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ORIGINAL ARTICLE

Physicochemical and functional properties of safflower protein isolate: Effect of drying methods and spray drying temperatures

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Abstract

In this study, the effects of the drying method and spray drying inlet air temperature on the color, surface morphology, hydrophobicity, powder and functional properties of safflower protein isolate were investigated. Safflower protein was extracted using the alkaline extraction and isoelectric precipitation method and then dried using freeze-drying and spray-drying at 140, 150, 160, 170, and 180 °C inlet air temperatures. When compared to the spray-dried samples, freeze-dried safflower protein isolate had lower cohesiveness (1.18) and better flowability (15.12%) with higher bulk density (0.31 g/cm^3) and lower tapped bulk density (0.37 g/cm^3). Additionally, its wetting time (9.00 s) was also found to be lower. On the other hand, spray-dried samples exhibited lighter color, higher foaming capacity (30.00–38.75%) and stability (87.50–89.17%), and emulsion activity (51.25–54.50%) and stability (85.76–87.51%) than the freeze-dried sample. Moreover, it was found that the foaming capacity and emulsion activity of safflower protein spray-dried at 140 °C were higher than those of other spray-dried samples. Overall, freeze-dried safflower protein showed better powder properties, while spray-dried samples had better functional properties. Consequently, it was shown that the choice of drying method and spray drying inlet air temperature were critical for the powder and functional properties of safflower protein.

Keywords Scanning electron microscopy · Surface hydrophobicity · Protein solubility · Foaming and emulsion properties · Least gelatinization concentration

Abbreviations

SPI	Safflower protein isolate
FD	Freeze-dried safflower protein isolate
SD-140 °C	Spray-dried at 140 °C inlet air temperature safflower protein isolate
SD-150 °C	Spray-dried at 150 °C inlet air temperature safflower protein isolate
SD-160 °C	Spray-dried at 160 °C inlet air temperature safflower protein isolate
SD-170 °C	Spray-dried at 170 °C inlet air temperature safflower protein isolate
SD-180 °C	Spray-dried at 180 °C inlet air temperature safflower protein isolate
SEM	Scanning electron microscopy
BPB	Bromophenol blue
PS	Protein solubility
WHC	Water holding capacity
OBC	Oil binding capacity
FC	Foaming capacity
FS	Foam stability
EA	Emulsion activity
ES	Emulsion stability
LGC	Least gelation concentration

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1 Introduction

Interest in proteins for human nutrition has grown as awareness of their nutritional and functional benefits increases. Plant proteins are more sustainable and cost-effective than animal-based proteins due to their abundance and lower resource requirements, including water, land, and fossil energy [1, 2]. Soy and pea proteins are the most widely used plant-based options [3]. Additional protein sources are thoroughly investigated to meet future needs for affordable and

high-quality plant protein ingredients [2]. Accordingly, agricultural industrial wastes and by-products have the potential to be used as protein sources [4]. Safflower (*Carthamus tinctorius* L.) is one of the oldest cultivated oilseed crops and has high oil yield and some agricultural advantages, such as tolerance to heat, salinity, and drought [5]. The high-protein safflower meal remaining after oil extraction from the seed has the potential as a raw material for deriving protein concentrates and isolates [6]. Additionally, safflower protein has a high essential amino acid content (41.55%) and shows good functional properties [7].

The effective incorporation of plant proteins into food processing relies on their functional characteristics, which are strongly affected by the extraction and drying methods employed [8, 9]. Several studies have been conducted on the effect of different extraction methods on the functionality of safflower proteins [10, 11]. Various methods are used in the production of protein concentrates and isolates from plants, but alkaline extraction-isoelectric precipitation method is the most commonly used due to high protein purity and application simplicity [12]. Also, drying methods and conditions have critical importance in determining protein functionality [2]. Freeze-drying and spray-drying are the most used methods for protein powder production [4]. Freeze-drying, the primary technique used in laboratory research for protein isolates, minimizes degradation and microbiological reactions, but it is both expensive and time-consuming [1, 4, 13]. Spray-drying, one of the most popular processes used in the food industry, saves time and has the ability to change particle properties [2, 14]. Moreover, spray-drying conditions are important as they affect the functionality of proteins by causing hydrophobic interactions, protein denaturation, and aggregation [15]. It has been reported that spray drying inlet air temperature significantly affects the powder properties of seed-watermelon seed protein and the functional properties of pea protein [15, 16]. However, to the best of our knowledge, there is no study on the effect of either drying methods or spray drying inlet air temperature on the physicochemical and functional properties of safflower proteins. Therefore, the objective of this study is to investigate the effect of drying methods and spray drying inlet air temperature on the color, surface morphology, hydrophobicity, powder and functional properties of safflower proteins.

2 Materials and methods

2.1 Material

Safflower seed (variety of Askon) meal was provided from a safflower oil-producing factory (Ripsa-Özşahin Tarımcılık) in Kayseri/Turkey. The safflower seed meal was ground to pass through a 1 mm sieve and then kept at 4 °C until protein extraction.

2.2 Preparation of safflower protein isolates (SPI)

2.2.1 Protein extraction

Alkaline extraction method with isoelectric precipitation was used for protein extraction from safflower seed meal. The optimum conditions for extraction of SPI were determined in our previous research [7]. For the defatting of safflower seed meal, it was mixed with hexane at a ratio of 1:3 (w/v) at room temperature for 1 h using an orbital shaker (Jeio Tech, Open-air shaker OS- 4000, Korea) at 200 rpm. After this was repeated 3 times, the solid was dried at room temperature overnight.

