Jurnal Teknologi

Full paper

Extraction and Identification of Vitamin E from *Pithecellobium Jiringan* Seeds Using Supercritical Carbon Dioxide

Mohd Azizi Che Yunus[a,b*], Salman Zhari[a], Saharudin Haron[b], Nur Husnina Arsad[a], Zuhaili Idham[a], Muhammad Syafiq Hazwan Ruslan[a]

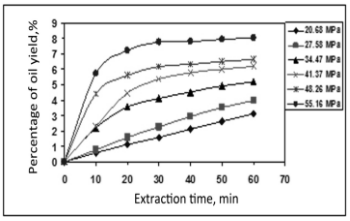
[a]*Centre of Lipids Engineering & Applied Research, Ibnu Sina Institute for Scientific and Industrial Research, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia*
[b]*Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia*

*Corresponding author: azizi@cheme.utm.my

Article history

Received : 2 March 2015
Received in revised form : 24 April 2015
Accepted : 10 May 2015

Graphical abstract



Abstract

Pithecellobium Jiringan (P. Jiringan) is traditionally known as natural herb consists of several medicinal compounds (vitamin E). Supercritical carbon dioxide extraction (SC-CO_2) has been proven as potential method to extract interest compound from herbs. By altering pressure and temperature, the specific compound can be extracted. In this study, the SC-CO_2 operating conditions are pressure (20.68 MPa to 55.16 MPa) and temperature (40°C to 80°C) in one hour extraction regime was used to extract vitamin E from *P. jiringan*. The quantification of vitamin E was analysed with Gas Chromatography Time of Flight Mass Spectrometry (GC-TOF-MS). The responses are overall oil yield and vitamin E yield. The overall oil yield was obtained at the highest condition of 55.16 MPa and 80°C with asymptotic yield of 8.06%. In contrast, the highest amount of vitamin E obtained is 0.0458mg/g sample (80.14 ppm) at the lowest extraction condition of 20.68 MPa and 40°C.

Keywords: Supercritical carbon dioxide; vitamin E; *Pithecellobium Jiringan*; optimization

1.0 INTRODUCTION

Supercritical fluid extraction process is introduced for better extraction quality and selectivity of the desired compound over the undesired product of the extraction. Supercritical fluid extraction is a technique of extraction that has been growing rapidly for the past 35 years [1]. This method of extraction is very common for the extraction of bioactive compound and natural product. The extraction is carried out in a condition where the solvent is above the critical temperature and critical pressure. The solvent possessed the characteristic of gas and liquid. It has higher diffusivity compared to the vapour phase and higher solubility compared to the liquid phase [2–6]. Supercritical fluid with high diffusivity has lower viscosity which can penetrate the sample more effectively than a normal liquid solvent. At the same time, the mass transfer is much faster which resulted in better extraction product [7].

Carbon dioxide (CO_2) is selected as a solvent because of its stable chemical properties, inflammable, radioactively stable, high purity, non-toxic and inert. In concern of the supercritical condition of the extraction process, CO_2 has a mild critical condition that will not affect the physiochemical properties of the extract. The extracts also will be free of solvent because CO_2 at the atmospheric condition is in a gaseous phase and the product will also be free of any inorganic salt, heavy metal and micro bacterial life which are better than the conventional method of extractions. Furthermore, the product of the extraction is accepted by European Food and Drugs Association [8] and World Health Organization [9]. Proven by previous research, supercritical CO_2 successfully extracted active compound such as tocopherol and carotens from palm oil at 500 bars and 80^{O}C. It has been discovered that the selectivity of supercritical CO_2 varies with pressure and temperature of the extraction condition [10].

Pithecellobium Jiringan (*P. Jiringan*) or known as 'Jering' in Malaysia and 'Jengkol' in Indonesia is a native plant that grows in the primary and secondary rainforest of South-East Asia. *P. Jiringan* has been used commonly for traditional medicinal purpose [11]. Traditionally, the seeds are boiled then drunk to treat high blood pressure and diabetes. The leaves are burnt and applied to skin to treat itch and cuts. The seeds contain high amount of glycosides, oligosaccharides and saponins [12]. It has high anti-oxidant activity that has been proven in previous study that 85% of the activity of the anti-oxidant present in two bioassay experiments using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. The seeds contain vitamin A, C and E that are useful for human to destroy the excess free radical and repair oxidative damage.

