogen-bond breakage are effective in these observed trends.

The results of Fig. 10 indicate 71.6% reduction in equilibrium partition coefficient by increasing operating temperature from 60 to 100°C.

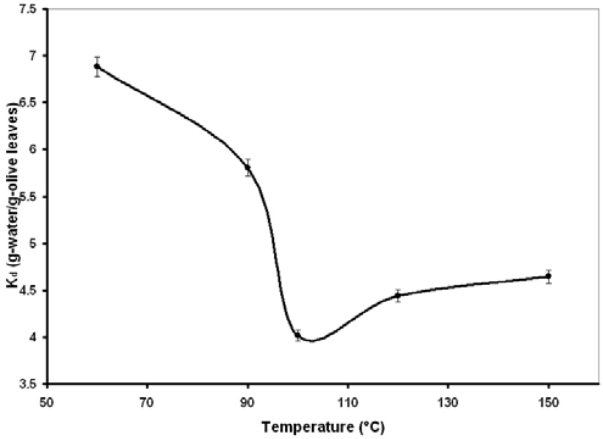

Fig. 10 Effect of temperature on the equilibrium partition coefficient at constant pressure of 50 bars, flow rate of 0.2 mL/min, and extraction time of 37.5 min

However, higher temperature of 100-150°C shows 15.7% increase in K_d. Operation in the subcritical water temperature range of 60-150˚C indicates that the equilibrium partition coefficient has an optimum condition (4.0 g of water per g of olive leaves) at the temperature of 100˚C. Operation of extraction column at optimum condition clearly shows a better economic incentive in terms of operating cost of the solvent, pump, heating, and electricity. For instance, the decrease of equilibrium partition coefficient (6.9 to 4.0 g of water per g of olive leaves) demonstrates that 2.9 g of less solvent is needed for extraction of Mannitol from 1g of olive leaves at 100˚C compared to 60˚C.

These results are attributed to the variation of the mass of subcritical phase to the solid phase which is a direct contribution of the mass transfer coefficient and solubility enhancement in contrast to hydrogen-bond breakage and the degradation of constituents by increasing temperature.

E. Effect of Subcritical Water Pressure on Yield and Equilibrium Partition Coefficient

Fig. 11 shows the effect of varying operating pressures on the extraction yield at constant temperature, flow rate, and extraction time of 100°C, 0.2 mL/min, and 37.5 min, respectively. It is clear that variation of the operating pressure results in an optimum point for the extraction yield at 50 bars. An increase in pressure from 30 to 50 bars boosts the extraction yield by 5.9%. However, increasing the pressure from 50 to 110 bars reduces the extraction yield by 33.5%. In the pressure range of 30-110 bars, even though density, viscosity, diffusion coefficient, and surface tension of water do not vary significantly, the experimental data of this study reveals that the extraction yield has two completely distinguished regions, 30-50, and 50-110 bars. The behavior of the extraction yield in different pressures at constant temperature for different polar and nonpolar adsorbates shows different increasing or decreasing trends. The findings of this study are in agreement with the results obtained in extraction of six herbicides [37].

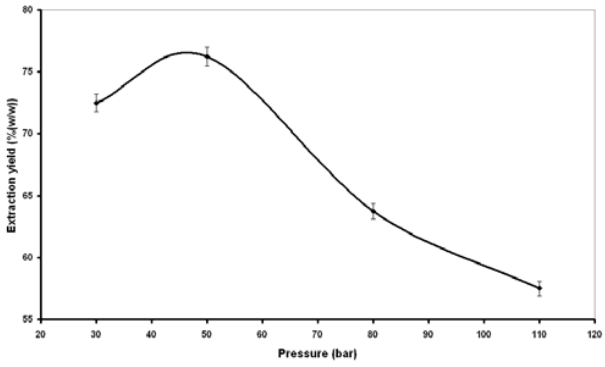

Fig. 11 Extraction yield of Mannitol as a function of pressure at constant temperature of 100°C, flow rate of 0.2 mL/min, and extraction time of 37.5 min

