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PERFORMANCE OF MENTHOL BASED DEEP EUTECTIC SOLVENTS IN THE EXTRACTION OF CAROTENOIDS FROM CRUDE PALM OIL

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ABSTRACT: Currently, alternative chemical methods that produce less adverse effects on the environment are being explored to replace the conventional chemical methods. One example is the use of green solvents to replace the conventional hazardous solvents that are used extensively in industry. Palm oil contains vegetable oil which has many types of small components such as carotenoids, which have a natural source of provitamin A and are able to supply a relatively expensive source of vitamin A. Carotenoid compounds related to palm oil are useful ingredients for users who are sought by antioxidants. In this study, carotenoids in palm oil was extracted using a solvent extraction method with methanol and a Deep Eutectic Solvent (DES) as a co-solvent. DES which used was DL-Menthol-based DES as a Hydrogen Bond Donor (HBD) and acetic acid and lauric acid as Hydrogen Bond Acceptors (HBA) which were reacted with 1: 1 and 2: 1 molar ratios. DES was formed in the characterization of its physicochemical properties which include: Viscosity, Density and pH while characterization of the functional groups using FTIR. The optimum conditions for carotene extraction in this study were 212.7 ppm using DES MLA, while for the concentration of β-carotene obtained 0.156 ppm. These results showed that the DES solvent as a co-solvent can increase the yield of carotene extraction in crude palm oil.

Keywords: Crude palm oil, Carotenoids, Deep eutectic solvents, Extraction

1. INTRODUCTION

Palm oil has been used for food reserves for years. Although, researchers and industries have shifted their focus to non-food applications, such biodiesel production, but almost 90% of palm oil production is in the food sector [1]. In the food industry, palm oil is used as cooking oil, making margarine and shortening, and to produce nutritional supplements. In addition, Crude Palm Oil (CPO) also contains about 1% of small components such as carotenoids, vitamin E (tocotrienol and tocopherol), sterols, phospholipids, glycolipids, terpenoids and aliphatic hydrocarbons and other impurities. The main components are vitamin E and carotenoids which have very important functions [2]. Carotenoids are organic pigments (natural dyes) in plants and other photosynthetic organisms such as algae, several types of fungi and some bacteria. Until now, there are 600 known carotenoids in the class of xanthophylls and carotenoids. Natural carotenoids (also known as carotene extracts) which can give color pigments to food.

In Palm oil carotenoids compound has pro-vitamin A activity. β-carotene has positive biological effects beneficial to the human body, to overcome blindness, prevent cancer and coronary heart disease, prevent premature aging enhance body immunity and can also act as antioxidants that destroy free radicals which can reduce the chance of degenerative diseases [3-6].

By realizing the benefits of carotenoid compounds and the high content of carotenoid compounds in crude palm oil, carotenoid isolation from crude palm oil received great attention from researchers. It has developed various methods to get carotenoid compounds from crude palm oil. Some methods that have been carried out to get carotenoids from crude palm oil are using the methods of saponification, solvent extraction, adsorption, urea, molecular distillation, iodine, and membrane [7].

ILS is an ionic compound comprising various cations and anions. Organic cations are preferred by ILS because they can interact with other organic compounds [8]. ILS has excellent performance in chemical synthesis and separation processes, but ILS cannot be considered a green alternative because of several issues related to environmental sustainability, especially during their preparation. The emerge of DES as a eutectic solvent, received great attention because of an increase in features compared to conventional ILS.

Most DES can be made from inexpensive, biodegradable, non-toxic components. Similar to ILS, DES is considered a solvent designed with a variety of cations and anions. DES is formed between salt and various Hydrogen Bonding

Donors (HBD) with different proportions. Many HBDs used are available, such as urea, glycerol, polyols which are carbohydrate derivatives, and renewable carboxylic acids [9]. DES is prepared by mixing selected cations and HBD at 80-100 °C and can be used without further purification. DES resulted has a much lower melting point than the two components used.

DES is an advanced generation of Ionic Liquid (IL) which are biodegradable, cheap and easy to prepare, while still having IL properties, such as low melting point, low volatility, high thermal stability, high polarity, the inflammable and wide range of solubility. Sometimes, DES offers a betteralternative to conventional ILS in many physical and chemical processes. DES performance has been showed in many separation processes, such as fuel purification, extraction of targeted compounds, and the development of analytical methods for example, proposed a new technique for separating glycerin from palm oil-based biodiesel using choline-based DES [10]. It is an inexpensive alternative to the complex and expensive refining of processes involved in conventional biodiesel production. Furthermore, investigated the extract of tools compounds by using the solvent extraction method using DES based on choline chloride with variations of hydrogen bond donors [11].

