

# The Effect of Extraction Method on the Extract Yield in the Carotenoid Pigment Encapsulation for Halal Natural Pigment

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**Abstract:** The Soxhlet and maceration methods were used to determine the extract yield in the carotenoid pigment encapsulation for halal natural pigment production. This study aims to obtain halal natural pigment by determining the highest extract yield from the encapsulation of  $\beta$ -carotene in carrots. The carrot was extracted using Soxhlet and maceration method and then continued by oven drying. The *n*-hexane was selected because of its better volatility than ethanol and provided less solvent residue after extraction. UV-Vis spectroscopy and Thin Layer Chromatography (TLC) were used to characterize the *n*-hexane yield extract. Encapsulation of the pigment was investigated by adding five grams of maltodextrin to extract *n*-hexane weights of 0.05, 0.50, 0.75, and 1.0 grams. The maceration method yielded a much higher yield than the Soxhlet extraction method, with 2.24% (w/w) and 0.88% (w/w), respectively. The *n*-hexane extract absorbed a maximum wavelength of 450 nm with a retention factor ( $R_f$ ) of 0.62. These values are confirmed by comparing the band's  $R_f$  values and absorption spectra with the standard's. Light absorption spectra at wavelengths 350-500 nm confirmed an intense color expression for encapsulation containing the highest pigment concentration.

**Keywords:** caretonoid, encapsulation, extraction, halal natural pigment, maceration, Soxhlet

## 1. Introduction

The demand for natural food dyes grows as food production diversifies. Natural food dyes are preferred because they are non-toxic, non-carcinogenic, slightly allergic, and easily biodegradable. Natural pigments can be derived from carotenoid group compounds abundant in plants. Carotenoids are found in chloroplasts and are responsible for the plant's aroma, flavor, and colors of yellow, bright red, and orange (Cazzonelli, 2011; Hermanns et al., 2020; Simkin, 2021). Carotenoids are soluble in a wide range of organic solvents, including alcohol group solvents (Kultys & Kurek, 2022; Saini & Keum, 2018). As a result, natural food coloring products have a critical halal point in the solvent used in the preparation process, primarily the potential to produce residues containing more than 0.5% alcohol.

The majority of carotenoids comprise eight isoprene units with 40 carbon chains. Carotenoids are composed of a polyene chain with nine conjugated double bonds and end groups at both ends of the polyene chain. Carotenoids are commonly found as  $\beta$ -carotene or pro-vitamin A. The  $\beta$ -carotene is a terpenoid hydrocarbon compound composed of isoprene units (isoprenoid). At high temperatures, it is unstable, easily oxidized, and degraded.  $\beta$ -carotene is an orange pigment found primarily in carrots (*Daucus carota* L) (Char, 2017; Nagraj et al., 2020; Wahyuni et al., 2020). Figure 1 depicts the molecular structure of  $\beta$ -carotene (Starek et al., 2015).

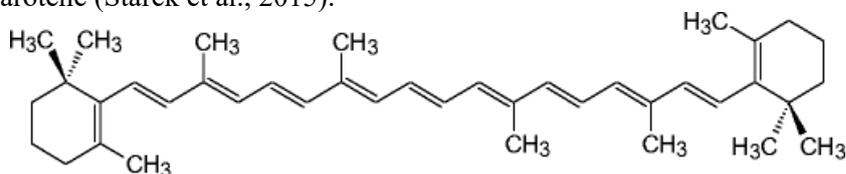


Figure 1. The Structure of  $\beta$ -Carotene (Starek et al., 2015).

