TAF
Journal of Advances in Technology and Engineering Research
2015, 1(1): 15-21
JATER

Extraction of beta-carotene from palm mesocarp via green sub-criticae Carbon Dioxide

Mohd Azizi CheYunus [1], Muhammad Syafiq Hazwan Ruslan [2], Jefry Bin Jamal [3], Wan Aminullah Wan Abdul Aziz [4,*], Zuhaili BintiIdham [5]

[1, 2, 3, 4,] Centre of Lipid Engineering and Applied Research (CLEAR), Faculty of Chemical Engineering, University Teknologi Malaysia

Index Terms:
Supercritical Fluid Extraction
Oil Yield
Antioxidant Activity
Beta-Carotene
Palm Mesocarp

Received: 28 September 2015
Accepted: 3 October 2015
Published: 15 October 2015

Abstract—This study talks about the experiments involving sub-critical carbon dioxide extraction of palm oil from treated palm mesocarp to determine palm oil yield and concentration of beta-carotene. For comparison, the conventional method of Soxhlet Extraction with six different solvents was used. The overall oil yield was measured as the weight of oil per weight of the sample, while anti-oxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl radical scavenging method. The sub-critical extraction was conducted at a constant temperature of 30˚C and four different pressures of 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.

I. 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 the history, palm oil has served as the primary source of dietary fat for countless numbers of applications and 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 extraction of solid materials or particles from a desired compound by using specific solvents. The extraction process can be applied either 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

*Corresponding author: Mohd Azizi CheYunus
E-mail: azizi@cheme.utm.my

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].

An 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]. 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 in 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]. This study focused on the extraction of beta carotene from palm mesocarp by using Soxhlet extraction with different types of solvent and sub-critical carbon dioxide extraction. In addition, the study also aimed to determine the optimum pressure for sub-critical extraction.

II. MATERIALS AND METHOD

A. Materials

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.

TABLE 1
CONDITIONS OF EXTRACTION PROCESS

Sub-CriticalFluid Extraction	Soxhlet Extraction
Temperature : 30C Pressure: 10Mpa, 15Mpa, 20Mpa, 25Mpa Flowrate: 5ml/min Time Extraction: 1 hour Weight Sample: 5 grams Particle Size:0.8±0.2mm	Temperature:70C Pressure: Ambient Pressure Time Extraction: 5 hours Weight Sample: 20 grams Solvents: Water, Methanol, n-Hexane, Chloroform, Isopropanol,Petroleum Ether Particle Size: 0.8±0.2mm

B. Extraction Process

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

C. 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 were diluted with 7ml of hexane. The solution was then transferred to a 1cm quartz cuvette and the absorbance was read at 446nm. The method was applied according to the PORIM Test Method, [6].

The carotene content was denoted as ppm beta-carotene by using the following formula:

$$\text{Concentration ?-carotene (ppm)} = 25 \times \frac{383}{100W} \times (a_s - a_b) \quad \text{(Eq 3.2)}$$

where a_s = absorbance of sample at 446 nm

a_b = the cuvette error

383 = the extension coefficient for carotenoids

W = weight of sample in grams

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

The radical scavenging ability of essential oil was determined as described [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:

$$AA\% = 100 - \left\{\frac{[(\text{Abs.sample} - \text{Abs.empty sample}) \times 100]}{\text{Abs.control}}\right\}$$

ISSN: 2414-4592
DOI: 10.20474/jater-1.1.2

III. RESULTS AND DISCUSSION

D. Soxhlet Extraction

Oil Yield Percentage

As seen from Figure 1, chloroform produced the highest percentage of oil yield (55.45%) than other solvents. [8] stated that oil is very soluble in chloroform because of its non-polar property, and chloroform is also an organic solvent that can be chemically used as solvent for the extraction of fat. 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. [8] also stated that oil is not soluble in water due to its high molecular weight and tendency to float.

