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Physicochemical, Functional and Rheological Properties of Soy Protein Isolates Prepared with Various Iranian Soybean Cultivars

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Abstract

Soybeans, a prominent legume, offer substantial health benefits due to their rich and beneficial nutritional profile. However, the food sector requires improved protein functions. The functional and physicochemical characteristics of isolates from four widely grown soybean cultivars in Iran, namely Katul, Sahar, Tellar, and Sari, were examined in this research. The proximate analysis revealed significant differences ($p<0.05$) among the cultivars in moisture, ash, protein, and fat contents, with Katul isolates showing the highest protein (90.75%) and lowest fat (3.67%) content. Color analysis indicated significant variations in brightness (L*), with Katul isolates being the brightest due to lower fat and ash content. Surface hydrophobicity varied significantly among cultivars, with Sahar showing the highest value (360.30 a.u.). Protein solubility was highest for Katul protein isolate (69.43%), influencing functional properties like emulsification and foaming. Cultivar-specific differences were observed in both water absorption capacity (WAC) and oil absorption capacity (OAC), with Tellar exhibiting the highest OAC (2.42 g/mL). Emulsifying properties, evaluated through emulsion stability (ES) and emulsion capacity (EC), were highest for Sari and Katul protein isolates. Foaming properties varied significantly among the samples, so that Katul protein isolate exhibiting the highest foaming capacity (180.50%) and foaming stability (66.3%), likely attributed to its high protein content. Rheological analyses revealed that Katul had the highest consistency index (*K*) and shear-thinning properties, while Sahar exhibited a more Newtonian-like flow behavior. Gelation studies identified Katul as the most efficient, with the lowest gelling concentration (10%), compared to Sahar's highest value (14%). These findings demonstrate the effect of soybean cultivar on the compositional and functional characteristics of protein isolates, suggesting potential applications in various food products depending on desired functional characteristics.

Keywords: Cultivar, Functionality, Plant protein, Soybean

Introduction

In recent years, global protein consumption has increased significantly. Future increases in protein consumption may be attributed to two factors: the expanding population and shifting

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dietary preferences, particularly the rising desire for healthful foods. It is estimated that by 2050, the world's protein consumption will have grown by 50%. In this sense, increasing food production and introducing valuable protein resources can guarantee food security (Westhoek *et al*., 2011; Henchion *et al*., 2017; Fasolin *et al*., 2019). Plant proteins have drawn a lot of interest lately due to their availability, affordability, and physicochemical characteristics (Abbou *et al*., 2019). Because of their beneficial qualities and ability to promote health, legumes are an important part of human nutrition. Furthermore, they are regarded as abundant providers of fiber, proteins, carbohydrates, and some minerals, as well as vitamins (B-vit.) (Boye *et al*., 2010). As a prominent member of the legume family, soybeans are significant because of their composition, which makes them necessary for a healthy diet. They have a significantly higher protein content (38–44%) than grains (8–15%) and other legumes (20–30%). This increases its value as a food and is one of the factors contributing to soybean's economic significance, along with its favorable amino acid profile. The physicochemical and functional characteristics of soy proteins, such as their ability to absorb water and oil and to emulsify, froth, and gel, make them valuable in food applications (Sui, Zhang, & Jiang, 2021; Yada, 2017). Soy protein isolate (SPI) is a high-quality plant-based protein with a protein content exceeding 90%, making it a suitable alternative to animal protein (Zheng *et al*., 2022).

Scientific evidence demonstrates that the molecular and chemical structures of soy proteins predominantly determine their physicochemical properties (Liu *et al*., 2015; Sui *et al*., 2021; Yada, 2017). Pulses cultivars have a major impact on the structure and composition, which in turn affects their functional qualities. Cui *et al*. (2020) examined the functional characteristics of pea protein isolate (PPI), under the influence of four different cultivars (Agasis, Spider, Trapeze and ND Trial). The findings demonstrated that cultivars significantly influenced on solubility, emulsifying capacity and stability, and foaming capacity and stability of PPI (Cui *et al*., 2020). The physicochemical and functional characteristics of the flour of six navy bean cultivars grown in two regions of Manitoba, Canada, were also studied by (Guldiken *et al*., 2021). The findings demonstrated that genotype had a significant impact on the total starch content, lipid content, and total phenolic content of raw navy bean flour. Additionally, the genotype had a significant effect on the oil holding capacity (OHC) and water hydration capacity (WHC) of navy bean flours.

Therefore, in order to manufacture quality functional ingredients, it is necessary to understand the way in which raw materials can influence the functionality of soy protein isolate (SPI). The impact of soy cultivars on the physicochemical and functional properties of SPI has not been investigated and different cultivars may have various functionalities. This study aims to investigate the physicochemical and functional properties of soy protein isolate (SPI) extracted from four prominent soybean cultivars cultivated in Iran. The research focuses on determining the physicochemical characteristics of SPI, including protein, fat, ash, moisture content, and color. Furthermore, the study evaluates the functional properties of SPI, encompassing surface hydrophobicity, water and oil absorption capacity, solubility, foaming, and emulsifying properties. This research endeavors to provide valuable insights for the development and optimization of protein-based products utilizing Iranian soybean cultivars.