The separation method of  $\beta$ -carotene from carrots is still crucial because  $\beta$ -carotene is bound in various matrices with high water content. There are several methods for isolating  $\beta$ -carotene, including conventional liquid extraction under ambient pressure, such as Soxhlet extraction, maceration, ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE), and supercritical fluid extraction (SFE). Soxhlet extraction and maceration methods are relatively preferred because they are simple, do not require complicated instruments, and produce a relatively high yield. However, Soxhlet extraction and maceration methods continue to have flaws. Soxhlet extraction and maceration necessitate many solvents and a lengthy extraction time (Saini & Keum, 2018; Srivastav et al., 2022). The disadvantages of both methods can be mitigated by selecting the appropriate solvent, extraction time, and extraction temperature.

The nature of  $\beta$ -carotene, which is not easily soluble in water, was considered when choosing a solvent. As a result, non-polar solvents like chloroform, *n*-hexane, acetone, petroleum ether, or ethanol are frequently used. Ethanol is the most commonly used solvent because of its food-grade solvent (Blanco-Llamero et al., 2022; Fikselová et al., 2008; Hermanns et al., 2020). Ethanol and propanol are suitable for extracting many phytochemical compounds because their polarity is close to that of  $\beta$ -carotene, making them easier to dissolve. However, using ethanol as a solvent can complicate the selectivity of the extracted substance because ethanol is more polar and has a higher boiling point (Jacotet-Navarro et al., 2018; Ko et al., 2011; Koca Bozalan & Karadeniz, 2011).

Furthermore, the choice of ethanol as a solvent is critical because the presence of ethanol residue in carotenoid extraction should be less than 0.5%. The Indonesian Ulema Council (MUI) fatwa number 10 of 2018 concerning food and beverage products containing alcohol/ethanol states: "The use of alcohol/ethanol derived from non-*khamr* industrial products (whether the result of chemical synthesis from petrochemicals or non-*khamr* fermented industrial products) for beverage product ingredients is legally permissible if the final ethanol content is less than 0.5% of the final product." The state said that foods containing ethanol cause a risky change in the halal status of these foods. In addition, the synthetic ethanol concentration must be less than 0.5%.

Food grade *n*-hexane is used in Soxhlet extraction and maceration. Hexane is a chemical mixture of branched and unbranched molecules with the formula  $C_6H_{14}$ . The primary distinction between hexane and *n*-hexane is that hexane has five structural isomers, each of which is either branched or unbranched. In contrast, *n*-hexane is an unbranched structure, and *n*-hexane is more food grade than hexane (Martiani et al., 2017). The use of *n*-hexane solvent refers to the Hazard Analysis Critical Control Point (HACCP) principle applied to the extraction and distillation process of  $\beta$ -carotene extract, which includes paying attention to the extraction composition. The solute maintains a solute ratio of 1:20 (w/v) in Kg and L (Muhammad et al., 2021).

*n*-hexane is more volatile and non-polar than ethanol, so it is easier to dissolve a non-polar  $\beta$ -carotene (Fikselová et al., 2008; Kua et al., 2016; Yara-Varón et al., 2016). The boiling point of *n*-hexane and ethanol is 55°C and 78.37°C, respectively. Therefore, *n*-hexane is easier to separate by evaporator from  $\beta$ -carotene than ethanol.  $\beta$ -carotene degrades at temperatures above 60°C (Saini & Keum, 2018). Thus, hexane as a solvent in  $\beta$ -carotene extraction is important in obtaining halal natural pigment from  $\beta$ -carotene (Handayani & Setyawati, 2020).

Soxhlet method is susceptible to enzymatic oxidation of  $\beta$ -carotene because it is performed at high temperatures. As a result, the extraction temperature is limited to no more than 60°C for 1–3 hours (Fikselová et al., 2008). This study investigates the effect of the Soxhlet extraction method and maceration in producing the highest extract yield using *n*-hexane as a solvent for the  $\beta$ -carotene encapsulation process. The  $\beta$ -carotene extract yield is then formed into encapsulates to protect the  $\beta$ -carotene from damage (Wagner & Warthesen, 1995). Encapsulate is a substance made up of a core, a coating of  $\beta$ -carotene pigment, and a polymer matrix of the polysaccharide group. The coating matrix dissolves easily in water. It forms a colloidal solution because the molecules have hydrophilic and hydrophobic groups that connect lipophobic pigment substances. Maltodextrin was used as the coating material in this study because of its low hygroscopicity, ability to form films and disperse pigment substances, and ability to prevent  $\beta$ -carotene crystallization (Chronakis, 1998; Eun et al., 2020). In addition, Maltodextrin is on the list of ingredients that do not have a critical halal point, so it already meets the halal criteria.

