Jurnal Teknologi

Full Paper

IMPROVED YIELD OF β-CAROTENE FROM MICROALGAE *SPIRULINA PLATENSIS* USING ULTRASOUND ASSISTED EXTRACTION

Article history
Received
2 May 2015
Received in revised form
31 July 2015
Accepted
1 October 2015

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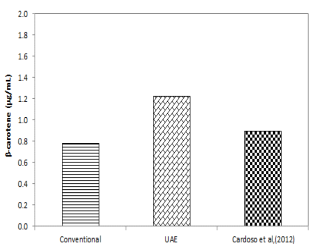
Graphical abstract



Abstract

Microalgae Spirulina has been considered as the rich nutrition microorganism including β-carotene. The β-carotene plays an important role in human's body as an antioxidant. The extraction of this compound from microalgae by using conventional method resulted low yield and required longer extraction time. The objective of this research was to investigate the use of ultrasound irradiation to assist extraction of β-carotene in order to accelerate the extraction process and increase the yield. The experiment was conducted by varying extraction variables such as solvent volume (20, 40, 60 ml) and extraction time (5, 15, 25 minutes) on a fixed temperature of 40°C. The result showed that the optimum condition was at ultrasound frequency of 40kHz, extraction time of 25 minutes and ratio of biomass to solvent 1:6, while the maximum yield was 1.38 µg/mL.

Keywords: Extraction, β-carotene, ultrasonic, anti-oxidant, Spirulina

1.0 INTRODUCTION

Among others, microalgae Spirulina has been considered as the main source of protein (>50%). The Spirulina biomass also contains various pigments contents such as phycocyanin, chlorophyll and carotenoids. Carotenoid is an organic pigment which naturally found in chromoplast of plants, photosynthetic microorganism such as microalgae and several types of mould and bacteria [1]. In photosynthetic microorganism, carotenoid has an important role in the main reaction especially in the energy transfer or protecting the main reaction from auto-oxidation [2]. In non-photosynthetic organisms such as human, carotenoid related to the mechanism of preventing oxidation. In microalgae Spirulina, β-carotene represents 67–79% of total carotenoids which is equivalent to 53% retinol present in carrot [3]. Previous studies reported that β-carotene plays a paramount role in human's body for an antioxidant, preventing myopia due to lack of vitamin A and also preventing cancer [4][5].

Conventionally, extraction of carotenoid compounds from natural biomass was conducted by using solvents methods such as maceration method. However, these methods led to several drawbacks i.e. large amount of solvent utilization, long extraction time and lower extraction yield. Several attempts have been reported to overcome these drawbacks such as by implementing ultrasound assisted extraction (UAE), microwave assisted extraction, supercritical fluid extraction and pressurized fluid extraction [6]. Amongst these methods, UAE is considered as an economically feasible and simple substitution to conventional extraction techniques [7].

The UAE system utilizes acoustic wave to produce cavitation bubbles which results high shear forces [8]. The forces help to disrupt the cell wall and then allow the solvent to penetrate into the material [9]. Consequently, the contact surface area between the solvent and bioactive compound of interest increase which resulting into an increase of mass transfer along with good mixing. Therefore, the UAE provides an increase of extraction yield and rate, reduced extraction time and higher processing throughput along with the advantage of the use of lower temperature and solvent volume [10] which is very useful for the extraction of heat sensitive compounds [11]. Despite above advantages, literature search to ultrasound assisted extraction of β-carotene from microalgae showed that studies of extraction related to β-carotene have been mostly focused on microalgae *Dunalliela salina*. Therefore, our research is focusing on the ultrasound assisted extraction of β-carotene from *Spirulina sp* as well as to determine optimum condition for this extraction method. Specifically, It purposes to determine the effect of time, biomass ratio to solvent, and wavelength frequency of ultrasonic extraction. The solvent of ethanol was chosen due to its high efficiency to extract high added value compound from microalgae [12].

2.0 EXPERIMENTAL

2.1 Materials

The biomass of Spirulina platensis was kindly provided by Bioprocess Laboratory of Chemical Engineering Department, Universitas Diponegoro. Ethanol 96% was used as the solvent for the extraction process. KOH was required as the mixing agent to dissolve fatty acid and other contents by saponification process.

