Optimization of Arecoline Extraction from Areca Nut Using Supercritical Carbon Dioxide

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ABSTRACT

The mass transfer of arecoline using supercritical carbon dioxide (SC-CO_2) from areca nut is studied by analyzing the diffusion coefficient derived from Crank equation for sphere model. Comparisons of the SC-CO_2 extracted yield under various conditions showed that the combination of pressure at 10.3 MPa and temperature of 50°C provided the optimum condition for extraction, followed by extraction at 40°C and 25.6 MPa. From the diffusion coefficient analysis, the optimum conditions also gave the highest coefficients. It was found that the yield is directly proportional to the values of the coefficients.

Key words: areca nut, arecoline, supercritical carbon dioxide, mass transfer, modeling

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INTRODUCTION

Supercritical Fluid Extraction (SFE) is a highly suitable technique to extract bioproducts from their natural matrices. Carbon dioxide is the most common solvent used in SFE and possesses several advantages compared to other solvents. SFE is a unit operation that exploits the unique properties of solvents above their critical values to extract soluble components from a mixture. The application of SFE in the extraction and separation of low vapor pressure chemicals and poly functional intermediate components from their natural matrices have been reported by many researchers[1-3].

One reason for the interest in the technique is that SFE offers the potential advantages of higher yields and better quality product especially in pharmaceutical, chemical and biotechnological processes. The supercritical solvent is chosen based on the ability to solubilize the desired product from the mixture, inert with respect to the raw material and itself under the extraction conditions which have a moderate critical pressure to minimize compression costs and have a low boiling point for ease of

removal after processing. It is also inexpensive, nontoxic, nonflammable and readily available in high purity[4,5].

Carbon dioxide meets most of the criteria outlined above. However, it is not a particularly powerful solvent for materials of low volatility when compared to the common organic liquid solvents. Carbon dioxide can be a relatively selective solvent. The selective extraction of low-to-medium volatility materials becomes possible by subsequent manipulation of the operating conditions. The critical point of CO_2 is at 31°C and 7.28 MPa, which are relatively moderate conditions in terms of the process equipment required and potential product damage[4-5].

Areca nuts are usually used in betel chewing common among the Indians. In India and China, areca nut has been used as an anathematic in man and animals for a long time. Taken with the juice from betel leaves, areca nut has a stimulating effect on the central nervous system. In addition to treating tapeworm and roundworm[6], areca nut also has astringent properties and induces bowel movement. Many Asians use this tonic agent to protect against the ill-effect of their food. The alkaline juice of the betel neutralizes the acid decomposition products of their food in the stomach, an act as an astringent hardening the mucous membrane of the stomach.

In traditional Indian medicine, the unripe fruit is reported to have cooling, laxative and carminative

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properties. Dried areca nuts are claimed to sweeten the breath, strengthen the gums, remove bad taste and produce a stimulant and exhilarant effect on the system to improve appetite and taste. The Chinese employed areca nuts in constipation and serious swelling. The use of areca nuts was recommended in calculus and urinary disorders and as an aphrodisiac in the form of a decoction with other aromatic and stimulant substances. When the nut is burnt and powdered, it was used as a dentifrice[6]. As early of 1886, it was discovered that the active principle of areca nut was alkaloidal in nature and since then six alkaloids have been characterized: arecoline, arecaidine, guvacine, guvacoline, isoguvacine and arecolidine. The structure of four alkaloids has been completely elucidated, but structure of arecolidine and the homogeneity of isoguvacine are uncertain.

Arecoline, $C_8H_{13}O_2N$, colorless oil with a strongly alkaline reaction, volatile in steam and forms well-defined salts, is the major alkaloid component in areca nut. The chemical structure of arecoline is shown in Figure 1. Arecoline is chemically the methyl ester of N-methyltetrahydronicotinic acid which contains a Δ3-ethylenic bond. This characteristic is the basis of the possibility that this alkaloid might have carcinogenic properties[7] Arecoline is a cholinomimetic agent which stimulates muscarinic receptors and therefore has a stimulating effect on the parasympathetic system. Typical parasympathomimetic effects observed after administration of arecoline are excessive salivation, stimulating of peristalsis and an increased tone and also rhythmicity of small and large intestines, as well as tremor. Arecoline also increase plasma free fatty acids and reduces the plasma blood sugar concentration. The aim of this study is to determine the mass transfer of arecoline using supercritical CO_2 and analyze the diffusion coefficient with Crank equation.

