Jurnal Teknologi

Full paper

Extraction of Beta Carotene from Palm Mesocarp via Green Sub-critical Carbon Dioxide

Mohd Azizi Che Yunus[a,b*], Zuhaili Idham[a], Jeffry Jamal[b], Muhammad Syafiq Hazwan Ruslan[a], Noor Azian Morad[c,d], Liza Md Salleh[a,b]

[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

[c]Centre of Lipid Engineering and Applied Research (CLEAR),Universiti Teknologi Malaysia, 54100 Kuala Lumpur, Malaysia

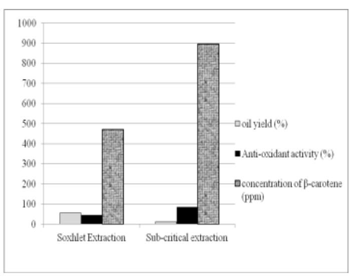
[d]Malaysia–Japan International Institute of Technology (MJIIT), Universiti Teknologi Malaysia, 54100 Kuala Lumpur, Malaysia

*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

Experiments involving subcritical carbon dioxide extraction of palm oil from treated palm mesocarp were done to determine palm oil yield and concentration of beta-carotene. As comparison, the conventional method of Soxhlet Extraction with six different solvents was used. The overall oil yield was measured as weight of oil per weight of sample, while anti-oxidant activity was determined using 2,2-diphenyl-1-picryihydrazyl (DPPH) radical scavenging method. The sub-critical extraction was conducted at a constant temperature of 30˚C and four different pressures at 10 MPa, 15 MPa, 20 MPa and 25 MPa. The sub-critical extraction produced high concentration of beta-carotene than soxhlet extraction, even though the oil yield was lower. Furthermore, the anti-oxidant analysis showed a similar trend as the concentration of beta carotene. The best condition of sub-critical extraction was obtained at 25 MPa and 30˚C.

Keywords: Supercritical fluid extraction; oil yield; antioxidant activity; beta-carotene, palm mesocarp

■1.0 INTRODUCTION

Palm oil is an edible vegetable oil derived from the mesocarp (reddish pulp) of the oil palm fruits. Palm oil is naturally reddish in color because of its high beta-carotene content. Throughout history, palm oil has served as the primary source of dietary fat for countless numbers of applications. Furthermore, its nutritional and healing properties have been recognized for generations. Carotenes are valuable nutrients which contain high antioxidants and essential nutrients.

Solid-Liquid Extraction can be described as the extraction of solid materials or particles containing the desired compound by using specific solvents. The extraction process can be applied whether on solid or liquid form. In the extraction process, in order to separate and remove one or more components from the original mixtures, each phase must be in contact with each other [1].

A pure component is considered to be in a sub-critical state if its pressure is higher than the critical pressure and its temperature is lower than the critical temperature. Critical temperature of gas is the highest temperature at which a gas can be converted into liquid by the increase of pressure. Meanwhile, the critical pressure of liquid is the highest pressure at which a liquid can be converted into gas by an increase in the liquid temperature [2-3]. After the critical point is achieved, no matter how much pressure is applied to the gas phase, it cannot be converted back to liquid phase.

Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers particular electrons from a substance to an oxidizing agent. Oxidation reactions produce free radicals, which start chain reactions that damage cells [4]. Antioxidants obtained from natural sources are much better in performance compared to the artificially synthesize antioxidant and most antioxidant compounds are obtained from plants which varies its concentration depending on the plant parts (e.g.: seeds, trunks, leaves, and roots).

Early researches on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity. Antioxidant activity can be measured simply by placing the fat in a closed container with the presence of oxygen and measuring the rate of oxygen consumption. However, it was the identification of vitamins A, C, and E as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in the biochemistry of living organisms [5].

The objective of the study is to focused on the extraction of beta carotene from palm mesocarp by using soxhlet extraction with different types of solvent and subcritical carbon dioxide extraction. In addition, the study also aimed to determine the optimum pressure for subcritical extraction.

■2.0 MATERIALS AND METHODS

2.1 Material

Palm mesocarp was obtained from Universiti Teknologi Malaysia Palm Oil Mill. Methanol, n-Hexane, Chloroform, Isopropanol, Petroleum Ether and 2-diphenyl-1-picrylhydrazyl were purchased from Merck.