In this study, carotenoid extraction from crude palm oil was carried out using the solvent extraction liquid-liquid extraction method with an alcohol solvent and menthol-based deep eutectic solvent as co-solvent. Then, impurities removal from the solvent in the carotene by washing and drying methods was conducted.

2. RESEARCH METHODS

2.1 Materials and Methods

The main raw material used in this study was Crude Palm Oil (CPO) got from PT. Perkebunan Nusantara IV Indonesia, lauric acid ≥ 98%, acetic acid > 99%, and Menthol 99% and 96% methanol solvent got from sigma-Aldrich.

2.2 DES Preparation

Two DESs were prepared by mixing menthol saltwith acetic acid, and lauric acid at different molarratios (Table 1). The respective molar ratios are required to form a eutectic mixture, where two carboxylic acid molecules are required to complex each in menthol [12]. The mixtures were heated at 50°C 15 minutes with 300 rpm until a clear viscous liquid formed and then used in the extraction procedure without further purification.

2.3 Physicochemical of DES

DES formed was tested for its physicochemical properties with 3 parameters: pH, density and viscosity. The pH of DES was measured using a pH meter and the density measured by pycnometric method and Ostwald’s viscometer measured the viscosity at 30 ºC (± 2 ºC).

Table 1 Molar ratio of DL-Menthol withHBD for DES synthesis

Deep eutectic solvents	Molar ratio [12]	Code
Menthol: Acetic acid	1: 1	MAA
Menthol: Lauric Acid	2:1	MLA

2.4 Characterization of DES

DES obtained was characterized using FTIR to see functional groups formed by Shimadzu IR-21 with a range of 450-4000 cm^{-1}

2.5 Carotenoid Extraction from CPO

10 grams of CPO were mixed with 50 ml of ethanol and DES solvents in a ratio of 1: 0.5 (W/W) (CPO: DES). Afterwards, the mixture was stirred with a stirrer for 3 hours at a speed of 200 rpm at room temperature. Then, the former product was separated for 8 hours which would form 2 layers where the top layer was ethanol, DES residue, and vitamin E. While the bottom layer was carotene, DES and the ethanol solvent residue. Then, the bottom layer formed was washed with Ethanol: Water with a ratio of 1: 2 (v/v) for 10 minutes to remove DES residue from the carotene products [13]. And then the drying process was then carried out at a temperature of 50-60 ºC for 3 hours to remove water content and DES residue.

2.6 Analysis of CPO and Carotene

CPO analysis as a raw material to investigate the content of monoglycerides, diglycerides, and triglycerides in palm oil used GC-MS and carotenoid content analysis using a UV-visible spectrophotometer as for the β-carotene compound used HPLC.

3. RESULT and DISCUSSION

3.1 Synthesis and Physicochemical Properties of Menthol-Based DES

The overcoming the high price and toxicity of Ionic Liquid Solvent (ILS), it made a new generation of solvent called Deep Eutectic Solvent. The preparation of deep eutectic solvents depends on the establishment of hydrogen bonds between the two compounds, one acting as a hydrogen bond donor and the other as the hydrogen bond acceptor. 2 types of Deep Eutectic Solvent (DES) were synthesized from menthol as HBA and 2 carboxylic acids with different carbon chain lengths, acetic acid and lauric acid as HBD reacted with certain molar ratios. Figs.1,2 show the structure of the HBA and HBD of DES.