MATERIALS AND METHODS

I. *Sample Preparation*

Six areca nuts were used to determine the most effective condition for the extraction of arecoline using supercritical carbon dioxide. The areca nuts were dehusked and the kernels were weighed and soaked in water for 24 hrs to facilitate the extraction of arecoline. After soaking, the kernels were weighed again. Ten grams of areca nuts were put into an extraction vessel together with the water used for soaking to ensure all traces of arecoline was being transferred.

II. *Extraction of Arecoline using SC-CO₂*

Commercial liquefied CO_2 (purity, 99.9%) was purchased from Malaysian Oxygen Ltd., Penang, Malaysia. CO_2 passed through a silica gel column was
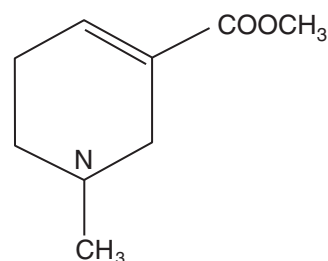

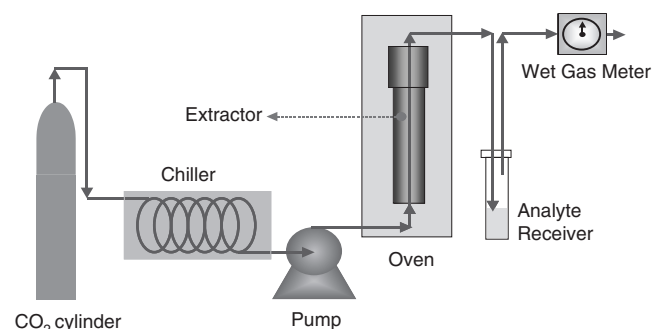**Figure 1.** Chemical structure of arecoline.

Figure 2. Schematic diagram of supercritical fluid extraction of arecoline.

cooled, compressed and pumped into the system by a high pressure pump. The temperature was controlled at 40°C and 50°C using a temperature controller (Yamatake Honeywell, COSMOPAK SA 30, Japan). Pressure in the cell varied from 10.0 MPa to 25.6 MPa by a back pressure regulator (Jasco Co., Model 880-81, Japan) shown in Figure 2. Six different combinations of temperature and pressure were investigated in the extraction of arecoline from the areca nuts over a period of 24 hours. The temperature and pressure combinations were 40°C and 25.6 MPa, 40°C and 20 MPa, 40°C and 10.3 MPa, 50°C and 25.6 MPa, 50°C and 20 MPa, and 40°C and 10.3 MPa A metering valve was used to regulate the flow rate at 2 mL/min. The gas flow rate was measured by a dry gas meter.

III. *Quantitative and Mass Transfer Analysis of Arecoline*

Arecoline was analyzed using Hewlett Packard gas chromatograph with a flamenionization detector (Hewlett Packard, USA). A standard calibration curve for arecoline was first prepared. Pure arecoline of known concentration was analyzed together with an internal standard, methyl *n*-caprylate ($CH_3(CH_2)_6COOCH_3$). Methyl *n*-caprylate was mixed with a series of concentrations of arecoline which ranged from 0.002 g/L to 0.0000125 g/L. From the chromatogram obtained, the ratio of the area of the peaks for arecoline and internal standard was determined. A standard calibration curve was constructed

by plotting the ratio of the arecoline area to the internal standard area ((arec.) / A(Int. Std.)) *versus* the ratio of the arecoline concentration to the internal standard concentration ([Arecoline] / [Int. std.]). The arecoline content extracted from the tested samples was determined from the standard calibration curve. Mass transfer of the removal of arecoline from its natural matrix, i.e. areca nut, was studied using the diffusion equation for sphere matrix derived by Crank[8] as we considered the areca nuts to be spheres with mean radius of 1.5 cm.