The defatted safflower seed meal was dispersed in distilled water at a ratio of 1:33 g/mL, and the pH of the dispersion was adjusted to 11.0 using 2 M NaOH. The dispersion was stirred at 23.3 °C for 31 min using a water bath (Nüve, ST 30, Turkey) at 125 rpm. At the end of the period, the dispersion was centrifuged (Nüve, NF 800, Turkey) at 9000 rpm for 5 min, filtrated through filter paper, and the supernatant was collected.

To precipitate the safflower protein, the pH of the supernatant was adjusted to 5.0 with 2 M HCl and centrifuged at 9000 rpm for 5 min. The precipitated safflower protein was re-suspended with deionized water, maintaining about 10% (w/v) total solid content, and the pH of the protein solution was adjusted to 7.0 using 2 M NaOH.

2.2.2 Drying

For freeze-drying of SPI, the protein solution was placed in petri dishes and frozen at − 18 °C for 12 h. Then, the sample was dried at − 90.6 °C for 48 h using a lyophilizer (Teknosem, TRST 4/4 DS, Turkey) at 0.0033 mbar vacuum pressure.

For spray-drying of SPI, a pilot-scale spray-dryer (Unopex, B15, Turkey) was used. SPI was spray-dried at five different inlet air temperatures: 140, 150, 160, 170, and 180 °C (with outlet temperatures of 67, 74, 82, 89, and 97 °C, respectively). The feed flow rate (16 mL/min), aspiration rate (52 m^3/h), compressed air flow rate (9 L/min), and compressed air pressure (0.8–1 MPa) were kept constant in all spray-drying processes.

Freeze-dried (FD) and spray-dried at different inlet air temperatures (SD-140 °C, SD-150 °C SD-160 °C, SD-170 °C, and SD-180 °C) SPI were stored in an amber package at − 18 °C until analyses.

2.3 Color characteristics

The color characteristics (L^*, a^*, b^*) of SPI were determined using a chromameter (CR- 400, Minolta Company, Japan).

2.4 Scanning electron microscopy (SEM)

After SPI was coated with a thin gold layer, the surface morphology was imaged using SEM (JEOL, JCM- 5000 NeoScope, Japan). During micrography, the accelerating potential was used as 10 kV [17].

2.5 Powder properties

2.5.1 Bulk density and tapped bulk density

The volume of 2 g SPI was measured in a graduated cylinder (10 mL) to determine bulk density. Then, the graduated cylinder was tapped on the smooth surface 100 times, and the volume of the sample was measured to determine the tapped bulk density. The results were determined by calculating the weight-to-volume ratio of the samples and were expressed in "g/cm^3" [18].

2.5.2 Hausner ratio and Carr index

Using the bulk and tapped bulk densities of the sample, Hausner ratio and Carr index values were calculated according to Eq. 1 and Eq. 2, respectively [4, 18].

$$\text{Hausner Ratio} = \frac{\rho_{tapped\ bulk\ density}}{\rho_{bulk\ density}} \quad (1)$$

$$\text{Carr Index} = \frac{\rho_{tapped\ bulk\ density} - \rho_{bulk\ density}}{\rho_{tapped\ bulk\ density}} \times 100 \quad (2)$$

2.5.3 Wettability

SPI (0.1 g) was dropped into 100 mL of distilled water in a 250 mL beaker from a height of 5 cm above the water surface. The time required for the powder to become completely wet was measured in seconds [4].

2.6 Surface hydrophobicity

The surface hydrophobicity of SPI was determined according to Tontul et al. [12]. Protein dispersion (5 mg/mL) was prepared in 20 mM phosphate buffer (pH 7.0). The protein dispersion (3 mL) was mixed with 1 mg/mL bromophenol blue (BPB) solution (600 µL) and kept in the dark for 15 min. Then, the mixture was centrifuged at 2000xg for 15 min, and the supernatant was diluted 1/10 with 20 mM phosphate buffer (pH 7.0). The control sample was prepared by mixing with 3 mL phosphate buffer and 600 µL BPB solution (1 mg/mL). The absorbance measurement was conducted using a spectrophotometer (Shimadzu, UV–VIS Spectrophotometer, UV- 1280, Japan) at 595 nm. The hydrophobicity of the SPI was determined based on the amount of BPB bound per 1 mL of protein solution, calculated using the following equation (Eq. 3).

$$\text{Bound BPB } (\mu g) = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}} \times (200\mu g) \quad (3)$$

2.7 Functional properties

2.7.1 Protein solubility (PS)

The method of Korkmaz [7] was used to determine the pH-dependent PS profile of SPI. Briefly, 200 mg SPI was mixed in 20 mL in distilled water, and the pH value was adjusted to a range of 2–12 using either 2 M HCl or NaOH. The protein dispersion was stirred at 200 rpm for 30 min and then centrifuged at 7500 rpm for 15 min. Lowry method [19] was used to measure the protein content of supernatant, and AACC standard method [20] was performed to determine the total protein content of SPI (96.08%, dry basis) (Nx6.25). The PS values were calculated as the percentage of the protein content of supernatant to the protein content of SPI.