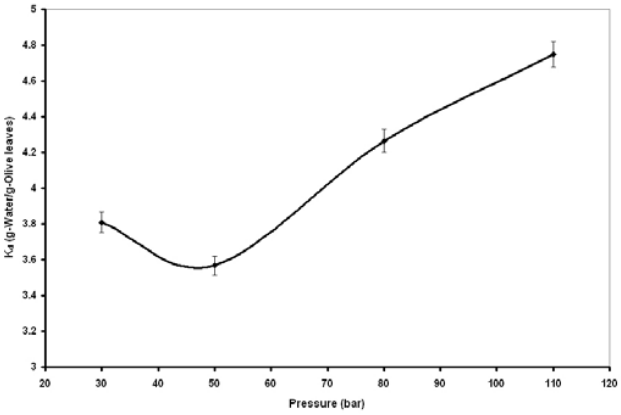

Fig. 12 Effect of pressure on the equilibrium partition coefficient at constant temperature of 100°C, flow rate of 0.2 mL/min, and extraction time of 37.5 min

The effect of different pressures on equilibrium partition coefficient is illustrated in Fig. 12 at constant temperature, flow rate, and extraction time of 100°C, 0.2 mL/min, and 37.5 min, respectively. The optimum (minimum) value of 4.0 g of water/g of olive leaves for K_d is obtained at pressure of 50 bars, which is consistent with the data of Fig. 11. It is observed that the equilibrium partition coefficient decreases 7.0% in the range of 30-50 bars and then increases 33.2% up to 110 bars. These results are attributed to the aforementioned findings in regard to the effect of pressure on extraction yield. It is imperative to realize that extraction at 100°C and 50 bars instead of ambient pressure provides a more preferred operating condition in which liquid water rather than its vapor is used as the solvent in the process.

F. Effect of Reynolds and Peclet Numbers on Yield

The effect of different Reynolds numbers of the packed column on the extraction yield at constant pressure of 50 bars, and different temperatures of 60, 90 and 100˚C is shown in Fig. 13.

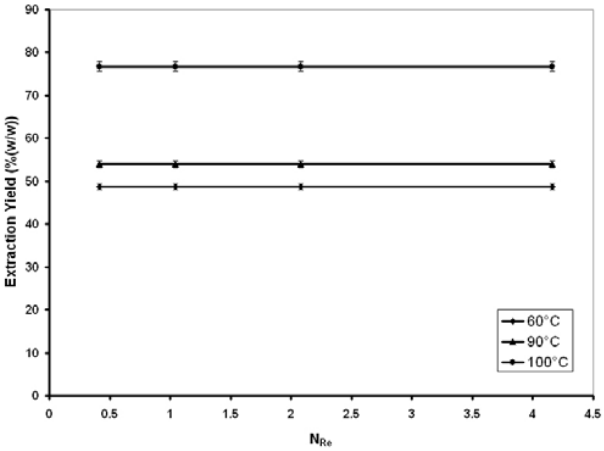

Fig. 13 Effect of Reynolds number on the extraction yield at constant pressure of 50 bars, and different temperatures of 60, 90 and 100˚C

The dimensionless Reynolds number was chosen as the independent variable (x-axis) because of the many advantages of using dimensionless parameters as it is common practice in the literature of chemical engineering to utilize dimensionless form so that the number of variables can be reduced. It is apparent that the extraction yield is independent of Reynolds number for each constant temperature. Considering the fact that the physical properties (density and viscosity of water) at each temperature and particle diameters are constant, therefore, the only parameter responsible for increasing the Reynolds number is variation of the fluid velocity through extraction bed. Since the Reynolds numbers range is well within the fully-developed laminar flow ($0.5 \le N_{Re} \le 4.2$) for this study, thus flow rate variation can not affect extraction yield. But of course, operation in much higher N_{Re}, for instance greater than 2100, causes axial and/or radial dispersions to hinder the extraction process; in the case of fully developed turbulent flow, lower yield is expected. Thus it is necessary to investigate and locate the optimum flow velocity in order to have an appropriate and reasonable production rate as well as avoiding any disadvantages because of dispersion creation.

