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# DETERMINATION OF THE CHARACTERISTIC ATTRIBUTES OF COTTONSEED PROTEIN CONCENTRATE

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## ABSTRACT

The current study focused on characteristic attributes of protein concentrate obtained from oil-free cottonseed. For this, the physicochemical properties namely moisture content, water activity, color, flowability, wettability, and protein solubility of cottonseed protein concentrate (CSPC) were investigated. Water holding capacity (WHC), oil binding capacity (OBC), foaming capacity, foam stability (10 and 30 min), emulsion activity index (EAI) and emulsion stability index (ESI) (10 and 30 min) of proteins were 2.75 g water/g protein, 2.59 g oil/g protein, 29.00%, 93.10% - 69.05%, 6.25 $m^2$/g and 29.27-87.81 min, respectively. Bands regarding CSPC in the 45 kDa molecular weight were detected by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) patterns. Fourier-transform infrared spectroscopy (FTIR) was used to verify the protein-specific structures. Sheet structures in the surface morphology of CSPC were dominant when scanning electron microscopy (SEM) images were investigated. Thermal gravimetric analyzer (TGA) results showed that the protein concentrate exhibited excellent stability to temperature.
**Keywords:** Cottonseed protein concentrate, techno-functional properties, SDS-PAGE, scanning electron microscopy, thermogravimetric analysis

# PAMUK ÇEKİRDEĞİ PROTEİN KONSANTRESİNİN KARAKTERİSTİK ÖZELLİKLERİNİN BELİRLENMESİ

## ÖZ

Mevcut çalışma, yağsız pamuk tohumundan elde edilen protein konsantresinin karakteristik özelliklerine odaklanmıştır. Bunun için pamuk tohumu proteini konsantresinin (CSPC) nem içeriği,

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su aktivitesi, rengi, akışkanlığı, ıslanabilirliği ve protein çözünürlüğü gibi fizikokimyasal özellikleri incelenmiştir. Proteinlerin su tutma kapasitesi (WHC), yağ bağlama kapasitesi (OBC), köpük oluşturma kapasitesi, köpük stabilitesi (10 ve 30 dakika), emülsiyon aktivite indeksi (EAI) ve emülsiyon stabilite indeksi (ESI) (10 ve 30 dakika) dâhil olmak üzere tekno-fonksiyonel özellikleri sırasıyla 2.75 g su/g protein, 2.59 g yağ/g protein, %29.00, %93.10-%69.05, 6.25 $m^2$/g ve 29.27-87.81 dk olarak bulunmuştur. 45 kDa moleküler ağırlıktaki CSPC ile ilgili bantlar, sodyum dodesil-sülfat poliakrilamid jel elektroforez (SDS-PAGE) modeli ile tespit edilmiştir. Proteine özgü yapıları tespit etmek için Fourier dönüşümü kızılötesi spektroskopisi (FTIR) kullanılmıştır. Taramalı elektron mikroskobu (SEM) görüntüleri incelendiğinde, CSPC'nin yüzey morfolojisindeki tabaka yapılarının baskın olduğu bulunmuştur. Termal gravimetrik analizör (TGA) sonuçları, protein konsantresinin sıcaklığa karşı mükemmel stabilite sergilediğini göstermiştir.
**Anahtar kelimeler:** Pamuk tohumu protein konsantresi, tekno-fonksiyonel özellikler, SDS-PAGE, taramalı elektron mikroskobu, termogravimetrik analiz

## INTRODUCTION

The nature of living organisms cannot give permission to synthesize essential amino acids. This deficiency could be eliminated with a balanced protein diet. Therefore, people consume protein-rich foods for growth and health benefits for centuries (Day et al., 2021). With the increasing population growth, the need for nutrients also increases and alternative protein sources are needed (Hua et al., 2019). The prevalence of animal-based proteins is high compared to plant-based ones in the human diet (Sá et al., 2020). However, some drawbacks regarding animal-based proteins are reported in the scientific literature. Their high cost and undesirable effects on health could be given as examples of these problems (Constable and Bange, 2015). Therefore, it cannot be ignored that the demand for plant-based ones has increased in the industry, recently.

