

## Comparison of Extraction Methods of Carthamin from Safflower Pulp

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### ABSTRACT

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Carthamin, a red pigment obtained from safflower (*Carthamus tinctorius* L.) pulp, is widely used in food colorants, dyes and medicines. This study was carried out to obtain red pigments in various forms by extraction and purification from industrial waste of safflower pulp. Green solvents, organic solvents and acidic/alkaline solvents were used for the extraction of carthamin from safflower pulp. pH adjustments during extraction were optimized using citric, lactic, and acetic acids, and the optimal concentration of 3% citric acid was determined for efficient extraction without deformation of the adsorbents. These extracts were adsorbed with various adsorbents and eluted according to the usage area. Among the adsorbents, cellulose has proven to be the most versatile and reusable. The structure of carthamin was elucidated with UV-Vis, FTIR and SEM. Carthamin was obtained in 5 different forms as powder, aqueous solution, green solvent solution, organic solution and adsorbed onto filler. After purification, carthamin in solution forms was detected using UV-Vis spectroscopy with a peak at 520 nm and the highest yield as 0.58% (5.78 mg carthamin /g dry plant) was obtained in the cellulose-adsorbed form. The fact that carthamin is obtained in different forms occupies an important place in the industry.

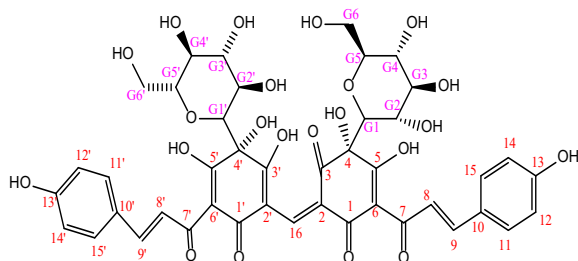
## 1. Introduction

The safflower plant (*Carthamus tinctorius* L.) contains yellow and red pigments (carthamin) in its petals. Safflower pigments have been traditionally used in paints and cosmetics for many years. Due to its natural origin and biological activities, carthamin is becoming increasingly used as an alternative to synthetic colorants in food, cosmetics and pharmaceuticals [1, 2]. While the water-soluble yellow pigment found in the safflower plant is used as a natural food colorant, the water-insoluble red pigment (Carthamin) has been used mainly in fabric dyeing. These pigments can be isolated from the safflower plant [3].

In recent years, the public has preferred the use of natural dyes rather than synthetic dyes in food. Industrially, yellow pigments are separated from the safflower plant by water extraction. The remaining pulp part is defined as by-product. It is known that this by-product contains a small

amount of yellow pigment and carthamin, which gives its red color. Hydroxysaflur yellow B, hydroxysaflur yellow C, safflower yellow A and carthamin show also strong antioxidant effect [4]. These pigments have pharmacological activities (such as cerebral thrombosis, myocardial infection, coronary heart disease) [5, 6]. Carthamin (chemical name: (6R,Z)-5,6-dihydroxy-4-((E)-3-(4-hydroxyphenyl)acryloyl)-2-(((3R)-2,3,4-trihydroxy-5-((E)-3-(4-hydroxyphenyl)acryloyl)-6-oxo-3-((3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)cyclohexa-1,4-dien-1-yl)methylene)-6-((3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)cyclohex-4-ene-1,3-dione), called safflower red, has an important role in traditional dyeing thanks to its ability to display different pink tones. Carthamin is a C-glucosylquinochalcone whose chemical structure is depicted in Figure 1 [7].

The C-glycosylquinochalcone forms a  $\pi$ -conjugated system by bonding with the carbon double bond. This conjugated system also produces a red color. Carthamin is composed of two chalcon moieties with conjugated bonds [8]. Carthamin is formed by oxidation with precarthamin. During carthamin biosynthesis, the color of the safflower flower gradually changes from yellow to red [9]. Depending on the species, water-soluble yellow dyes represent 25% to 36% of the chemical composition of the leaves, while carthamin is alkali-soluble and constitutes only 0.3–0.6% of the leaves [10, 11]. In addition, carthamin in red form has various using area such as a cosmetic, textile dye, toys, cakes, and biscuits [12].