At 45–65°C, several drying methods, including spray-drying, freeze-drying, coating, and spray granulation, can be used to complete the encapsulation process. The coating method is reported to be better than others for producing mass fractions because the coating method does not require preheating. The method employs non-toxic polysaccharide compounds with high solubility, high binding capacity,

and ease of film formation (Eun et al., 2020; Rifqi et al., 2020). However, the accuracy of the rotational speed of the spraying time in the rotary atomizer significantly impacts the coating method's success. Temperature regulation in the inlet and outlet systems is also required for the coating method (Chranioti et al., 2016; Li et al., 2019).

This study reported a simple method for obtaining halal natural pigment by combining the oven drying method with an adjustable and maintainable drying temperature. The oven-drying method combined maltodextrin and  $\beta$ -carotene in solution to form an emulsion system, which was then dried in an oven at 50°C for 10 hours. TLC and UV-Vis Spectrophotometer were used to determine the weight of the extract in encapsulation of 0.05, 0.50, 0.75, and 1.00 grams.

## 2. Materials and Methods

The research was conducted in three stages: 1) Soxhlet extraction and maceration, 2) characterization of *n*-hexane extract and 3) encapsulation by oven drying.

### 2.1. Apparatus and Materials

This study made use of a set of laboratory glassware, an analytical balance (Ohaus), an evaporator (Heidolph), a UV-Vis spectrophotometer (U-1800 spectrophotometer), a TLC plate with Fs Silica gel 60 F<sub>254</sub>, a stirrer and hotplate (Cimarec), a vacuum pump, and an oven. Standard  $\beta$ -carotene (Sigma Aldrich), *n*-hexane (C<sub>6</sub>H<sub>14</sub>), methanol, acetone (C<sub>3</sub>H<sub>6</sub>O), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), maltodextrin, and distilled water were the chemicals used. All of the chemicals used were of analytical quality. In addition, fresh carrots were purchased at the local market.

### 2.2. Soxhlet Extraction and Maceration

The dried carrots were extracted by washing, cleaning, grating, and drying fresh carrots in the oven for 8–10 hours. Soxhlet extraction was performed using dried carrots that were weighed 40 grams, wrapped in filter paper, and placed in the Soxhlet apparatus's F tube. The extraction was carried out in two cycles at 55°C. Forty grams of dried carrots were used to test maceration extraction. The extraction was carried out at room temperature with *n*-hexane solvent, covered with aluminum foil, and stored in a dark place for 24 hours. At 55°C, the Soxhlet extraction and maceration results were continuously filtered and evaporated. This procedure was repeated until there were no more solvent drips. Equation 1 was used to calculate the yield of each extract (Dewatisari et al., 2018).

$$\frac{\text{Extract yield obtained}}{\text{Weight of extracted simplicia}} \times 100\% \quad (1)$$