2.7.2 Water holding capacity (WHC) and oil binding capacity (OBC)

WHC and OBC analyses were performed according to Mutlu and Korkmaz [17]. SPI (0.25 g) and either 5 mL distilled water or sunflower oil were vortexed for 5 min in a pre-weighed centrifuge tube. After the protein dispersion was centrifuged at 5000xg for 10 min, supernatants were discarded. The weight of residues and centrifuge tube was weighed, and the WHC and OBC of SPI were calculated according to Eq. 4.

$$\text{WHC or OBC (g/g)} = \frac{final\ weight\ of\ residue\ and\ centrifuge\ tube - (weight\ of\ sample + weight\ of\ centrifuge\ tube)}{weight\ of\ sample} \quad (4)$$

2.7.3 Foaming capacity (FC) and foam stability (FS)

The method specified by Kasapoğlu et al. [21] was used to determine the FC and FS of SPI. The protein dispersion prepared with 0.2 g SPI and 20 mL distilled water was whipped at 12,000 rpm for 2 min with a homogenizer (Witeg HG-15D, Germany). The total and foam volumes of the sample were recorded before and after homogenization and after standing for 30 min at room temperature. The FC and FS were calculated according to Eq. 5 and Eq. 6, respectively.

$$\text{FC}\ (\%) = \frac{\textit{Volume after whipping}\ (mL) - \textit{Volume before whipping}\ (mL)}{\textit{Volume before whipping}\ (mL)} \times 100 \quad (5)$$

$$\text{FS}\ (\%) = \frac{\textit{Volume of foam after}\ 30\ \textit{min}\ (mL)}{\textit{Initial foam volume}\ (mL)} \times 100 \quad (6)$$

2.7.4 Emulsion activity (EA) and emulsion stability (ES)

EA and ES were measured based on the method of Özdemir et al. [4]. First, 1 g of SPI, 10 mL of distilled water, and 10 mL of sunflower oil were whipped at 11,000 rpm for 30 s with a homogenizer (Witeg HG- 15D, Germany). Then, the mixture was centrifuged at 1200xg for 5 min, and the volume of the emulsion layer was recorded. The EA was obtained with the following equation (Eq. 7)

$$\text{EA}\ (\%) = \frac{\textit{Volume of emulsion layer}\ (mL)}{\textit{Total volume}\ (mL)} \times 100 \quad (7)$$

After incubation in a water bath at 80 °C for 30 min, the mixture was centrifuged at 1200xg for 5 min. The volume of the remaining emulsion layer was recorded, and the following equation (Eq. 8) was used to calculate the ES.

$$\text{ES}\ (\%) = \frac{\textit{Volume of remaining emulsion layer}\ (mL)}{\textit{Volume of the original emulsified layer}\ (mL)} \times 100 \quad (8)$$

2.7.5 Least gelation concentration (LGC)

The LGC of SPI was determined by employing the method of Korkmaz [7]. Protein dispersion was prepared at concentrations between 2- 20% (w/v) in two-unit intervals. After incubation in boiling water for 1 h, the sample was incubated at 4 °C for 2 h. When the test tubes were inverted, the lowest concentration at which no fall or slide was recorded as LGC.

2.8 Statistical analysis

All analyses were performed in triplicate, and the results were calculated on a dry basis. The data were evaluated by one-way ANOVA using MINITAB Statistical Software (ver. 17.0, USA), and the Tukey test was used to determine the differences among the groups. The results were expressed as mean ± standard error.

3 Results and discussions

3.1 Color properties and surface morphology

The color characteristics of SPI samples are shown in Table 1. It was found that both the drying method and spray drying inlet air temperature significantly affected the color characteristics of SPI samples ($p < 0.05$). FD showed the lowest L^* and the highest a^* value among all SPI samples ($p < 0.05$). As seen in Fig. 1, FD had a brownish color, while SD samples had a creamy white color. These results were

Table 1 Color and powder properties of safflower protein isolates

	Color properties			Powder properties				
	L^*	a^*	b^*	Bulk density* (g/cm^3)	Tapped density* (g/cm^3)	Hausner ratio*	Carr index (%)*	Wettability (s)*
FD	36.93 ± 0.54^{c}	6.41 ± 0.08^{a}	22.57 ± 0.37^{a}	0.31 ± 0.01^{a}	0.37 ± 0.01^{b}	1.18 ± 0.01^{c}	15.12 ± 0.46^{c}	9.00 ± 1.00^{e}
SD-140 °C	69.35 ± 0.12^{a}	2.03 ± 0.01^{c}	21.42 ± 0.11^{c}	0.25 ± 0.01^{b}	0.44 ± 0.01^{a}	1.76 ± 0.02^{a}	43.13 ± 0.63^{a}	121.50 ± 2.50^{a}
SD-150 °C	69.08 ± 0.27^{a}	2.04 ± 0.01^{c}	22.27 ± 0.10^{ab}	0.27 ± 0.01^{b}	0.45 ± 0.01^{a}	1.67 ± 0.02^{b}	40.13 ± 0.66^{ab}	115.00 ± 2.00^{ab}
SD-160 °C	68.95 ± 0.42^{a}	2.01 ± 0.01^{c}	21.52 ± 0.13^{c}	0.27 ± 0.01^{b}	0.45 ± 0.01^{a}	1.68 ± 0.01^{ab}	40.51 ± 0.51^{ab}	106.00 ± 1.00^{bc}
SD-170 °C	66.08 ± 0.42^{b}	2.46 ± 0.02^{b}	22.75 ± 0.10^{a}	0.26 ± 0.01^{b}	0.45 ± 0.02^{a}	1.68 ± 0.01^{ab}	40.51 ± 0.51^{ab}	97.00 ± 3.00^{cd}
SD- 180 °C	66.74 ± 0.24^{b}	2.49 ± 0.06^{b}	23.17 ± 0.20^{a}	0.26 ± 0.01^{b}	0.42 ± 0.01^{a}	1.65 ± 0.01^{b}	39.51 ± 0.49^{b}	89.50 ± 2.50^{d}

