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Effects of Organic Solvent and Temperature on the Extraction of Lutein from *Scenedesmus sp* Biomass

(Kesan Pengunaan Pelarut Organik dan Suhu dalam Proses Pengekstrakan Lutein dari biomas *Scenedesmus sp*)

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ABSTRACT

Lutein is a valuable bioactive compound that has various industrial applications. In nature, lutein is a yellow coloured isoprenoid polyene pigment, being produced by many photosynthetic organisms. With regard to Scenedesmus sp as the studied organism, this study aims to investigate the efficiency of microalgae-derived lutein extraction process. Repetitive solvent extraction method had been examined with four different organic solvents under different treatment temperatures for their lutein extraction effectiveness. Results showed that diethyl ether was the most effective organic solvent to extract lutein as compared to acetone, ethanol and dichloromethane. Under the extraction temperature of 60°C, diethyl ether was able to extract a total of 14.61 ± 0.31 mg/g of lutein from microalgae biomass. All the studied extraction treatments showed that lutein content in the extractants decreased as the number of extraction repetitions increased. By increasing the extraction repetition to 7 cycles using diethyl ether, treatment temperature at 60 °C resulted in a total of 16.07 ± 0.26 mg/g of lutein being extracted. Therefore, by selecting the most suitable organic solvent in lutein extraction, higher extraction treatment temperature shall provide sufficient energy required for the extraction process, thus further enhancing the overall extraction performance.

Keywords: Microalgae; Scenedesmus sp; Organic Solvent Extraction; Temperature

ABSTRAK

Lutein adalah sejenis bioaktive kompound yang kerap digunakan dalam pelbagai aplikasi industri. Dari sudut kimia, lutein adalah sejenis poli-isoprenoid pigmen yang berwana kuning. Pigmen ini dapat dihasilkan daripada pelbagai jenis fotosintetik organisma dengan cara semula jadi ataupun secara organik. Scenedesmus sp adalah sejenis mikro-alga yang menghasilkan lutein. Dengan itu, matlamat kajian ini ingin menyelidik keberkesanaan cara pengekstrakan lutein dari sumber mikro-alga. Cara pengekstrakan berulangan "Repetitive solvent extraction" telah dilaksanakan dengan menggunakan empat jenis pelarut organik yang berbeza. Selain itu, keberkesanan proses pengekstrakan lutein turut dikaji dari segi suhu pengekstrakan yang berbeza. Hasil kajian menunjukkan bahawa, dietil eter merupakan pelarut organik yang paling berkesan untuk pengekstrakan lutein. Susunan ini diikuti dengan aseton, etanol dan akhirnya diklorometana. Apabila proses pengekstrakan dilaksanakan pada suhu 60°C, sampel ekstrak dari dietil eter mengandungi jumlah lutein sebanyak 14.61 ± 0.31 mg/g. Dari segi pengekstrakan secara berulangan, semua pelarut organik yang dikaji menunjukkan bahawa kuantiti lutein yang dapat diekstrak dari biomas semakin menurun apabila proses pengulagan semakin lanjut. Selepas kitaran pengekstrakan yang ketujuh dimana proses dilaksanakan pada suhu 60°C, jumlah lutein yang dapat diperolehi daripada sampel dietil eter adalah 16.07 ± 0.26 mg/g. Secara keseluruhan, suhu proses pengekstrakan memainkan peranan yang penting dengan membekalkan tenaga haba yang secukupnya dalam kegiatan aktiviti pengekstrakan lutein dari biomas. Dengan itu, keberkesanan cara pengekstrakan lutein dari mikro-alga dapat ditingkatkan dengan menggunakan pelarut organik yang bersesuaian serta dengan pengubahsuaian proses pengekstrakan kepada suhu yang lebih tinggi.