### 2.3. Characterization of Carrot Carotenoid Pigment Extraction Yield

Soxhlet and maceration methods are based on Malgorzata's modified method. UV-Vis spectrophotometer and Thin Layer Chromatography (TLC) were used to characterize the yield of carotenoid pigment (Starek et al., 2015). The radiation absorption was measured after the extract was evaporated to remove the remaining solvent clinging to the  $\beta$ -carotene completely. The extract's absorbance in the UV-Vis wavelength range was measured to determine its yield (300–700 nm). Thin Layer Chromatography (TLC) was used to identify carotenoid pigments, with silica gel 60 F<sub>254</sub> as a stationary phase and acetone: diethyl ether: hexane (2:3:6 v/v) as the mobile phase. Spraying a concentrated H<sub>2</sub>SO<sub>4</sub> solution on the spot on the TLC plate performed a qualitative test. UV-Vis light at 254 nm and 365 nm was used to identify  $\beta$ -carotene in carotenoid pigments. Furthermore, the retardation factor (R<sub>f</sub>) of standard  $\beta$ -carotene and sample in carotenoid pigments was determined using TLC with silica gel plate 60 F<sub>254</sub> and hexane: methanol as mobile phase (95:5 v/v).

### 2.4. Encapsulation with Oven Drying

Encapsulation was performed using an oven drying method based on Charikleia's (Chranioti et al., 2016) and Chen (Chen & Tang, 1998). The yield of the extract with a weight of 0.05, 0.50, 0.75, and 1.0 g plus maltodextrin was dissolved with distilled water and then dried in an oven at 50°C for 10–15 hours. The encapsulated product was determined and characterized by UV-Vis Spectrophotometer at  $\lambda_{\text{max}}$  of 450 nm.

## 3. Results and Discussion

### 3.1. Extraction Yield of Carotenoid Pigments in Carrots

Table 1 shows that the yield and weight of the extract obtained by the maceration method were greater than those obtained by the Soxhlet method. The lower yield of carrot carotenoid extract from Soxhlet extraction was due to the higher temperature than in the maceration method. The high

temperature of the Soxhlet extraction method damages and breaks the carotenoid double bonds. High temperatures endanger carotenoid pigments because they are more stable at low temperatures (Fikselová et al., 2008). Furthermore, because carotenoid pigments have long chains, they are easily oxidized or degraded due to double bond breaking caused by high-temperature changes (Lubis et al., 2016). Light exposure can also cause oxidative degradation of the carotenoid matrix structure.

Table 1. Weight and Yield of Extract in Carrots

Extraction Method	Carrot Weight (g)	Extraction time (Hour)	Extract Weight (g)	Yield (%)
Soxhlet	40.00	1.5	0.253	0.88
Maceration	40.00	24	0.468	2.24

The maceration extraction method was used 24 hours in a closed container at room temperature and without light. It allows for enough contact between  $\beta$ -carotene and *n*-hexane solvent, resulting in a higher yield of carrot  $\beta$ -carotene extract than the Soxhlet extraction method. (Eun et al., 2020) confirmed that increasing the temperature in the  $\beta$ -carotene isolation technique increases the yield by up to 6%. When deciding on an extraction method, it is critical to consider the material's heat stability (Abubakar & Haque, 2020). The extraction time has an impact on the yield of  $\beta$ -carotene. The increase in waiting time of up to 300 minutes contributed to a 6% increase in yield (Gul et al., 2015). The non-heat-resistant plant materials were extracted using maceration or percolation. While heat-resistant materials were extracted using Soxhlet or microwave. The maceration method is appropriate for extracting materials requiring lengthy contact time. Soxhlet extraction takes much less time than a maceration. Soxhlet extraction is a heating-based separation method. Because the solvent *n*-hexane evaporates quickly and runs out in the flask, the separation of  $\beta$ -carotene with Soxhlet was completed in 1.5 hours at 55°C. As a result, each extraction cycle is completed relatively quickly.

### 3.2. Characterization by UV-Vis Spectroscopy

Characterization was performed using a UV-Vis spectrophotometer at 350–600 nm to determine the yield's color intensity and maximum wavelength. Figure 2 shows the spectral results.

