Food Safety Analysis and Improvement Concept of \( \beta \) – Carotene Extraction from Fungal Fermented Palm Oil Empty Fruit Bunches (EFB); Extraction Method and Solvent Selection

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Abstract. Palm oil is one of the main agricultural commodities in Indonesia. Beside CPO and PKO as the main downstream product, the palm oil industry also produces solid wastes such as shells, fibers, and palm oil empty fruit bunches (EFB). Palm oil EFBs are often dumped nearby the palm oil plantations and being left to be decomposed, but several literature studies indicated that palm oil EFB still contained oil residue rich in \( \beta \) – carotene as well as other lipids components dissolved on it. \( \beta \) – carotene is among the major products of the food industry which have been widely employed as nutrients, food colorants, and additives. It serves as antioxidants and so-called pro-vitamin A. The previous research used n-Hexane as a solvent in \( \beta \) – carotene extraction from fungal – fermented palm oil EFB, whereas n-Hexane has a moderate toxicity level and low solubility of \( \beta \) – carotene which is only 600 ppm. Quality and food safety aspects of \( \beta \) – carotene extract haven’t been reviewed or analyzed yet. This paper will be focused on the food safety analysis and improvement concept that can be applied in the extraction method and appropriate solvent selection to obtain high-quality extracts of food-grade \( \beta \) – carotene from fungal – fermented palm oil EFB. Toxicity level and the Hansen Solubility Parameter (HSP) simulation results are the main criteria for solvent selection, while thermal stability, operational cost, and practical aspects are being considered to choose a better extraction method. Based on the HSP simulation results and all criteria mentioned before, n-Hexane can be substituted with 3 recommended solvents; D-Limonene, Tetrahydrofuran (THF), or Tetrahydrofurfuryl Alcohol. According to the economic analysis, the maceration method using THF at room temperature is being preferred to the soxhletation method. Moreover, food safety analysis is being reviewed based on HACCP principles.

1. Introduction
Indonesia is one of the top palm oil producers in the world with an average production of 45.861.121 tonnes in 2019 and will be predicted to 49.117.260 tonnes in 2020. A ton of palm fruit will produce about 230 kilograms of palm oil EFB (Empty Fruit Bunches) solid waste [1]. Commonly, a large portion of palm oil EFB has dumped nearby the palm oil plantations and left to be decomposed. Several palm
oil industry uses them as mulch, but the abundance of palm oil EFB offers the potential for being a raw material of fine chemicals production. One of them is the carotenoid compound.

Carotenoids contained in palm oil residue are dominated by β – carotene (60%) and α – carotene (34%). β – carotene is among the major products of the food industry which have been widely employed as nutrients, food colorants, and additives [2]. It serves as antioxidants and so-called pro-vitamin A. Several kinds of research have been conducted for carotenoids extraction from palm oil biomass. In his paper, reviewed carotenoids content in several kinds of palm oil biomass represented in Table. 1 [3].

Table 1. Carotenoid Composition in Several Kinds of Palm Oil Biomass.

| Oil Palm Biomass          | Carotenoid Content (ppm) | References                |
|---------------------------|--------------------------|---------------------------|
| Palm Oil                  | 500 – 1600               | Choo et al. (1992)        |
|                           | 800 – 1000               | Lau et al. (2006)         |
|                           | 800 – 1000               | Chiu et al. (2009)        |
|                           | 330 – 780                | Mustapa et al. (2011)     |
| Palm Pressed Fiber (PPF)  | 6000 – 7000              | Choo et al. (1996)        |
|                           | 2992                     | Lau et al. (2008)         |
| Palm Oil Mill Effluent    | 287 – 1665               | Ahmad et al. (2009)       |
| (POME)                    |                          |                           |
|                           | > 1160                   | Hudiyono and Septian.     |
|                           |                          | (2012)                    |
| Palm Kernel Cake (PKC)    | 1.6                      | Ogbuagu. (2008)           |
| Oil Palm Leaves (OPL)     | 1900                     | Ng and Choo. (2010)       |