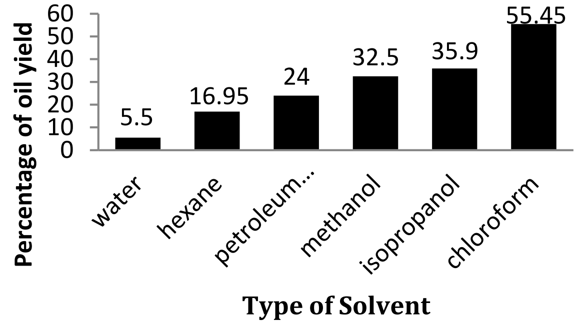

Fig. 1. Percentage of oil yield versus different types of solvent

E. Concentration of Beta-Carotene

The concentration of beta-carotene against types of solvent is shown in Figure 2. The highest concentration of beta-carotene was obtained by choloroform (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 scale. [9] Defined polarity as the ability of a molecule to engage in strong interaction with other polar molecules.

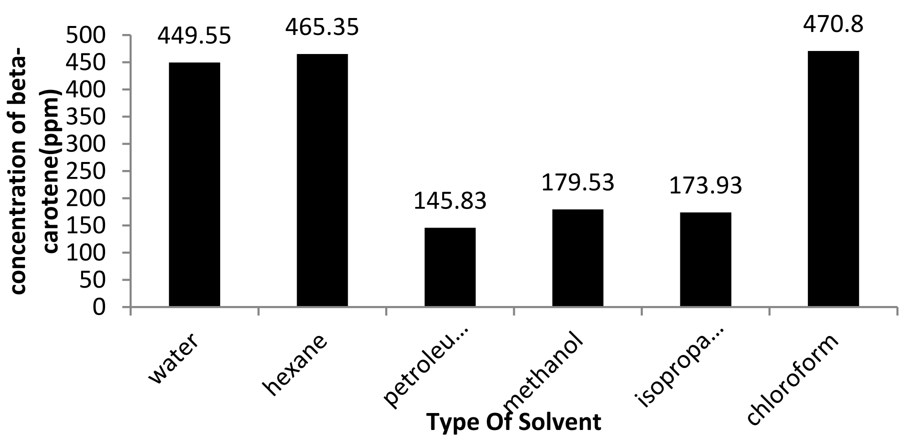

Fig. 2. Concentration of beta-carotene in different types of solvent.

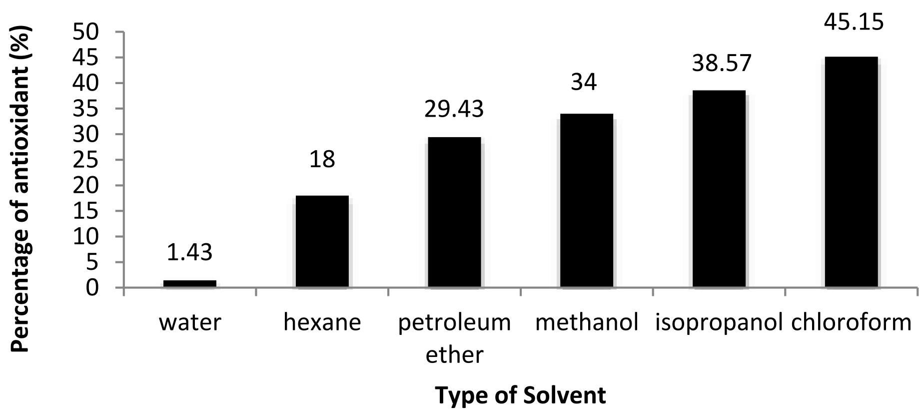

Fig.3. Antioxidant percentage with the use of different solvents.

F. 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, due to ability of oil to penetrate into the interaction of relative polarity of solvents as compared to water [10].

G. Sub-Critical Fluid Extraction

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, the oil yield directly increased with the increase in pressure. The highest overall oil yield was obtained at pressure 25 Mpa (10.22 %), while the lowest pressure of 10 Mpa generated only 1.92%. This phenomenon can be easily explained by the basic principle of sub-critical fluid extraction which states that as the pressure increases the density of sub-critical CO2 approaches of a liquid, it will result in an increase in the solvating power [11]. Futhermore, as the density of solvent increased, the inter-molecular interaction of solutes also rose [12]. As a result, oil and solvent dissolution were promoted, thereby increasing the oil extraction [3]. However, the increase in the oil-solvent dissolution with pressure during the solubility-dependent stage was very slow as compared to the increase in the diffusability-dependent stage [12].