The objective of this study is to determine the SC-CO_2 conditions on the extraction of oil yield and vitamin E during 60 minutes of the extraction regime. The effect of temperature and pressure for both, oil yield and vitamin E was investigated as well.

2.0 MATERIALS AND METHODS

2.1 Material

P. Jiringan seeds were bought at a local market at Kepala Batas, Penang, Malaysia. 30 kg was bought to ensure the standardization of the sample [13]. The seeds were cut open and sliced to small size and sun dried. Grinder was used to reduce the particle size to several sizes (215 µm, 275 µm, 362.5 µm and 637.5 µm). After pre-treatment, the best particle size was identified as 215 µm. Water and n-hexane were used as comparison solvent.

2.2 Soxhlet Extraction

Soxhlet apparatus is setup by n-hexane as solvent. 10 grams of sample is used as the extraction sample. The volume of solvent used is 250 mL and operates at atmospheric pressure with 6 hours of extraction time. Rotary vacuum evaporator (RVE) was used to remove excess solvent. The final product is then weighed and stored at temperature below -30°C to preserve the volatile compound.

2.3 Water Extraction

For water extraction, 20 g of sample is placed into a 500 mL conical flask and filled with 250 mL of distilled water. The mixture is macerated overnight and at the same time shakes using shaking apparatus. The mixture is then filtered through a filter paper and freeze dried (FDX Heto) to get water free product. The product is then weighed and stored at temperature below -30^{O}C to preserve the volatile compound.

2.4 Supercritical Fluid Extraction (SFE)

The apparatus used is SFX^{TM} 220 extraction system (ISCA, Lincoln, NE, US). It consists of high pressure syringe pump that can operate at maximum pressure of 58.95 MPa, extractor chamber with extraction cell, capillary restrictor that reduce analyte disposition with maximum operating temperature at 150°C and collection vial.

Blank extraction is done to purge all contaminant from the system at 34.47 MPa, 60°C for 15 minutes. 1.75 g of sample is placed into the extraction cell and then capped at both end with cartridge filter frits to make sure none of the powder from the sample plug the tube and valve and also to maintain the flow rate. The extraction cell is placed into its chamber and switched on the temperature controller to allow the extractor to reach its desired temperature. The extraction pressure and temperature are set according to the experimental layout. The experiment starts by weighing the collection vial every 10 minutes for 60 minutes of extraction time. The volume of CO_2 used is recorded at the extractor control panel. The used CO_2 gas is vented out if the system is using the vent valve. After the 60 minutes extraction process has completed, the extracted oil is wrapped using aluminum foil to prevent photo degradation. The extracted oil is then stored at temperature below -30^{O}C to preserve the volatile compound for chromatographic analysis.

SO-CO_2 conditions are pressured at 20.68 MPa, 27.58 MPa, 34.47 MPa, 41.57 MPa, 48.26 MPa and 55.16 MPa and put under temperature at 40°C, 50°C, 60°C, 70°C and 80°C. The extracts were collected per 10 minutes fraction of oil over 60 minutes of extraction time.

2.5 Gas Chromatography-Time of Flight-Mass Spectrometer (CG-TOF-MS) Analysis

For chromatogram analysis for identification and quantification of vitamin E, the gas chromatography used is GC 6890N 7683 series with auto sampler injector and controller. The system is coupled with LECO Pegasus III reflection retention TOF-MS with electron impact ionization and Chrom TOF mass data analysis. The oven temperature is set to 100°C hold for 1 minute and increased to 200°C with the increasing rate of 20°C/min and finally to 280°C with the same rate. The injection volume is 1 µL and helium is used as the carrier gas with the flow rate of 1 mL/min. Parameters for LECO Pegasus III are set as stated: mass range of 30–700 amu, acquisition rate of 20 spectra/second, detection temperature of 225°C, total acquisition time of 27.50 minutes, signal to noise ratio (S/N) of 10:1, baseline offset of 0.5 through the middle of noise.