*Means followed by different letters in each column are significantly different ($p < 0.05$)

FD, freeze-dried safflower protein isolate; SD-140 °C, spray-dried at 140 °C inlet air temperature safflower protein isolate; SD-150 °C, spray-dried at 150 °C inlet air temperature safflower protein isolate; SD-160 °C, spray-dried at 160 °C inlet air temperature safflower protein isolate; SD-170 °C, spray-dried at 170 °C inlet air temperature safflower protein isolate; SD-180 °C, spray-dried at 180 °C inlet air temperature safflower protein isolate

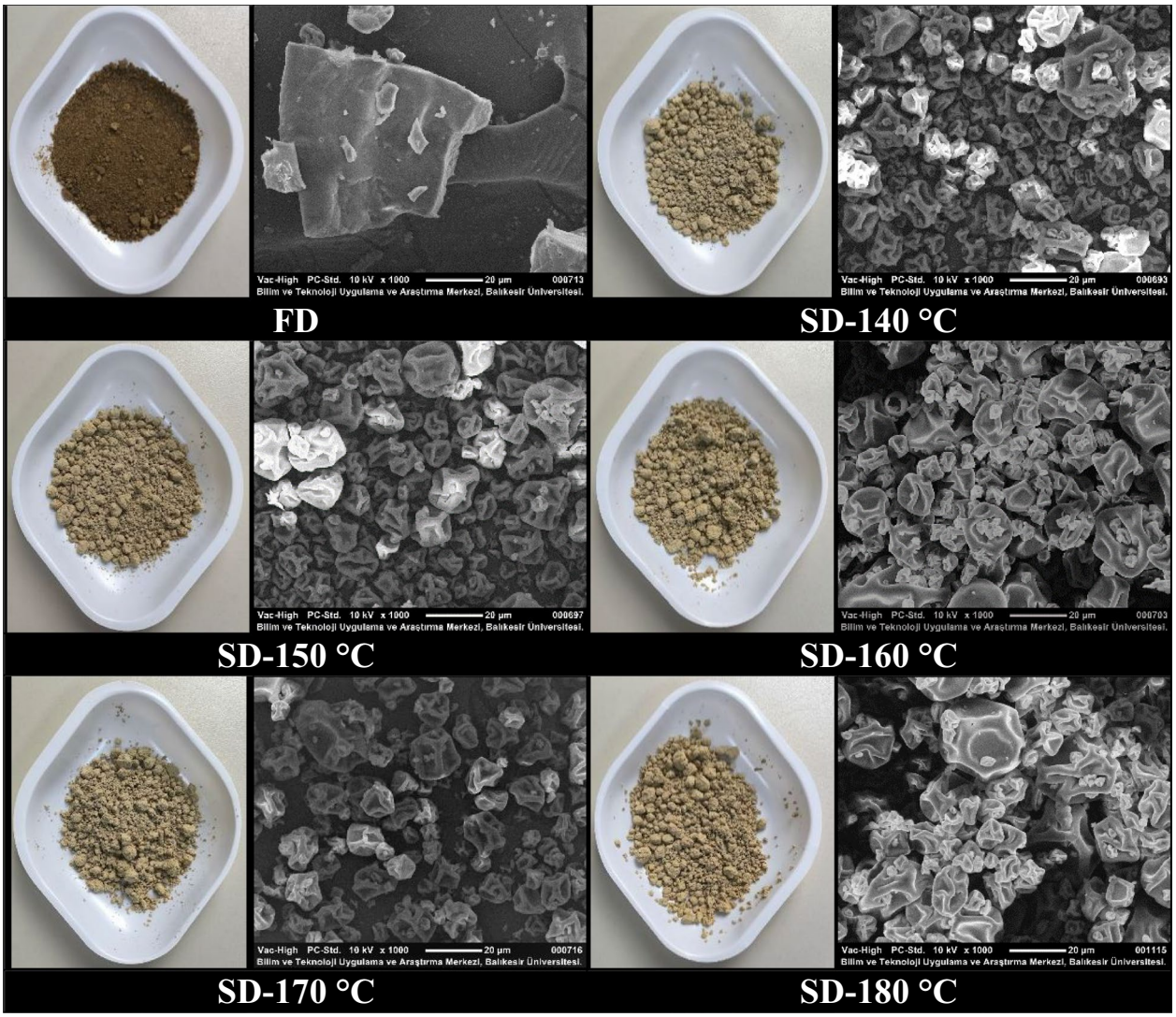

Fig. 1 Powder and scanning electron microscope (SEM) images belonging to the safflower protein isolates. FD, freeze-dried safflower protein isolate; SD-140 °C, spray-dried at 140 °C inlet air temperature safflower protein isolate; SD-150 °C, spray-dried at 150 °C inlet air temperature safflower protein isolate; SD-160 °C, spray-dried at 160 °C inlet air temperature safflower protein isolate; SD-170 °C, spray-dried at 170 °C inlet air temperature safflower protein isolate; SD-180 °C, spray-dried at 180 °C inlet air temperature safflower protein isolate