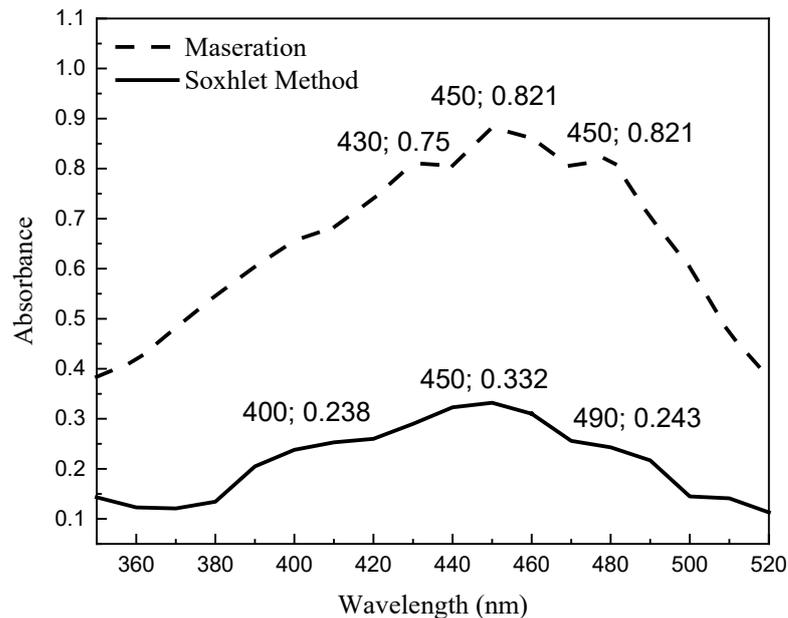


Figure 2. Spectra of Extract Solution Using (---) Soxhlet Method and (—) Maceration Method.

Figure 2 shows that the maximum of the two extraction yields at 450 nm with the expression of a yellow solution. It is because most carotenoid group compounds have maximum absorption in the visible region, with a wavelength of 400–500 nm. It is because the chromophore group of carotenoid compounds undergoes an electronic transition at  $\pi$ - $\pi^*$  (Hermanns et al., 2020). Generally, the electrons in carotenoid compounds are delocalized due to the double bonds in the long chain. Therefore, carotenoid compounds absorb visible light wavelengths in the reddish-yellow color spectrum due to their low electronic transition energy (Bhaumikkumar et al., 2016). Therefore, the absorptivity at the maximum wavelength was used to calculate the color intensity of the two extracts, as shown in Figure 3 and Table 2.

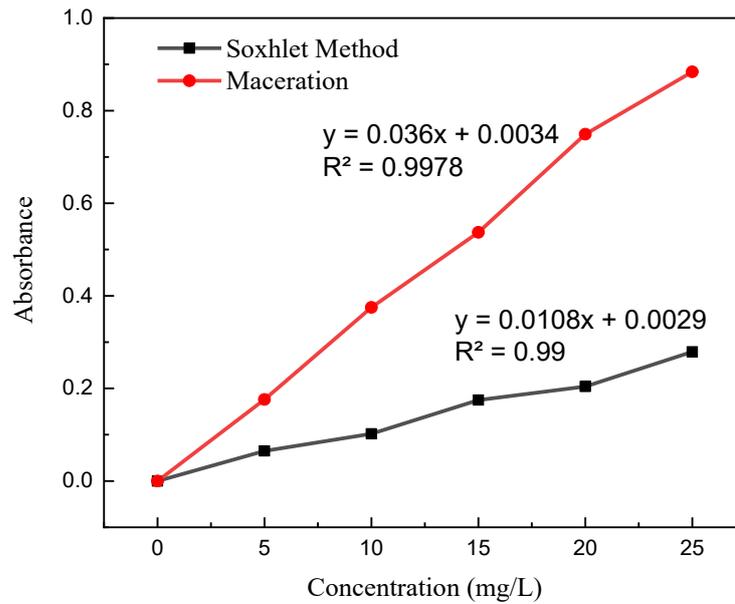


Figure 3. Determination of the Absorptivity of Soxhlet and Maceration Extraction.