Mass ionization spectrum obtained from the analysis is compared with the National Institute of Standard and technology (NIST) library and Willey database for identification. Any similarity greater than 79% is considered detected. GC-TOF-MS is more advanced than conventional GCMS. It uses a micro-channel plate detection that has high sensitivity. Due to this matter 79% similarity is good enough [14].

Calibration curve for vitamin E is constructed by plotting graph of peak area (µV.s) versus concentration, ppm using four different concentrations on vitamin E standards: 10 ppm, 25 ppm, 50 ppm and 100 ppm. These standards create a linear curve: $Y = 7.696 \times 10^5X - 6.0 \times 10^6$ with regression correlation, $R^2 = 0.9991$ that is used to determine the concentration of vitamin E in each extraction oils. The retention time observed is at 1057.20 second for all concentration.

For the identification of vitamin E from extraction oil, the retention time must be similar with the standard. However, slight difference is tolerated but must be complied with the international guideline: US FDA 2003 (FDA Method for GC/HPLC-MS determination of volatile or semi volatile compound): tolerance of ±30 seconds, EC Commission Decision 2002: ±0.5% deviation and World Anti-Doping Agency (WADA) Technical Document 2003: ±1.0% deviation or ±12.0 seconds (whichever smaller).

3.0 RESULTS AND DISCUSSION

3.1 Oil Yield, %

The highest oil yield was generated at the SC-CO_2 condition of 55.16 MPa and 80°C as shown in Figure 1. The oil yield obtained at asymptotic yield is 8.07%. The oil yield increases and reaches plateau after 30 minutes and then slowly reaches the asymptotic yield. Generally, the extraction yield from natural herbs of 8.00% is good enough [15]. The results were in good agreement with previous research on the extractions of natural products. The highest extraction condition of pressure and temperature causes higher yield to be obtained [10, 16, 17].

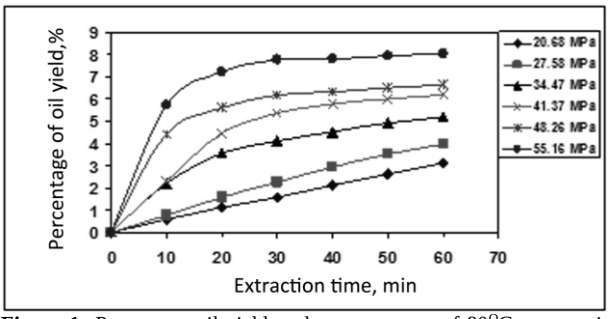


Figure 1 Percentage oil yield at the temperature of 80^{O}C at operating various pressure using average particle size of 215 µm

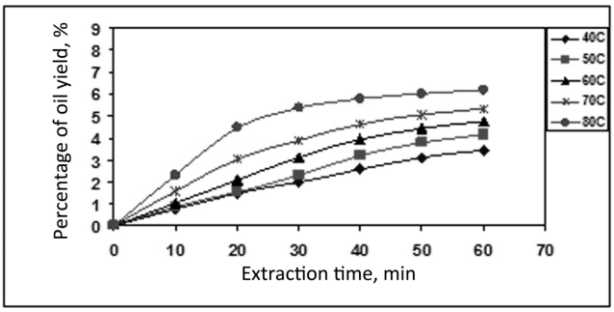


Figure 2 Effect of temperature on percentage overall oil yield at constant pressure 41.37 MPa in 60 minutes extraction

Figure 1 shows that, the extraction process can be divided into three phases: equilibrium control phase, intermediate control phase and diffusion control phase. The first stage is equilibrium control phase that occur averagely during the first 10 minutes of extraction for the pressure between 34.47 MPa to 55.16 MPa. During this phase, the extraction rate is constant whereby the extraction profile is in linear form. The concentration of oil in supercritical CO_2 is almost constant. In this phase, the extraction process is limited by equilibrium solubility of oil in the solvent. The outer layer of oil is completely extracted by the solvent.