Material and methods

Sample Preparation

Four soybean varieties (Katul, Sahar, Tellar, and Sari) were obtained from the Oilseeds Research Institute (Gorgan, Iran). The soybeans were manually cleaned to remove broken seeds and foreign objects. Seeds were then crushed in an electrical miller (type M20IKA) to produce full-fat flour. To obtain defatted soybean flour (DFSF), full-fat soybean flour (FFSF) was defatted with hexane at a 1:5 (w/v) mixing ratio with constant stirring for six hours at room

temperature. The defatted flour was air-dried in a fume hood at room temperature, ground in a blender to ensure homogeneity, sieved through a 40-mesh screen, and stored in polyethylene bags at -20°C before further analysis.

SPI Preparation

The SPI was prepared based on the method previously described by Shokrollahi Yancheshmeh *et al*. (2022) with some modifications (Yancheshmeh *et al*., 2022). To summarize, 1M NaOH was used to adjust the pH to 9.5, 50 g of defatted flour was agitated for 1 hour at 25℃ (1:20 w/v), and the mixture was centrifuged for 20 minutes at 5000×g. After collecting the supernatant, the pH was adjusted to 4.5, which is the isoelectric point for soy protein. In order to precipitate the protein, the mixture was centrifuged at 5000×g for 20 minutes. The protein was centrifuged at 5000×g for 10 minutes after being cleaned with deionized water and 1M NaOH was used to bring the pH to 7. The extraction procedure was performed at 25°C. The SPI was then freeze-dried and kept for further examination at 4°C.

Physicochemical Properties

Proximate Composition

The protein content of the SPI was determined through the Kjeldahl method (N×6.25). Its fat content was measured based on AOAC 922.06 via the Soxhlet method using the extraction apparatus of B- 811 (Buchi, Switzerland). The moisture and ash contents were quantified through AOAC 925.1 and 923.03, respectively (AOAC, 1990). All results have been expressed on dry weight basis (d.b.).

Color Measurement

In order to obtain the color indices of the samples, a Hunter Lab digital colorimeter (Color Slex, 45Reston VA, and the USA) was employed. The instrument was calibrated using a white plate that was considered as standard color (L*, a*, and b* were 98.84, − 0.73, 1.27 respectively) (Yancheshmeh *et al*., 2022).

Functional Properties

Surface Hydrophobicity Measurement

The surface hydrophobicity was determined based on the method explained by Ding *et al*. (2019) using a fluorometer (Agilent Technologies Inc., Santa Clara, CA, the USA) at λ_{ex} = 390 nm and λ_{em} = 480 nm with a slit width of 2.5 nm. Six concentrations (1-5 mg/mL) were prepared for each SPI sample. Next, 100 µL of anilino-8-naphthalenesulfonate fluorescence (ANS) solution was incorporated into 4 mL of the SPI solution. After the solution was incubated in darkness for 15 min, the absorbance value was measured. The surface hydrophobicity (H_0) was quantified as the slope of the linear regressions of the relative fluorescence intensity against protein concentration (Ding *et al*., 2019).

Protein Solubility (PS)

To determine the protein solubility (mg/ml), at first 1% (w/v) protein dispersion was made in deionized water and stirred (30 min) at ambient temperature. The solution was then centrifuged at 5000 g for 15 min. The protein content of the supernatant was measured through the Biuret method using the UV-2601 spectrophotometer (RayLeight, China) at 540 nm (Feyzi *et al*., 2015). Bovine serum albumin (BSA) was used as an external standard.

Water and Oil Absorption Capacity

The oil absorption capacity (OAC) and water absorption capacity (WAC) of the SPI samples were measured based on (Chandi & Sogi, 2007) with some modifications. For this purpose, 0.5 g (W) of the SPI was dissolved in 5 g of sunflower oil (or 5 g of distilled water for measuring WAC) in 15-mL centrifugal tubes. The tubes were vortexed 30 min at 5-min intervals for 10 s. They were subsequently centrifuged at 2000g for 20 min. Afterwards, the supernatant was removed, and finally, the sediment was weighed (W_1). The values of OAC and WAC were expressed as g/g using Eq. 1:

$$\text{WAC (or OAC)} = (W_1 - w)/\text{w}\times 100 \qquad (1)$$

Least Gelling Concentration

Determination of LGC was carried out following the method described by Boye *et al.* (2010). Protein isolate solutions with specific concentrations (6, 8, 10, 12, 14, 16, 18, and 20% w/v) were prepared. After stirring for 1 hour, 8 mL of each suspension was transferred into test tubes. These samples were heated in a boiling water bath, then immediately cooled to 4°C and refrigerated overnight. Determination of LGC was performed visually by observing the gel behavior during the inversion test.