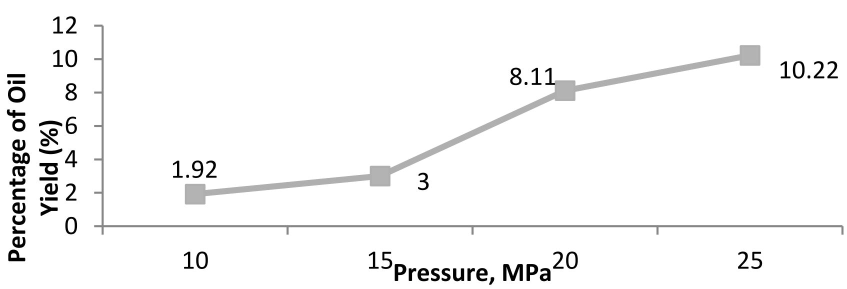

Fig. 4. Oil yield percentage for sub-critical extraction.

H. 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 25MPa (896.22 ppm), while the lowest concentration was at 418.91ppm at 10MPa. The increase might be due to the high concentration of beta-carotene compared to other components co-existing in carotenoids. [13] noted that the presence of other components being co-extracted with carotene could result in low solubility of β-carotene.

ISSN: 2414-4592
DOI: 10.20474/jater-1.1.2

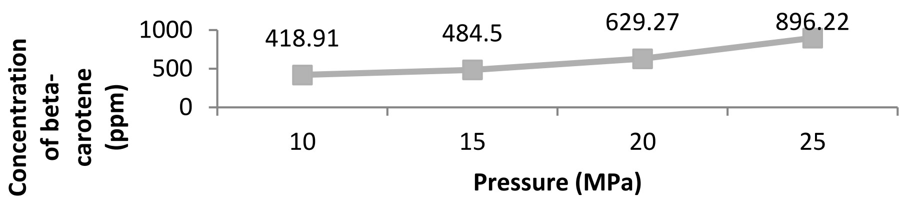

Fig. 5. Concentration of beta-carotene by sub-critical extraction.

In addition, there are unofficial 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 [14].

I. 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 of 25 MPaobtained 86.20% of antioxidant activity. On the other hand, the minimum pressure of 10 MPaobtained 83.37% of antioxidant activity.

This phenomenon was due to the increase in pressure as the density of CO2 was higher, causing the penetration of CO2 into matrix solid to become easier[15]. Therefore, the mass transfer increased according to the increase of pressure [1].

J. Comparison between Soxhlet Extraction and Sub-Critical Fluid Extraction

The comparison between the strength of sub-critical Carbon dioxide extraction and Soxhlet Extraction was done with respect to the oil yield, concentration of beta-carotene and antioxidant activity as shown in Figure 7 to 9.

As observed, sub-critical 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 sub-critical extraction which took less than 1 hour.

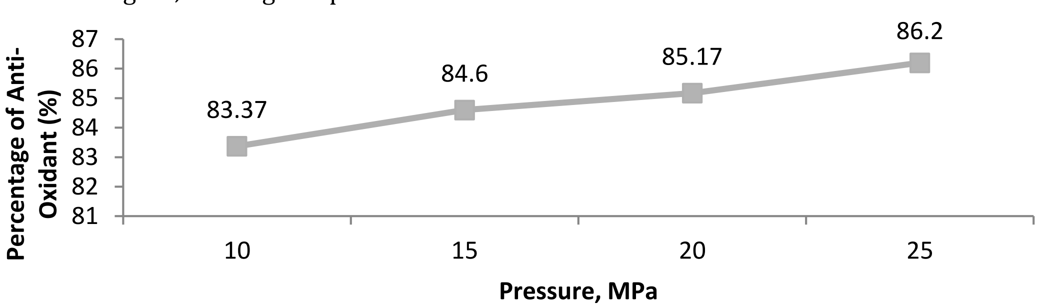

Fig. 6. Anti-oxidant activity for oil extracted by sub-critical fluid extraction.