The equation is

$$m/m_o = (6/\pi^2)\sum_{n=1}^{\infty}(1/n^2)\exp(-n^2\pi^2 Dt/r^2)$$

where m is the mass of extractable material in the sphere that remains in the matrix sphere after extraction for time, t; m_o is the initial mass of extractable material; n is an integer and *D* is the diffusion coefficient of the material in the sphere (arecoline in this case). This equation was solved using a Turbo Pascal program to get the *D* value. From the data obtained, graphs of Log [Arecoline] *vs.* time, and graphs of Log *D vs.* time were plotted. The performance of each condition was evaluated from those graphs.

RESULTS AND DISCUSSION

I. *Extraction at Constant Pressure*

Three different pressures were tested for the extraction procedure: 10.3, 20.0 and 25.6 MPa which are higher than the critical pressure of CO_2 at 7.3 MPa. These pressures were similarly used for the extraction of palm kernel oil from palm kernels by Nik Norulaini *et al.*[21]. Arecoline was easily extracted within this low to middle pressure range when 40°C and 50°C were applied to each pressure. At a constant pressure of 10.3 MPa, higher arecoline concentration was extracted at 50°C than 40°C (Figure 3A). Corollary to that the *D* value was higher at 50°C (Figure 3B). The increase in the diffusivity could enhance the ability of CO_2 to diffuse through the sample and dissolve more solutes.

Conversely, at a constant pressure of 20.0 MPa, extraction at 40°C resulted in higher arecoline concentration and consequently greater *D* value than for the extraction at 50°C (Figures 4A and 4B). Similar trend in the retrograderbehavior was also observed by Saldana *et al.*[9]. in the extraction of caffeine from guarana seeds at 10.3 MPa. The CO_2 density increased with temperature and thus lowered its diffusivity. In addition, higher density of CO_2 might also result in the channeling effect where the solvent is pushed through the sample matrix without actually diffusing through the sample pores.