Emulsifying Properties

The emulsifying activity (EA) and emulsion stability (ES) of SPI were determined using the method described by Zhu *et al.* (2020), with some modifications. In brief, 45 ml protein suspensions (0.5% w/v or 5 mg/mL) were prepared, and pH was set at 7.0 with 0.1M NaOH or HCl. Then 15 ml of sunflower oil was added, and the mixture was gently stirred using a magnetic stirred for 5 min, followed by homogenization using an Ultratorax (IKA T25, Staufen, Germany) at 20000 rpm for 2 min. Afterwards, 50 µL of the emulsions were diluted 100 times using 0.1% SDS. Eventually, the absorbance value was immediately read at 500 nm (A_0) and after 10 min (A_{10}) using a spectrophotometer (UV–2601, RayLeight, China)

The EAI and ESI were calculated based on the following equations:

$$\text{EA } (m^2/g) = \frac{2\times 2.303 \times A_0 \times DF}{10000\times \phi \times L \times C} \quad (2)$$

$$\text{ES (min)} = \frac{A_0 \times 10}{A_0 - A_{10}} \quad (3)$$

where D stands for the dilution factor (100); C represents the concentration of the SPI (g/ mL); L denotes the cuvette optical path length (1 cm); ϕ shows the emulsion oil phase fraction (0.25); and A_0 and A_{10} respectively indicate the emulsion's absorbance values at the times 0 and 10 min.

Foaming Capacity and Stability

The foaming properties of SPI were determined according to Shokrollahi Yancheshmeh *et al.* (2022) with some modifications. For this urpose, the SPI suspensions were prepared at 0.5% (w/v), and their pH values were set at 7.0 with 0.1M NaOH or HCl before the homogenization. Then suspensions were poured into a 50-mL graduated cylinder to measure the volume (V_0). The suspensions were homogenized with Ultratorax homogenizer (25 digital Model, IKEA Company) at 10,000 rpm for 2 minutes and immediately recorded foam volume (V_1) (Yancheshmeh *et al.*, 2022). The foam capacity (FC) was calculated as follows:

$$\text{FC } (\%) = (V_1/V_0) \times 100 \quad (4)$$

Foam stability (FS) was calculated after 60 min based on Eq.3:

$$\text{FS } (\%) = (V_t/V_1) \times 100 \quad (5)$$

Where V_1 represent the volume of the foam after whipping at time 0 min; and V_t denotes the volume of the foam after 60 min.

Time-independent Steady Shear Rheological Measurements

Samples (10% protein content; pH 7) were subjected to shear rates ranging from 1 to 200 s^{-1} and the resulting shear stress was recorded (at 25°C). To ascertain the samples' shear-dependent rheological properties, the Power law model (Eq.6) and Herschel-Bulkley model were (Eq.7) fitted to the experimental shear stress-shear rate data:

$$\tau = K(\dot{\gamma})^n \quad (6)$$

$$\tau = \tau_0 + K(\dot{\gamma})^n \quad (7)$$

Where, τ is the shear stress (Pa); τ_0 is the yield stress (Pa); k indicates the consistency index (Pa s^n); γ represents the shear rate (s^{-1}); and n is the flow behaviour index (dimensionless) (Steffe, 1996).

Statistical Analyses

All the measurements were at least triplicated, and the data have been expressed as mean ± standard deviation. In order to analyze the obtained data, one-way analysis of variance (ANOVA) and Duncan's multiple-range test were performed at $p<0.05$ using SPSS version 22.

Result and Discussion

Proximate Composition

The compositions of soybean seeds and protein isolates of four soybean cultivars (Katul, Sahar, Tellar, and Sari) are presented in Table 1. The moisture and ash contents of soybean seeds lay in the range of 7.56- 8.40% and 4.87-6.10% respectively. Fat and protein contents varied from 20.89-21.78% and 37.15-41.59% respectively. Moreover, the ash and moisture contents of soybean isolates were in the range of 7.94-9.04% and 2.32-3.91%, respectively. The fat and protein contents of soybean isolates varied between 3.67-4.61% and 84.83-90.75% respectively. There were significant ($p<0.05$) differences between the moisture, ash, fat, and protein contents of the four soybean cultivars. Katul protein isolates had more protein (90.75%) and less fat (3.67%), moisture (7.94%) and ash (2.32%) than others. An analysis of variance found that cultivars played a significant role in the determination of the SPI yield ($p < 0.05$), which was maximized in the case of Katul at 28.12%, followed by Sari, Sahar, and Tellar gave lower yields at 27.12, 26.89 and 26.40 g/100 g, respectively (Table 1).

Table 1- Chemical compositions and color parameters of soybean seeds and soy protein isolates determined for different cultivars