Kata kunci: Mikro-alga; Scenedesmus sp; Pelarut Organik; Suhu

INTRODUCTION

Carotenoids are widely distributed in nature. They are synthesized *de novo* by a vast group of photosynthetic organisms, from higher plants to many unicellular microorganisms. These colored carotenoid pigments play an important role in photosynthesis to ensure the survival of the living cells. Lutein ((3R, 3'R, 6'R)-β,ε-carotene-3,3'-diol)) is a yellow pigment in oxygenated carotenoids group or xanthophyll. Lutein has been regarded as a high value bioactive compound due to its various industrial applications in aquaculture, poultry farming, cosmetic, food processing and health-care industries (Lin et al. 2015 and Del Campo et al. 2001). Additionally, lutein has anti-oxidant property and reported to possess nutraceutical benefits such as preventing cardiovascular diseases (Dwyer et al. 2001), age-related blindness (Fernandez-Sevilla et al. 2010), and some types of cancers (Barbara and William. 2002; Del Campo et al. 2001; Heber and Lu. 2002). In present day, lutein is a common food additive labelled as E161b, and its market size in the United States has been estimated for more than 150 million dollar (Chan et al. 2013).

Today, lutein that is available in the current market has been derived from the petals of marigold flowers (Lin et al. 2015). Marigold flowers are usually being reported containing 0.3 mg/g of lutein (Gong and Bassi 2016). On the other hand, the lutein content in microalgae can achieve over 4mg/g in various studies (Ho et al. 2014) which is several folds higher than that extracted from marigold flowers. The increasing interest for lutein thus gives a new dimension for more alternative source like microalgae which have the advantages such as minimizing the use of limited agricultural land and intense human labour requirement in conventional lutein harvesting from plant resources (Fernandez-Sevilla et al. 2010).

The advance of fermentation technology play a critical role in the synthesis of various bio-products (Safri et al. 2017). Microalgae species are known to produce various bioactive compounds and potentially a new biological resources in many applications. Chlorophyceae, or commonly known as the green algae is a group of photosynthetic microorganism with their carotenoids distribution resemble those of green tissue in higher plants. Carotenoids with ε-end groups are present in these cells with lutein as the most abundant carotenoid pigment. In bioprocessing, the selection of competent lutein production strain is vital. Studies have been reported using various microalgae species to synthesize lutein. Among these, Scenedesmaceae family microalgae are known to produce carotenoid pigments (Minhas et al. 2016). However, local strains of *Scenedesmus sp* in Malaysia are not well documented for their lutein synthesis application.

Currently, lutein extraction process applied to microalgae study are derived generally from those established phytochemical extraction techniques on higher plants and macroalgae (Pasquet et al. 2011). Extraction and other downstream processing steps are critical components for the overall bioprocessing of microalgae lutein production. Although various downstream processes had been proposed, each and every processes have their own strength and weakness (Gong et al. 2016). In cell disruption stage, process choices ranged from mechanical aided treatments (bead beating, grinding, and cryogenic grinding), non-mechanical aided treatments (autoclave, microwave, and ultrasound), chemical treatments (acid, alkaline, hydrolytic enzymes) and others (pulse electric shock, osmotic pressure).Some of the major concerns including drawbacks in high energy demand in the multiple extraction steps, process efficiency, and cost-effectiveness; had urged the need for better downstream technologies.

Solvent extraction method is effective in extracting various valuable compounds from biological resources. Due to the "like dissolve like" property, organic solvents give a better extraction performance to extract slightly polar organic compounds. Other benefits of organic solvent mediated extraction process include their product selectiveness, enhance the purity of the final product, and without sophisticated purification downstream steps (Chen et al. 2016). Research work had been carried out intensively to identify the ideal solvent capable for intended compound extraction. Since lutein is the compound of interest, different types of organic solvent is expected to provide different strength of intermolecular forces of attraction which will result in different extraction performances. Heat energy is required in the extraction process. However, lutein contains many conjugated double bonds which are susceptible to degradation from heat, light, and oxidation. Currently, most of the reported studies carried out carotenoid solvent extraction under the treatment temperature of 40 ^{o}C. There is a lack of information to justify the effect of extraction treatment temperatures on the extraction of microalgae lutein. Therefore, this study aims to examine a better microalgae lutein extraction through organic solvent selection and heat treatment temperature determination using repetitive solvent extraction method.