Several literature studies indicated that palm oil EFB still contained oil residue rich in carotenoids as well as other lipid components dissolved on it. Carotenoids can be synthesized biochemically by plants, bacteria, fungi, and algae [4]. They have conducted re-extraction of oil residue from palm oil EFB by maceration using n-hexane at room temperature, which yields carotenoids content of 915,25 ± 300,28 ppm [5]. Improving the previous work studied the effect of natural fungal growth on the carotenoid content of palm oil EFB. They performed carotenoid extraction by using the soxhlet apparatus using n-hexane at its boiling point [6]. According to the obtained results, the extraction process yields 1,87 – 3,08 ppm of β – carotene, and the presence of fungi can improve β – carotene content up to 36% after 3 – 8 days of growth. However, quality and food safety aspects of obtained β – carotene extract haven’t been reviewed or analyzed yet. In this work, the explanation will be focused on the food safety analysis and improvement concept that can be applied in the extraction method and appropriate solvent selection to obtain high-quality extracts of food-grade β – carotene from fungal – fermented palm oil EFB.

2. Food Safety Analysis

Food safety refers to all hazards that can cause several drawbacks to consumer health. A lack of safety can result in serious injury or even death for the consumer. Safety differs from many other quality attributes since it is almost difficult to be observed. A product can appear to be of high quality (i.e. well colored, flavourful, appetizing, etc). However, unsafe food consumption causes many acute and life-long diseases, ranging from diarrhoeal diseases to various forms of cancer [11]. Moreover, complete guidelines for the Food Safety Management System (FSMS) have been listed in ISO 22000:2018 Standard.

Technical process control of all possible risks and the critical points monitoring provide a solution to prevent the quality or safety defects. Such a procedure for hazard determination and avoidance is presented by the Hazard Analysis and Critical Control Point (HACCP) method [12]. There are seven main principles of HACCP will be explained in this following table:
Table 2. Seven Principles of HACCP [9], [12].

| No. | Principle                                           |
|-----|-----------------------------------------------------|
| 1   | Perform a hazard analysis / Risk Assessment          |
| 2   | Determine the Critical Control Point (CCP)           |
| 3   | Determine One or Several Critical Limits             |
| 4   | Establish a CCP Monitoring System                    |
| 5   | Establish Corrective Action                          |
| 6   | Establish Verification Procedure                     |
| 7   | Establish Documentation                              |

2.1 Risk Assessment

In hazard analysis, the main point was being objected to incidents, events, or situations that could lead to hazards being introduced to the whole process. Risk assessment is the key to identify entire process risks, assessing their significance, and the controls in the system which systematically manage those risks starting from the whole production process [12].

Almost all of the previous work represented in section 1 uses n-hexane for the extraction solvent. Five potential hazards have been identified in the whole β-Carotene extraction and distillation process using n-hexane; Direct exposure of the solvent, bacterial contamination in raw material, fire and explosion hazard, health hazard, and the excess of solvent usage.

Nowadays, petroleum-based solvent as n-hexane is now strictly regulated by European Directives and “Registration, Evaluation, Authorisation, and Restriction of Chemicals / REACH” [15]. This is because n-hexane can cause several health hazards to the human. In 1975, Spencer et al. (1975) showed that 2,5-hexanedione, which was considered to be one of the n-hexane metabolites could cause polyneuropathy. The previous paper showed the biological monitoring of n-hexane exposure on vinyl-sandals factory workers. The result was 2,5-dimethylfuran, 2,5-hexanedione, and γ-valerolactone, which are considered to be n-hexane metabolites, were detected in the urine of workers by gas chromatography [16]. It has estimated the carcinogenic risk of some organic vapors indoors based on the value of carcinogenic potency [17]. According to his work, n-hexane has a carcinogenic potency of $2.6 \times 10^{-5}$ (mg/kg.day)$^{-1}$. In the extraction process, health hazards can be controlled by considering solvent IDLH (Immediately Dangerous to Life or Health) and PDE (Permitted Daily Exposure) value before choosing it as the solvent. n-Hexane has the IDLH and PDE value of 5000 ppm and 0.1 mg/day respectively [14], [18].

Excessive solvent usage is a potential hazard that affects the extraction efficiency and operational cost directly. Moreover, it can raises the carcinogenic potency value due to increasing solvent exposure concentration in the air. This hazard can be controlled by choosing the appropriate green solvent. Hence, “green” is used for describing different types of solvents including the ones which are produced from biomass feedstock and eco-friendly petrochemical-based solvents that are non-toxic and/or biodegradable. The potential of alternative green solvents instead of petrochemical-based solvent has been widely studied. In this work, the selection of the most appropriate solvent among several green solvents for β-Carotene extraction is carried out using Hansen Solubility Parameters (HSP) modeling.