Physicochemical properties	Cultivar			
	Katul	Sahar	Tellar	Sari
Protein content of raw seed (%)	38.50±0.22 ^{c}	41.59±0.41 ^{a}	37.15±0.35 ^{d}	39.16±0.14 ^{b}
Fat content of raw seed (%)	21.35±0.11 ^{b}	21.65±0.09^{a}	20.89±0.18 ^{c}	21.78±0.16 ^{a}
Moisture content of raw seed (%)	8.26±0.20 ^{b}	7.56±0.15 ^{d}	8.02±0.17 ^{c}	8.40±0.12 ^{a}
Ash content of raw seed (%)	5.12±0.15 ^{b}	5.02±0.19 ^{c}	6.10±0.23 ^{a}	4.87±0.17 ^{d}
Protein content of isolate (%)	90.75±0.54 ^{a}	89.14±0.48 ^{b}	84.83±0.35 ^{d}	85.70±0.42 ^{c}
Fat content (%)	3.67±0.12 ^{d}	3.86±0.10 ^{c}	4.61±0.18 ^{a}	4.32±0.14 ^{b}
Moisture content (%)	7.94±0.10 ^{d}	8.49±0.18 ^{b}	9.04±0.20 ^{a}	8.14±0.19 ^{c}
Ash content (%)	2.32±0.32 ^{d}	3.91±0.19 ^{a}	2.84±0.20 ^{c}	3.12±0.15 ^{b}
Yield (g/100 g)	28.12±0.23 ^{a}	26.89±0.36 ^{c}	26.40±0.24 ^{d}	27.12±0.36 ^{b}
Color				
*L**	84.34±0.17 ^{a}	81.86±0.31 ^{b}	68.57±0.45 ^{d}	78.05±0.21 ^{c}
a*	-2.07±0.07 ^{c}	-2.12±0.10 ^{c}	-0.92±0.05 ^{a}	-1.71±0.14 ^{b}
b*	25.31±0.21 ^{c}	26.53±0.37 ^{a}	16.39±0.16 ^{d}	26.27±0.45 ^{b}

a–d: Means sharing the same letter in the same row do not differ significantly ($p > 0.05$).

Color

An important parameter about protein isolates is their color. The *L** parameter indicates the degree of brightness and can take values from 0 to 100. The higher the *L** value the brighter the color (Nielsen, Wrolstad, & Smith, 2010). As seen in Table 1, there was a statistically significant difference between the *L** parameter of protein isolates. The highest *L** parameter was related to Katul, probably due to the lower amount of fat and ash in Katul protein isolate. Parameter *a** ranges from negative values (indicating green color) to positive values (indicating red color) and parameter *b** also ranges from negative values (blue color) to positive values (yellow color) (Nielsen *et al.*, 2010). According to Table 1, there was a significant difference between the parameters *a** and *b** of the isolates ($p < 0.05$). The highest absolute value of a* and the amount of *b** was related to Sahar protein isolate. In general, protein isolates that partially cause a brown color are desirable for use in breads and cakes, and isolates that help make the product colorless can be used in another group of light-colored breads (Singh *et al.*, 2008). Based on this, it is possible to use Katul, Sahar and Sari protein isolates in some bakery products in which a brighter color is desired, and Tellar isolate to create a brown color for the bread crust in colored bread or pasta.

Surface Hydrophobicity

Cultivar had a substantial impact ($p>0.05$) on surface hydrophobicity values, according to the analysis of variance (Table 2). Tellar, Katul, and Sari produced isolates with lower surface hydrophobicities (337.85, 252.60, and 240 a.u., respectively), whereas Sahar produced an isolate with a greater surface hydrophobicity (360.30 a.u.). The differences in surface hydrophobicity values among the cultivars could be attributed to variations in their protein composition, structure, or amino acid profiles, which influence the exposure of hydrophobic groups. These intrinsic differences affect how the proteins interact with their environment, leading to the observed variations in surface hydrophobicity. According to Cserhalmi *et al*. (1998), surface hydrophobicity can vary depending on the variety of pea. The surface hydrophobicity values of the mixed globulin fractions obtained from five distinct pea varieties lay in the range of 21.81-43.11 a.u (Cserhalmi *et al*., 1998).

Protein Solubility

Table 2 displays the solubility values of the four SPI samples at pH 7.0. Solubility plays a key role in the functional properties of a protein, including emulsification, gelation, and foaming (Kinsella & Melachouris, 1976). Tellar had the lowest solubility, with an average value of 57.82%, based on the data. Compared to Sari (61.52%) and Sahar (64.11%), Katul (69.43%) had a higher solubility. The higher solubility observed in Katul compared to the other cultivars could be explained by differences in protein structure and composition. Proteins from Katul may have a higher proportion of hydrophilic groups exposed on their surface, facilitating better interaction with water. Additionally, variations in amino acid composition and protein folding may enhance the ability of Katul proteins to remain soluble, as solubility is closely linked to the balance between hydrophilic and hydrophobic regions. Different pea genotypes display varying solubility values at pH 7, according to Barac *et al*. 2010. Of the three experimental lines studied, two showed both the lowest (L2=70%) and highest (L1=85%) solubility values.

Table 2- Functional properties of soy protein isolates determined for different soybean cultivars

Functional properties	Cultivar			
	Katul	Sahar	Tellar	Sari
WAC (g/mL)	3.48±0.11 ^{a}	3.13±0.21 ^{c}	3.33±0.23 ^{ab}	3.22±0.30 ^{bc}
OAC (g/mL)	2.13±0.22 ^{b}	1.89±0.15 ^{c}	2.42±0.10 ^{a}	2.12±0.09 ^{b}
Solubility (%)	69.43±0.54 ^{a}	64.11±0.36 ^{b}	57.82±0.41 ^{d}	61.52±0.27 ^{c}
H0	337.85±1.01 ^{b}	360.30±2.05 ^{a}	240±0.94 ^{d}	252.60±0.97 ^{c}
LGC (%)	10.00±1.41 ^{c}	14.00±1.74 ^{a}	12.00±1.22^{b}	12.00±1.45 ^{b}

a–d: Means sharing the same letter in the same row do not differ significantly ($p > 0.05$).