2.2 Extraction Process

a. Conventional Extraction

The conventional extraction was conducted with variable of biomass to solvent ratio (1:2, 1:4, 1:6 w/v) and the temperature was set constant at 40°C for 5,15 and 25 min. After extraction, phycocyanin was filtered and centrifuged before it was being stored in a closed room and protected from the light.

b. UAE experiments

The ultrasound assisted extractions were performed in a ultrasonic bath, SONOREX RK type 100 H, (25-40 kHz, 320 W) produced by Bandelin Electronic GmbH & Co.KG, Berlin, Germany using biomass/solvent ratio of 1:2,1:4 and 1:6 w/v. Extractions were carried out at 45 °C for 5, 15, and 25 minutes. The obtained extracts were centrifuged at 2000 rpm for 8 min in a centrifuge Hettich Rotofix 32 A type. The supernatant was collected and analyzed

2.3 Analysis

After the extraction process, analysis using UV-Vis Spectrophotometer was applied. The extract was put in a centrifuge tube and saturated KOH and n-heptane were added into it, and then wait until the saponification process occurred. After centrifugation was done, the tube was shaken slowly before it was centrifuged at 4200 rpm. The extract was then mixed with n-heptane to optimize the β-carotene dissolved. The procedure was done until a clear-yellowish layer was obtained and be analyzed using UV-Vis Spectrophotometer at a wavelength of 436 nm.

3. RESULTS AND DISCUSSION

3.1 The effect of extraction time to β–Carotene concentration

The effect of extraction time (5-25 minutes) towards β-Carotene is shown in Figure 1. The extraction was applied using Ultrasound Assisted Extraction (UAE) method at low frequency (28 KHz) in 40°C with variation of solvent: sample ratio. The result shows that at constant solvent: sample ratio, increasing the extraction time leads to an increase of β-carotene concentration.

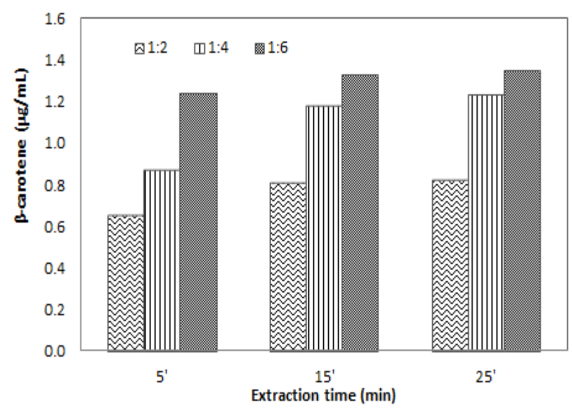


Figure 1 Effect of extraction time to the yield of β-Carotene with variation of extraction time

It is caused by high contact intensity between solvent and sample, which increase the diffusion due to carotene concentration gradients between inside and outside biomass. This process continues until the solvent is saturated in which there will be no effect anymore of increasing time on mass transfer [10].

Figure 1 also shows that the increase of solvent volume could increase the β-carotene concentration during extraction. The amount of solvent will be corresponded to the amount of β-Carotene could be extracted from the biomass [13].

3.2. The Effects of Ultrasonic Frequency to β-Carotene Concentration

The effect of wavelength frequency towards β-Carotene concentration is shown in Figure 2. The extraction was applying UAE method in 40°C for 25 minutes. The result shows that the yield of β-Carotene extracted at frequency of 28 KHz is lower than at frequency of 40 KHz. Ultrasonic with high frequency leads active cavitation which physically distrupt the sample14].