Similarly at 25.6 MPa, higher yield of arecoline could be extracted with larger *D* value at 40°C than at 50°C, although extraction at 40°C started with lower arecoline content and lower *D* value (Figures 5A and 5B). This was in contrast to the caffeine extraction findings by Saldana *et al.*[9]. who reported that the quantity of yield increased with temperature at higher pressure. Specifically, the increase in vapor pressure of caffeine due to the increase in temperature compensated the decrease in the solubility arising from the diminishing
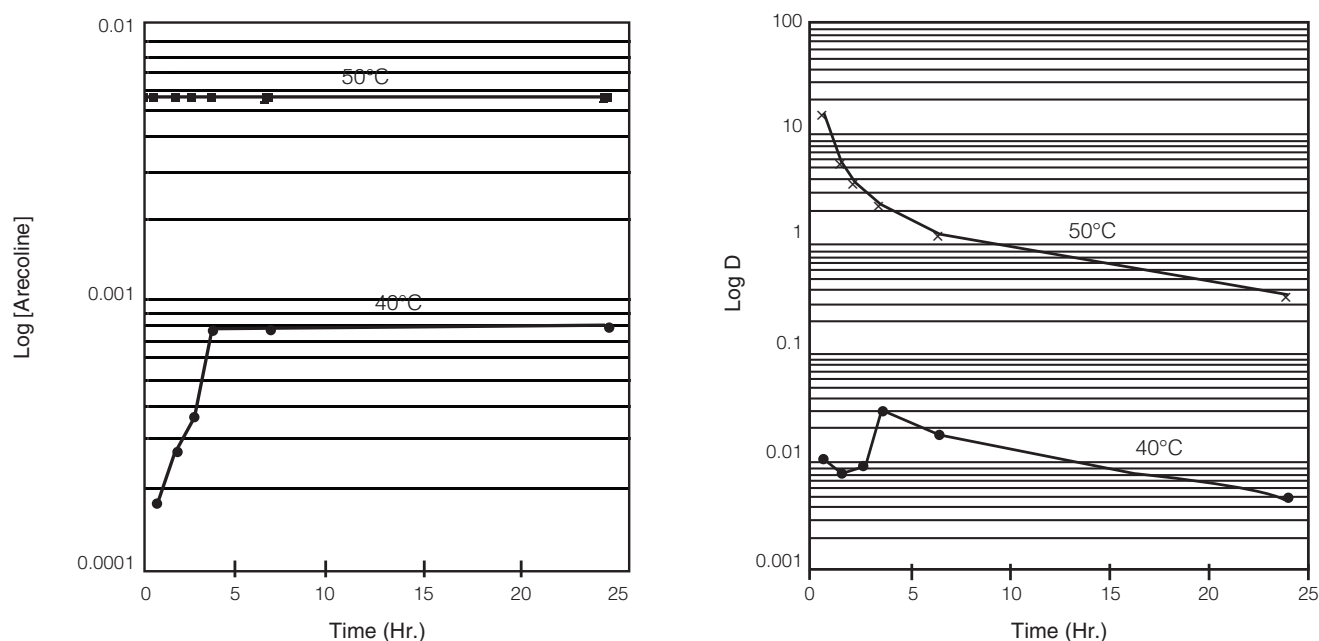
Figure 3. (A) Cumulative Log [Arecoline] (g/L) *vs.* time (hr) at a constant pressure of 10.3 MPa and extraction temperatures 40°C and 50°C. (B) Log D *vs.* time (hr) for extraction at a constant pressure of 10.3 MPa and extraction temperatures 40°C and 50°C.