Water and Oil Absorption Capacity

SPI's water absorption capacity (WAC) ranged from 3.13 to 3.48 g/mL of isolates, with a statistically negligible difference ($p > 0.05$) (Table 2). WAC is essential for certain product qualities, including moisture content and staling. Proteins and carbohydrates, due to their hydrophilic elements such as polar or charged side chains, are the primary chemical components that enhance the WAC of SPI. The range of values for the WAC of kidney bean flour reported by Siddiq *et al*. (2010) and Aguilera *et al*. (2011) were 2.2 to 2.7 kg/kg and 2.2 to 2.7 L/kg, respectively. Variations in a protein's ability to absorb water are connected to changes in its structure. Proteins with a higher surface concentration of hydrophilic groups typically exhibit enhanced water-binding capacity (Feyzi *et al*., 2015). Oil absorption capacity (OAC), which is essential for enhancing mouthfeel and maintaining flavor, is another important functional feature of flours (Kinsella & Melachouris, 1976). OAC varied from 1.89 to 2.42 g/mL for SPI (Table 2). The OAC of Tellar was substantially ($p<0.05$) higher than those of the other isolates.

The higher OAC of Tellar (2.42 g/mL) compared to other isolates may be attributed to its higher hydrophobic amino acid content and protein structure. Hydrophobic amino acids enhance the ability of proteins to bind non-polar oil molecules. This factor allows Tellar to interact more effectively with oil, resulting in a significantly higher OAC ($p<0.05$). OAC is primarily influenced by protein comprising both hydrophobic and hydrophilic moieties. Lipid hydrocarbon chains and the side chains of non-polar amino acids may have hydrophobic interactions (Wani *et al*., 2013).

Least Gelling Concentration

Significant variations were observed in the least gelling concentration (LGC) among soy protein isolates (SPI) derived from different soybean cultivars (Katul, Sahar, Tellar, and Sari). As presented in Table 2, Katul exhibited the lowest gelling concentration (LGC) (10%), suggesting superior gelation efficiency compared to the other cultivars .

The lower LGC of Katul can be attributed to its higher protein solubility (69.43%) and better water absorption capacity (WAC, 3.48 g/mL), as shown in Table 2. Higher protein solubility ensures a greater availability of protein molecules in the solution, which facilitates the formation of intermolecular interactions, such as hydrogen bonds and hydrophobic interactions, during gelation. Additionally, a higher WAC allows for better hydration of protein molecules, which is crucial for unfolding and aligning the proteins to create a stable and cohesive gel network. These combined factors enable Katul to form a stronger gel network at lower protein concentrations, resulting in its lower LGC compared to other cultivars. In contrast, Sahar exhibited the highest LGC (14%), suggesting weaker gelation properties potentially due to lower levels of hydrogen bonding and hydrophobic interactions compared to Tellar and Sari, which both demonstrated intermediate gelling concentrations (12%).

These differences could be attributed to the varying ratios of glycinin and β-conglycinin, which are the major protein components of SPI and strongly influence gelling behavior. Higher glycinin content is often associated with enhanced gelation properties due to its stronger intermolecular interactions during heat-induced gelation. Additionally, environmental factors during soybean cultivation and post-harvest processing can affect the protein composition and, consequently, functional properties like gelation. The results highlight the significance of cultivar selection for specific applications, especially in food systems that demand precise textural and structural properties. For example, Katul's lower gelling concentration makes it ideal for formulations where strong gel formation is required with minimal protein content.

Emulsifying Properties

Fig. 1A shows the emulsion capacity (EC) results for the four isolates. Sari (74%), Sahar (71.5%), and Katul (70%) exhibited similar EC values, while Tellar (53%) had the lowest. This variation in EC can be attributed to differences in protein structure, which influence the ability to stabilize oil-water interfaces. Sari, Sahar, and Katul likely have more favorable surface properties, such as a higher proportion of hydrophilic groups or better protein conformations, which enhance their ability to form stable emulsions. In contrast, Tellar's lower EC may be due to less effective interaction between the proteins and the oil phase, possibly due to a higher presence of hydrophobic regions that hinder emulsion formation.

Barac *et al*. (2010) demonstrated that emulsifying stability (ES) does not always correlate directly with emulsifying activity (EA) (Barac *et al*., 2010). Fig. 1B presents the findings of the cultivar's effect on ES. All isolates showed ES values greater than 35%, and cultivar selection had a significant impact on these values ($p<0.05$). Katul and Sahar cultivars exhibited significantly higher ES compared to the others, which differed markedly from each other ($p > 0.05$). These differences suggest that Katul and Sahar cultivars possess proteins that, due to their molecular structure, provide better stabilization

of emulsions. The enhanced ES is likely due to sufficient steric hindrance and/or charge repulsion between oil droplets, which prevents coalescence and maintains the stability of the emulsion. This stability is crucial for applications in food systems, as it ensures uniform dispersion of oil in the aqueous phase.