HSP modeling provides a convenient and efficient way to characterize solute-solvent interactions according to the classical “like dissolves like” rule. HSP is based on the concept that the total cohesive energy density ($\delta^2_{\text{total}}$) is approximated by the sum of the energy densities required to overcome atomic dispersion forces (London Parameter, $\delta^2_\text{D}$), molecular polar forces arising from dipole moments (Polar Parameter, $\delta^2_\text{P}$) and hydrogen-bonds between molecules (Electron Transfer Parameter, $\delta^2_H$).

$$\delta^2_{\text{total}} = \delta^2_\text{D} + \delta^2_\text{P} + \delta^2_\text{H}$$

A simple affinity parameter, the relative energy difference (RED) number, has been calculated using Eq. (2) to determine the solubility between solute and solvent,

$$\text{RED} = \frac{R_A}{R_o}$$
reduce the chemical, microbiological, and physical hazard, because preventive action—corrective action can be taken by checking the temperature controller, and then reset the temperature indicators during the distillation process. When the temperature deviates extremely from the setpoint, corrective action can be taken by choosing 2 methods; changing back the solute content of β-carotene which can affect the microbiological contamination. This CCP can be monitored from microbiological analysis results. When the monitoring result deviates extremely from the setpoint or CCP, corrective action must be taken by performing chemical analysis, an inspection of wash water and finding another supplier of wash water if necessary. To overcome the chemical hazard, the operator should maintain the solute solvent ratio at 1:20 (w/v). The proper solute-solvent ratio must be maintained because this affects the residual solvent level at the end of the product. This can be monitored by doing the frequent inspection and chemical analysis of the obtained β-Carotene extract. When the monitoring result deviates extremely from the setpoint or too much solvent being used, corrective action can be taken by choosing 2 methods; changing back the solute-solvent ratio to the setpoint, or replacing the current solvent (n-hexane) with the alternative green solvent. However, considering the restriction of n-hexane usage for food products by European Directives and REACH, low n-hexane PDE value of 0.1 mg/day from FDA standard, and several carcinogenic risks study of n-hexane, replacing n-hexane with the alternative green solvent is more favorable, because according to the HSP simulation results, there are several potential green solvents can be used to replace n-hexane. Then, the distillation process uses two temperature settings, the first one by normal distillation is 70⁰C and the second one by vacuum distillation is 50-55⁰C. In this process, the temperature level is a CCP and must be controlled properly. Otherwise, it may affect the quality of β-carotene which allows degradation if the temperature is too high. This CCP can be monitored by regular inspection of temperature indicators during the distillation process. When the temperature deviates extremely from the setpoint, corrective action can be taken by checking the temperature controller, and then reset the temperature indicators.

where \( R_o \) is the radius of a Hansen solubility sphere, and \( R_A \) is the distance of a solvent from the center of the Hansen solubility sphere, which can be calculated using Eqn. (3),

\[
R_A^2 = 4(\delta_D A - \delta_D B)^2 + (\delta_P A - \delta_P B)^2 + (\delta_H A - \delta_H B)^2
\]

where \( A \) refers to the solute and \( B \) refers to the solvent. The lower the \( R_A \) value, the greater the affinity between solute and solvent because of their similar properties. It means that good alternative solvents have a RED of less than 1, while medium and poor solvents have RED values of from 1 to 3 and more than 3, respectively [15].

2.2 Critical Control Points (CCPs)

The CCPs are being determined by the decision tree method. An application of the CCP decision tree on the extraction and distillation process is depicted in Table 3.

| Process Step | Hazard Category | Q1 | Q2 | Q3 | Q4 | CCP |
|--------------|----------------|----|----|----|----|-----|
| Extraction   | Physical       | Yes| No | No | -  | NOT CCP |
|              | Chemical       | Yes| Yes| -  | -  | CCP |
|              | Microbiological| Yes| Yes| -  | -  | CCP |
| Distillation | Physical       | Yes| No | Yes| Yes| CCP |
|              | Chemical       | Yes| No | No | -  | NOT CCP |