METHODOLOGY

MICROALGAE STRAIN

Local strains of *Scenedesmus sp* were isolated from a fresh water pond (latitude: 1.5546892, longitude: 103.6379231) located at Johor, Malaysia. Isolated *Scenedesmus sp* was maintained on modified Bold's Basal Medium agar (1.6% w/v) in a light equipped incubator. The medium consisted of, 2.5×10^{-1} g $NaNO_3$, 7.5×10^{-2} g $MgSO_4.7H_2O$, 2.5×10^{-2} g NaCl, 7.5×10^{-2} g K_2HPO_4, 1.75×10^{-1} g KH_2PO_4, 2.5×10^{-2} g $CaCl_2.2H_2O$, 8.82×10^{-3} g $ZnSO_4.7H_2O$, 1.44×10^{-3} g $MnCl_2.4H_2O$, 2.04×10^{-3} g $MoNa_2O_4.2H_2O$, 1.57×10^{-3} g $CuSo_4.5H_2O$, 4.9×10^{-4} g $Co(NO_3)_2.6H_2O$, 1.14×10^{-2} g H_3BO_3, 5×10^{-2} g EDTA, 3.1×10^{-2} g KOH, 4.98×10^{-3} g $FeSO_4.7H_2O$, concentrated 1×10^{-3} ml H_2SO_4, and distilled water to make up in 1 L. Plates containing isolated strains were sub-cultured once a month to ensure the continuous growth of *Scenedesmus sp*.

GROWTH PROFILE DETERMINATION

Scenedesmus sp growth profile was determined in order to identify the optimum harvest point with highest lutein content accumulated in the biomass (Data not shown). Optical density measurements using Shimadzu UVmini-1240 UV-vis spectrophotometer at 680nm of *Scenedesmus sp* was converted to dry cell weight (g / L). Nitrate concentration in the medium was measured using spectrophotometer at 220nm (Ho et al. 2014).

MICROALGAE CULTIVATION

Cultivation inoculum was prepared initially by inoculating a loop of *Scenedesmus sp* from agar into 200 ml Bold's Basal Medium in a conical flask. Inoculated flask was then incubated under continuous illumination of cool white fluorescent light at 120 $\mu molm^{-2}s^{-1}$, aerated with bubbling gas flow. When biomass reached mid log growth phase (4-5 days cultivation) it served as a seed culture for photobioreactor cultivation. In order to ensure the uniform quality of the biomass for extraction study, *Scenedesmus sp* was cultivated in a 5L airlift photobioreactor illuminated with external light source mounted at the sides of photobioreactor vessel. Airlift photobioreactor was operated according to the following conditions: inoculum concentration, 100 mg/L; working volume, 5L; pH control, 7; temperature control, 30°C; CO_2 concentration, 3.5%; aeration; 0.4 vvm and continuous light illumination of 300 $\mu molm^{-2}s^{-1}$ as optimized in previous study (Data not shown).

Upon nitrate depletion in the cultivation medium, *Scenedesmus sp* biomass was harvested by the mean of centrifugation method at 4000 rpm for 10 min. Microalgae cells pallet was then washed twice with deionised water to remove any residual medium content. Cell pallet was lyophilised for 48 hours using freeze dryer, then stored at -20°C for the later lutein extraction study.

LUTEIN EXTRACTION

The whole extraction process was performed under dim light condition. 15 mg of lyophilised biomass was mixed with 1ml of 60% KOH solution (w/w) and then subjected to homogenizer treatment for 1 minute. The cell mixture was then transferred to a screw capped test tube (with septum) and added with 2 ml of organic solvents (diethyl ether, dichloromethane, acetone or ethanol). Cell mixture was then incubated in water bath of different treatment temperatures at 40°C, 50°C, and 60°C for 40 min to extract lutein out from the biomass. After the heat treatment, cell mixture was vortexed for 10 seconds before centrifuged for 2 min at 2000 rpm. The organic layer containing lutein was then removed from the test tube, new organic solvent was added into the test tube and the extraction process was repeated from the heating process. All the lutein extracts were purged with stream of nitrogen gas to remove organic solvent. Dried lutein precipitate was then re-suspended in acetone and kept in -20°C for later quantification analysis.