2.2 Extraction Process

The extraction of beta carotene from palm mesocarp was conducted using two methods of extraction which were Subcritical Fluid Extraction and Soxhlet Extraction methods. Table 1 shows the condition set for both extraction processes.

For sub-critical fluid, the parameters were selected based on the sub-critical region of carbon dioxide. Extraction time was fixed in one hour due to the pre treatment result shows that one hour of extraction regime was an adequate time for the extraction of palm oil. Different types of solvent were used for soxhlet extraction which is water, *n*-hexane, petroleum ether, methanol, isopropanol and chloroform. This variation of solvent also varies the polarity of solvent and also will affect the extraction yield and content. Particle size that was used for both processes remained constant because different particle size has different amount of free and intact oil. The amount of free and intact oil needs to be kept constant throughout the entire experiment.

Table 1 Condition of extraction process

Extraction Paramter	Sub-Critical Fluid	Soxhlet
Temperature (°C)	30	70
Pressure (MPa)	10, 15, 20, 25	Ambient
Flowrate (mL/min)	5	n/a
Extraction Time (hr)	1	5
Weight of sample (g)	5	20
Particle Size (mm)	0.8 ± 0.2	0.8 ± 0.2
Type of Solvent	Carbon dioxide	Water, Methanol, n-Hexane, Chloroform, Isopropanol,Petroleum Ether

2.3 Concentration of Beta Carotene

The concentration of β-carotene in the extracted oil was measured using a spectrophotometer (model UV-Vis, Shimadzu, Tokyo, Japan). About 20 mg of the extracts was diluted with 7 ml of hexane. The solution was then transferred to a 1 cm quartz cuvette and the absorbance was read at 446 nm. The method was applied according to [6]. The β-carotene content was denoted as ppm beta-carotene by using the following formula:

$$\text{Conc. of } \beta\text{-carotene (ppm)} = 25 \times (383/100W) \times (a_a - a_b) \quad (1)$$

Where a_a and a_b are the absorbance of sample at 446 nm and the cuvvette error respectively, W is the weight of sample in gram and 383 is the extention coefficient of β-carotene.

2.4 Analysis of Antioxidant Activity using Ultraviolet-Visible Spectrometry (UV-VIS)

The radical scavenging ability of essential oil was determined as described by [7]. Briefly, one ml from 5 mg/L alcohol solution of DPPH was added into 2.5 ml of the samples. The samples were kept in the dark at room temperature, and after 30 min the optic density was measured at 518 nm using UV-VIS. The antiradical activity (AA) was determined by using the following formula:

$$AA\ (\%) = \{(Abs_{blank} - Abs_{sample}) / Abs_{blank}\} \times 100\% \quad (2)$$

Where AA is the antioxidant activity in percentage, abs_{blank} and abs_{sample} are the absorbance of DPPH solution without and with the sample added to it respectively.

■3.0 RESULTS AND DISCUSSION

3.1 Soxhlet Extraction

3.1.1 Oil Yield Percentage

Figure 1 shows that chloroform produced the highest oil yield (55.45%) than other solvents. Previous research stated that oil is very soluble in chloroform because of its non-polar property whereby the non polar solutes tend to be easily extracted when the solvent used is a non-polar solvents, and chloroform is an organic solvent that can be chemically used as solvent for the extraction of fat [8]. Meanwhile, the lowest oil yield was produced by water which was only 5.5%. This was due to the polarity of water. Water is a highly polar solvent, whereas oil is a non-polar compound, thus the difference in polarity caused water to produce less significant yield than chloroform. Oil is not soluble in water due to its high molecular weight and tendency to float. Moreover, oil is naturally a hydrophobic compound that repels contact with polar solvents such as water. Other solvents shows the similar trends of results. As the polarity scale decreases, the oil yield obtain increases.