The HACCP application plan of previous plan, works has been described in Table 2 [5]. It explains the β-carotene extract production process step, identified hazard category, preventive measures, CCP parameter, monitoring procedure, and corrective actions. In the extraction process, it should be designed to eliminate or reduce the chemical, microbiological, and physical hazard, because preventive action probably can’t be managed well on the next process. Before the extraction process, wash water quality for washing the raw palm oil EFB must be specified based on WHO guidelines of WFI (Water For Injection) quality standards. It is a critical control point (CCP) because it can affect the microbiological content of β-carotene, so that preventive action can be taken to reduce microbiological contamination of wash water. The CCP can be monitored from microbiological analysis and inspection results. When the monitoring result deviates extremely from the setpoint or CCP, corrective action must be taken by performing a comprehensive evaluation of the water treatment system for the wash water, an inspection of wash water analysis results, and find another supplier of wash water if necessary. To overcome the chemical hazard, the operator should maintain the solute-solvent ratio at 1:20 (w/v). The proper solute-solvent ratio must be maintained because this affects the residual solvent level at the end of the product. This can be monitored by doing the frequent inspection and chemical analysis of the obtained β-Carotene extract. When the monitoring result deviates extremely from the setpoint or too much solvent being used, corrective action can be taken by choosing 2 methods; changing back the solute-solvent ratio to the setpoint, or replacing the current solvent (n-hexane) with the alternative green solvent. However, considering the restriction of n-hexane usage for food products by European Directives and REACH, low n-hexane PDE value of 0.1 mg/day from FDA standard, and several carcinogenic risks study of n-hexane, replacing n-hexane with the alternative green solvent is more favorable, because according to the HSP simulation results, there are several potential green solvents can be used to replace n-hexane. Then, the distillation process uses two temperature settings, the first one by normal distillation is 70⁰C and the second one by vacuum distillation is 50-55⁰C. In this process, the temperature level is a CCP and must be controlled properly. Otherwise, it may affect the quality of β-carotene which allows degradation if the temperature is too high. This CCP can be monitored by regular inspection of temperature indicators during the distillation process. When the temperature deviates extremely from the setpoint, corrective action can be taken by checking the temperature controller, and then reset the temperature indicators.
If a serious error happens to the temperature controller or indicator, a replacement can be done to fix the problem.

### Table 4. Principle of HACCP Applied to Extraction and Distillation Process of $\beta$-carotene Extract.

| Process Step | Hazards Category | Preventive measures | CCP parameter | Monitoring procedure | Corrective actions |
|--------------|------------------|---------------------|---------------|----------------------|-------------------|
| Extraction   | Microbiological  | Wash water certificate of analysis (CoA) | WHO guidelines of WFI (Water for Injection) quality standards | Microbiological analysis result and inspection | • A comprehensive evaluation of water treatment system for the wash water  
• Inspection of wash water analysis result  
• Replace wash water supplier if necessary |
|              | Chemical         | Maintain the optimum solute-solvent ratio | The solute-solvent ratio of 1:20 (w/v) in kg and lt | Frequent chemical analysis and inspection | • Change the solute-solvent ratio back to the setpoint  
• Replace the current solvent with the alternative green solvent |
| Distillation | Physical         | Controlling the temperature level | The temperature of 70°C for normal distillation and 50-55°C for vacuum distillation | Regular inspection of temperature indicator | • Check the temperature controller  
• Reset the temperature controller  
• Replace the temperature controller/indicator if a serious error happens |

### 3. Improvement Concept

#### 3.1. Solvent Selection

Improvement is the key to achieve operational excellence, while it aims to improve product quality and process efficiency, maintain process safety, and minimize waste, hazards, and contamination. As have been mentioned before, n-hexane must be replaced by new alternative solvents. The appropriate solvent must have a better affinity with the solute, which represented by RED value. Using the obtained $\beta$-carotene solubility parameter ($\delta_D, \delta_P, \delta_H, R_o$) on section 2.1, 6 solvents are being tested by HSP simulation: Benzene, Methanol, Ethanol, d-Limonene, Tetrahydrofuran, and Tetrahydrofurfuryl alcohol.
Table 5. New Solvent Testing by HSP Simulation Method.