Table 2. Absorptivity of *n*-Hexane Extract Solution from Soxhlet Method and Maceration

Extraction Method	$\lambda_{maks}$ (nm)	Absorptivity ( $cm^{-1}.g^{-1}.L$ )	$R^2$
Soxhlet	450	0.0108	0.9900
Maceration	450	0.0360	0.9978

Figure 3 shows that the absorptivity of the uptake of *n*-hexane extract from the maceration method was higher than that of the *n*-hexane extract from the Soxhlet method. It indicates that the macerated extract's reddish-yellow color expression was stronger than the Soxhlet method's. Separation at room temperature by maceration extraction did not harm  $\beta$ -carotene. It ensures that the extract structure is stable and does not degrade.

### 3.3. Characterization by Thin Layer Chromatography (TLC)

TLC was used to identify carotenoid pigments in Soxhlet and maceration extracts. Spraying a concentrated  $H_2SO_4$  solution on spots on the TLC plate resulted in qualitative tests, as shown in Figure 4 and Table 3.

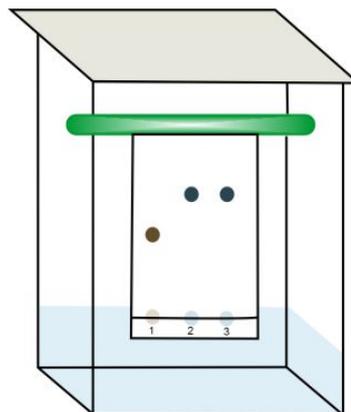


Figure 4. The Results of the Qualitative Test of Carotenoid Pigments from Soxhlet Extraction (1) and Maceration Extraction (2,3) Using TLC (Silicate Gel Plate 60  $F_{254}$  with the Mobile Phase of Acetone: Diethyl Ether: Hexane (2:3:6 v/v).

Table 3. Qualitative test of carotenoid pigments from Soxhlet extraction and maceration extraction

Extract type	Reactant	Reaction result	Color
1. Soxhlet Extraction	Concentrated $H_2SO_4$	Negative (-)	Yellow
2. Maceration Extraction	Concentrated $H_2SO_4$	Positive (+)	Blue
3. Maceration Extraction	Concentrated $H_2SO_4$	Positive (+)	Blue

Figure 4 shows the qualitative test of carotenoid pigments. TLC was used in this qualitative test technique to ensure the presence of  $\beta$ -carotene, not to separate  $\beta$ -carotene. TLC was used in this test because  $\beta$ -carotene compounds in carotenoid pigments can be identified after the carotenoid pigments interact with the polar stationary phase of silica gel. Detention traces appear on the TLC plate due to non-polar mobile phase elution. Table 3 shows that after spraying concentrated  $H_2SO_4$ , the maceration extraction results changed color from orange to dark blue. It indicates that the carotenoid pigment contains  $\beta$ -carotene (Britton et al., 2004). The color of the Soxhlet extraction results remained unchanged. It indicates that the structure of the carotenoid pigment compound changed during the extraction process. The conjugated double bonds in oxidized carotenoid compounds at high temperatures were responsible for these results. Epoxy compounds are formed during the oxidation of carotenoid compounds (Wahyuni et al., 2020). Excessive light exposure, which initiates the oxidation reaction, is the primary cause of damage to the structure of carotenoids.

Figure 5 identifies  $\beta$ -carotene in carotenoid pigments using UV-Vis light and the retardation factor ( $R_f$ ) value. It demonstrates that the maceration extracted  $\beta$ -carotene follows the  $R_f$  value of standard  $\beta$ -carotene (0.62) reported by Monitasari (Monitasari, 2020).