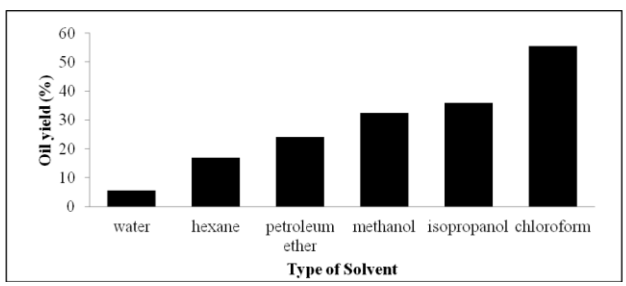


Figure 1 Percentage of oil yield versus different type of solvents

3.1.2 Concentration of Beta-Carotene

The concentration of beta-carotene against the type of solvents is shown in Figure 2. The highest concentration of beta-carotene was obtained by chloroform (470.8 ppm), followed by hexane and water. On the other hand, petroleum ether, methanol and isopropanol produced less likely amount of beta-carotene. This scenario was due to the effect of polarity which is the ability of a molecule to engage in strong interaction with other polar molecules [9]. Similarly to several researches conducted using supercritical carbon dioxide which is a mild polar solvent. High amount of β-carotene was able to be extracted through the extraction process using a non to mild polar solvent as an extraction medium [10, 11].

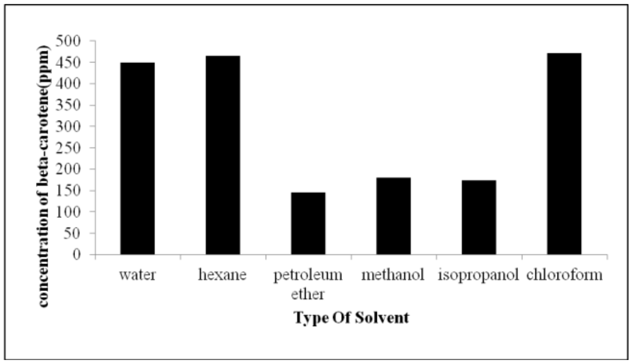


Figure 2 Concentration of beta-carotene in different type of solvents

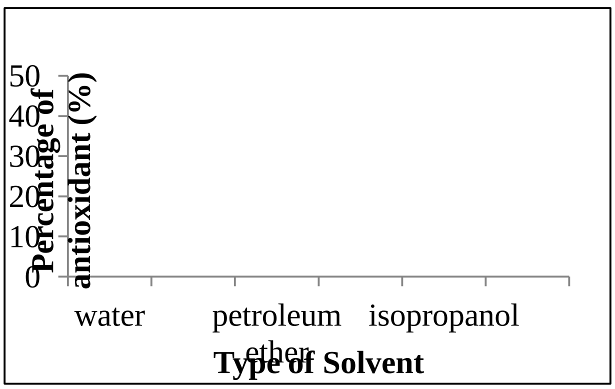


Figure 3 Antioxidant percentage with the use of different solvent

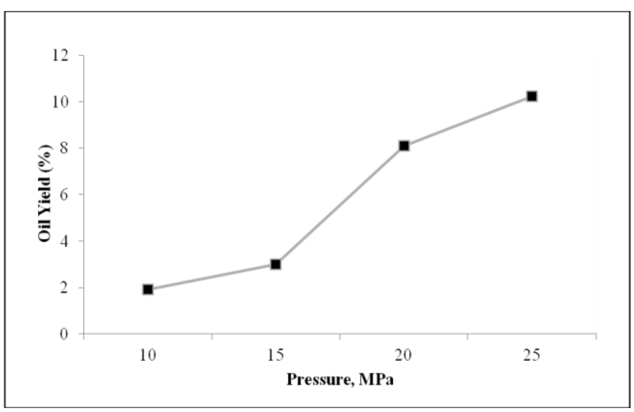


Figure 4 Oil yield percentage for sub-critical extraction

3.1.3 Antioxidant Activity

Figure 3 shows the value of antioxidant activity percentage in different solvents used. The antioxidant activity showed a similar profile as shown in Figure 1. The highest antioxidant property was obtained when using chloroform (45.15%) while water showed the lowest antioxidant activity with 1.43% activity, due to ability of oil to penetrate into the interaction of relative polarity of solvents compared to water [12]. Antioxidant components are usually non-polar compound which in this case the presence of β-carotene plays a major role in inducing the antioxidant properties to the extracts. However, high concentration of β-carotene in the extracts such as in water does not primarily contributed to the anti-oxidant activity. This is due to the collective effect of other side components in the extracts that give the chloroform extracts higher activity compared to the polar extracts of water which clearly have lower activity.