The intermediate phase is the transition phase before the diffusion phase. During this phase, the extraction rate decreases rapidly because a large amount of oil was extracted during the equilibrium control phase. The mass transfer during the intermediate phase decreases due to the depletion of oil content in the sample resulting to the decreases of extraction rate. This intermediate phase can be clearly seen on the extraction at constant pressure of 41.37 MPa when temperature slowly increases as shown in Figure 2. At 60°C, it can be observed that diffusion region dominates the extraction process. When the temperature increases to 70°C, both diffusion and solubility phase control the extraction process. Intermediate phase can be seen averagely at 20 to 30 minutes after the extraction process started and the result complies with previous research conducted using supercritical CO_2 extraction process [7, 17, 18].

At the third phase, the extraction process is controlled by diffusion. This phase starts after 30 minutes of extraction when the profile approaches asymptotic yield. The extraction greatly depends on the diffusion rate and the capability of solvent to penetrate deep into the sample matrix to extract the remaining solute [19]. The extraction profile progresses slowly compared to the solubility region which increased linearly.

Other thermodynamic property that helps the extraction process is the volatility of oil, where it functions as temperature. At constant pressure, diffusivity of supercritical CO_2 increases with the increase of temperature. Fluid characteristic is more to gas-like state. Therefore, combining the diffusivity of solvent and the increased volatility of solute makes the extraction process faster and reaches the asymptotic yield in shorter time.

Table 1 Extraction Parameters used for optimization purpose using Statistica 6.0 Software

Parameters	+1	0	-1
Temperature (^{O}C)	80	60	40
Pressure (MPa)	55.16	37.92	20.68
Time, t	60	35	10

Based on the experimental data, a simple mathematical model can be suggested to relate the percentage oil yield with time, pressure and temperature of extraction. The suggested model is derived using STATISTICA 6.0 software using Box-Behnken design. Parameters that have been used are tabulated in Table 1 where, +1 shows the maximum value, 0 shows the middle value and -1 shows the minimum value. Using a second-order polynomial with linear-linear interaction, the suggested mathematical model is as follows:

$$Y = 171.77 - 4.89T + 1.30P - 2.96t + 0.03T^2 - 0.02P^2 + 0.006t + 0.02TP + 0.008Pt + 0.04Tt \quad (1)$$

Where Y is the percentage oil yield (%), T is the temperature (°C), P is the pressure (MPa) and t is the time of extraction for the extraction process.

3.2 Comparison between Extraction Method

The result from the extraction using SC-CO_2 was compared with Soxhlet and water extraction as shown in Figure 3. The effect of thermodynamic properties is the most significant. The highest yield is obtained with using SC-CO_2 at highest operating condition: 8.06% yield. Meanwhile, the lowest yield was obtained at the lowest condition of extraction with 3.12%. The extraction time contributes in the overall oil yield obtained. Although supercritical extraction has proven to be the best extraction method, the time constrain is limiting the amount of yield obtained. Soxhlet and water extraction get a higher yield because of longer time of extraction. The final parameter that plays a role is polarity of the solvent. Water is used for preliminary stage for the selection of solvent [20], while n-hexane is a non-polar solvent. CO_2 polarity varies with pressure and temperature. It is naturally a non-polar solvent but can be mild polar at high temperature and pressure. From results obtained, it shows that the solute inside the sample matrix is mild polar because the extraction of solute is more favourable when the polarity scale of solute and solvent is the same.