**Figure 1.** Chemical structure of carthamin

Studies showed that carthamin is chemically unstable under high temperature and different pH conditions, rapidly decomposing in acidic and neutral mediums but relatively stable in slightly alkaline conditions [13]. Similarly, it has been determined that it maintains color stability for a limited time in alkaline media before gradual fading occurs. Therefore, pH control and solvent selection are critical for maximizing pigment stability [14]. Various extraction strategies have been proposed for carthamin, including alkali-acid isolation, aqueous two-phase systems, and solvent-ultrasound hybrid processes.

It has been determined that both chemical and physical extraction parameters strongly affect the yield and quality of carthamin [15, 16]. Organic solvents or strongly alkaline conditions, which may degrade the pigment or raise environmental concerns, are mostly used for extraction of safflower pigment [7, 11]. Therefore, researches continue to attract attention in order to increase the extraction efficiency by using environmentally friendly solvents and to develop extraction and purification methods to ensure the stability of the obtained pigment.

In recent years, the use of biodegradable, low-toxicity, environmentally friendly "green solvents" has become widespread. Green solvents have shown promising results in the recovery of natural pigments regarding yield or stability [17]. In addition, mild organic acids (citric, lactic, acetic) have been used for pH adjustment to prevent pigment degradation, which is usually caused by strong mineral acids [8]. Adsorbents such as cellulose, starch, and amylopectin contribute to purification and stabilization by forming pigment-polymer complexes that protect the chromophore from oxidation and light degradation [16, 17]. This present study focuses on the extraction and identification of red color pigment from safflower pulp as by-product. This study will contribute to the literature by testing different solvents, pHs and different adsorbents (e.g., polymeric natural adsorbents, amylopectin or industrial waste sawdust).

## 2. General Methods

### 2.1. Chemicals

All chemicals and solvents used in analysis were obtained from Sigma Aldrich (Sternheim, Germany), Alfa Aesar (Karlsruhe, Germany) and Merck (Darmstadt, Germany). Solvents and chemicals used such as DMF, acetone, DMSO,  $\text{KNO}_3$ ,  $\text{Na}_2\text{CO}_3$ ,  $\text{NaOH}$ , ethyl alcohol, ethyl lactate, pyridine, citric acid, acetic acid and lactic acid are of analytical purity.

### 2.2. Plant material

Safflower plant was supplied in the form of industrial waste by Döhler Food. Safflower is an industrial by-product from which the yellow pigment has been removed by the Company. This by-product was dried by using the freeze-dry method. The dried by-product was used in the extraction of carthamin.

### 2.3. Extraction

Carthamin extraction from the safflower plant was carried out using 3 different solvent groups: Organic solvents (DMF, acetone, DMSO), acidic/alkaline solutions ( $\text{KNO}_3$ ,  $\text{Na}_2\text{CO}_3$ ,

NaOH) and green solvents (ethyl alcohol, ethyl lactate).

5 grams of dry safflower plant is weighed and extracted with 100 ml of water in a waring blender at 18000 rpm for 2 minutes. The extract is filtered to dryness under vacuum in a Buchner funnel. This process is repeated 2 times. Yellow pigment-free safflower pulp is washed with 1-5% aqueous ethyl alcohol. The yellow pigments remaining in the plant were removed. The pulp filtered until dry is extracted with approximately 100 mL of organic solvents (DMF, acetone, DMSO) in a Waring blender at 18000 rpm for 2 minutes. It is filtered under vacuum with Whatman-1 filter paper until dry.

Likewise, the pulp filtered until dry is extracted with approximately 100 mL of one of the acidic/alkaline solutions ( $\text{KNO}_3$ ,  $\text{Na}_2\text{CO}_3$ , NaOH) in a Waring blender at 18000 rpm for 2 minutes. It is filtered under vacuum with Whatman-1 filter paper until dry. The pH values of the obtained extracts were adjusted with 1% citric acid, 3% citric acid, 5% citric acid, 10% citric acid, 1-5% lactic acid and 1-5% acetic acid. Another extraction was carried out using green solvents (ethyl alcohol, ethyl lactate:water) with the same method. The obtained extracts are collected to be used in the second stage.