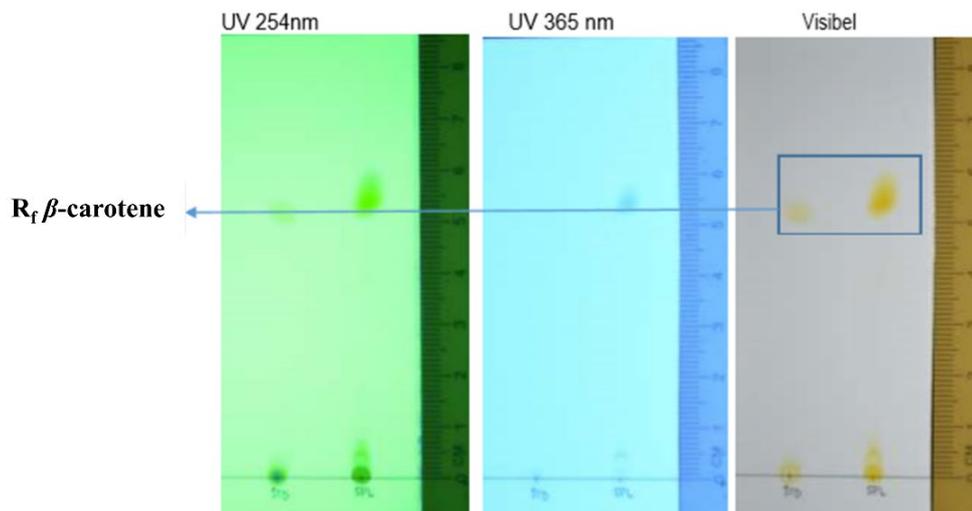


Figure 5.  $R_f$  Value from Maceration Extraction and Standard  $\beta$ -Carotene Standard using TLC (60 F<sub>254</sub> Silica Gel Plate with Hexane: Methanol as Mobile Phase [95:5 v/v]).

The  $R_f$  value of the macerated extract and standard  $\beta$ -carotene was the same. It confirmed the presence of  $\beta$ -carotene compounds in the macerated extract. Different antioxidant compounds have different pigments, so they have different  $R_f$  values.  $\beta$ -carotene pigment has a higher  $R_f$  value than anthocyanin pigment (0.32) and chlorophyll (0.42), according to (Starek et al., 2015). It shows that the solubility of each pigment differed during the TLC identification process and that this solubility directly impacts the  $R_f$  value. The less soluble pigments were left behind, and the more soluble pigments moved faster onto the paper.

### 3.4. Carotenoid Pigment Encapsulation

Encapsulation of maceration extraction results containing  $\beta$ -carotene was accomplished using maltodextrin as an emulsifier. Figures 6 and 7 show the encapsulation of carotenoid pigments with 5 g of maltodextrin. In addition, Figures 6 and 7 show that increasing the carotenoid pigment extracts in the encapsulated extracts increased the absorbance of the encapsulate in UV-Vis light with a wavelength of 350–500 nm. However, the excess maltodextrin may reduce its ability as an emulsifier. It results in droplet particles that agglomerate on the chamber wall (Eun et al., 2020).

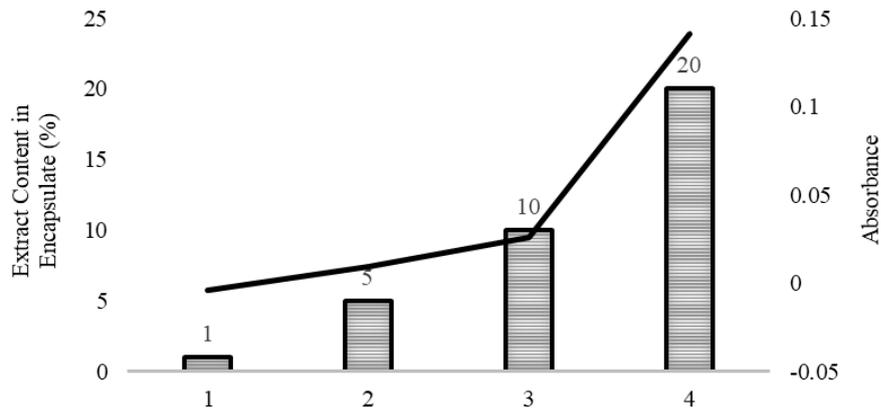


Figure 6. The Absorbance of  $\beta$ -Carotene Encapsulates at  $\lambda_{max}$  of 450 nm.