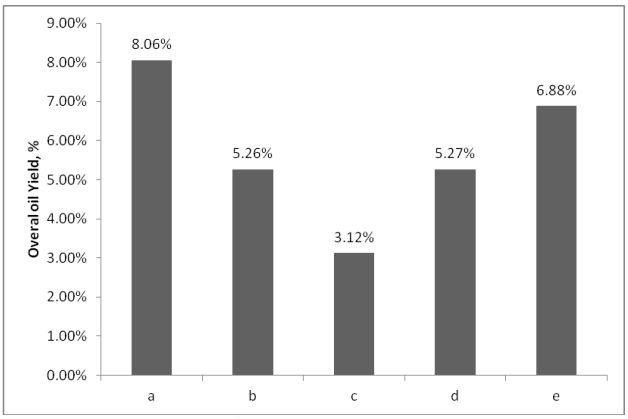


Figure 3 Comparison of extraction methods and their extraction conditions; (a) SC-CO_2 at temperature of 80°C, pressure of 55.16 MPa for 60 minute extraction time, (b) SC-CO_2 at temperature of 70°C, pressure of 41.37 MPa for 60 minute extraction time, (c) SC-CO_2 at temperature of 60°C, pressure of 20.68 MPa for 60 minute extraction time, (d) Soxhlet extraction at using n-hexane as solvent, operated at atmospheric pressure for 6 hours, (e) water extraction at atmospheric temperature and pressure overnight

3.3 Chromatography Analysis

Figure 4 and 5 shows the profile of chromatogram analysis for all temperatures at the pressure of 20.68 MPa and 55.16 MPa respectively. Observed at constant pressure, the maximum yield of vitamin E is 0.045 mg/g sample (80.14 ppm) obtained at the lowest temperature of 40^{O}C while the minimum yield, 0.0064 mg/g sample (11.28 ppm) is obtained at the highest temperature of 80^{O}C. This is due to the competitive extraction of other compound at higher temperature. At higher extraction temperature, the extraction of vitamin E which has a relatively lower molecular weight must compete with compound that is easily extracted at higher extraction temperature: long chain hydrocarbon and long chain fatty acid. The extraction profile is similar with some previous researches of extraction using supercritical CO_2 of soy bean flakes [16, 21] and rice bran [22] which obtained the highest amount of vitamin E at lower extraction pressure and temperature. For the extraction of vitamin E, which is easily extracted at low temperature [23], it is advised to be operated at low pressure and temperature near critical temperature of solvent [24].

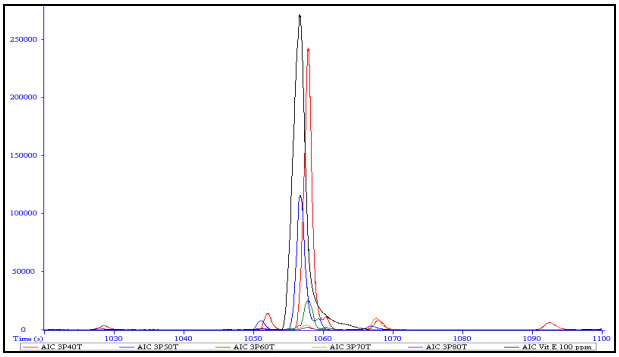


Figure 4 GC-TOF-MS profile of detection and quantification of vitamin E standard (100 ppm) and extracted oil at constant pressure 20.68 MPa for all operating temperatures (40°C, 50°C, 60°C, 70°C and 80°C)

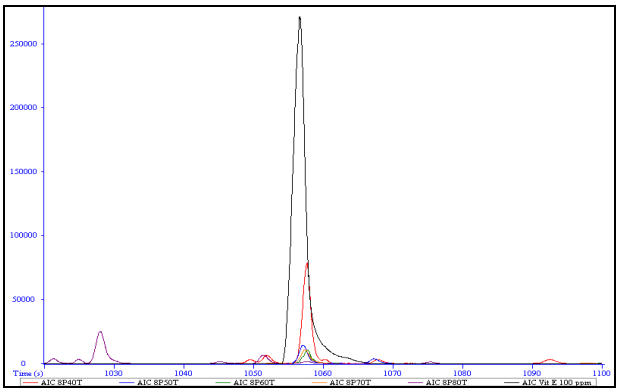


Figure 5 GC-TOF-MS profile of detection and quantification of vitamin E standard (100 ppm) and extracted oil at constant pressure 55.16 MPa for all operating temperatures (40°C, 50°C, 60°C, 70°C and 80°C)