| Solvent                  | δD  | δP  | δH  | RED   | Recommended / Not |
|--------------------------|-----|-----|-----|-------|-------------------|
| Hexane*                  | 14.90 | 0.00 | 0.00 | 1.126 | Not               |
| Benzene                  | 18.40 | 0.00 | 2.00 | 0.964 | Not               |
| Methanol                 | 14.70 | 12.30 | 22.30 | 1.230 | Not               |
| Ethanol                  | 15.80 | 8.80 | 19.40 | 1.148 | Not               |
| d-Limonene               | 17.20 | 1.80 | 4.30 | 1.017 | Yes               |
| Tetrahydrofuran (THF)    | 16.80 | 5.70 | 8.00 | 1.043 | Yes               |
| Tetrahydrofuranyl Alcohol| 17.80 | 8.20 | 12.90 | 1.020 | Yes               |

*: Reference Value

According to the simulation results, benzene has the greatest affinity with β-carotene. However, benzene is a petroleum-based solvent which is not environmentally friendly and has bigger carcinogenic potential compared to n-hexane. Benzene has the carcinogenic potential value of $1 \times 10^{-3}$ (mg/kg/day)$^{-1}$, which is bigger than n-hexane [17]. It can be inferred that benzene is not a green solvent and can’t be used to replace n-hexane. Methanol has the largest value of RED, which means it has the lowest affinity with β-carotene and can be eliminated from the choice. Ethanol, which is categorized as the green and food-grade solvent has the RED value not far from n-hexane. However, ethanol RED value is higher than n-hexane and this will lower the extraction efficiency.

D-Limonene, a monocyclic mono-terpene listed as a flavoring agent in the Code of Federal Regulation, is a major constituent of several kinds of citrus peel essential oil, such as lemon, orange, mandarin, lime, and grapefruit [19]. It has been categorized as a green biodegradable and non-toxic solvent instead of hazardous petroleum based-solvents, because it can effectively be used for lipid extraction from microalgae or plant matrices [20], [21]. Moreover, D-Limonene has the antioxidant potential, anti-diabetic effect and other clinical applications, so that it is recommended to be used for n-hexane substitute [22]–[25].

Tetrahydrofuran (THF) is a multi-purpose polar solvent which has been used for decades in a wide range of applications. It can be synthesized from furfural by hydrogenation reaction using a catalyst consisting of 5% palladium (Pd) on microporous carbon and carried out at 100°C and 20 bar (Godawa et al, 1986). Furfural itself synthesized by dehydrating pentose sugars; xylose and arabinose, or by acid hydrolysis of lignocellulosic biomass obtained from cornstalks, corncobs, wood chips, and other biomass waste products [26]. Toxicological and environmental hazards of THF have been studied comprehensively. Bowles, in his review paper, explained that the acute toxicity of THF in the human body is low to moderate by all routes. The in vitro and in vivo studies have shown that THF is non-mutagenic [27]. THF has been categorized as “Class-3 Solvent” with the PDE value of ≤ 50 mg/day, which has low toxicity and safe to be employed in the manufacturing of drug or food products compared to n-hexane. Nevertheless, it should be limited by GMP or other quality-based requirements [18]. Because of this advantage, THF can be chosen as the alternative solvent replacing n-hexane.

Tetrahydrofuranyl alcohol (THFAL) is a green industrial solvent because it is less toxic, degradable, and more stable compared to unsaturated furan compounds [29]–[31]. It can be derived from furfural which produced from renewable biomass resources by acid-catalyzed pentose dehydration. Similar to THF, THFAL can be synthesized by furfural catalytic hydrogenation. Nakagawa et al. (2014) has synthesized THFAL from furfural using Ni-Pd/SiO$_2$ alloy catalyst and yielded 94% of THFAL under 8 MPa hydrogen pressure and 40°C for 2 hours with acetic acid solvent. Improving the previous work, have successfully synthesized THFAL from biomass-derived furfural with 100% conversion and yield over a hydroxyapatite-supported Pd catalyst (Pd-HAP) under relatively mild condition (40°C, 1 MPa H$_2$, and 3 hours) in 2-propanol [32]. However, the research of THFAL utilization as the extraction solvent for food and pharmaceutical product processing hasn’t been explored yet. The toxicological and carcinogenic effects of THFAL in human still need to be studied comprehensively, for making sure that it will not be harmful to the human body.
3.2. Extraction Method
Several common extraction methods are being used for phytochemical and bioactive compound extraction from plants; Maceration, Soxhletation, Supercritical Fluid Extraction (SFE), and Subcritical Water Extraction (SWE). Thermal stability of the extract, operational cost, and practical aspects are the main considerations for choosing the best method of extraction. Thermal stability can be defined as how difficult the chemical compound changing from its initial molecular form due to the stimulus of heat. The insight about β-carotene thermal stability has been created and applied using multiresponse modeling because its unsaturated structure is very fragile and can degrade via two reaction pathways: isomerization and oxidation [38], [39]. Isomerization of β-carotene involves a configuration change from its natural trans-form to various cis-isomer species. Although the resulting isomers still able to be converted into vitamin A, the yield will be lower than before isomerization [40].