Cotton (*Gossypium hirsutum L.*) is a perennial plant species that belongs to the systematic mallow family (*Malvaceae*) (Constable and Bange, 2015). It is one of the main agricultural products known worldwide and produced for commercial purposes (Baran, 2016; Gao et al., 2022). China, Brazil, Egypt, the USA, Mexico, India, and Pakistan are the leading producing countries (Seal et al., 2015; Kumar et al., 2022; Wei et al., 2022). The annual production in the world is approximately 25 million tons (Khan et al., 2020). Cotton production in Turkey is at a level that cannot be ignored. Cotton farming is intense in certain regions namely Çukurova, Aegean, and South-eastern Anatolia (Yılmaz et al., 2005; Baran, 2016; Khan et al., 2020; Çullu et al., 2022). This product, which has a very high importance for the world, is widely evaluated as a raw material in different industries (Yılmaz et al., 2005; Van Atta, 2009). A high amount of waste is generated during the production of cotton-derived products. Among these by-products, seeds possess the largest proportion. The cottonseed production, which was 1.064,189 tons (2020), increased by 26.85%, and reached 1.350,000 tons (2021) in Turkey (Anonymous, 2022). The cottonseeds contain approximately 18-25% oil, and 20-25% protein (Saxena et al., 2011). In this context, cottonseeds gain importance because of their nutritional value and different value-added product groups for the economy/society could be produced by using them (Wei et al., 2017; Tepecik et al., 2022). They have been widely evaluated as raw material in the oil industry. After being used in the oil production, oil-free seeds contain a high amount of protein. Therefore, datasets on the extraction of proteins from related materials have been reported in previous studies (Ory and Flick, 1994; Tsaliki et al., 2002; Tunç and Duman, 2007; Ma et al., 2018). In addition to these, the secondary metabolite gosypol is present in cottonseed (Chenoweth et al., 2000). Studies have shown that gossypol is not suitable for human and animal consumption due to its toxic nature (Kumar et al., 2021a; Jacobs et al., 2022). The World Health Organization (WHO) has set the limit for the consumption of gossypol in cotton protein-based products as 450 ppm (Kumar et al., 2021a). Recently, studies have been carried out to reduce gossypol in cotton protein and it has been determined that the proteins obtained by alkaline

extraction method contain gossypol below the desired level (Kumar et al., 2021b).

However, the number of studies on cottonseed protein should be increased to exactly put forth protein attributes. In line with this approach, research has been continued on the extraction of cottonseed protein concentrate (CSPC) and its use as a sustainable protein supplement in recent years (He et al., 2020; Kumar et al., 2021b; Kumar et al., 2022). The current study was conducted to provide innovative datasets related to CSPC and support these previous studies. Therefore, in the present study, it was aimed to obtain CSPC by alkaline extraction method, to determine the physicochemical composition, investigate the techno-functional properties, molecular weights with SDS-PAGE, evaluate certain groups with Fourier-transform infrared spectroscopy (FTIR), morphological structure with scanning electron microscopy (SEM) and thermal behaviour by thermal gravimetric analyzer (TGA) of proteins.

## MATERIALS AND METHODS

### Materials

Cottonseeds were supplied from a local market in Şanlıurfa province of Turkey. The fibers of the seeds were allocated by treatment with sulfuric acid. The cleaned seeds were ground, then the oil was removed with hexane (1:10, w/v). Defatted seeds were kept at +4 ℃ until protein extraction. All chemicals were obtained either from Sigma-Aldrich or Merck and were standard analytic grade.

### Protein extraction

The method reported by Yücetepe et al. (2021) was applied for protein extraction (Yücetepe et al., 2021). Briefly, 1 gram cottonseed was mixed with 0.12 mol/L NaOH at a ratio of 1:10 (w/v). Samples were held at 37 ℃ in a water bath for 1 h occasionally mixing. At the end of the period, they were centrifuged at 1420 *x g* for 15 min at +4 ℃ and the supernatant was collected. The pH of the supernatant was adjusted to 4.5 with HCl (0.1 mol/L). After samples were centrifuged at 1420 *x g* for 15 min at 4 ℃, the precipitate was dried using a freeze dryer (Armfield SB4, Ringwood, England). The resulting samples were stored in a refrigerator at +4 ℃ until analyses.

### Analyses

#### Moisture content

The moisture content of the samples was calculated gravimetrically. One g sample was left in an oven at 105 ℃ for 24 hours until it reached a constant weight. Moisture content was calculated by proportioning the weights noted before and after the drying process (Samuelsson et al., 2006).

#### Total mineral content

To determine the total mineral content of the samples, 1-4 g of the samples were weighed and incinerated for 16 h in a 600 ℃ muffle furnace until they reached a constant weight. The total amount of mineral matter was calculated by dividing the initial and final weights by each other (Liu, 2019).