## 2.4. Adsorption and desorption/elution

In the adsorption stage, artificial adsorbents (resin, silica gel), polymeric natural adsorbents (starch, cellulose) and industrial waste with adsorbent properties were used. In the desorption/elution stage, organic solvents (e.g. 60% acetone), green solvents (ethyl alcohol, isopropyl alcohol) or acidic/alkaline solutions (1%  $\text{Na}_2\text{CO}_3$  solution) were used.

### 2.4.1. Column chromatography

Adsorption: The pH of the extract obtained from acidic/alkaline and organic solution is adjusted to 4.5-5.5 with 1% citric acid, 3% citric acid, 1-5% lactic acid and 1-5% acetic acid solution. The pH adjusted solution is cooled to approximately 5-10 °C in an ice bath. The cold solution is added directly onto the cellulose (approximately 1.5 g)

placed in a 2.5 cm diameter column with a stationary phase height of approximately 1 cm.

Desorption/Elution: Carthamin in the solution loaded onto the column is adsorbed by cellulose. Approximately 200 ml of cold water is passed through the column to remove the yellow pigments in the mixture. After that, the carthamin adsorbed by cellulose is removed with solvents according to the usage form. Organic solvents (pyridine, DMSO, 60% acetone) for Form-1, green solvents (ethyl alcohol, isopropyl alcohol) for Form-2 or acidic/alkali solutions ( $\text{KNO}_3$ , NaOH, 1%  $\text{Na}_2\text{CO}_3$ ) for Form-3 were used. A powder form of carthamin can be obtained by evaporating the organic solvent (60% acetone) at low temperatures (about 25-30 °C).

### 2.4.2. Adsorbent selection

Cellulose, amylopectin or industrial waste sawdust is added to the extract obtained by using green solvents (ethyl alcohol, ethyl lactate: water) without any pH adjustment. The carthamin in solution is adsorbed by cellulose or sawdust. Pure carthamin adsorbed by cellulose, amylopectin or sawdust is eluted with propyleneglycol:alcohol. The alcohol in the elution solution is removed with the evaporator and the product is obtained in the propylene glycol phase.

In addition, after adjusting the pH of the extract obtained from acidic/alkaline and organic solution, it is adsorbed by polymeric natural adsorbents (starch, cellulose) and industrial waste with adsorbent properties. The adsorbed substance is eluted with organic solvents (e.g. 60% acetone) for Form-1, green solvents (ethyl alcohol, isopropyl alcohol) for Form-2, or acidic/alkaline solutions (1%  $\text{Na}_2\text{CO}_3$  solution) for Form-3.

## 2.5. Spectroscopy (UV-Vis, FTIR and SEM)

The structure of carthamin was elucidated using UV-Vis spectroscopy. In the measurements made with UV-Vis spectrophotometer, the absorption peak in the band of approximately 520-540 nm, which is a specific wavelength of carthamin, was detected and the obtained products were controlled. Additionally, the

carthamin compound was characterized by FTIR spectroscopy. SEM (Scanning Electron Microscope, Thermo Scientific Axia ChemiSEM) was used to determine the morphological properties of carthamin in powdered and carthamin on cellulose form for SEM-EDX analysis.

## 2.6. Statistical analysis

All experimental data were obtained in three parallel replicates and expressed as mean  $\pm$  standard deviation (SD). Statistical evaluation of differences between groups was performed using one-way analysis of variance (One-Way ANOVA) and Minitab Statistical Software 19.0 (State College, PA, USA). When a generally significant difference ( $p < 0.01$ ) was observed as a result of ANOVA, the Tukey's Test was applied to determine individual differences between group means.

## 3. Results and Discussion

EtOH:hexane,  $\text{KNO}_3$  and alcohol:water were used to separate the yellow pigment from the safflower pulp. 1%  $\text{Na}_2\text{CO}_3$ ,  $\text{K}_2\text{CO}_3$  (aq), 1% NaOH, DMF and ethyl lactate: water (1:4 and 1:2) were used in the extraction of carthamin from yellow pigment-free pulp. The pH was adjusted between 4.5 and 6 in all extraction processes except for ethyl lactate. The pH values of this used extraction solvents were determined as 10.3-10.9, 11.3, 12.8 and 10.5, respectively. 1% citric acid, 3% citric acid, 5% citric acid, 10% citric acid, 1-5% lactic acid, 1-5% acetic acid and 1% HCl were used for pH adjustment in carthamin extraction.