3.2 Sub-Critical Fluid Extraction

3.2.1 Oil Yield Percentage

The yield curve of percentage oil yield against pressure at constant temperature of 30°C is shown in Figure 4. In general, oil yield increases with the increase of operating pressure. The highest overall oil yield was obtained at the pressure of 25 MPa (10.22%), while the lowest pressure of 10 MPa generated only 1.92% yield. This phenomena can be easily explained by the basic principle of subcritical fluid extraction which states that as the pressure increases the density of subcritical CO_2 approaches of a liquid, it will result in an increase in the solvating power [13]. Futhermore, as the density of solvent increased the inter-molecular interaction of solutes also rose [14]. As a result, oil and solvent dissolution was promoted, thereby increasing the oil extraction [15]. However, the increase in the oil-solvent dissolution with pressure during the solubility-dependent stage was very slow compared to the increase in the diffusivity-dependent stage [16]. This result is in good agreement with previous research which reported that the solubility of β-carotene increases as pressure increases at constant temperture [17]

3.2.4 Concentration of Beta-Carotene

Concentration of beta carotene against pressure at a constant temperature of 30° C is shown in Figure 5. It was observed that the concentration of beta carotene showed the same trend as oil yield. The highest concentration was obtained at 25 MPa (896.22 ppm), while the lowest concentration was at 418.91ppm at 10 MPa. The increase might be due to the high concentration of beta-carotene compared to other components co-existing in carotenoids. Puah *et al.* in 2005 noted that the presence of other components being co-extracted with carotene could result in low solubility of *β*-carotene [18]. Higher yield also affect the concentration of *β*-carotene in the extract. Higher amount indicates the extraction process is more effective in withdrawing solute from solid matrices that contain the interest compound.

In addition, there are unoffical reports on high pressure processing having detrimental effects on the concentration or total carotenoids in vegetables or fruits, although some studies found an increase in total carotenoids concentration after high pressure treatment [19].

3.2.3 Antioxidant Activity

The graph on the effects of extraction pressure on antioxidant activity in constant temperature is shown in Figure 6. At constant temperature, the maximum pressure was obtained at 86.20% of antioxidant activity at 25 MPa. On the other hand, the minimum pressure was obtained at 83.37% of antioxidant activity at 10 MPa. This phenomena was due to the increase in pressure as the density of CO_2 was higher, causing the penetration of CO_2 into matrix solid become easier [20].

Therefore, the mass transfer increases according to the increase of pressure [21]. High quality of extract is often reported to be obtain at higher extraction conditions. Sub-critical extraction tends to be very selective extration process that targeted a certain compound at certain operating condition. Clearly from Figure 6, it can be seen that bio-active antioxidant compound tends to be extracted at higher operating pressure compared to the low pressure.

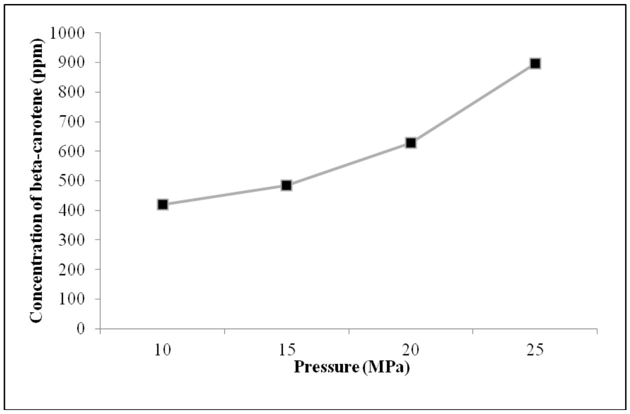


Figure 5 Concentration of beta-carotene by sub-critical extraction

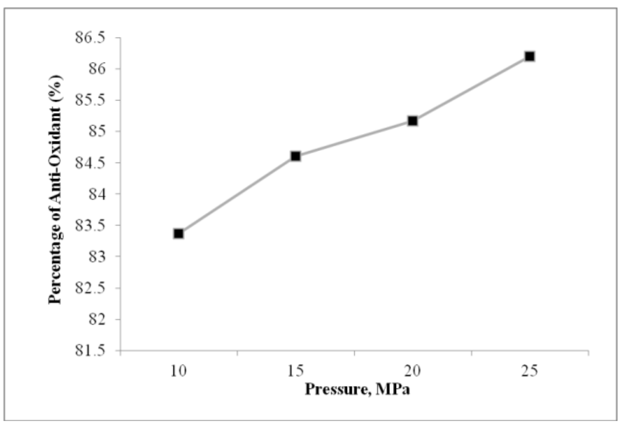


Figure 6 Anti-Oxidant Activity for oil extracted by sub-critical fluid extraction