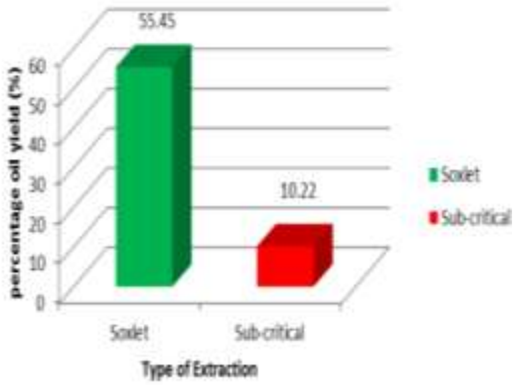

Fig. 7. Oil yield percentage

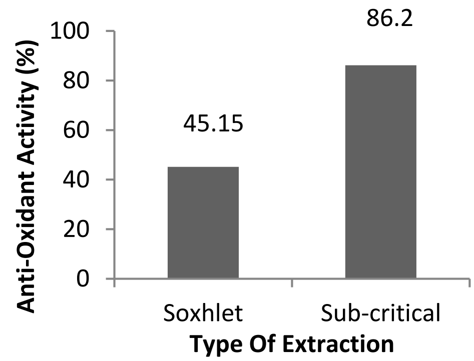

Fig. 8. Percentage of antioxidant activity in different extraction

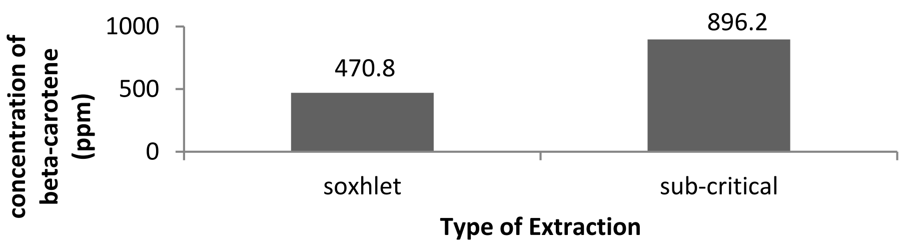

Fig. 9. Concentration of beta-carotene at different methods.

IV. CONCLUSION

From the experiments conducted in this study, the following conclusions can be made:

1. Sub-critical extraction is a promising method for the extraction of desired compound, beta-carotene and their properties. In this study, the optimum pressure at 25MPa produced 896.22 ppm of beta-carotene and 86.2 % antioxidant activity in less than 1 hour of extraction regime.
2. Soxhlet extraction with choloroform is potential as best extraction process. In this study, the process produced 55.15% oil yield in 5 hours regime extraction time.
3. The study can be further explored by increasing the polarity of sub-critical CO2 with the addition of modifiers.

ACKNOWLEDGEMENT

This research is funded by Malaysian government under Exploratory Research Grant Scheme (ERGS) (Vote no:R.J130000.7844.4L037), 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] M. Akgün, A. N. Akgün and Dinçer, S. 2000," Extraction and modeling of lavender flower essential oil using supercritical carbon dioxide," *Industrial &Engineering Chemistry Research,* vol. 39, no. 2, pp. 473-477. **DOI**: 10.1021/ie9904798

[2] M. Atanassova, S. Georgieva and K. Ivancheva, "Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs," *Journal of the University of Chemical Technology & Metallurgy*, vol. 46, no.1, pp. 81-88, 2011.

[3] A. de Lucas, A. García, A. Alvarez and I. Gracia, "Supercritical extraction of long chain *n*-alcohols from sugar cane crude wax," *The Journal of Supercritical Fluids*, vol. 41, no. 2, pp. 267-271, 2007. **DOI:** 10.1016/j.supflu.2006.09.013

[4] B. Díaz-Reinoso, A. Moure, H. Domínguez and J. C. Parajó, "Supercritical CO_2 extraction and purification of compounds with antioxidant activity. *Journal of Agricultural and Food Chemistry*, vol. 54, no. 7, pp. 2441-2469, 2006. **DOI:** doi.org/10.1021/jf052858j