All acids except 1% HCl are suitable for use during the desorption stage; however, 1% HCl, 5% and 10% citric acid are not suitable. After the extraction method, the target pH point was reached by using approximately 50-75 mL of 3% citric acid solution. In addition, acid solutions of different concentrations (1, 2, 3, 5 and 10%) were used in the experiments. When solutions with higher concentrations (e.g. 5% and 10%) were used, the amount of solution decreased even further (approximately 15-20 mL). However, problems such as degradation of the adsorbent were encountered in subsequent purification

steps. Therefore, the optimum acid concentration was determined as 3% citric acid.

The adsorbents (cellulose, starch, amylopectin, sawdust) and suitable solvents (1%  $\text{Na}_2\text{CO}_3$ , 60% acetone: water, 50-70% ethyl lactate:water, 50% EtOH, 50-80% isopropyl alcohol, pure acetone, pure ethyl alcohol, pure ethyl lactate and pure isopropyl alcohol) were used for the adsorption/desorption of the obtained carthamin solution. It was determined that all adsorbents were suitable when 50% EtOH and pure acetone were used as elution solutions. 60% acetone solution elutes carthamin adsorbed to cellulose, starch and sawdust. When 1%  $\text{Na}_2\text{CO}_3$  and 50-80% isopropyl alcohol are preferred as elution solutions, cellulose, starch and amylopectin can be used as adsorbents. While elution with 50-70% ethyl lactate is suitable for cellulose and sawdust adsorbed carthamin. However, only cellulose adsorbent is suitable for pure ethyl lactate. Additionally, adsorbed carthamin on starch can be eluted with pure ethyl alcohol and pure isopropyl alcohol. Apart from cellulose, amylopectin, starch and sawdust, silica gel and amberlite XAD-7 were also used in the column. However, carthamin could not be adsorbed by silica gel and amberlite XAD-7. After desorption, it was determined that the cellulose in the column can be reused in other purification processes.

The obtained carthamin in different five forms are given in Figures 2-5. The products obtained as Form-1 were desorbed with organic solvent. These are elution solutions made from cellulose, starch and sawdust with pure and 60% acetone.



**Figure 2.** Form-1: Organic solvent solution of carthamin

The Form-2 is used after being desorbed with green solvents (%50-70 ethyl lactate:water, %50 EtOH, pure ethyl alcohol, pure ethyl lactate) from cellulose, amylopectin, starch and sawdust adsorbents.

The desired product as Form-3 is obtained by eluting by 1% Na<sub>2</sub>CO<sub>3</sub> solution from cellulose, amylopectin and starch and then acidifying with citric acid/lactic acid solution.



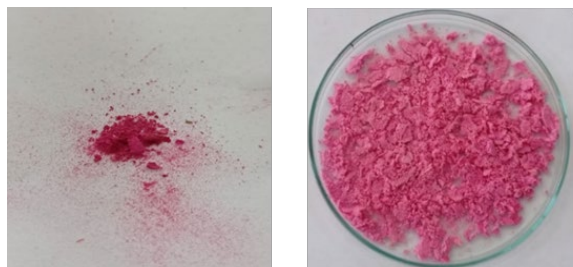
**Figure 3.** Form-2: Green solvent solution of carthamin



**Figure 4.** Form-3: Aqueous solution of carthamin

The Form-4 (powder form of carthamin) is obtained by evaporation of the organic solvent (acetone, anhydrous ethyl alcohol, isopropyl alcohol, etc.) at low temperatures (about 25-30 °C). The Form-5 is obtained by drying only by-passing air, without any desorption after washing with water. A stable form was obtained by absorption of carthamin into cellulose.

The absorbance values and yields of carthamin are given in Table 1. Yields of the powder form (Form-4) and cellulose adsorbed form (Form-5) are also given in mg/g dry plant.