The effect of particles and packed bed Peclet numbers (Pe_p is Peclet number for shredded olive leaves ($(2R_pv)/D_L$), and Pe_b is Peclet number for the bed ($(Lv)/D_L$)) on the extraction yield at constant pressure of 50 bars and different temperatures of 60, 90, and 100˚C is shown in Figures 14 and 15, respectively. The results of both Figures show that by increasing Peclet numbers via changes in fluid velocity, the extraction yield does not vary. Even though it is obvious that by increasing velocity, higher dispersion lowers the extraction efficiency, but in this case no such phenomenon is observed. The explanation of this behavior is due to the fact that the overall hydrodynamic process conditions is well within the fully developed laminar flow and in this region dispersion coefficient is the same as diffusion coefficient and therefore, the obtained experimental data is compatible and consistent with theoretical analysis. In both Figures, higher temperature increases yield, which is expected because of enhanced mass transfer coefficient and solubility.

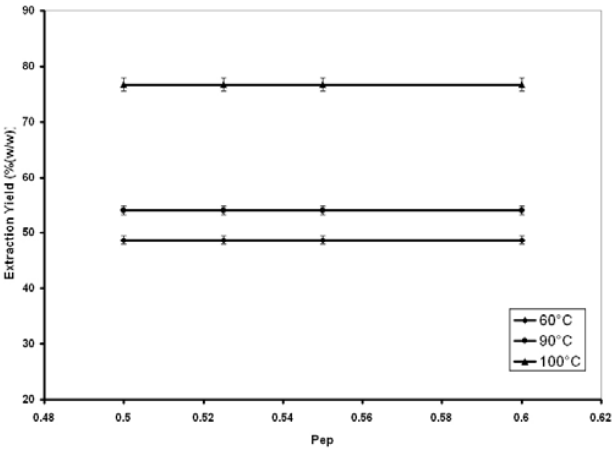

Fig. 14 The extraction yield as a function of particle Peclet number at constant pressure of 50 bars, and different temperatures of 60, 90 and 100˚C

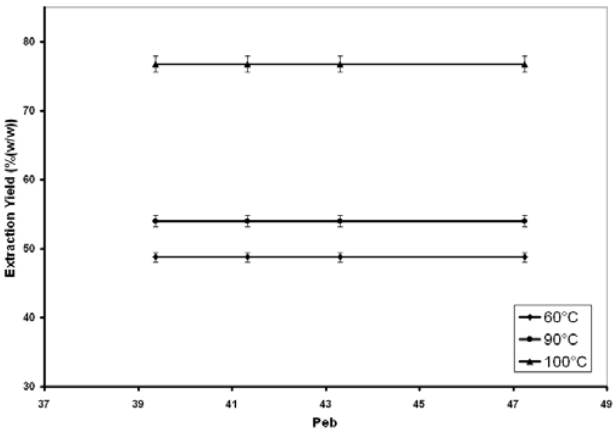

Fig. 15 Effect of packed bed Peclet number on the extraction yield at constant pressure of 50 bars, and different temperatures of 60, 90 and 100˚C

G. Modeling Versus Experimental Measurements

The predicted extraction curve and the profile of three independent experimental measurements are shown in Fig. 16. In order to investigate the applicability of the mathematical model, the theoretical results are compared with experimental measurements obtained at optimum conditions (50 bars, 100˚C, and 0.2 mL/min). Fig. 16 shows that the modeling extraction yield profile is increasing rapidly in the period of 0-37.5 min and thereafter in the second region (37.5-93 min) the slope reduces until reaches a constant trend in the third period of 93-250 min. In the first region, because of high Mannitol concentrations in the olive leaves and therefore, high mass transfer driving force, high desorption rate of Mannitol from solid matrix occurs.