#### Oil content

The hexane extraction was carried out with minor modifications (Koubaa et al., 2017). Ten g ground samples were mixed with 100 mL n-hexane in a glass bottle. Solutions were left in the shaker at 150 rpm for 5 h. At the end of the period, the samples were centrifuged at 1420 *x g* for 10 min. The supernatant was evaporated with a rotary evaporator system at 55 ℃. The amount of oil obtained at the end of the extraction was noted and the oil content (%) was calculated according to Equation (1) below:

$$\text{Oil content (\%)} = \frac{oil\ weight}{total\ sample\ weight} \text{x100} \qquad (1)$$

#### Protein content

The protein content of the cottonseed and CSPC was defined by using an organic element analyzer (FlashSmart Elemental, ThermoFisher Scientific, USA). CHNS reactor temperature of 950 ℃, GC oven temperature of 65 ℃, helium carrier flow of 140 mL/min, helium reference flow of 100 mL/min, oxygen flow of 250 mL/min, oxygen injection time of 5 s, sample delay of 12 s, and total run time of less than 600 s were implemented as fixed parameters for analysis. The protein

content was calculated using a conversion factor of 6.25 (Nx6.25) (Başyiğit et al., 2020a).

**Water activity**

Water activity device (model 4TE, Aqualab, Decagon Devices Inc., Pullman, WA, USA) was used for definition of water activity according to the operating manuals (Turchiuli et al., 2005).

**Color**

Color was defined by using a chroma meter (Color Quest, Hunter Associates Laboratory, Inc., Reston, VA 22,090, USA) (Duangmal et al., 2008).

**Hausner ratio and Carr index**

For the Hausner ratio and Carr index analyses, the bulk and tapped density values of the proteins were used. Briefly, 5 g CSPC was placed in a 25 mL measuring cylinder and the volume was recorded. The bulk density was defined by dividing the sample weight by the measured volume. For the tapped density, the measuring cylinder was struck by hand 200-hold on flat ground to a constant volume. The tapped density was calculated by the weight of the samples to the final volume ratio (Turchiuli et al., 2005). Hausner ratio and Carr index values calculated using by following Equations (Eqs. 2 and 3):

$$\text{Hausner ratio} = \frac{tapped\ density}{bulk\ density} \quad (2)$$

$$\text{Carr index} = \frac{tapped\ density - bulk\ density}{tapped\ density} \times 100 \quad (3)$$

**Wettability**

One gram sample was transferred manually to 100 mL distilled water (20 ℃) in the beaker and the time required for the samples to completely leave the aqueous phase surface was recorded (Turchiuli et al., 2005).

**Protein solubility**

Protein solubility was achieved by applying some modifications according to a previous study (Xia et al., 2023). CSPCs were prepared at 15 mg/mL and the pH was adjusted to 7.0. The solution was stirred in a shaker at 150 rpm for 1 h. At the end of the time, the sample was centrifuged at 1420 *x g* for 10 min. The Lowry method was used to detect protein in the supernatant. Protein solubility was calculated using the following Equation (Eq 4):

$$\text{Protein solubility (\%)} = \frac{\text{Protein content of the supernatant}}{\text{Total protein of the original sample}} * 100 \quad (4)$$

**Water holding and oil binding capacity**

To calculate the water holding (WHC) and oil binding capacities (OBC) of the samples, 1 gram sample was mixed with 10 mL water (for WHC) or 10 mL corn oil (for OBC) in a centrifuge tube. The samples were vortexed manually every 15 min at room temperature. At the end of the period, the tubes were centrifuged for 15 min at 1420 *x g* at 24 ℃. The supernatant was filtered, and the tubes were left for 30 minutes at a 45° angle. The final weights of the samples were noted. WHC and OBC were calculated by the ratio of the final weights of the samples to their initial weights (Cho et al., 2004).

**Foaming properties**

The foaming activity and stability were achieved by protein solutions at a concentration of 1 g/100 mL (pH 7.0). For this, 50 mL solution was transferred into a 100 mL measuring cylinder and homogenized at 12000 rpm for 1 min. Volumes were noted before and after the homogenization to use in the calculation of foaming activity and stability values. The foaming activity and stability were calculated according to the following Equations (Eqs 5 and 6):

$$\text{Foaming capacity (\%)} = \frac{(Va - Vb)}{Vb} * 100 \quad (5)$$

$$\text{Foaming Stability (\%)} = \frac{Vr}{Va} * 100 \quad (6)$$

Where, Vb is the volume of solution before homogenization, Va is the volume of solution after homogenization, and Vr is volume of solution at related time (Li et al., 2021).