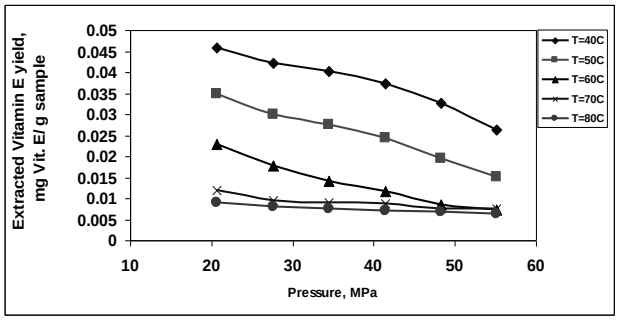


Figure 6 Effect of operating pressure on amount of vitamin E extracted from sample

3.4 Effect of Extraction Condition on Vitamin E Extraction Process

Figure 6 shows the effect of extraction pressure at constant temperature on the amount of vitamin E yield. At high temperatures of 70°C and 80°C, the extraction of vitamin E as the interest compound is not suitable because it has the lowest yield regardless of the extraction pressure. At other extraction temperature, the extraction yield decreases as pressure increases. Due to the increasing density of CO_2, the diffusion rate and interaction between solute and solvent decrease results in the decrease of amount of vitamin E extraction. Low diffusivity of CO_2 results in difficulty to break the linkage of interest compound in sample matrix [20].

■4.0 CONCLUSION

The extraction of Vitamin E from *P. Jiringan* is best performed at the pressure and temperature of 20.68 MPa and 40^{O}C respectively. The amount of Vitamin E extracted using this condition was found to be the highest, which was 0.0458 mg vitamin E/g sample (80.16 ppm). In contrast, the highest overall oil yield (8.07%) is obtained at the extraction pressure of 55.16 MPa and temperature of 80°C. Vitamin E was extracted in the equilibrium control phase in the early stage of extraction. Identifications and understanding on the controlling step are essential for determining the efficiency of the extraction process. Solvating powers of SC-CO_2 and vaporing pressure of oil were very important properties that can enhance the quality of extraction products.

Acknowledgement

This research is funded by Malaysian government under Fundamental Research Grant Scheme (FRGS) (Vote no:Q.J130000.2525.00H28), Ministry of Higher Education Malaysia with collaboration of Centre of Lipids Engineering and Applied Research (CLEAR), Faculty of Chemical Engineering, Universiti Teknologi Malaysia (UTM), Malaysia.

References

[1] Mohd Azizi, C. Y. 2007. Extraction, Identification and Separation of Vitamin E and Djenkolic Acid from *Pithecellobium Jiringan* (Jack) Prain Seeds Using Supercritical Carbon Dioxide. Ph.D Thesis. Univerisiti Sains Malaysia, Penang, Malaysia.

[2] Hassan, M. N. 2000. Extraction and Fractionation of Palm Kernel Oil Using Supercritical Carbon Dioxide as Solvent. Ph.D Thesis, Universiti Sains Malaysia, Penang, Malaysia.

[3] Mastura, M., S. Harcharan, and H. Mashitah. 2001. SC-CO_2 Fractionation of Crude Palm Oil. *Journal of Supercritical Fluids*. 20: 45–53.

[4] Anuar, O. 2002. Modification of Palm Kernel Cake Composition by Using Supercritical Carvon Dioxide Extraction Method. MS.c Thesis, Universiti Sains Malaysia, Penang, Malaysia.

[5] Harcharan, S., S. C. Ng, T. Y. Hoon, and S. S. On. 2002. Supercritical Carbon Dioxide Extraction of Black Pepper Oil. *Proceedings of the 2nd World Engineering Congress*. Sarawak, Malaysia. August 2002, 72–75.

[6] Zaidul, I. S. M. 2003. Supercritical Carbon Dioxide as a Process Fractionation Technology of Palm Kernel Oil for Cocoa Butter Quality Fat, Ph.D Thesis, University Sains Malaysia, Penang, Malaysia.

[7] Norhuda, I. 2005. Studies on Mass Transfer Characteristics of Palm Oil Kernel Oil Extraction Using Supercritical Carbon Dioxide Extraction. Ph.D Thesis. Universiti Sains Malaysia, Penang, Malaysia.