3.2.1 Maceration. Maceration is the method while the coarsely powdered raw material is kept in contact with the solvent in a stoppered container for a defined period with frequent stirring at a certain speed and temperature until the soluble matter is dissolved. This method is best suitable for the extraction of several compounds that have low thermal stability. It is the best method to be used in β-carotene extraction.

Table 6. Economic Analysis of Maceration Method using Several Extraction Solvent.

| Extraction Solvent | Fixed Cost | Variable Cost | Total Cost |
|--------------------|------------|---------------|------------|
|                    | Electrical-Stirring (0.408 kWh) | Electrical-Heating | Electrical-Pumping | Cooling Water | Solvent (1 L) | Cost per 1 L of Crude Extract | Cost per mg of β-Carotene |
| n-Hexane*          | Rp 423     | -             | -           | Rp 23,000    | Rp 23,423    | Rp 925 |
| D-Limonene         | Rp 423     | -             | -           | Rp 150,000   | Rp 150,423   | Rp 3,701 |
| THF                | Rp 423     | -             | -           | Rp 66,675    | Rp 67,098    | Rp 1,880 |
| THF AL             | Rp 423     | -             | -           | Rp 1,183,000 | Rp 1,183,423 | Rp 26,275 |

* : reference

The maceration process only needs electrical power about 8,5 W for stirring purposes, because this process is done in ambient temperature without solvent recirculation (no heating, cooling, and pumping needed). Because the extraction time takes 48 hours, electrical power requirements for stirring purposes will be 0,408 kWh. Variable cost depends directly on the extraction yield. According to the economic analysis presented in Table 6, the total cost of the maceration method is ranging from Rp 1.880 to Rp 26.275 per mg of β-carotene. The maceration using tetrahydrofuran (THF) has the lowest total operational cost, i.e. Rp 1.880 / mg of β-carotene, and considered to be the best extraction method.

3.2.2 Soxhletation. Soxhlet extraction method can be chosen while the desired compound has limited solubility in the used solvent and the impurities are insoluble in that solvent. The advantage of this system is that instead of many portions of the warm solvent being passed through the sample, only one batch of solvent is recycled. Nevertheless, this method cannot be used for the compounds having low thermal stability, which degradation or isomerization will easily happen at the high processing temperature [42]. Because of the low β-carotene thermal stability, this extraction method is not suitable to be used.
Different from the maceration method, the soxhletation method needs electrical power about 550 W for heating purposes, because this process is done in boiling point with solvent recirculation. Because the extraction time takes 3 hours, electrical power requirements for heating purposes will be 1.65 kWh. Cooling water supply from tap water is needed to condense the solvent which partially vaporizes during extraction. Assuming tap water flow rate is about 2 L/min, the total cooling water being used during extraction is 360 L. The cooling water cost calculation refers to the PDAM (Perusahaan Daerah Air Minum) tariff for 4A Industrial group, i.e. Rp 4,900 / m³. According to the economic analysis presented in Table 7, the total cost of the soxhletation method is more expensive than maceration and ranging from Rp 3,976 to Rp 29,325 per mg of β-carotene.

4. Conclusion
The food safety analysis of β-carotene extraction has been reviewed using HACCP principles. Food safety of β-carotene extract can be improved by replacing n-Hexane with green solvents and using the low-cost extraction method. Based on the HSP simulation results, n-Hexane can be substituted with 3 recommended solvents: D-Limonene, Tetrahydrofuran (THF), or Tetrahydrofurfuryl Alcohol. According to the economic analysis, maceration using THF at room temperature is being preferred to the soxhletation method.

5. Acknowledgment
Further experimental research of β-carotene extraction using proposed green solvents (D-Limonene, THF, THFAL), solubility measurement, and extract purification method is needed for testing the validity of HSP simulation results and the effectiveness of the proposed improvement concept.

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