**Emulsifying properties**

The protein sample was prepared with 10 mM potassium buffer (pH 7.0) at a concentration of 0.5 g/100 mL. To form oil-in-water emulsions,

the solution and corn oil were mixed at a ratio of 3:1 (v/v) and homogenized with Ultra-Turrax at 12000 rpm for 1 min. After homogenization, 50 μL emulsion was added to the tubes containing 5 ml SDS (0.1%, v/v). The absorbance of solution was read at 500 nm ($A_1$). The same procedure was repeated at the 10th and 30th min and the emulsion features were calculated by using the Equations (Eqs 7 and 8) below:

$$\text{Emulsifying activity (EAI) } (m^2/g) = \frac{2*2.303*A1*DF}{C*\varphi*\theta*1000} \quad (7)$$

$$\text{Emulsifying stability (ESI) (min)} = \frac{A1}{A1-Ar} * t \quad (8)$$

Where, $A_1$ is absorbance of after homogenization, DF is dilution factor, C is protein concentration, $\varphi$ is optical path, $\theta$ is volume fraction of oil, $A_r$ is absorbance at related time (10 or 30 min), and t is 10 or 30 min (Lee et al., 2021).

**Sodium dodecyl sulfate polyacrylamide gel electrophoresis**

The CSPC was dissolved in sample buffer containing 0.1 M Tris-HCl, 0.1 M NaCl and 10% (w/v) SDS at a concentration of 10 mg/mL (Ata et al., 2022). The prepared samples were stirred with sample loading buffer (5% Tris-HCl (pH 6.8), 4% glycerol, 0.8% SDS, 0.02% bromphenol blue and 2% β-mercaptoethanol) at a ratio of 1:1 (v/v). SDS-PAGE analysis was performed according to the Laemmli (1970) by using 12% separation gel and 5% stacking gel (Laemmli, 1970). Samples were kept at 95 ℃ for 10 min before being placed in gel lanes. A standard protein mixture (11-190 kDa) was utilized as the molecular weight marker. A 0.1% Coomassie Brilliant Blue G-250 was applied to stain the electrophoresis gel.

**FTIR analysis**

For the FTIR analysis, a FTIR spectrophotometer (IRTracer-100, Shidmadzu Corporation, Kyota, Japan) was used. The transmittance spectra of protein concentrate were detected in the wavelength 4000 to 500 $cm^{-1}$ by applying the data range of 1 $cm^{-1}$ at ambient temperature (Andrade et al., 2019).

**SEM analysis**

The morphological structures of the sample coated with palladium before analysis were monitored using a SEM (ZEISS Sigma 300 Field Emission SEM, Oberkochen, Germany) at 30 kV (Ortiz et al., 2009).

**Thermal analysis**

TGA (DTG-60H 60H, Shimadzu) were used to detect the thermal behavior of the protein sample. The sample (2.5-3 mg) was weight and placed in the corresponding part of the device. The analysis was carried out at a heating rate of 10 K/min, in an inert atmosphere ($N_2$), between 30 °C and 1000 °C (Başyiğit et al., 2022a).

**Statistical analysis**

All analyses were performed in three replications. The graphics were drawn using the OriginPro 2021b (Origin Lab Inc.).

## RESULTS AND DISCUSSIONS

**Chemical composition of cottonseed and cottonseed protein concentrate**

The chemical composition of cottonseed and CSPC was investigated, and the results are presented in Table 1. The content of moisture and minerals was found to be 5.72% and 5.12%, respectively. The oil content was 16.85% in the seed. As for protein content, the seed contained 17.09% protein. The standard values for CSPC are <8% for moisture content, <0.35% for total mineral substance content, and >46% for protein content (Kumar et al., 2021b). The values (moisture content: 3.46%, mineral content: 4.92%, protein content: 65.72%) obtained for CSPC in the present study were in line with the standard. Additionally, the oil content in CSPC was detected as 2.02%.