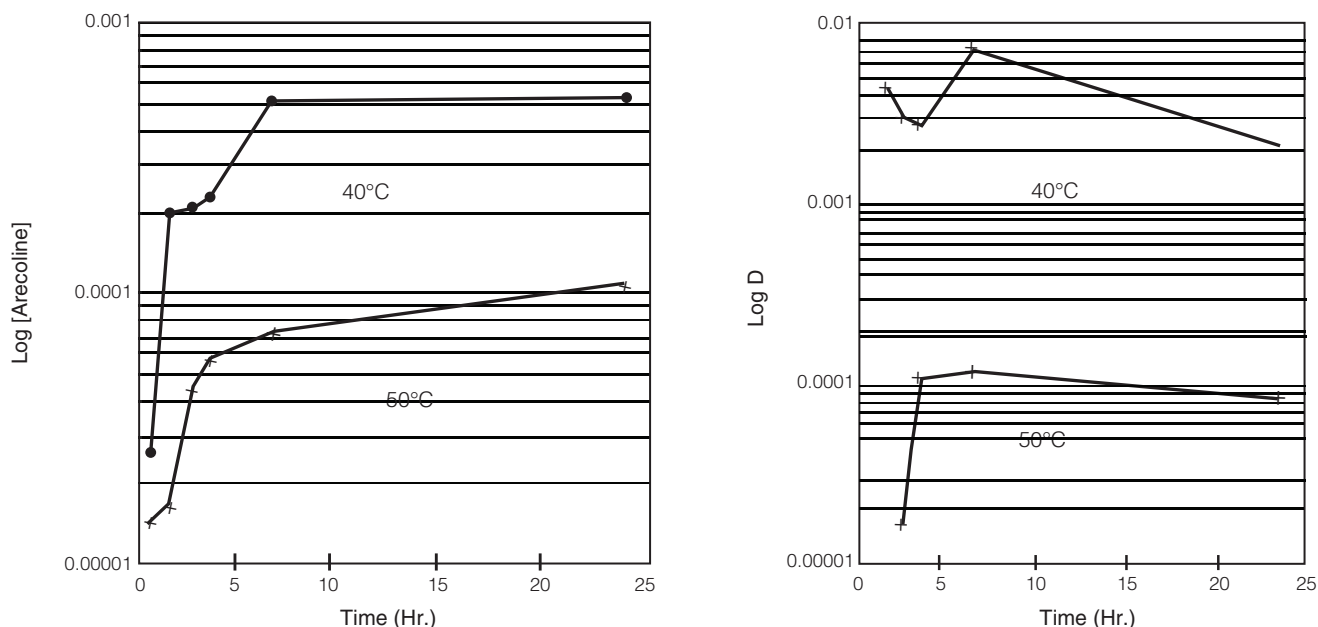
Figure 4. (A) Cumulative log [Arecoline] (g/L) *vs.* time (hr) at a constant pressure of 20.0 MPa and extraction temperatures 40°C and 50°C. (B) Log D *vs.* time (hr) for the extraction at a constant pressure of 20.0 MPa and extraction temperatures 40°C and 50°C.
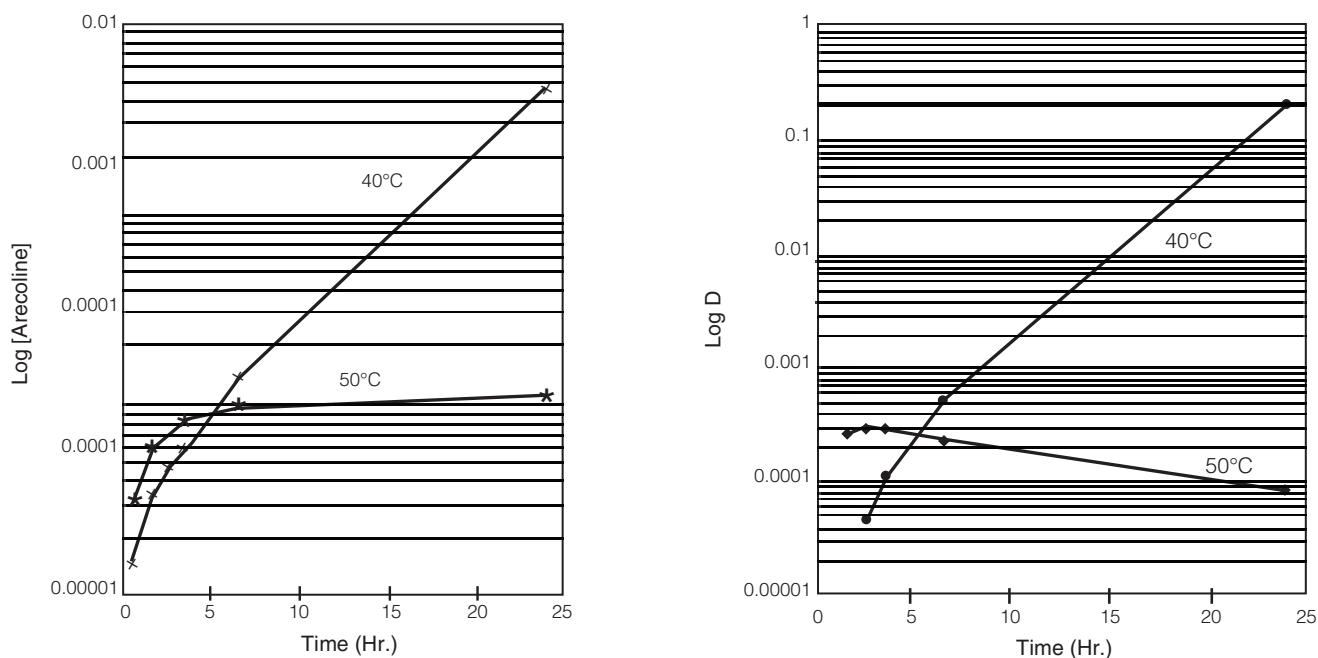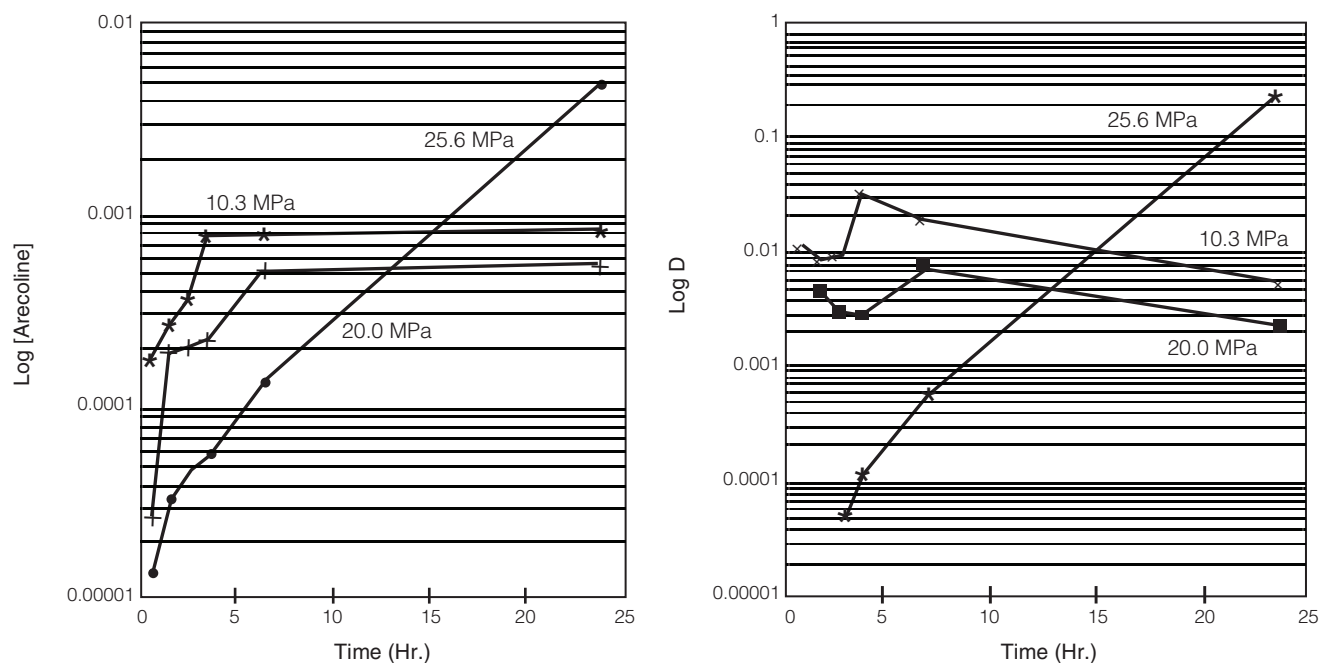