consistent with previous studies on hempseed [22], quinoa [2], sesame [4], camelina [17], and sour cherry seed [8] proteins. The processing time of freeze drying is long, and cold burn may occur due to the samples being exposed to low temperatures for a long time, which may cause a dark color in the sample [4]. On the other hand, removing heat-sensitive pigments during spray drying may enhance brightness and result in a lighter color [22]. It has also been noted that samples with small particle size and large surface area may appear lighter in color due to greater light refraction [23]. The b^* value of FD displayed slightly higher than that of SD samples dried at lower inlet air temperatures (140–160 °C); however, it was similar to samples dried at higher inlet air temperatures (170 and 180 °C). On the other hand, compared to samples dried at lower inlet air temperatures, the L^* value of samples dried at higher inlet air temperatures was lower, while a^* and b^* values were higher ($p < 0.05$). It was stated that a non-enzymatic browning reaction may occur during drying at high temperatures, resulting in color changes [16].

The SEM micrograph of SPI samples is represented in Fig. 1. FD had inhomogeneous particle distribution and had some large particles. Particles of FD exhibited a crumbled multilayer sheet-like structure with loose porous surface morphology. Due to the lack of force to form droplets during the sublimation process in freeze drying, aggregation may occur after a long processing time at low temperatures

[14, 17]. This may be the reason for the large particles and sheet-like structure. On the other hand, SD samples had more uniform particle distributions when compared to FD. The particles of SD samples had a spherical-shaped structure with some collapses and a smooth surface structure. Some wrinkled particles were also found in SD samples. It was stated that the collapses in structure may be related to the irregular shrinkage of particles [24]. Moisture diffusion occurs very slowly in aqueous protein solutions. The concentration gradient between the particle and the drying environment is high during spray-drying. For easier moisture evaporation, the diffusion path for water vapor is attempted to be minimized, which may cause wrinkling of the particle surface [14].

3.2 Powder properties

The powder properties of SPI are shown in Table 1. Bulk density and tapped bulk densities are important parameters that determine the processing, packaging, and storing requirements of a product [18]. FD had the highest bulk density, while it also had the lowest tapped bulk density among the SPI samples ($p < 0.05$). Similarly, it was also found that freeze-dried camelina protein powder exhibited lower bulk density and higher tapped bulk density when compared to the spray-dried sample [17]. Lower tapped bulk density indicated that the higher area required to store FD [8]. The bulk and tapped bulk densities of SD samples ranged between 0.25–0.27 g/m^3 and 0.42–0.45 g/m^3, respectively ($p > 0.05$). The bulk density of protein isolate can be affected by particle size and shape, size and number of connection points each other, attractive forces between particles, and moisture content of samples [17, 25].

Hausner ratio and Carr index parameters are used to interpret the cohesiveness and flowability of powders, respectively [8, 18]. The lowest Hausner ratio and Carr index values were found in FD as 1.18 and 15.12%, respectively, among all SPI samples. These parameters indicated that FD exhibited low cohesiveness and good flowability. On the other hand, the Hausner ratio and Carr index parameters were in a range of 1.65–1.76 and 39.51–43.13, respectively, indicating that the SD samples had high cohesiveness and bad flowability. The cohesiveness and flowability of protein powders may be affected by surface characteristics and particle size and shape [26]. It was stated that cohesiveness may tend to increase, and flowability may tend to decrease due to the increase in the contact surface between particles as the particle size decreases [18, 26].

The wettability property provides information on the interaction between protein powder and water [18]. The wettability value of FD (9.00 s) was substantially lower than that of SD samples (89.50–121.50 s) ($p < 0.05$). Başyiğit et al. [8] found that drying methods had a significant effect on the wettability value of proteins, and freeze-dried sour cherry seed protein had a lower wettability than the spray-dried sample. Similarly, a lower wettability value was determined in freeze-dried sesame protein when compared to that of spray-dried [4]. The authors attributed this to differences in surface properties [8] and particle size [4]. Moreover, the wettability of the SD samples decreased with the increase in inlet air temperature ($p < 0.05$). Malik et al. [27] reported that the wettability of protein powder is influenced by particle size, surface charge and characteristics (area, density and porosity), amphipathic compounds, and the contact angle between the powder surface and water. It was stated that proteins wet more quickly when surface hydrophobicity decreases [27]. This was compatible with the surface hydrophobicity values of SPI samples in Fig. 2.