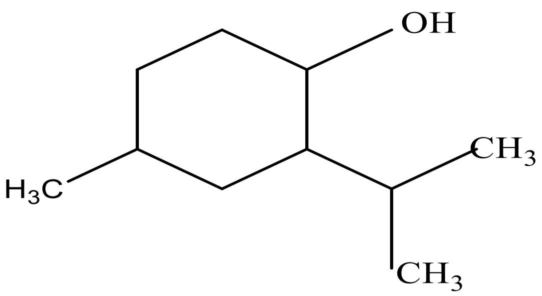

Fig.1 Menthol as HBA used for DES synthesis

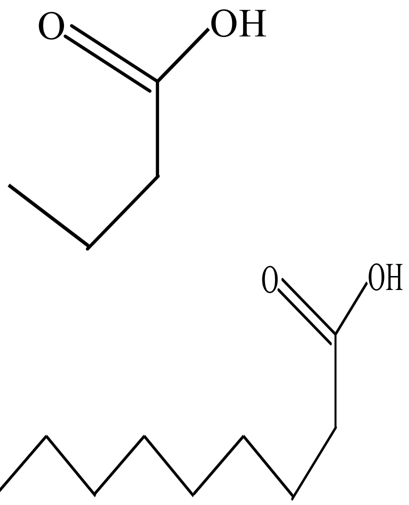

Fig.2 Acetic acid and Lauric acid as HBD used for DES synthesis

The mechanism of interaction between the Hydrogen Bond Acceptor menthol and the Hydrogen Bond Donor (R-OH) can be seen in Fig.3. The interaction between menthol and HBD occurs through the hydrogen bonding between salt halide anions and hydrogen group donors [14]. The reason it is called Deep Eutectic Solvent (DES) is when two components are mixed in the right ratio so that the eutectic point of the mixture will be obtained. This eutectic point is the lowest melting point from the molar ratio of the two components.

3.2 Physicochemical Properties of DES

DES is a chemical solvent that design according to its use. Therefore, DES with certain characteristics can be produced [9]. Research conducted by Ribeiro, et al., reports that the physical properties of DES differ depending on the salt molar ratio and HBD [15]. The following are the characteristics of DES obtained:

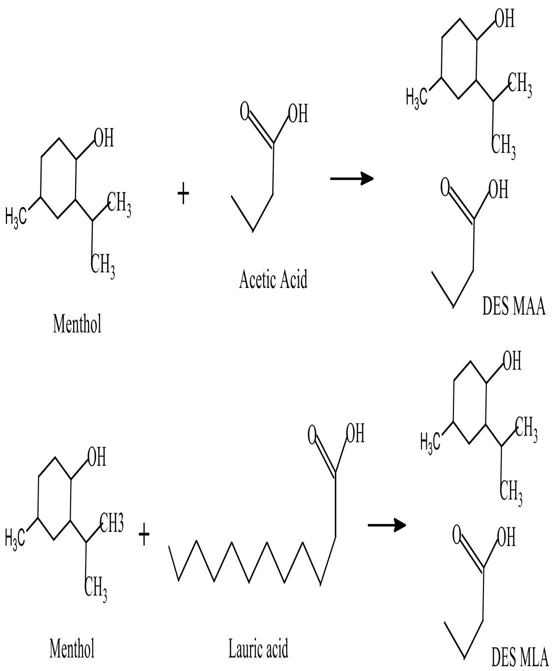

Fig.3 Interaction mechanism of HBA (menthol) and HBD (Carboxylic acid).

Table 2 Physiochemical properties of DES obtained

Physicochemical	MAA	MLA
DES formed (30ºC)	Colourless liquid	Colourless liquid
pH	5.0	6.0
Density 30ºC (g/ml)	0.950	0.890
Viscosity 30ºC (mPa.s)	6.910	18.975

From the results of the study, after the heating process completed the DES was in the form of a clear liquid (colorless liquid), then the DES cooled to room temperature ($30^0C \pm 2\ ^0C$). At room temperature DES is still in the form of clear liquid as shown in Fig.4. DES in the form of clear liquid at room temperature shows that the freezing point of DES is below room temperature ($30^0C \pm 2^0C$) because there is no change in the form of DES that occurs.

According to the research by Zhang et al. DES in the form of clear liquid was formed by mixing salt and HBD at certain molar ratios. In the molar ratio, HBD has the ability to form the interaction of hydrogen bonds with Menthol [9]. The DES clear liquid shows a decrease in freezing point and characterized by freezing point, which is lower than both of its constituent solids [9,16]. The low freezing DES value is caused by hydrogen bonds and the complex interactions of HBD and salt halide anions reducing the lattice energy in the mixture which leads to a decrease in freezing point [16,17]. Whereas for DES that does not have a clear liquid form, maybe the molar ratio of HBD and salt is incorrect.

Fig.4 DES Colourless liquid

From the results of the 2 menthol-based DES research, there was a decrease in freezing point and the DES results obtained were in the form of clear liquid. It can be concluded that menthol-based DES is a DES that has potential as a solvent that can be used in this study. Density values are needed to know the behavior of a liquid [18]. Measurement of density is very important in the field of fluid mechanics, calculation of mass transfer and to design chemical processes [16,19]. Density is one of the important physical properties of ionic liquids and DES [20].