Figure 5. (A) Cumulative Log [Arecoline] (g/L) *vs.* time (hr) at a constant pressure of 25.6 MPa and extraction temperatures 40°C and 50°C. (B) Log D *vs.* time (hr) for the extraction at a constant pressure of 25.6 MPa.

supercritical fluid density at higher temperature.

II. *Extraction at Constant Temperature*

Two extraction temperatures of 40°C and 50°C were examined since arecoline is stable at these temperature

which are higher than 31°C, the critical temperature of carbon dioxide. For extraction temperature of 40°C, the highest yield was obtained at 25.6 MPa followed by extraction at 10.3 MPa and then 20.0 MPa. The increment in pressure was not proportional to the yield; the pattern change was unpredictable (Figure 6A). Similarly the

Figure 6. (A) Log [Arecoline] *vs.* time (hrs.) for extraction at a constant temperature of 40°C. (B) Log D *vs.* time (hr) for extraction at a constant temperature of 40°C.
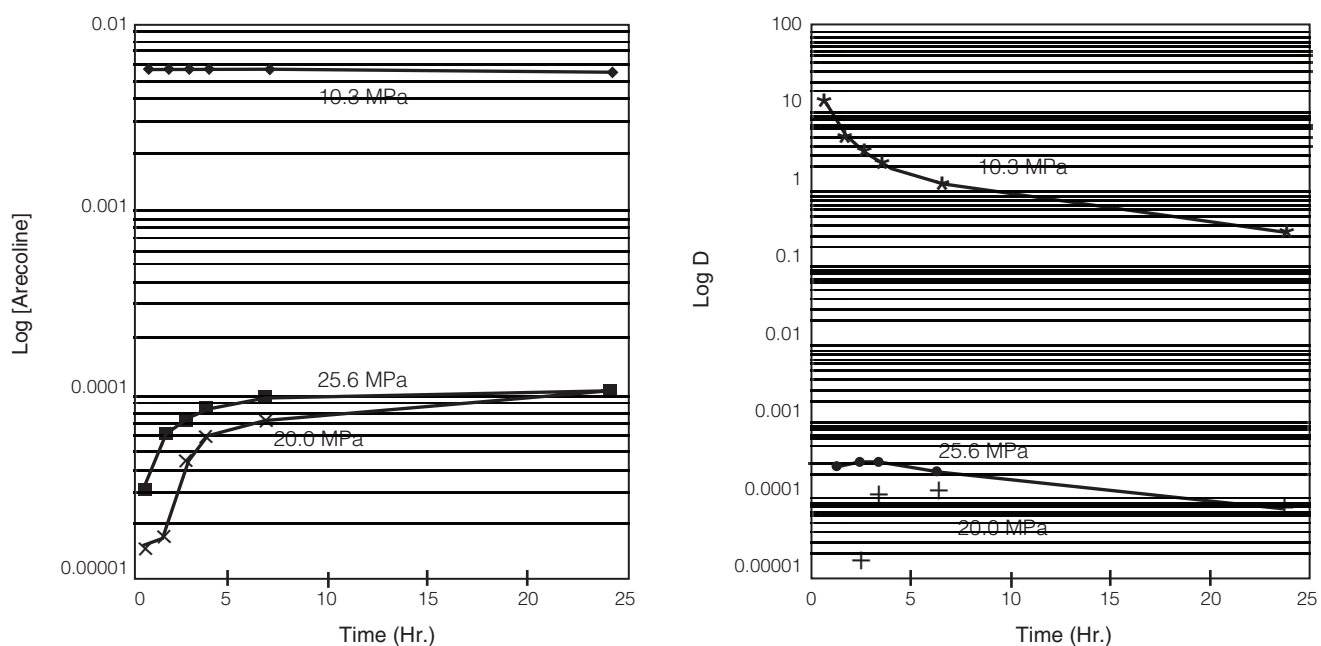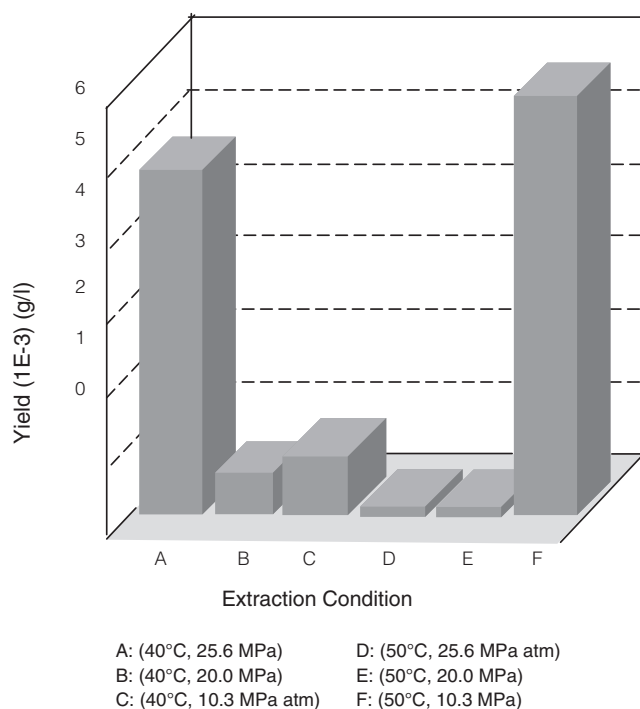

Figure 7. (A) Cumulative Log [Arecoline] *vs.* time (hr) for extraction at a constant temperature of 50°C. (B) Log D *vs.* time (hr) for extraction at a constant temperature of 50°C.

D value was highest at 25.6 MPa followed by 10.3 MPa and 20.0 MPa. The change of pressure also resulted in no predictable effect to the *D* values (Figure 6B).

Extraction at 50°C resulted in higher yield at 10.3 MPa followed by 25.6 MPa and 20.0 MPa. The pressure increment did not have a direct effect on the yield.

The *D* value for the extraction at 10.3 MPa was the highest. At a constant temperature of 50°C, higher *D* value was initially achieved at pressure of 25.6 MPa compared to 20.0 MPa (Figures 7A and 7B). Subsequently, the *D* value at 25.6 MPa diminished and attained equivalent *D* value as for 20.0 MPa Hence, extraction at constant temperature of

A: (40°C, 25.6 MPa)   D: (50°C, 25.6 MPa atm)
B: (40°C, 20.0 MPa)   E: (50°C, 20.0 MPa)
C: (40°C, 10.3 MPa atm) F: (50°C, 10.3 MPa)

Figure 8. Amount of arecoline extracted for 24 hours for each condition.