3.3 Surface hydrophobicity

Protein hydrophobicity, which is an important property, is related to the hydrophobic groups located on the protein surface [9]. Protein denaturation and aggregation are two

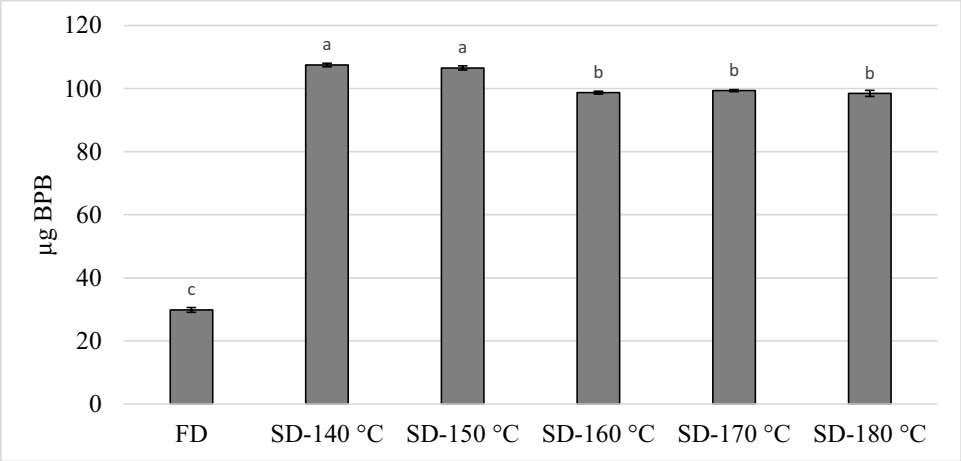

Fig. 2 Surface hydrophobicity of safflower protein isolates. FD, freeze-dried safflower protein isolate; SD-140 °C, spray-dried at 140 °C inlet air temperature safflower protein isolate; SD-150 °C, spray-dried at 150 °C inlet air temperature safflower protein isolate; SD-160 °C, spray-dried at 160 °C inlet air temperature safflower protein isolate; SD-170 °C, spray-dried at 170 °C inlet air temperature safflower protein isolate; SD-180 °C, spray-dried at 180 °C inlet air temperature safflower protein isolate

main factors influencing surface hydrophobicity [17]. Surface hydrophobicity could determine the interactions of proteins with other proteins and lipids and thus affect functional properties such as solubility, foaming, and emulsifying properties [2]. The surface hydrophobicity of SPI samples is represented in Fig. 2. It was observed that the drying method and spray drying inlet air temperatures had significant effects on the surface hydrophobicity of SPI ($p < 0.05$). FD exhibited the lowest surface hydrophobic in all SPI samples ($p < 0.05$). Mutlu and Korkmaz [17] also reported that freeze-dried camelina protein powder showed lower surface hydrophobicity than the spray-dried sample. The authors noted that this may be due to less protein denaturation and unfolding occurring with freeze-drying [17]. On the other hand, the surface hydrophobicity of SD samples tended to decrease with increasing inlet air temperature ($p < 0.05$). The reason may be associated with protein aggregation induced by higher temperatures [14, 17]. Protein–protein interactions may cause a reduction of the exposed hydrophobic group, and thus a decrease in surface hydrophobicity [17].

3.4 Functional properties

PS is one of the most important parameters of proteins due to its effect on foaming, emulsifying, and gelling properties [7]. Moreover, it can affect the texture, color, and flavor characteristics of the food [1]. The PS of SPI samples exhibited similar U-shaped curves, and the pH-dependent protein solubility of SPI samples is shown in Fig. 3. In SPI samples, the highest PS values (86.48–99.40%) were found in extremely acidic and alkaline (pH 2 and 12), while the lowest PS values (6.11–11.77%) were observed in pH 4. Korkmaz reported that the isoelectric point of SPI was reported as pH 5 [7]. At pH values near the isoelectric point, positive and negative charges are in balance. It reduces the electrostatic repulsion between protein molecules and causes aggregation and precipitation, thus decreasing the PS of protein [1]. When compared to FD, the PS of SD samples was lower at pH 4–6, while it was higher at pH 7–10 ($p < 0.05$). It was shown that the interactions between SD samples and water were stronger in neutral and alkaline environments, which led to higher PS. Spray-dried soy protein isolate and rice pulp protein were found to have higher solubility than freeze-dried proteins when pH was above 5 [28, 29]. The higher solubility of spray-dried proteins was attributed to less surface tension and smaller particle size [28, 29]. Moreover, it was reported that differences in solubility may be related to hydrophobic residues, protein surface charges, and ionic hydration and electrostatic repulsion at different pH values [14]. On the other hand, the effect of inlet air temperature on PS at pH 2, 10, and 12 was insignificant for SD samples ($p > 0.05$). The highest PS at pH 7 was observed in SD-160 °C (47.99%) among SPI samples ($p < 0.05$). Cao et al. [16] dried seed-watermelon seed hydrolyzed protein in a spray-dryer at different inlet air temperatures (150–180 °C). The authors found that the sample spray-dried at 160 °C had the highest solubility. Another study found the spray drying inlet air temperature (165–195 °C) had a slight effect on the solubility (52.2–56.5%) of pea protein isolate [15]. It was reported that this may be related to the amount of charged amino acids, which is affected by temperature, conformation, and ionic strength [4].