Table 1. Biochemical composition of cottonseed and cottonseed protein concentrate

| Analysis | Cottonseed | Cottonseed protein concentrate |
|---|---|---|
| Moisture content (%) | 5.72±0.04 | 3.46±0.16 |
| Ash content (%) | 5.12±0.17 | 4.92±0.21 |
| Protein content (%) | 17.09±0.00 | 65.72±0.00 |
| Oil content (%) | 16.85±0.26 | 2.02±0.04 |

**Physicochemical properties of cottonseed protein concentrate**

The physicochemical features of CSPC are given in Table 2. The water activity in proteins was 0.35. This means that the unbound water was evaporated at the desired level during drying process. Also, this result was supported by the moisture content of proteins. The lower water activity indicates high stability (the safe-zone category) in CSPC. In other words, proteins are stable against biological and microbiological deterioration (Mathlouthi, 2021). Color is one of the quality parameters playing an important role in consumer preferences. L* (lightness-darkness), a* (red-green), and b* (yellow-blue) values of CSPC were 54.83, 6.77 and 25.04, respectively. The L* values for other plant seeds-based proteins including canola, hemp and flaxseed were reported as 26.82, 29.48 and 44.89, respectively (Teh et al., 2014). The darker color for CSPC could be ascribed to raw material type. Also, this phenomenon could be associated with the extraction conditions (Das purkayastha et al., 2015). Positive b* value was related to the yellowish color of CSPC. Hausner ratio and Carr index values are parameters that give information about the flow properties and stickiness of samples (Calafato and Picó, 2006). Both are affected by moisture content (Goula et al., 2004). If these values are not at the desired level, the deterioration of the products occurs faster due to the high level of oxygen molecule between the grains (Koç et al., 2011). The Hausner ratio and Carr index values of CSPC were 1.34 and 34.31, respectively (Table 2). Hausner ratio of 1.34 is determinative of reasonable fluency characteristic (Calafato and Picó, 2006). In other words, CSPC was acceptable in terms of flow properties and stickiness (Patil et al., 2013). Wettability is a parameter that shows the settling time of the powder samples by adding the water to their structure. The wettability value of CSPC was found to be 7.79 sec. The low wettability value may be associated with the high hydrophilic groups in protein structure (Turchiuli et al., 2005).

**Protein solubility**

Protein solubility affects the texture, color, emulsifying properties, foaming ability and sensory properties of food products. Therefore, it is one of the most important functions of proteins (Haque et al., 2016). The protein solubility was discussed in this part and results are presented in Table 2. This value for CSPC was 28.75%. The solubility value of CSPC varies in the range of 20-45% at pH 7.0 (Ma et al., 2018). The lower solubility could be related to the pH value. This approach was supported by a previous study. Tsaliki et al. (2002) examined the effect of pH-shifting on CSPC solubility. The authors reported that the solubility increased in parallel with the increase in pH and also, soluble protein content was between 0-20% at pH 7 (Tsaliki et al., 2002). As well as pH, protein solubility depends on temperature, ionic strength, protein structure, and protein concentration (Wang et al., 2019). The results clearly showed that after the proteins are dissolved in alkaline conditions, incorporation into food systems seems to be a more logical approach.

Table 2. Physicochemical properties of cottonseed protein concentrate

| Analysis | Cottonseed protein concentrate |
|---|---|
| Water activity | 0.35±0.00 |
| | 54.83±1.19 |
| Color (L*, a*, b*) | 6.77±0.93 |
| | 25.04±0.19 |
| Carr index | 34.31±1.39 |
| Hausner ratio | 1.34±0.01 |
| Wettability(sec) | 7.79±0.33 |
| Solubility (pH 7.0) (%) | 28.75±0.95 |

**Techno-functional properties of cottonseed protein concentrate**

WHC/OBC, foaming activity/stability, and emulsifying activity/stability of proteins were performed, and the results are presented in Table 3. WHC and OBC are associated with hydrophilic and hydrophobic groups in powder products. (Wu et al., 2009). Also, both are directly related to the nature of protein (Hadidi et al., 2021). In our study, WHC value was found to be 2.75 g water/g protein. This feature, which is related to the functionality of proteins, was 1.77 g water/g protein for CSPC in a previous study (Delgado et al., 2019). The rehydration rate of samples

obtained in the current study was superior than that of the previous finding (Haque et al., 2016). As for OBC, proteins exhibiting superior behavior in terms of the relevant property are effective in preserving the flavor and stability of the oil in emulsified foods (Özdemir et al., 2022). OBC of CSPC was 2.59 g oil/g protein. Delgado et al. (2019) reported lower values for CSPC in terms of OBC (Delgado et al., 2019). These differences could be related to the nonpolar side chains and different conformations in the proteins (Khalid et al., 2003). The nonpolar side chains and different conformations might be affected by extraction methods and environmental conditions in which the raw materials are grown.