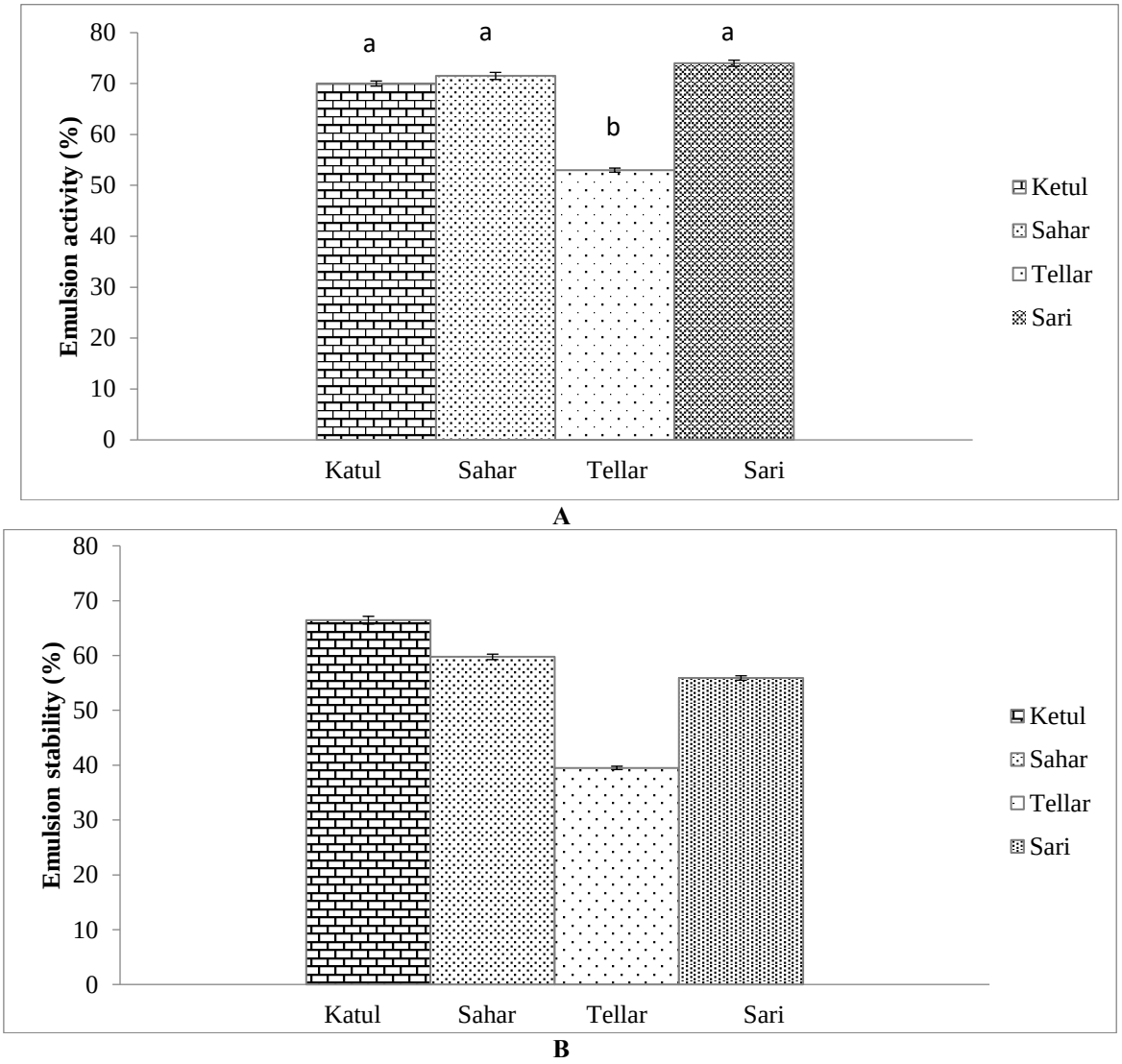
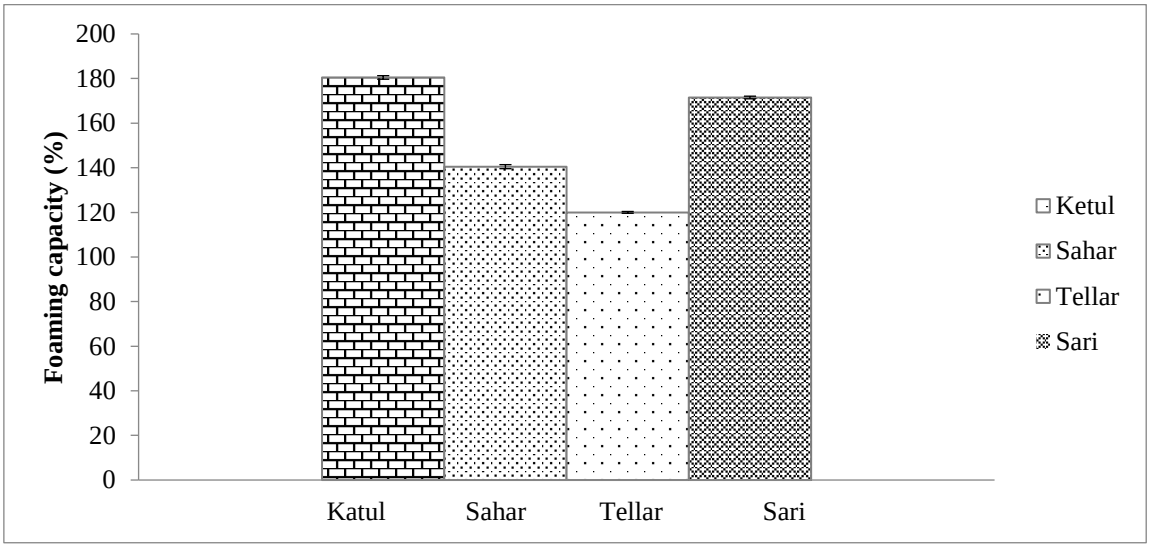