[5] B. M. Gauthier, S. D. Bakrania, A. M. Anderson and M. K. Carroll, "A fast supercritical extraction technique for aerogel fabrication," *Journal of Non-Crystalline Solids*, no. 350, pp. 238-243, 2004. **DOI:** 10.1016/j.jnoncrysol.2004.06.044

[6] N. Itoh, M. Numata, Y. Aoyagi and T. Yarita, "Comparison of low-level polycyclic aromatic hydrocarbons in sediment revealed by Soxhlet extraction, microwave-assisted extraction, and pressurized liquid extraction. *Analytica Chimica Acta*, vol. 612, no. 1, pp. 44-52, 2008. **DOI:** 10.1016/j.aca.2008.02.012

[7] A. C. Kumoro, M. Hasan, and H. Singh, "Effects of solvent properties on the Soxhlet extraction of diterpenoid lactones from Andrographis paniculata leaves," *Science Asia,* vol. 35, pp. 306-309, 2009. **DOI:** 10.2306/scienceasia1513-1874.2009.35.306

[8] M. Z. Islam and G, Ali, "Antioxidant activity of winter melon (Benincasa Hispida) seeds using conventional soxhlet extraction technique," *International Food Research Journal*, vol. 19, no. 1, pp. 229-234. 2012.

[9] L. L. Mensor, F. S. Menezes, G. G. Leitão, A. S. Reis, T. C. D. Santos, C. S. Coube and S. G. Leitão, "Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phyto therapy Research*, vol. 15, no. 2, 127-130, 2001. **DOI:** 10.1002/ptr.687

[9] N. A. Nik Norulaini, W. B. Setianto, I. S. M. Zaidul, A. H. Nawi, C. Y. M. Azizi and A. K. Omar, "Effects of supercritical carbon dioxide extraction parameters on virgin coconut oil yield and medium-chain triglyceride content, "*Food Chemistry*, vol. 116, no. 1, pp. 193-197, 2009. **DOI:** 10.1016/j.foodchem.2009.02.030

[10] Anonymous, *PORIM: Test Methods*. Palm Oil Research Institute of Malaysia, 1995.

[11] C. W. Puah, Y. M. Choo, A. N. Ma and C. H. Chuah, "Supercritical fluid extraction of palm carotenoids," *American Journal of Environmental Science*, vol. 1, no. 4, pp. 264-269, 2005. **DOI:** 10.3844/ajessp.2005.264.269

[12] B. C. Roy, M. Sasaki and M. Goto, "Effect of temperature and pressure on the extraction yield of oil from sunflower seed with supercritical carbon dioxide," *Journal of Applied Sciences,* vol. 6, no. 1, pp. 71–75, 2006. **DOI:** 10.3923/jas.2006.71.75

[13] S. Sporring, S. Bøwadt, B. Svensmark and E. Björklund, "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,* vol. 1090, no. 1, pp. 1-9, 2005. **DOI:** 10.1016/j.chroma.2005.07.008

[14] I. Stoilova, A. Krastanov, A. Stoyanova, P. Denev and S. Gargova, "Antioxidant activity of a ginger extract (Zingiber officinale)," *Food Chemistry,* vol. 102, no. 3, pp. 764-770, 2007. **DOI:** 10.1016/j.foodchem.2006.06.023

[15] P. Tangkanakul, P. Auttaviboonkul, B. Niyomwit, N. Lowvitoon, P. Charoenthamawat and G. Trakoontivakorn, "Antioxidant capacity, total phenolic content and nutritional composition of Asian foods after thermal processing," *International Food Research Journal*, vol. 16, no. 4, pp. 571-580, 2009.

[16] C. Yunus, M. Azizi, Z. Salman and N. A. Nik Norulain, "Extraction and identification of compounds from Parkia speciosa seeds by supercritical carbon dioxide," *Journal of Chemical and Natural Resources Engineering*, vol. 2, pp. 153-163, 2008.

[17] M. A. Yunus, "Extraction, identification, and separation of vitamin e and djenkolic acid from pithecellobium jiringan (jack) prain seeds using Supercritical Carbon Dioxide," Doctoral dissertation, University Sains Malaysia, 2007.

— This article does not have any appendix. —