LUTEIN QUANTIFICATION USING HPLC

Prior to sample quantification, all the extracts were filtered through 0.22µm syringe filter. Lutein concentration in each extract was then determined using High Performance Liquid Chromatography 1220 infinity gradient LC system VL (Agilent, Germany). Compounds separation was performed using a reverse phase C-18 column (Lune 3.0 mm C18, 250 mm x 4.6 mm, 5 µm, Phenomenex, Torrance, California, USA). Binary solvent system which consist of 99% solvent A (Acetonitrile : Methanol, 7:3) and 1% solvent B (Ethyl Acetate : Water, 2:0.04) was used to elute the analytical sample with the solvent flow rate of 1 ml/minute. HPLC system was equipped with Photodiode Array Detector (PDA) set with absorbance wavelengths at 450 nm. Lutein standard from Sigma Chemical Co. (St. Louis, Missouri, USA) was purchased for quantification purpose.

The concentration of lutein extracted per gram of lyophilized biomass, mg/g was calculated based on equation (1):

$$\text{Lutein Concentration, mg/g} = \frac{\text{Lutein Concentration (mg/L) x Solvent (L)}}{\text{Cell Weight of Treated Biomass(G)}} \quad (1)$$

STATISTICAL ANALYSIS

Statistical analyses of data was carried out using Statistical Package for the Social Sciences, Version 22 (IBM). Data collected was subjected to analyses of variance (ANOVA).

RESULTS AND DISCUSSION

Temperature is an important factor which affects the extraction performance of biological compounds (Markom et al, 2002). As shown in results presented in both Figure 1 and Table 1, extraction temperature is a critical factor that affects lutein extraction efficiency.

The overall results showed that the amount of lutein extracted was significantly higher when the process was conducted at 60°C, compared to 50°C, and 40°C. Microalgae synthesized lutein and stored it in intracellular for its role in photosynthesis activity. In order to obtain such intracellular biological compound, extraction process is required. Some of the energy demanding processes include the breaking of the microorganism cell wall, breaking of the chlorophyll organelles and even the thylakoid membrane to release lutein from microalgae biomass. Besides, studies showed that saponification process which is another energy demanding process is required in the extraction of lutein from biological sources (Dineshkumar et al. 2015; Ho et al. 2014). Therefore, the outcome of this study suggests that extraction process performed at 60°C resulted in higher lutein content because the amount of energy provided was sufficiently higher to facilitate the breaking of microalgae cellular cell walls.

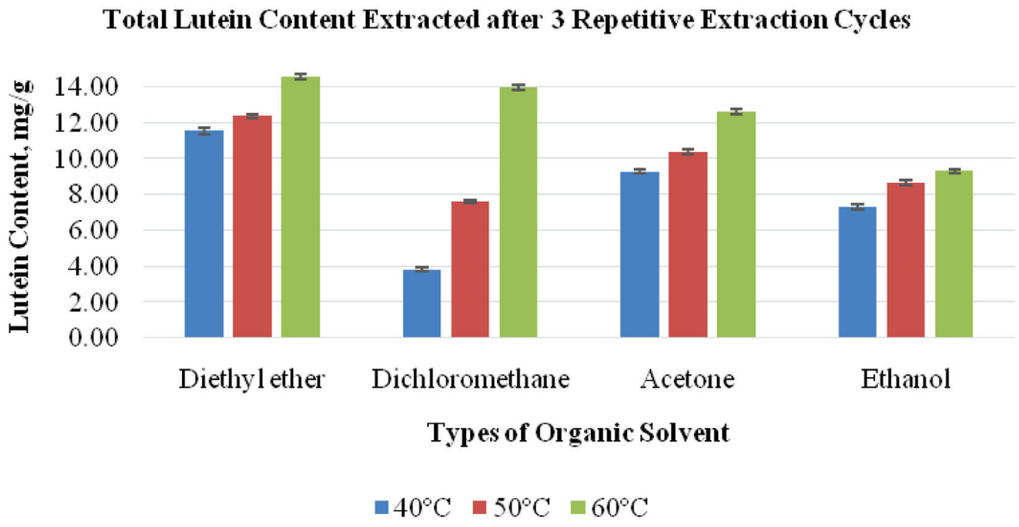

FIGURE 1. Total Amount of Lutein Extracted after 3 Repetitive Extraction Cycles (Data collected in Triplicates)

TABLE 1. Amount of Lutein Collected after Three Repetitive Extraction Cycles (Means ± Standard Deviation in Triplicates)