WHC and OBC are two key parameters of the functional properties of proteins and indicate the amount of water or oil that the protein can hold per unit weight [13]. The WHC of protein affects the textural and sensorial properties of food products, while OBC has an impact on the emulsifying properties of proteins and the flavor and shelf life of food

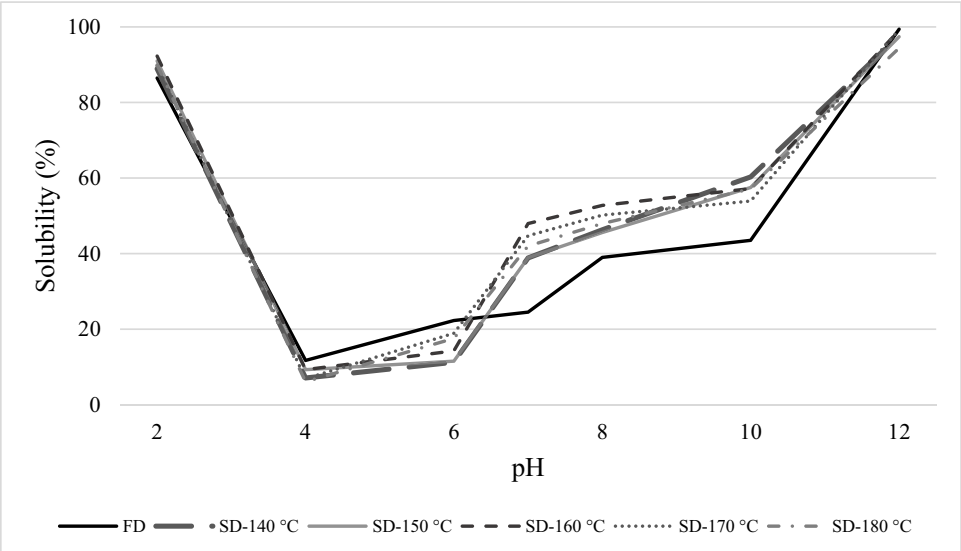

Fig. 3 Solubility of safflower protein isolates at different pH values. FD, freeze-dried safflower protein isolate; SD-140 °C, spray-dried at 140 °C inlet air temperature safflower protein isolate; SD-150 °C, spray-dried at 150 °C inlet air temperature safflower protein isolate; SD-160 °C, spray-dried at 160 °C inlet air temperature safflower protein isolate; SD-170 °C, spray-dried at 170 °C inlet air temperature safflower protein isolate; SD-180 °C, spray-dried at 180 °C inlet air temperature safflower protein isolate

products [1]. The WHC of FD was significantly higher than that of SD samples ($p < 0.05$) (Table 2). Zhao et al. [29] and Liu et al. [30] reported similar findings for rice dreg and peanut proteins, respectively. The authors attributed the higher WHC to the amorphous and porous morphology of freeze-dried proteins [30]. Moreover, it was stated that this may be due to less loss of soluble proteins and less protein denaturation by freeze-drying [14]. The WHC of SD samples showed a linear increasing trend with increasing inlet air temperatures ($p < 0.05$). Vinayashree and Vasu [31] indicated that the WHC value of proteins depends on their amino acid composition, conformation, ionic strength, hydrophobicity, pH, and temperature. WHC is related to the accessibility of hydrophilic groups to bind with water [32]. Therefore, increasing binding sites with changes in protein conformation at high temperatures could lead to better WHC [2]. Additionally, decreasing surface hydrophobicity with increasing inlet air temperature may contribute to the increase in WHC. The effect of drying method on the OBC of SPI was found to be insignificant ($p > 0.05$). However, FD had a slightly higher OBC value than SD samples. It was noted that the reason for this may be that the porous structure of the FD facilitates oil absorption by altering interactions between protein and lipid [4, 9]. The OBC of SPI samples ranged from 2.46 to 2.54 g/g ($p > 0.05$). The similar surface properties of the SD samples may be the reason that the difference between the OBC of the samples was insignificant.

The foaming properties, including FC and FS, indicate the ability of the protein to create and hold the air bubble [2]. The effect of drying method on the foaming properties of SPI was significant ($p < 0.05$) (Table 2). FD exhibited the lowest FC and FS values among SPI samples. Consistent with our result, Başyiğit et al. [8] and Mutlu and Korkmaz [17] found that freeze-dried sour cherry and camelina seed proteins had lower FC and FS values compared to spray-dried ones. Also, Zhao et al. [29] demonstrated that freeze-dried rice dreg protein possessed lower FC than spray-dried. According to the authors, weaker foaming properties may be related to lower surface hydrophobicity and lower solubility (at pH 7) of freeze-dried proteins [17, 29]. Moreover, the highest FC was displayed by SD-140 °C among SD samples ($p < 0.05$), which may be attributed to its higher surface hydrophobicity. Similarly, soy protein spray-dried at 130 °C was found to have higher FC than samples dried at 170 and 200 °C [9]. The effect of spray drying inlet air temperature on FS of SD samples was insignificant, and it ranged between 87.50–89.58% ($p > 0.05$). The stable foam structure of SD samples may be assigned to the strength of the multilayer interfacial film formed as a result of a large protein–protein interaction at the interface [3].