Foam is defined as a structure formed by a continuous phase consisting of liquid or solid surrounding the air. While the proteins form foam, air bubbles and liquid phase settle at the interface. They prevent air bubbles from coming together and play an effective role in the foam formation. Foam ability of proteins plays an important role in the production of foamy food (Were et al., 1997). They are used especially in the bakery industry as foaming agents (Muthukumaran et al., 2008). Foam capacity and foam stability are the most commonly used parameters when examining the foaming properties of proteins (Yavuz and Özçelik, 2016). Foaming capacity and foaming stability ($10^{th}$ and $30^{th}$ min) values (pH 7.0) of CSPC were found to be 29.00%, 93.10% and 69.05%, respectively (Table 3). The similar results were reported by a previous study (Tsaliki et al., 2002).

The emulsion capability of proteins is a desirable feature in the food industry, especially in areas such as emulsified meat products (Özdemir et al., 2022). Emulsion abilities vary according to surface amphiphilicity of proteins, amount of soluble/insoluble protein content and other substances (impurities) (Zielińska et al., 2018). The EAI was detected as 6.25 $m^2/g$ in CSPC (Table 3). Lower EAI could be attributed to the undesired protein solubility (Table 3). ESI at $10^{th}$ and $30^{th}$ min was determined to be 29.27 and 87.81 min, respectively. Ma et al. (2018) also investigated EAI (13.3-23.1 $m^2/g$) and ESI (17.3-29.6 min, at $10^{th}$ min) in CSPC (Ma et al., 2018). In addition to these, the high ESI value at the $30^{th}$ min might be interpreted as the dissolution of some protein and the improvement of the emulsion stability property due to protein passing to the oil-water (Delgado et al., 2019). The techno-functional properties of proteins such as emulsion behavior and foam formation are important features that support each other (Yavuz and Özçelik, 2016). In the food industry, the stability of the product is increased, and the shelf life is extended by using proteins with high emulsion capacity in the production of emulsified foods such as sausage and mayonnaise (Jideani, 2011; Mirzanajafi-Zanjani et al., 2019). In addition, the use of plant proteins in the daily diet for the nutritional needs of the increasing population comes to the fore (Fresco, 2009). For this reason, it is predicted that the use of plant proteins in products such as protein-rich protein bars and protein drinks will become widespread.

Table 3. Techno-functional properties of fruit seed protein concentrate

| Analysis | Cottonseed protein concentrate |
|---|---|
| Water holding capacity (g water/g protein) | 2.75±0.00 |
| Oil binding capacity (g oil/ g protein b) | 2.59±0.01 |
| Foaming capacity (%) | 29.00±1.41 |
| Foaming stability (%) ($10^{th}$ min) | 93.10±0.34 |
| Foaming stability (%) ($30^{th}$ min) | 69.05±3.37 |
| Emulsifying activity EAI ($m^2/g$) | 6.25±0.09 |
| Emulsifying stability (ESI10) (min) | 29.27±0.28 |
| Emulsifying stability (ESI30) (min) | 87.81±0.86 |

**Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)**

Molecular weight distribution of CSPC was confirmed by SDS-PAGE analysis and the related images are shown in Fig 1A. Two intense specific bands were detected in the 45 kDa molecular weight band. These two bands are the main components of cottonseed proteins (King, 1980). When the CSPC molecular weight fractions in the literature were compared, the two bands were found to overlap (Ma et al., 2018; Delgado et al., 2019). Smaller thin bands were reported in the SDS-PAGE images regarding the CSPC in the previous band (Ma et al., 2018). However, these bands were not observed in our study. The differences could be attributed to the deformation level of disulfide bonds in the proteins depending on extraction methods (Ma et al., 2018). Also, this phenomenon could be ascribed to low protein solubility or solution used to dissolve proteins in the analysis phase.

**FTIR analysis**

FTIR analysis is based on the vibrational movements of atoms between molecules. The vibrations forming with the modify of the dipole momentum of the molecules, seem like peaks in the spectrum. Therefore, the FTIR spectrum of a protein is formed as a result of vibrational modes originating from functional groups (peptide bonds, amino acid side chains) in the protein (Haris, 2013).