	Diethyl Ether	Dichloromethane	Acetone	Ethanol
40°C	11.58 ± 0.35	3.82 ± 0.18	9.31 ± 0.19	7.36 ± 0.26
50°C	12.41 ± 0.21	7.63 ± 0.17	10.42 ± 0.26	8.66 ± 0.29
60°C	14.61 ± 0.31	14.00 ± 0.31	12.68 ± 0.25	9.32 ± 0.23

The selection of suitable organic solvent is another factor being investigated. In this study, it was found that different organic solvent posed different attraction affinity towards lutein compound as represented in their lutein extraction efficiency. Diethyl ether was found to be the most effective organic solvent in lutein extraction at all temperatures as compared to dichloromethane, acetone and ethanol. These results were consistent with that reported by Chan et al. (2013) in his study of lutein extraction. Another possible explanation for this observation was that pressure factor was involved in the lutein extraction process. The boiling point of the organic solvents in used were 35°C for diethyl ether, 39°C for dichloromethane, 56°C for acetone, and 78°C for ethanol. As stated by Gay-Lussac's law, the pressure of gas accumulated at a constant volume is directly proportional to the Kelvin temperature. Therefore, when the extraction temperature is higher, the pressure accumulated in a constant volume is higher.

In the case of different extraction solvent, diethyl ether which had the lowest boiling point had created a higher pressure in the closed extraction system, thus increased the amount of lutein being extracted as compared to other type of organic solvents. Halim et al. (2012), concluded that high pressure treatment resulted a more effective cell disruption process while Chen et al. (2016) found that low level of pressure is sufficient to enhance lutein extraction performance. Further study could be done to investigate the relationship between extraction pressures with that of lutein extraction efficiency.

Since diethyl ether was the best organic solvent to extract lutein, the number of extraction cycle was examined to recover the total lutein content from its biomass. As tabulated in Table 2, results indicated that approximately 16mg of lutein can be extracted from 1g of *Scenedesmus sp* regardless of any extraction temperature.

TABLE 2. Total Amount of Lutein Collected after Seven Repetitive Extraction Cycles using Diethyl Ether (Means ± Standard Deviation in Triplicates)

Extraction Temperature	40°C	50°C	60°C
Total Amount of Lutein, mg/g	15.98 ± 0.21	15.99 ± 0.18	16.07 ± 0.26

However, it took only 6 repetition cycles to achieve this result when the extraction process was carried out at 60°C, where else it took 7 cycles to achieve the same result when the extraction process was carried out at 50°C and 40°C. This means that extraction process carried out at higher temperature stands a chance to decrease its processing time. From the presented Figure 2 as well, extraction cycles 1, 2

and 3 showed that the amount of lutein extracted was higher when the extraction temperature was higher; while the later cycles 4 to 7 showed that the amount of lutein extracted was lower when the extraction temperature was higher. The curve shifted to its left when the extraction temperature increased was another explanation that lutein extraction efficiency was better when the extraction temperature was higher.

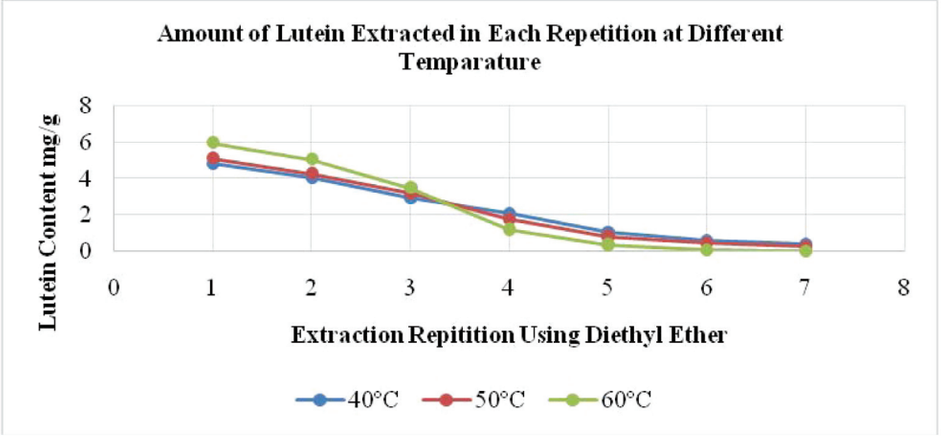

FIGURE 2. Amount of Lutein Extracted in Each Repetition at Different Temperature

CONCLUSION

The findings revealed that extraction temperature and choice of organic solvent were important factors in the process of developing lutein extraction protocol. In repetitive solvent extraction method using diethyl ether as the extraction solvent, 60°C was the better extraction temperature due to sufficient heat energy provided for the extraction process. Since the current study covered the extraction temperature only from 40°C to 60°C, future study can examine temperature factor in a higher range to identify the optimum extraction temperature for the selected extraction solvent.