EA, which is the ability of proteins to emulsion form, and ES, which shows the stabilization of the formed emulsion, are critical for the role of protein as food ingredients [33]. The EA of SPI ranged between 46.00–54.50%, and FD had significantly lower EA compared to proteins from spray-drying ($p < 0.05$) (Table 2). Similar to EA, the ES of FD was significantly lower than that of SD samples ($p < 0.05$). Many studies reported that freeze-dried proteins, including soy [28], mung bean [9], peanut [30], sesame [4], camelina [17] and sour cherry seed [8], showed weaker emulsifying properties than spray-dried proteins. The authors attributed this finding to lower solubility and surface hydrophobicity of freeze-dried proteins [2, 8, 14, 17, 28, 30]. Moreover, it was stated that the bigger particle size of freeze-dried proteins may be another reason for weaker emulsifying properties [2, 17, 29]. When comparing SD samples, SD-180 °C exhibited slightly lower ES, which might be related to its lower surface hydrophobicity. Lower surface hydrophobicity may result in weaker binding between the protein and the oil droplet, leading to weaker emulsifying properties [2, 34]. The ES of SD samples ranged between 85.76–87.51% and there was no

Table 2 Functional properties of safflower protein isolates

	Water holding capacity* (g/g)	Oil binding capacity* (g/g)	Foaming capacity* (%)	Foaming stability* (%)	Emulsion activity* (%)	Emulsion stability* (%)	Least gelation concentration (%)
FD	2.03 ± 0.02^{a}	2.91 ± 0.01	20.00 ± 0.01^{b}	66.67 ± 0.01^{b}	46.00 ± 0.50^{b}	77.18 ± 0.84^{b}	12.00 ± 0.01
SD-140 °C	1.11 ± 0.03^{c}	2.46 ± 0.21	38.75 ± 1.25^{a}	88.33 ± 5.00^{a}	54.50 ± 0.50^{a}	87.15 ± 1.04^{a}	12.00 ± 0.01
SD-150 °C	1.18 ± 0.02^{c}	2.49 ± 0.12	32.50 ± 2.50^{ab}	89.17 ± 2.50^{a}	52.75 ± 1.25^{a}	86.70 ± 1.26^{a}	12.00 ± 0.01
SD-160 °C	1.41 ± 0.01^{b}	2.54 ± 0.09	35.00 ± 5.00^{ab}	89.58 ± 2.08^{a}	52.00 ± 0.50^{a}	87.51 ± 0.84^{a}	12.00 ± 0.01
SD-170 °C	1.49 ± 0.09^{b}	2.49 ± 0.05	35.00 ± 5.00^{ab}	87.50 ± 4.17^{a}	52.50 ± 1.50^{a}	85.76 ± 1.50^{a}	12.00 ± 0.01
SD-180 °C	1.65 ± 0.04^{b}	2.48 ± 0.01	30.00 ± 0.01^{ab}	87.50 ± 4.17^{a}	51.25 ± 1.25^{ab}	85.83 ± 0.83^{a}	12.00 ± 0.01

*Means followed by different letters in each column are significantly different ($p < 0.05$)

FD, freeze-dried safflower protein isolate; SD-140 °C, spray-dried at 140 °C inlet air temperature safflower protein isolate; SD-150 °C, spray-dried at 150 °C inlet air temperature safflower protein isolate; SD-160 °C, spray-dried at 160 °C inlet air temperature safflower protein isolate; SD-170 °C, spray-dried at 170 °C inlet air temperature safflower protein isolate; SD-180 °C, spray-dried at 180 °C inlet air temperature safflower protein isolate

significant difference ($p > 0.05$). Similarly, Burger et al. [15] found that the effect of spray drying inlet air temperature (165–195 °C) was insignificant for the ES of pea protein isolates.

LGC of protein indicates heat-induced gelation ability, and it is a very important property to produce gel-like or emulsion-type foods. When a protein dispersion at a critical concentration is heated, the molecular structure unfolds, followed by aggregation and gelation, respectively [21]. From Table 2, it can be seen that the LGC of all SPI samples is 12%. These results showed that the LGC of SPI was not affected by the drying method and spray drying inlet air temperatures. Freeze- and spray-dried proteins, including lentil [24] and mung bean [9] proteins, were also reported to have similar LGC values. It was stated that many factors can affect LGC, such as material genotype, protein extraction conditions, and purity of the protein extract [24, 33], which were similar for SPI samples in the present study.

4 Conclusion

In this study, SPI powders were produced using two different drying methods, including freeze-drying and spray-drying (at inlet air temperatures ranging between 140–180 °C), and color, surface morphology, hydrophobicity, and powder and functional properties were compared. FD had a brownish color and crumbled multilayer sheet-like structure, while SD samples had a creamy white color and spherical-shaped structure with some collapses. Compared with SD samples, FD showed lower cohesiveness and better flowability with higher bulk density and lower tapped bulk density and also had a lower wetting time. Although FD showed better powder properties, its functional properties were poorer than SD samples because it exhibited lower FC, FS, EA, and ES. On the other hand, there was no significant effect of spray-drying inlet air temperatures on the bulk and tapped bulk density of SD samples. However, cohesiveness tended to increase, and flowability and wettability tended to decrease with increasing inlet air temperature. SD-140 °C displayed higher FC and EA values than other SD samples due to its higher surface hydrophobicity. However, it was found that the stability of foaming and emulsion of SD samples were not significantly different. In conclusion, this study showed that the drying method and spray-drying inlet air temperatures affected the color, surface morphology, hydrophobicity, powder and functional properties of SPI. Thus, it is important to select optimum drying methods and spray drying inlet air temperatures for SPI to provide the powder and functional properties needed for different food applications.

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Author contribution Fatma KORKMAZ: Methodology, Validation, Formal analysis, Investigation, Writing–original draft, Project administration.

Necati Barış TUNCEL: Methodology, validation.

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Data availability Datasets are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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