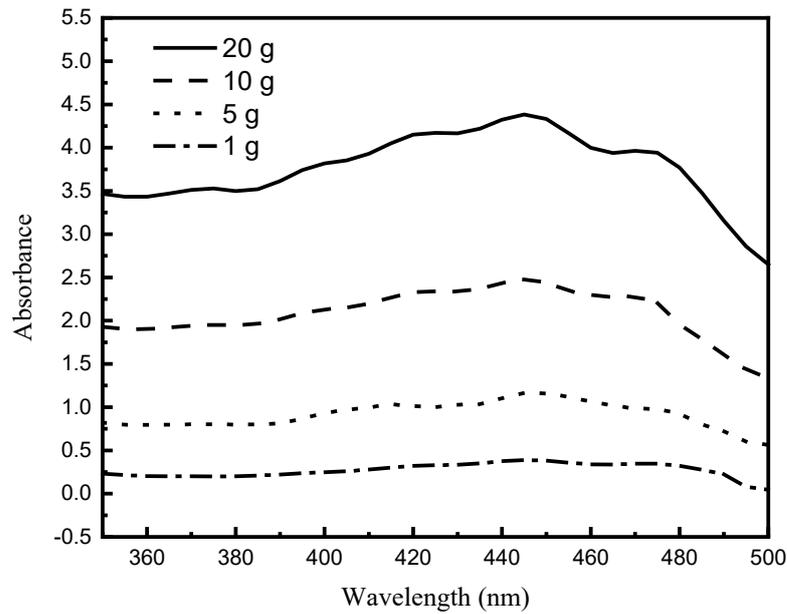


Figure 7. The Absorbance Spectra of the Encapsulated at the Wavelength between 350–500 nm with the Weight of the Carotenoid Pigment Extract in the Encapsulated (a) 20 g, (b) 10 g, (c) 5 g, and (d) 1 g.

Figure 8 shows the results of dye encapsulation from carotenoid pigments with varying extract levels. The expression of orange color became stronger with increasing levels of carotenoid pigment extracts in the encapsulate. Maltodextrin can form an emulsion film on the surface of carotenoid pigments and stabilize  $\beta$ -carotene in the extract, increasing its water solubility. The particles' hydrophobic core would serve as cargo space, while the hydrophilic outer shell would keep the particles stable in a water dispersion (Pan et al., 2007). It suggests that carotenoids could be a natural pigment.



Figure 8. The Results of Encapsulation of Carotenoid Pigments Containing  $\beta$ -Carotene with Different Extract Levels; (1) 1 g, (2) 5 g, (3) 1 g, and (4) 20 g.

#### 4. Conclusion

Halal natural food dyes can be obtained by producing carotenoid pigments from carrots (*Daucus carota* L). Although carrots are a halal raw material, other natural materials that meet halal criteria can also be used. In this research, the optimum yield of the extract obtained by maceration and Soxhlet extraction methods was 2.24% and 0.88%, respectively. The extract from the maceration method had higher absorptivity values than the Soxhlet extraction. Furthermore, it reacted positively with concentrated H<sub>2</sub>SO<sub>4</sub>, indicating the presence of  $\beta$ -carotene. UV-Vis spectrophotometry and TLC characterization yielded the highest absorption and R<sub>f</sub> value at 450 nm and 0.62, respectively. These values are confirmed by comparing the band's R<sub>f</sub> values and absorption spectra with the standard's. In addition, the encapsulation results revealed that the color expression increased for the encapsulates containing the highest levels.

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