3.3 Comparison between Soxlet Extraction and Sub-Critical Fluid Extraction

The comparison between the strength of subcritical Carbon dioxide extraction and Soxhlet Extraction were done with respect to the oil yield, concentration of beta carotene and antioxidant activity as shown in Figure 7. As observed, subcritical extraction was more significant for producing beta carotene and antioxidant activity. Meanwhile, Soxhlet Extraction was more preferable for oil yield. However, the Soxhlet extraction took longer extraction time; more than 5 hours compared to subcritical extraction which took less than 1 hour. This also shows that sub-critical extraction can produce very high quality extracts. Even tough the yield was very low compared to soxhlet extraction, the concetration of β-carotene and the anti-oxidant content was clearly higher than soxhlet extraction extracts. Furthermore, sub-critical extraction was more rapid with only 1 hour of extraction regime compared to 5 hours of soxhlet extraction.

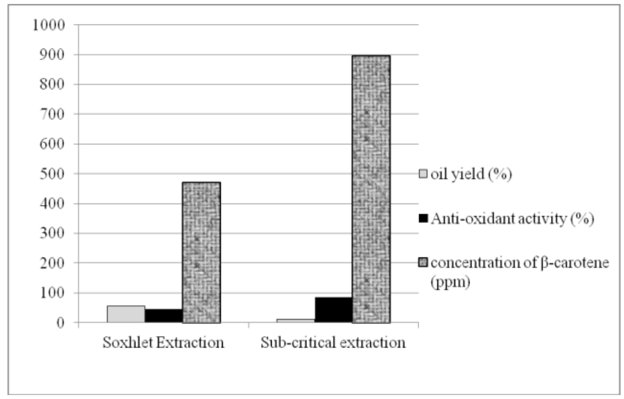


Figure 7 Comparison between soxhlet and sub-critical extraction method

4.0 CONCLUSION

From the experiments conducted in this study sub-critical extraction is a promising method for the extraction of interest compound, β-carotene and their properties. In this study, the optimum pressure at 25 MPa produced 896.22 ppm of beta carotene and 86.2 % antioxidant activity during less than 1 hour extraction regime while soxhlet extraction with chloroform is potential as best extraction process. In this study, the process produced 55.15% oil yield in 5 hours regime extraction time. The study can be further explored by increasing the polarity of subcritical CO_2 with the addition of modifiers.

Acknowledgement

This research is funded by Research University Grant, UTM (Vote No. Q.J130000.2544.04H05) from Ministry of Higher Education Malaysia with collaboration of Centre of Lipids Engineering and Applied Research (CLEAR), Universiti Teknologi Malaysia (UTM), Malaysia.

References

[1] Itoh, N., M. Numata, Y. Aoyagi, and T. Yarita. 2008. Comparison of Low-level Polycyclic Aromatic Hydrocarbons in Sediment Revealed By Soxhlet Extraction, Microwave-Assisted Extraction, and Pressurized Liquid Extraction. *Analytica chimica Acta.* 612(1): 44–52.
[2] Akgün, M., N. A. Akgün, and S. Dinçer. 2000. Extraction and Modeling of Lavender Flower Essential Oil Using Supercritical Carbon Dioxide. *Industrial & Engineering Chemistry Research.* 39(2): 473–477.
[3] Mohd Azizi, C. Y., Z. Salman, and N. A. Nik Norulain. 2008. Extraction and Identification of Compounds from Parkia Speciosa Seeds by Supercritical Carbon Dioxide. *Journal of Chemical and Natural Resources Engineering*. 2: 153–163.
[4] Atanassova, M., S. Georgieva, and K. Ivancheva. 2011. Total Phenolic and Total Flavonoid Contents, Antioxidant Capacity and Biological Contaminants in Medicinal Herbs. *Journal of the University of Chemical Technology & Metallurgy*. 46(1): 81–88.
[5] Mandana, B., A. R. Russly, S. T. Farah, M .A. Noranizan, I. S. Zaidul, and G. Ali. 2012. Antioxidant Activity of Winter Melon (Benincasa Hispida) Seeds Using Conventional Soxhlet Extraction Technique. *International Food Research Journal*. 19(1): 229–234.
[6] *PORIM: Test Methods*. Palm Oil Research Institute of Malaysia, 1995.
[7] Mensor, L. L., F. S.Menezes, G. G. Leitão, A. S. Reis, T. C. D. Santos, C. S. Coube, and S. G. Leitão. 2001. Screening of Brazilian Plant Extracts for Antioxidant Activity by the Use of DPPH Free Radical Method. *Phytotherapy Research*. 15(2): 127–130.