Fig. 1. Emulsifying capacity (A) and emulsion stability (B) of SPI at different cultivars
Different letters show significant differences between the cultivars ($p < 0.05$).

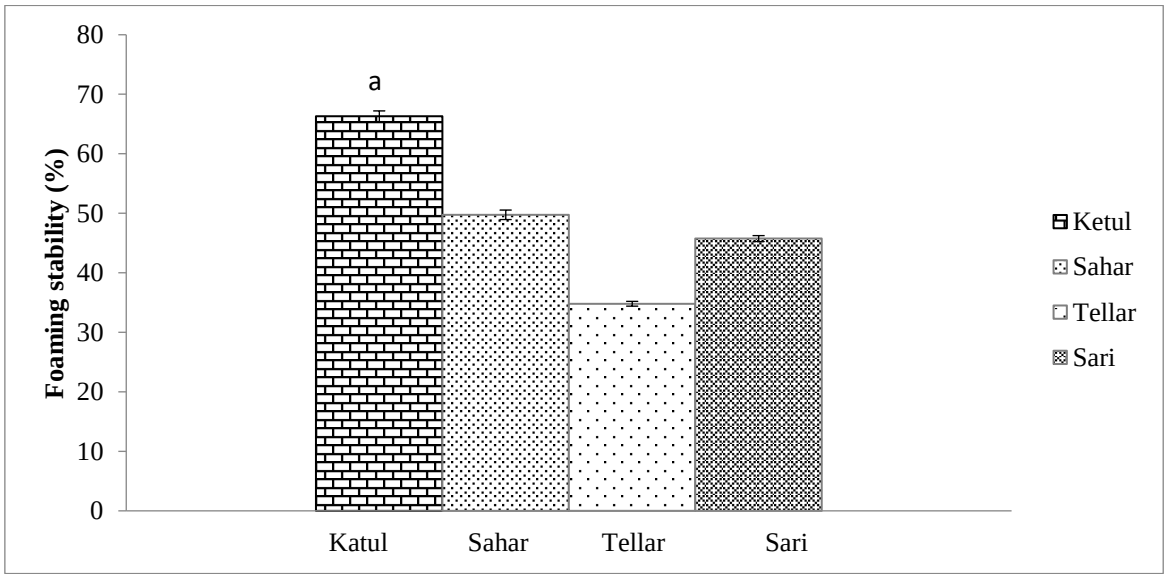
Foaming Properties

Foaming capacity (FC) is the ability of a protein to create foam; on the other hand, foam stability (FS) is the protein's ability to retain the foam volume for a certain duration. Flours can produce foam because of surface active proteins (Adebowale & Lawal, 2003). An overview of the effect of cultivar on FC is shown in Fig. 2A. Tellar (120%) had the lowest amount of FC, while Katul (180.50%) had the highest one, followed by Sari (171.5%) and Sahar (140.5%). Based on pea genotype, Barac *et al.* (2010) found that there are variations in FC, with Calvedon having the lowest FC (235%) and Maja having the highest (325%) (Barac *et al.*, 2010). The assessed foaming capacities of the research were higher than those of two reported commercial pea protein isolates (104% and 96%) (Soral-Smietana *et al.*, 1998). A significant association was found between

solubility and FC ($p<0.05$), suggesting that a higher concentration of protein will move to the air-water interface and generate more foam. In the investigation of foam volume stabilities after 60 minutes conducted in this study, Sari and Tellar had the lowest values (45.75% and 34.80%) (Fig. 2B). The Katul and Sahar cultivars have higher protein contents, more initial protein molecules were presumably added to the foaming mechanism, explaining their superior foaming qualities (Feyzi *et al.*, 2015).



A



B

Fig. 2. Foaming capacity (A) and foam stability after 60 minute (B) of SPI at different cultivars
Different letters show significant differences between the cultivars ($p < 0.05$).