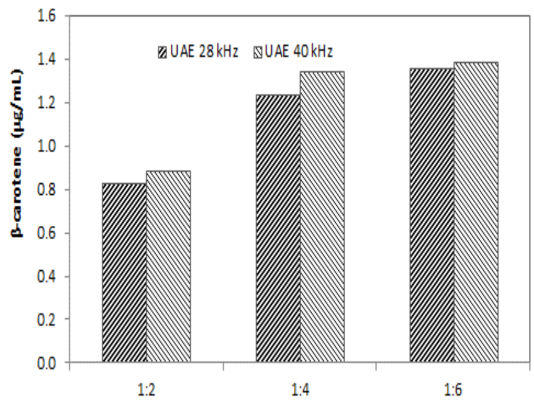


Figure 2 Effect of biomass: solvent ratio to the yield of β-Carotene with variation of ultrasound frequencies

The higher ultrasonic frequency will create more cavitation bubbles, thus the energy releases will be higher. Moreover, the small bubbles lead to the increase of surface area of contact between solvent and biomass. The energy produced is important to increase the turbulence in the solution such that the mass transfer rate will be higher. High mass transfer rate will stimulate the diffusion process of β-Carotene in Spirulina into solvent.

3.3 The effect of Extraction Method to β-Carotene Concentration

The research result in the effect of extraction method towards β-Carotene concentration is shown in Figure 3. The extraction was running at 40°C for 25 minutes. The result shows that β-Carotene concentration is significantly increased in UAE method under varied solvent ratio, which reaches twice larger than conventional method.

This also proves that the ultrasonic wave eases the preparation steps such as dissolution, fusion, and leaching. The ultrasonic cavitation also creates local temperature and movement of interface between solid and liquid, so it leads the increase of mass transfer rate As compared to conventional method, ultrasonic method has more advantages and one of them is its ability to increase the yield of product [16].

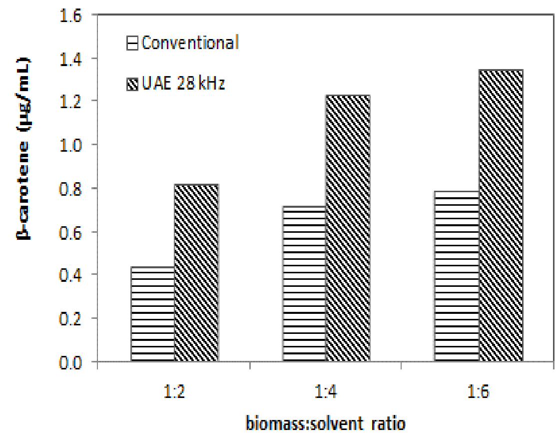


Figure 3 Effect of ultrasound to the improvement of yield of carotene at variation of solvent

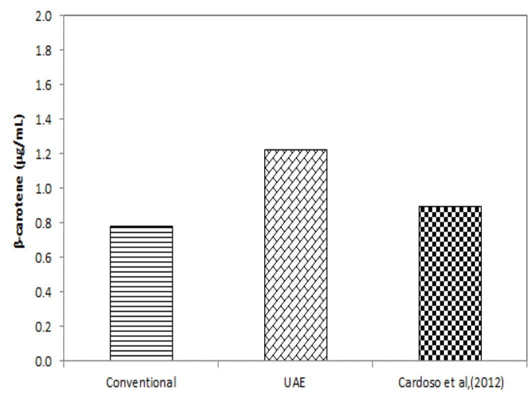


Figure 4 Comparison extraction methods and reference result

Figure 4 shows the comparison between the current extraction and reference result [17]. The UAE method obtained yield of 1.38 µg/mL which was more than the conventional method: 0.8 µg/mL. Cardoso *et al.* [17] used super critical assisted extraction method and obtained the maximum concentration at 0.9 µg/mL, which was very much lower than the UAE. Overall, the UAE also proves that the method was really powerful for increasing extraction efficiency and therefore it will be economical benefits for industries.

4.0 CONCLUSION

The microalgae Sprilunia has been evaluated as a potential source of β-Carotene. The ultrasound assisted extraction could enhance the yield of extraction with 50% higher than conventional.

The optimum concentration of pigment β-Carotene was achieved when using UAE with frequency 40 kHz and ratio of biomass and solvent up to 1:6 under 40°C with the yield of 1.38 µg/mL.

Compared to supercritical extraction method, the UAE results better performances and less energy used.

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