From Table 2, the density values of the 2 DES produced were 0.950 and 0.890 gr/ml. Where this value lies between the density of menthol and the density of acetic and lauric acid at room temperature, 0.792 gr/ml and 1.050 and 0.880 gr/ml. That the measurement of DES density in the temperature function, the DES density lies between the density of the menthol and the constituent of HBD [15]. Therefore, it can be concluded that the density of DES produced is feasible to use. The development of low viscosity DES is expected because DES has the potential to be an environment friendly media [21]. The lower the viscosity value of DES, the better the DES is used as a solvent [22]. From Table 2, the viscosity values of the 2 DESs produced were 6.950 and 18.975 mPa [15].

3.3 FTIR Analysis

FTIR is a measuring tool used to determine the functional group or type of bond of a compound based on the value of the wavelength.

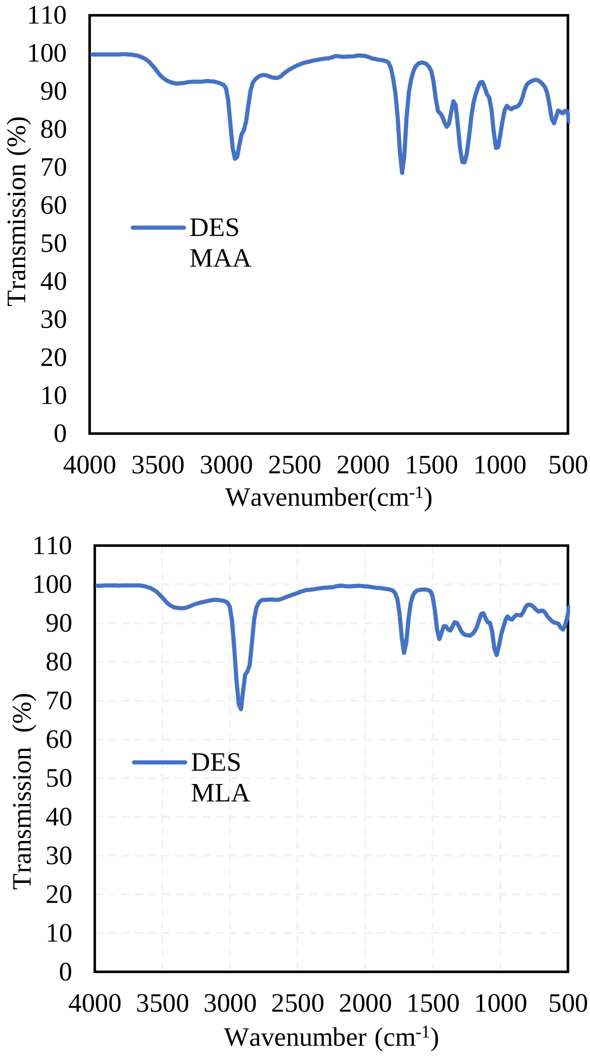

Fig.5 IR spectra of DES MAA and DES MLA

The FTIR spectrum of the hydrogen bond acceptor, menthol, is only one representative band corresponding to the hydroxyl group (-OH) at around wave-number 2925 and 2931 cm^{-1}. Figure 5 shows that all hydrogen bond donors used in this eutectic mixture have a carboxylic acid group in their structure, which presents a representative band of ketones or carbonyl groups around the wave number 1712.11 and 1731.31 cm^{-1}[15].

3.4 Analysis of CPO

In this study, CPO was characterized by using Gas Chromatography to get triglyceride, diglyceride, and monoglyceride components in CPO, while UV-Visible Spectrophotometer with a wavelength method of 470 nm characterized the amount of carotene. The results are presented in Table 3 below.

Table 3 Characteristics of CPO

Parameter	CPO
Triglyceride (%)	78.6516
Diglyceride (%)	8.0316
Monoglyceride (%)	0.0738
Fatty acid (%)	11.1463
Carotene (ppm)	108.6

Table 3 shows that the carotene content in CPO is still low because the carotene compounds bind with triglycerides. High triglyceride concentrations can cause effects on the carotene extraction process because triglyceride compounds bind with carotene.

3.5 The Effect of CPO: DES Ratio on Carotene Concentration

The poor solubility of DES in water showed that DES in this study was non-polar or hydrophobic molecules. On the other hands CPO is a non-polar organic substance. This information is important for making a biphasic system in solvent extraction [23]. In this study, DES as a co-solvent by using the main solvent of methanol, which is semi polar.