**Figure 5.** Form-4: Powder form of carthamin, Form-5: Carthamin in cellulose adsorbance form

The powder and cellulose adsorbed form was obtained 4.8 mg/g dry plant and 5.78 mg/g dry plant, respectively. Additionally, the yields % of all forms were calculated according to absorbance values. Absorbance values varied

between 0.09 and 0.531. The yields % of the obtained forms (Form 1-5) were determined as 0.52%, 0.25%, 0.09%, 0.48% and 0.58%, respectively ( $p < 0.01$ ). Form-5 has the highest yield (0.58%). It is seen that Form-1 (0.52%) and Form-4 (0.48%) also have quite high efficiency. The lowest yield in the extraction of carthamin was determined in the aqueous solution form (0.09%). As shown in Table 2, previously reported extraction yields of cartamin ranged from 0.2% to 2.6% depending on the extraction system. The obtained yields in this study are lower than the reported study by Sun et al. [15], while many of them are higher yields than the other reported study [5, 6, 8, 17], given in Table 2.

**Table 1.** UV-Vis absorbance values and calculated yields of the obtained forms

Obtained form	Calculated yields (mg/ g dry plant (%))	UV-Vis absorbance value (at 520 nm)
Organic solvent solution of carthamin	- (0.52%)* <sup>A,B</sup>	0.482
Green solvent solution of carthamin	- (0.25%)* <sup>C</sup>	0.234
Aqueous solution of carthamin	- (0.09%)* <sup>D</sup>	0.090
Powder	4.8 mg/ g dry plant (0.48%)* <sup>B</sup>	0.451
Carthamin on cellulose absorbance	5.78 mg/ g dry plant (0.58%)* <sup>A</sup>	0.531

\* yields% was calculated according to absorbance values. Different uppercase letters in the same row indicate significant differences ( $p < 0.01$ ).

Compared to different extraction systems, the cellulose-adsorbed form of this present study stands out in terms of efficiency. The acetone-water-based ATSS method reported by Sun et al. [15] achieved a high efficiency of 2.65%; however, it is not environmentally friendly due to the use of high amounts of organic solvents. In contrast, the cellulose-adsorbed system used in this study offers an alternative method with environmental perspectives thanks to use a reusable adsorbent and green solvent, although achieving lower yield. The green solvent form (0.25%) yields very similar results to the ethanol:water-based system (0.21%) reported by Ghorbani et al. [8].

**Table 2.** Carthamine purification methods and yields in the literature

Source Materia	Yield (% or mg/g)	Remarks	Ref.
Fresh florets	2.65 %	ATSS yielded highest carthamin recovery.	[15]
Florets	0.21 %	Obtained with low yield but high pigment purity.	[8]
Dried petals	0.43 %	Traditional alkaline method; pigment degraded after drying.	[6]
Safflower by-products	0.37 %	Cellulose adsorption enhanced pigment stability; reusable.	[17]
Florets	0.31 %	Using ethanol-water gave positive results in terms of yield and antioxidant.	[5]