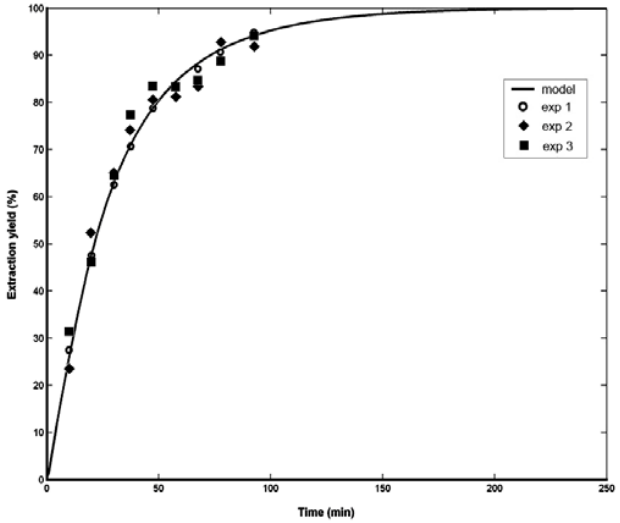

Fig. 16 Comparison of three independent experimental measurements at optimum conditions (50 bars, 100˚C, and 0.2 mL/min) and modeling data

Subsequently, lower mass transfer flux in the second period results in milder slope of Mannitol desorption. Finally, no more driving force exists in the last period of extraction, which results in a flat desorption profile. The obtained theoretical and experimental profiles suggest that 37.5 min could be the optimum extraction time in order to achieve an economic incentive yield in which the slope of extraction curve becomes steady and further extraction beyond 37.5 min, despite of more cost, does not bring about appropriate higher yield.

TABLE I
ADJUSTABLE PARAMETERS AND INPUT DATA FOR EXTRACTION MODEL

Input Data				*Adjustable Parameters*			
$c_{s0}(mol/cm^3)$	C_0	ε	L(cm)	u(cm/min)	$k_f a(min^{-1})$	h	AAD(%)
1	0	0.4	12.5	0.314	0.0098	0.55	1.2

The experimental data obtained up to 100 min is quite compatible with the modeling prediction, which is the indication of viability and authenticity of the developed model.

The estimated model parameters are presented in Table I, including mass transfer coefficient (k_f) and equilibrium coefficient (h). Table I also shows the modeling input data which are needed to initiate the software program. The 1.2% absolute average deviation (AAD) is calculated using model's data and the average experimental data obtained from three independent measurements. Using the first and second moments of experimental measurements, the standard deviation of $\pm 1.5\%$ for the worst case is reported. Utilizing this validated model, the investigation of the effect of different operating conditions on extraction yield and equilibrium partition coefficient can be conducted without performing any additional time-consuming and expensive experiments.

VIII. Conclusion

In this study, the experimental and theoretical extraction of Mannitol from olive leaves using subcritical water was investigated. In this procedure, the optimum extraction yield of 76.75% (w/w) was obtained at temperature of 100°C, pressure of 50 bars, extraction time of 37.5 min. In addition, Soxhlet solvent extraction yield was obtained 57.342% (w/w) at extraction time of 8 hr. It is clear that SWE method provides a higher extraction yield than Soxhlet method. Furthermore, water in contrast to ethanol as the solvent is cost effective and also superior in terms of toxicity, flammability, and availability. Equilibrium partition coefficients for SWE at optimum condition and for Soxhlet method were obtained 4.01 g water/g olive leaves and 194.39 g ethanol/g olive leaves, respectively. Therefore, equilibrium partition coefficient as an economical advantage based on lower use of solvent is another incentive of SWE method.

It is concluded that SWE is a viable method for extraction of Mannitol from olive leaves. The moderately high temperature of SWE process needed to increase the solubility of Mannitol in water may be pinpointed as the only shortcoming of this process. However, the results indicated that the SWE process conditions for a large-scale industrial extraction system do not require a high pressure to obtain optimum extraction yield.

Acknowledgment

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