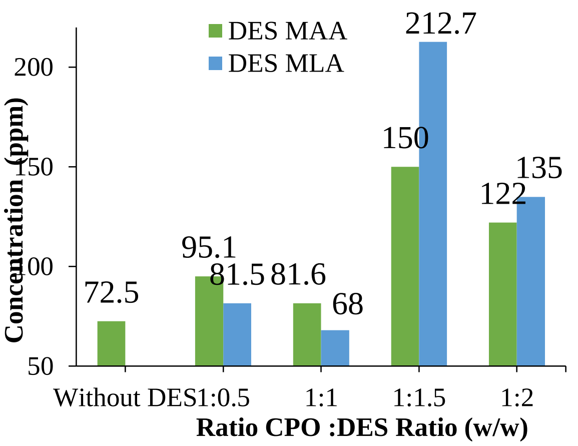

Fig.6 Effect of CPO: DES ratio on carotene concentration

Using methanol in this study because of an organic compound so it is non- toxic and does not pollute the environment compared to the use of polar compounds hexane. It shows the carotene concentration results obtained in Fig.6.

The influence of different DES uses in extraction can be explained when the same proportion from the weight of CPO to DES (ratio 1: 0.5, w/w) is used. In this study, the highest concentration of carotene in extraction was in MAA (95.1 ppm) while MAA (81.5 ppm) in the separation process using DES, the extraction efficiency of most compounds highly depended on HBD of DES [20]. In this study, this phenomenon was explored by testing two HBDs with different carbon chain lengths in the formation of DES. MMA which has lower carbon chain bonds in the molecule produced the highest concentration of carotene in the product compared to MLA, which has a longer double bond even though both are from the carboxylic acid group. However, this only applied in extraction with the ratio of CPO to DES 1: 1.05 and 1: 1. The critical point occured in the ratio of CPO to DES from 1: 1.5 and, where MLA gave a higher tochol concentration compared to MAA. The highest carotene concentration results were obtained with a ratio of CPO to DES 1: 1.5 (212.7 and 150 ppm). Furthermore, the concentration of CPO: DES 1: 2 had a decreasing process of carotene concentration high viscosity causes, which in DES.

3.6 Effect Without DES and DES Addition to β-Carotene Concentration

Carotenoid compounds can be grouped into two, which are carotene and xanthophyll. Carotene is a hydrocarbon compound composed of C and H, while xanthophyll is a compound derived from carotene that contains oxygen in its molecular structure, so that the constituent elements of xanthophyll are C, H and O. Examples of carotene compounds α, β, ɣ-carotene. Examples of xanthophyll carotene compounds cryptoxanthine, capxanthine and zeaxantin. β-carotene as one of the bioactive components in palm oil has several biological activities are beneficial to the body, to overcome blindness, prevent cancer, prevent premature aging, and reduce the occurrence of degenerative diseases [24,25]. In this study the HPLC method analyzed the β-carotene component. The analysis results are shown in Fig.7 below.

In fig.7, the concentration of β-carotene was 0.052 ppm with DES MAA solvent from acetic acid as HBD which has a shorter carbon bond than DES MLA which by using concentrate 0.041 ppm. The results of concentrate of β-carotene without DES 0.025 was ppm. This is caused because the methanol solvent is hydrophilic, while β-carotene is a hydrophobic compound.

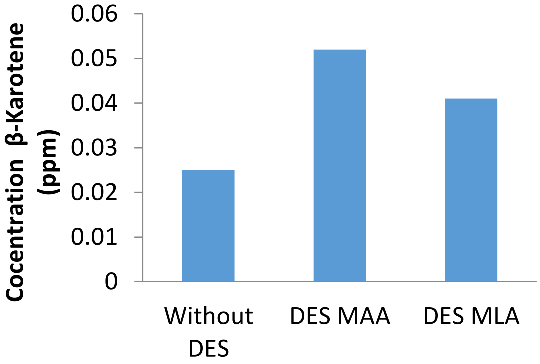

Fig.7 Influence without DES and DES addition to β-carotene concentration.

4. CONCLUSION

The performance of the DESs—prepared from menthol as stable salt and various carboxylic acids, acetic acid and lauric acid as HBDs—in the extraction of carotene and β-carotene content for vitamin A from CPO is reported here for the first time. Optimal extraction conditions were achieved using a CPO to MLA ratio of 1: 1.5 (w/w), with carotene concentration of 0.2127 ppm while extraction conditions of β-carotene had 0.052 ppm this result is proportional to carotene extraction. This is an important finding because β-carotene in chemoprevention against cancer and other degenerative diseases. The potential of the DES in the separation of carotenes from palm oil was well tested in this study. It paves the way for the separation of other components from vegetable oil by establishing use of the tunable DES, by generating other task-specific DESs to suit the separation process based on demands.

5. ACKNOWLEDGMENTS

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