50°C at these two pressures failed to manifest any direct and positive correlation between pressure and the D value.

III. *The Overall Evaluation of the Extraction*

From the graphs obtained, we can see certain patterns to the lines. Figures 3A, 4A, 5A, 6A and 7A show the correlation between the yield and time whereas Figures 3B, 4B, 5B, 6B and 7B exhibit the changes in the D values with time. Most of the graphs of Log [Arecoline] *vs.* time, show an increment of Log [Arecoline] at the initial stage of the extraction and eventually became constant or reach a maximum value. According to McHugh and Krukonis[10] there are three distinct extraction regions which are solubility dependent, intermediate and diffusion controlled regions. The first region in Figure 7A shows thermodynamic solubility as observed in the first 2 to 3 hours of extraction at 25.6 MPa and 20.0 MPa at 50°C. Afterwards, the extraction was more dependent on arecoline diffusion through the areca nut internal matrix. In Log D *vs.* time graphs, D value increased initially, reached a maximum value and then reduced with the time. It shows that the diffusivity of arecoline from the areca nuts increased at the initial stage of the extraction, reached a maximum value and reduced diffusivity until the end of extraction. The reduction in the D value corresponded to the reduction of the amount available to be extracted after the maximum amount was attained. Based on the preceding graphs, the diffusion coefficient value has a positive influence on the amount

of arecoline extracted. From the data on the D value, the optimum execution of extraction could be achieved under combined temperature and pressure of 50°C and 10.3 MPa. At 50°C and 25.6 MPa, compaction of the areca nut might limit the diffusion of CO_2, through the sample. Compaction of the sample CO_2 movement in and out of the areca matrix will inadvertently reduce the efficiency to extract the arecoline from the areca matrices.

As seen in Figure 8, the highest yield of arecoline was achieved at 50°C and 10.3 MPa, followed by 40°C at 25.6 MPa. Both temperature and pressure appeared to influence yield, with higher temperature requiring lower pressure. On the other hand, similar yield could be achieved at higher temperature and lower pressure. Theoretically, each compound possesses different optimum extractability conditions under the supercritical fluid temperature, pressure and time factors[10]. Zaidul *et al.*[11] reported that separation and fractionation of triglycerides could be done by conducting SFE at various temperature and pressure regime. At higher pressure of 25.6 MPa, the density of CO_2 decreased with temperature at constant pressure that reduces the contact time and thus solubility of arecoline in the solvent. However, at lower pressure of 10.3 MPa, diffusivity was higher than that of higher temperature at 50°C. The higher temperature in SFE could have altered the areca matrix to make it more diffusible by CO_2. According to Xu & Godber[12], the solubility of solutes increases with increasing temperature if pressure is sufficiently greater than the critical pressure of CO_2, which is 7.3 MPa, owing to increased vapour pressure of the solute in the sample. As for the extraction of palm kernel oil from palm kernels using pressure swing technique, Zaidul *et al.*[13] reported that higher yield at higher pressure could be attributed to the disruption of the oil glands. Chen and Ling[14] stated that the selection of modifier (co-solvent) is a critical factor to be considered.

CONCLUSIONS

This study focused on the optimum yield of arecoline from areca nut using supercritical carbon dioxide (SC-CO_2). In this study, there was no direct effect of the temperature on the yield and the D value at each pressure applied. Efficiency of supercritical fluid extraction was influenced by specific combination of temperature and pressure.

Higher yield of arecoline could be extracted at 10.3 MPa and 50°C with high diffusivity followed by at 25.6 MPa, at 40°C. The extractions and analyses of arecoline showed that solubility and diffusivity of arecoline in supercritical carbon dioxide are affected by both temperature and pressure. Changes in the temperature and pressure have effects on the yield and value of the diffusivity coefficients. Analyses also show that the concentration of arecoline extracted from areca nut and the diffusivity coefficient are the highest at 50°C and

10.3 MPa. Thus, this condition could be regarded as the most efficient condition for the extraction of arecoline from areca nut using SC-CO_2.

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