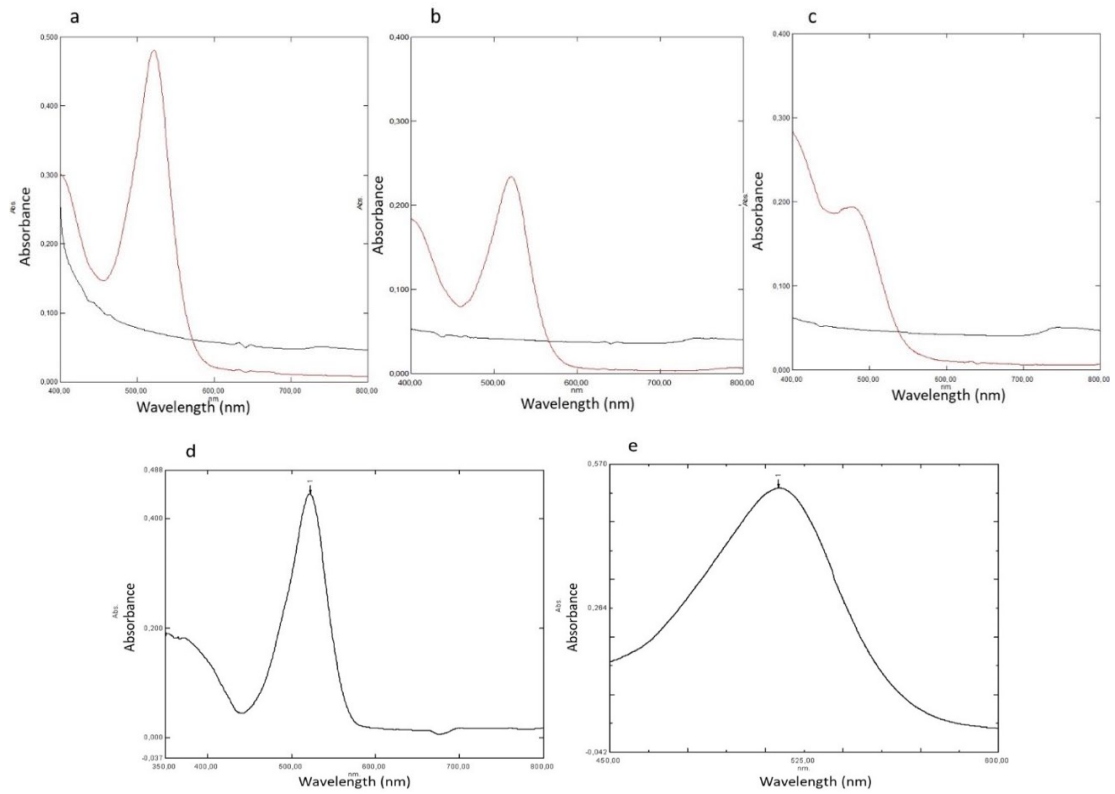
Similarly, Satrianegara et al. achieved a yield of 0.31% with an ethanol–water mixture; our results obtained in this study close to reported study, confirming the environmental advantages of green solvent systems [5]. The lowest yield (0.09%) is due to carthamin's limited solubility in water and sensitivity to pH changes. Although the yield obtained in the extraction with  $\text{Na}_2\text{CO}_3$  aqueous system (0.43%) reported by Jadhav and Joshi is higher than the obtained value in this study. It is considered that the use of fresh petals as raw material and less enzymatic degradation may have increased the yield [6]. Conversely, the partially oxidized pigment in industrial waste materials may reduce the solubility in aqueous systems.

The obtained forms of carthamin were confirmed by using UV-Vis spectrophotometer. The UV-Vis spectrums of carthamin obtained in 5 different forms are given in Figure 6. The carthamin peak is seen at 520 nm wavelength.

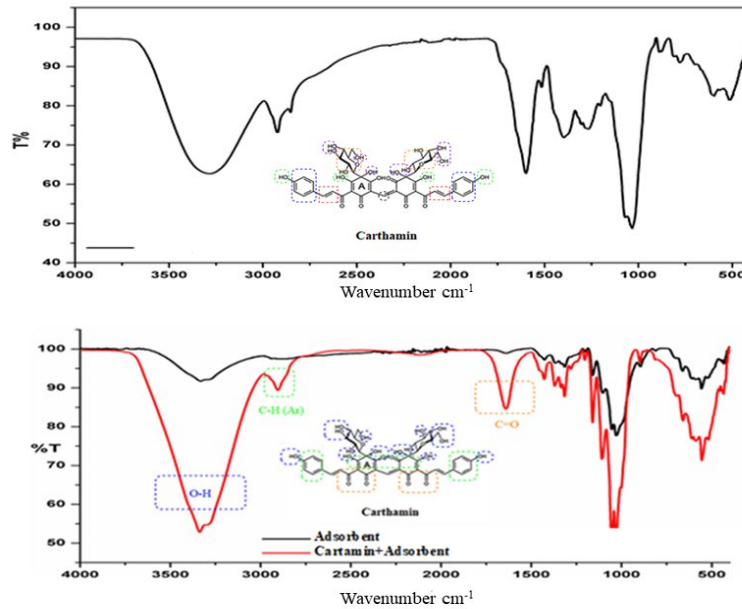
The structure of the obtained carthamin was elucidated by FTIR spectroscopy. The FTIR spectrum is given in Figure 7. -OH bands are wide and located in the 3700-3100  $\text{cm}^{-1}$  region. The carbonyl (C=O) stretching vibration is characterized by an absorption of approximately 1700  $\text{cm}^{-1}$ . In addition, aromatic C-H bonds are characterized by an absorption of approximately 3000-3100  $\text{cm}^{-1}$ . Figure 8 shows distinct structural features in the scanning electron microscope (SEM) image of carthamin. The SEM image diagram of carthamin obtained from safflower pulp shows that the absorbent material has a different shape morphology.