The absorption bands for CSPC are presented in Fig 1B. The characteristic structures including Amide A (3000-3500 $cm^{-1}$), Amide B (2850-2980 $cm^{-1}$) (Ebrahimi et al., 2016), Amide I (1700-1600 $cm^{-1}$), Amide II (1600-1500 $cm^{-1}$), and Amide III (1300-1200 $cm^{-1}$) (Kong and Yu, 2007) were exactly observed in the Fig 1B. Amide A and Amide B bands associated with symmetrical stretching of C–H and $NH_2$ groups appeared in the wavelengths of 3276 $cm^{-1}$ and 2985 $cm^{-1}$, respectively. The bands representing Amide I related to the C=O stretching vibration were observed in the wavelengths of 1649 $cm^{-1}$. The amide II band is important in protein structure analysis and arises from vibrations of the N—H bond at a wavelength of 1522 $cm^{-1}$. Both C=O and N-H bonds directly give an idea about the secondary structure of a protein (Cheng et al., 2017; Liu et al., 2018). Amide III was represented in 1212 $cm^{-1}$ and the spectrum in amide III is governed by the C–N and N–H stretch (Aewsiri et al., 2009; Yücetepe et al., 2021). The presence of these specific groups indicated that the protein extraction process was successful. Also, the confirmation was showed in the SDS-PAGE part (Fig 1A).

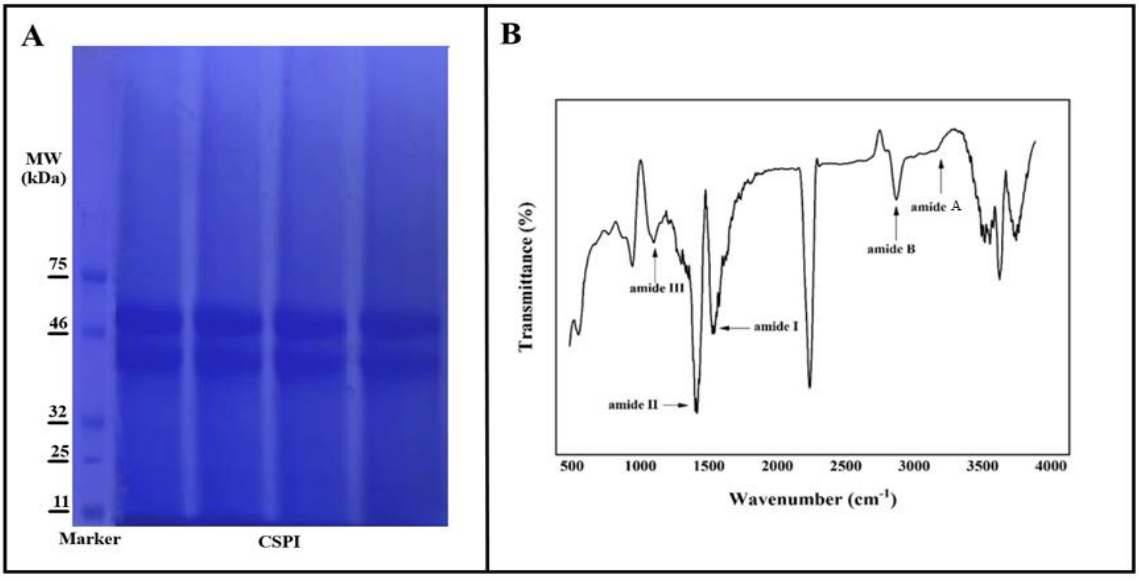


Figure 1. SDS-PAGE pattern (A) and FTIR spectrum (B) of cottonseed protein concentrate

**Morphological structure**

Surface morphology of CSPC was identified by SEM and the related appearance is given in Fig 2A**.** Protein sample possessed a relatively smooth surface. Moreover, the sample was in the form of hard sheet-like structure and these sheets were multi-layered forms. This phenomenon could be explained by the interaction of protein molecules because of long processing time in the freeze-drying method. (Özdemir et al., 2022). Similar images were observed in protein samples dried by freeze-drying system, *e.g.*, the sesame bran protein (Özdemir et al., 2022), sour cherry seed protein (Başyiğit et al., 2022a), peanut protein (Liu et al., 2019), moringa stenopetala seed protein (Kebede et al., 2019), soybean protein (Zhao et al., 2015), and rice dreg protein (Zhao et al., 2013).