[8] Kumoro, A. C., M. Hasan, and H. Singh. 2009. Effects of Solvent Properties on the Soxhlet Extraction of Diterpenoid Lactones From *Andrographis Paniculata* Leaves. *Science Asia*. 35: 306–309.

[9] Sporring, S., S. Bøwadt, B. Svensmark, and E. Björklun. 2005. Comprehensive Comparison of Classic Soxhlet Extraction with Soxtec Extraction, Ultrasonication Extraction, Supercritical Fluid Extraction, Microwave Assisted Extraction and Accelerated Solvent Extraction for the Determination of Polychlorinated Biphenyls in Soil. *Journal of Chromatography A*. 1090(1): 1–9.

[10] Markom, M., H. Singh, and M. Hasan. 2001. Supercritical CO_2 Fractionation of Crude Palm Oil. *Journal of Supercritical Fluid*. 20(1): 43–53.

[11] Franca, L. F. D., G. Reber, M. A. A. Meireless, N. T. Machado, and G. Brumer. 1999. Supercritical Extraction of Caroteniod and Lipid from Buriti (*Mauriria Flexuosa*) a Fruit from the Amazon Region. *Journal of Supercritical Fluid.* 14(3): 247–256.

[12] Tangkanakul, P., P. Auttaviboonkul, B. Niyomwit, N. Lowvitoon, P. Charoenthamawat, and G. Trakoontivakorn. 2009. Antioxidant Capacity, Total Phenolic Content and Nutritional Composition of Asian Foods After Thermal Processing. *International Food Research Journal*. 16(4): 571–580.

[13] de Lucas, A., A. García, A. Alvarez, and I. Graci. 2007. Supercritical Extraction of Long Chain *N*-Alcohols from Sugar Cane Crude Wax. *The Journal of supercritical fluids*. 41(2): 267–271.

[14] Nik Norulaini, N. A., W. B. Setianto, I. S. M. Zaidul, A. H. Nawi, C. Y. Mohd Azizi, and A. K. Omar. 2009. Effects of supercritical Carbon Dioxide Extraction Parameters On Virgin Coconut Oil Yield And Medium-Chain Triglyceride Content. *Food Chemistry*. 116(1): 193–197.

[15] Yunus, M. A. 2007. Extraction, Identification, and Separation of Vitamin E and Djenkolic Acid from *Pithecellobium jiringan* (Jack) Prain Seeds using Supercritical Carbon Dioxide (Doctoral dissertation, Thesis of Doctor of Philosophy University Sains Malaysia).

[16] Roy, B. C., M. Sasaki, and M. Goto. 2006. Effect of Temperature And Pressure on the Extraction Yield of Oil from Sunflower Seed with Supercritical Carbon Dioxide. *Journal of Applied Science.* 6: 71–75.

[17] Mendes, R. L., B. P. Nobre, J. P. Coelho, and A. F. Palavra. 1999 Solubility of β-carotene in Supercritical Carbon Dioxide and Ethane. *Journal of Supercritical Fluids.* 16(2): 99–106.

[18] Puah, C. W., Y. M. Choo, A. N. Ma, and C. H. Chuah. 2005. Supercritical fluid Extraction of Palm Carotenoids. *American Journal of Environmental Science*. 1(4): 264–269.

[19] Gauthier, B. M., S. D. Bakrania, A. M. Anderson, and M. K. Carroll. 2004. A Fast Supercritical Extraction Technique for Aerogel Fabrication. *Journal of Non-Crystalline Solids.* 350: 238–243.

[20] Stoilova, I., A. Krastanov, A. Stoyanova, P. Denev, and S. Gargova. 2007. Antioxidant Activity of a Ginger Extract (*Zingiber Officinale*). *Food Chemistry*. 102(3): 764–770.

[21] Díaz-Reinoso, B., A. Moure, H. Domínguez, and J. C. Parajó. 2006. Supercritical CO_2 Extraction and Purification of Compounds With Antioxidant Activity. *Journal of Agricultural and Food Chemistry*. 54(7): 2441–2469.