[8] Dick, A. J., and H. N. Herry. 1996. Extraction of Secondary Metabolites from Plant Material: A Review. *Trend in Food Science and Technology*. 7: 191–197.

[9] Tiek, N. L., and M. I. Mohd Azmi. 1997. Trade in Medicinal and Aromatic Plants in Malaysia. Forest Research Institute of Malaysia, FRIM Report. 71: 52–67.

[10] Bisunadan, M. 1993. Extraction of Oil From Oil Palm Fruit Fiber Using Supercritical Carbon Dioxide Extraction. MS.c Thesis, Universiti Sains Malaysia, Penang, Malaysia.

[11] Abdullah, H. 1999. Pengendalian Lepas Tuai Buah-Buahan Dan Sayur-Sayuran Tropika. Institut Penyelidikan dan Kemajuan Pertanian Malaysia, MARDI, Malaysia.

[12] Vimala, S., A. Abdul Rashid, A. Mohd Ilham, and S. Rohana. 2003. Nature's Choice to Wellness Antioxidant Vegetables/Ulam, Volume 7. *Forest Research Institute of Malaysia, FRIM.*

[13] Lazarowych, N. J., and P. Pekos. 1986. Use of Fingerprint and Marker Compounds for Identification of Botanical Drugs: Strategies for Applying Pharmaceutical HPLC Analysis to Herbal Product. *Journal of Drug Information*. 32: 297–512.

[14] Sakamoto, T., A. Yamamoto, M. Owari, and Y. Nihei. 2003. Development of a Supercritical Fluid Extractor Couple with a Time of Flight Mass Spectrometer for on-Line Detection of Extracts. *Journal of Analytical Science*. 19: 853–857.

[15] Del Valle, J. M., M. Jimenez., and J. C. De La Fuente. 2003. Extraction Kinetics of Pre-Pelletized Jalapeno Peppers with Supercritical CO2. *Journal of Supercritical Fluids*. 28: 11–19.

[16] Paretti, G., E. Miniati, L Montanari, and P. Fantozzi. 2003. Improving The Value of Rice by Products by Supercritical Fluid Extraction, *Journal of Supercritical Fluids*. 26: 1–9.

[17] Favati, F., J. W. King, and M. Mazzanti. 1991. Supercritical Carbon Dioxide Extraction of Evening Primrose Oil. *Journal of American Oil Chemists Society*. 68(6): 422–427.

[18] Özkal, S. G., U. Salgin, and M. Yener. 2005. Supercritical Carbon Dioxide Extraction of Hazelnut Oil. *Journal of Food Engineering*. 69: 217–223.

[19] Kiriamati, H. K., E. Rascol, A. Marty, and J. S. Condoret. 2001. Extraction Rates of Oil from High Oleic Sunflower Seeds with Supercritical Carbon Dioxide. *Journal of Chemical Engineering and Processing*. 41: 711–718.

[20] Harborne, J. B. 1973. *Phytochemical Methods: A Guide to Modern Technique of Plant Analysis*. Chapman and Hall Ltd., London.

[21] King, J. W., F. Favati, and S. L. Taylor. 1996. Production of Tocopherol Concentrates by Supercritical Fluid Extraction and Chromatography. *Journal of Separation Science and Technology*. 13(31): 1840–1847.

[22] Han, N. M., C. Y. May, M. A. Ngan, and C. Y. Hock. 2000. Analysis of Palm Oil Minor Components by Supercritical Fluid Chromatography. *Proceedings of the 2000 National Seminar on Palm Oil Milling, Refining Technology, Quality and Environment*. Genting Hotel, Malaysia July 2000. 41–47.

[23] Lucas, D. A., O. E. Martines De La, J. Rincon, M. A. Blanco, and I. Garcia. 2002. Supercritical Fluid Extraction of Tocopherol Concentrates from Olive Tress Leaves. *Journal of Supercritical Fluids.* 22: 45–53.

[24] Dean, J. R. 1993. *Application of Supercritical Fluids in Industrial Analysis*. Blackie Academic & Professional Publisher, Glasgow.