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REFERENCES

Barbara, D. A. & William, W. A. 2002. Antioxidants in photosynthesis and human nutrition. *Science* 298: 2149-2153.

Chan, M. C., Ho, S. H., Lee, D. J., Chen, C. Y., Huang, C. C. & Chang, J. S. 2013. Characterization, extraction and purification of lutein produced by an indigenous microalga Scenedesmus obliquus CNW-N. *Biochemical Engineering Journal* 78: 24-31.

Chen, C. Y., Jesisca, Hsieh, C., Lee, D. J., Chang, C. H. & Chang, J. S. 2016. Production, extraction and stabilization of lutein from microalga Chlorella sorokiniana MB-1. *Bioresource Technology* 200: 500-505.

Del Campo, J. A., Herminia, R., José, M., Ángeles, V. M., Joaquín, R. & Miguel G. G. 2001. Lutein production by Muriellopsis sp. in an outdoor tubular photobioreactor. *Journal of Biotechnology* 85: 289-295.

Dineshkumar, R., Dhanarajan, G., Dash, S. K. & Sen, R. 2015. An advanced hybrid medium optimization strategy for the enhanced productivity of lutein in Chlorella minutissima. *Algal Res* 7: 24-32.

Dwyer, J. H., Navab, M., Dwyer, K. M., Hassan, K., Sun, P., Shircore, A., Hama-Levy, S., Hough, G., Wang, X., Drake, T., Merz, C. N. & Fogelman, A. M. 2001. Oxygenated carotenoid lutein and progression of early atherosclerosis: the Los Angeles atherosclerosis study. *Circulation* 103: 2922-2927.

Fernandez-Sevilla, J. M., Acien-Fernandez, F.G. & Molina-Grima, E. 2010. Biotechnological production of lutein and its applications. *Appl Microbiol Biotechnol* 86: 27-40.

Gong, M. & Bassi, A. 2016. Carotenoids from microalgae: A review of recent developments, *Biotechnology Advance* 34: 1396-1412.

Heber, D. & Lu, Q. Y. 2002. Overview of mechanisms of action of lycopene. *Exp Biol Med* 227: 920-923.

Halim, R., Harun, R., Danquah, M. K. & Webley, P. A. 2012. Microalgal cell disruption for biofuel development. *Appl. Energy* 91: 116-121.

Ho, S. H., Chan, M. C., Liu, C. C., Chen, C. Y., Lee, W. L., Lee, D. J. & Chang, J. S. 2014. Enhancing lutein productivity of an indigenous microalga Scenedesmus obliquus FSP-3 using light-related strategies. *Bioresource Technology* 152: 275-282.

Lin, J. H., Lee, D. J. & Chang, J. S. 2015. Lutein production from biomass: Marigold flowers versus microalgae. *Bioresource Technology* 184: 421-428.

Markom, M., Singh, H. & Hasan, M. 2002. Extraction of crude palm oil using supercritical CO_2. *Jumal Kejuruteraan* 14: 53-62.

Minhas, A. K., Peter, H., Barrow, C. J., Sashidhar, B. & Adholeya, A. 2016. The isolation and identification of new microalgal strains producing oil and carotenoid simultaneously with biofuel potential. *Bioresource Technology* 211: 556-565.

Pasquet, V., Chérouvrier, J. R., Farhat, F., Thiéry, V., Piot, J. M., Bérard, J. B., Kaas, R., Serive, B., Patrice, T., Adoret, J. P. & Picot, L. 2011. Study on the microalgal pigments extraction process: Performance of microwave assisted extraction. *Process Biochemistry* 46: 59-6

Safri, N. A., Jalil, R. & Kalil, M. S. 2017. Fermentable sugars from agrowastes using cellulase enzymes from local white rot fungi *Pycnoporus sanguineus*. *Jurnal Kejuruteraan* 29(2): 105-111.

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