Among the extraction systems using in this study, it is considered that cellulose adsorption method not only improved pigment yield and stability but also reduced the cost due to higher recovery efficiency and adsorbent reusability. In contrast, powder- and organic solvent-based extractions methods exhibited higher energy and solvent costs due to additional evaporation steps. Given the low cost or no purchasing value of safflower pulp as an industrial waste, the overall process demonstrates favorable economic potential compared to methods using fresh safflower reported in the literature

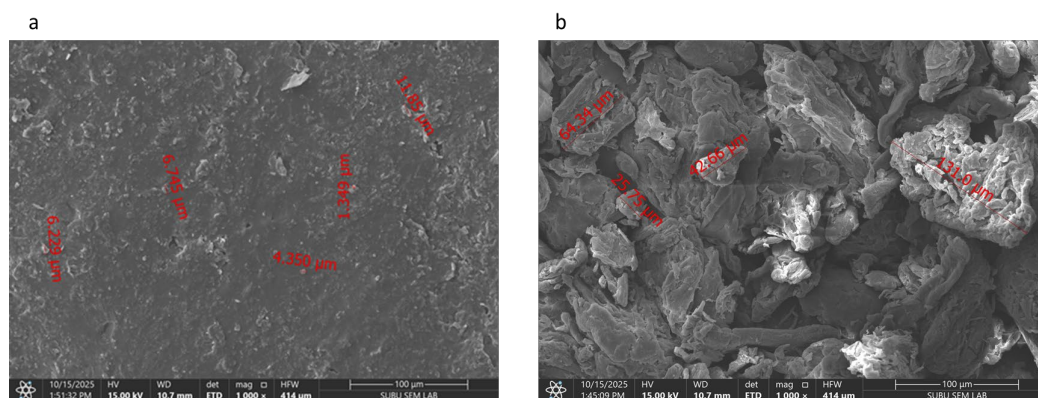
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**Figure 6.** UV-Vis spectra of carthamin; a) organic solvent solution of carthamin, b) green solvent solution of carthamin, c) aqueous solution of carthamin, d) powder form of carthamin, e) Carthamin on cellulose adsorbed form



**Figure 7.** FTIR spectra a) powdered carthamin, b) adsorbent and carthamin + adsorbent



**Figure 8.** Scanning electron microscope (SEM) image of a) powder form of carthamin, b) Carthamin on cellulose

#### 4. Conclusions

This study presents an efficient and environmentally friendly approach for the extraction and purification of carthamin from safflower pulp. Through systematic evaluation of extraction solvents, pH adjustment methods and adsorbent materials, five different forms of carthamin (powder, aqueous solution, green solvent solution, organic solution, and adsorbed onto filler) were successfully obtained. The form adsorbed on to cellulose (Form-5), having the highest yield (0.58%), demonstrates that cellulose is an effective and reusable adsorbent for carthamin purification. Acid concentration was found to be critical in preventing adsorbent degradation and ensuring product stability and 3% citric acid was determined as the optimum acid concentration. The structure of carthamin was elucidated with UV-Vis, FTIR and SEM spectroscopy. The methodology developed here supports the use of green solvents and recyclable materials in natural pigment isolation. This study contributes to safflower plant research.

#### Article Information Form

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##### *The Declaration of Conflict of Interest/ Common Interest*

No conflict of interest or common interest has been declared by author.

##### *Artificial Intelligence Statement*

No artificial intelligence tools were used while writing this article.

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