**Thermal analysis**

Thermal analysis is important for the changes that occur with processes such as drying, heat exchange and cooking of foods (Batista et al., 2013). Generally, thermal processes are widely used in packaged food products. Therefore, it is desirable that the ingredients in related foods possess high temperature stability. In this context, TGA is conducted to put forth the dataset regarding the thermal behavior of materials. With this analysis method, the mass loss in the relevant sample depending on the temperature is revealed. TGA curve of CSPC is presented in Fig 2B. The thermal decomposition of proteins occurred in three main phases in the temperature range of 30–1000 °C. The first mass loss in the protein concentrate was approximately 6% at temperatures below 200 °C. This event might be ascribed to the loss of moisture (Başyiğit et al., 2020; Başyiğit et al., 2021). A major degradation was observed at phase two (200–600 °C). A mass loss of approximately 83% was reported in this phase. Thermal changes occurring in this range may be associated with the degradation or decomposition of polypeptide chains in the protein (Mshayisa et al., 2021). Similar curves for plant-based proteins were noted in previous studies (Yu et al., 2015; Timilsena et al., 2016; Başyiğit et al, 2022a; Başyiğit et al., 2022b). In conclusion, TGA analysis showed that CSPC possessed thermal stability up to 600 °C. Considering the temperatures used in food production systems in industry, this temperature level will provide significant advantages to proteins.

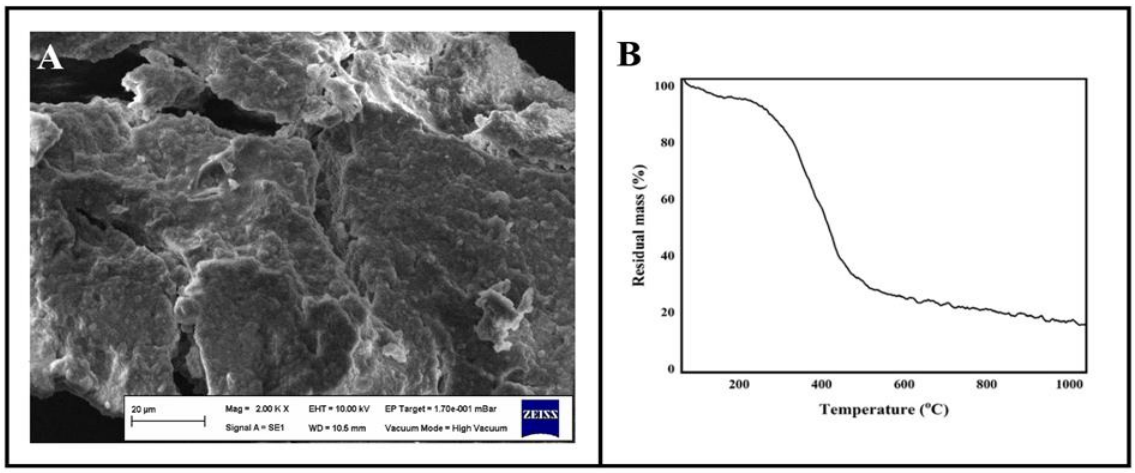


Figure 2**.** SEM image (A) and TGA curve (B) of cottonseed protein concentrate

**CONCLUSIONS**

In recent years, there has been an undeniable trend towards the use of plant proteins in different sectors. However, the resources in the market do not meet the demands sufficiently and it is predicted that this problem will increase in the coming years. Therefore, extracting proteins from suitable sources and placing them on the market are among issues of global importance. In this context, different perspectives to the literature

and industry were presented with the data obtained from this study. Moreover, this study has guided the evaluation of different food processing by-products as a source of protein. However, further studies are needed to clearly see the effectiveness of cottonseed proteins compared to their counterparts. Therefore, they could be used as an emulsifier and/or ingredient in the food formulations in future applications.

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**CONTRIBUTIONS OF ALL AUTHORS**

Melike Yücetepe: Formal analysis, Data curation, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. Merve Akalan: Formal analysis, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing. Kamile Bayrak Akay: Investigation, Writing – original draft, Writing – review & editing. Mehmet Şükrü Karakuş: Investigation, Software, Writing – original draft, Writing – review & editing. Asliye Karaaslan: Methodology, Visualization, Writing – original draft. Bülent Başyiğit: Formal analysis, Investigation, Software, Writing – original draft, Writing – review & editing. Mehmet Karaaslan: Conceptualization, Data curation, Funding acquisition, Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing.

**CONFLICTS OF INTEREST**

The authors have declared no conflicts of interest for this article.

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