Steady Shear Flow Behavior

Table 3 illustrates the rheological parameters of soy protein isolates (SPI) extracted from four soybean cultivars (Katul, Sahar, Tellar, and Sari) at a 10% protein concentration. The coefficients of determination (R2) were consistently high across all cultivars, ranging from 0.954 to 0.997, signifying an excellent fit of the Power law model to the experimental data. Furthermore, the root mean square error (RMSE) values were minimal for all cultivars, with Sari displaying the lowest error (0.001),

confirming the model's precision. The consistency index (K) varied significantly among cultivars, with Katul exhibiting the highest value (0.297 Pa.s^n), indicating the greatest viscosity, while Sahar showed the lowest value (0.133 Pa.s^n), representing the least viscous

behavior. The flow behavior index (n), which reflects the fluid's non-Newtonian characteristics, was highest for Sahar (0.957), suggesting its flow behavior was closest to Newtonian, whereas Katul and Sari had the lowest n values (0.887 and 0.888, respectively), indicative of a more pronounced shear-thinning behavior.

These findings reveal significant differences in the rheological properties of SPI among soybean cultivars ($p<0.05$). Katul demonstrated the highest viscosity and model reliability, whereas Sahar's SPI exhibited lower viscosity and a flow behavior closer to Newtonian fluids. These variations underscore the influence of soybean cultivar on the functional and rheological characteristics of SPI, which may have implications for their application in food systems.

Table 3- Rheological parameters of soy protein isolates determined for different soybean cultivars

Samples	Power law model			Herschel-Bulkley model			
	K (Pa s^n)	n (-)	R^2	K (Pa s^n)	n (-)	τ_0 (Pa)	R^2
Katul	0.297±0.01 ^{a}	0.887±0.00 ^{c}	0.997	0.285±0.07^a	0.897±0.00^c	0.086±0.00^a	0.999
Sahar	0.133±0.02 ^{d}	0.957±0.01 ^{a}	0.954	0.113±0.06^d	0.931±0.00^b	0.053±0.00^b	0.998
Tellar	0.183±0.00 ^{c}	0.917±0.00 ^{b}	0.987	0.145±0.09^c	0.960±0.00^a	0.031±0.00^c	0.997
Sari	0.198±0.01 ^{b}	0.888±0.00^c	0.974	0.156±0.003^b	0.927±0.00^b	0.048±0.00^b	0.989

a–d: Means sharing the same letter in the same row do not differ significantly ($p > 0.05$).

The rheological parameters of the Herschel-Bulkley model for SPI extracted from four soybean cultivars (Katul, Sahar, Tellar, and Sari) are also presented in Table 3. The coefficients of determination (R^2) for this model were consistently high, ranging from 0.989 to 0.999, indicating a superior fit of the Herschel-Bulkley model to the experimental data compared to the Power law model. This highlights the model's capability in accurately describing the rheological behavior of SPI. The yield stress (τ_0) values, which represent the minimum stress required to initiate flow, varied among cultivars. Katul exhibited the highest yield stress, indicating stronger structural resistance to flow, while Tellar showed the lowest yield stress, reflecting a weaker internal structure. The consistency index (K) values were consistent with those observed in the Power law model, with Katul demonstrating the highest viscosity and Sahar the lowest. The flow behavior index (n) closely aligned with those observed under the Power law model, with values ranging from 0.897 to 0.931. Sahar exhibited the highest -value (0.931), indicating flow behavior closest to Newtonian, whereas Katul showed the lowest-value (0.897), emphasizing its pronounced shear-thinning nature. These results reinforce the significant influence of soybean cultivar on SPI rheological properties ($p<0.05$). Katul's SPI demonstrated the highest structural integrity and viscosity, making it more suitable for applications requiring higher resistance to deformation. In contrast, Sahar's SPI showed a lower viscosity and a flow behavior closer to Newtonian fluids, making it more applicable in systems requiring easier flow.

Conclusion

The effects of four soybean cultivars (Katul, Sahar, Tellar, and Sari) on various physicochemical, functional, and rheological properties were examined in this study. The findings demonstrate that soybean cultivar significantly influences the quality and functionality of soy protein isolates (SPI). Katul and Sahar exhibited superior solubility, emulsifying, and foaming capacities compared to Tellar and Sari. These differences are consistent with their physicochemical profiles, particularly their higher protein content and

lower residual fat levels, despite the removal of oil during the production of the protein isolate. In terms of rheological behavior, Katul displayed the highest consistency index (K) and significant shear-thinning properties, highlighting its potential for thickening applications. Similarly, Sahar showed the highest flow behavior index (n), indicative of a more Newtonian-like flow suitable for beverage formulations. Regarding gelation properties, Katul required the lowest gelling concentration (10%), making it the most efficient in forming gels, while Sahar needed the highest concentration (14%). Overall, this study emphasizes the importance of cultivar selection in optimizing SPI functionality for specific food industry applications. The combination of physicochemical, functional, and rheological insights provides a comprehensive understanding of how different soybean cultivars influence the final SPI product, enabling tailored applications based on desired properties.

Author Contributions

Behdad Shokrollahi Yancheshmeh: Done the experiments, analyzed and interpenetrated the data, prepared the manuscript, and revised the paper. **Mehdi Varidi:** As the corresponding author, designed the research, provided the fund and laboratory facilities, checked the data, controlled the analysis, edited the paper, revised the paper, and submitted the paper**. Seyed Mohammad Ali Razavi:** Writing review, supervision, provided the fund and laboratory facilities, checked the data, controlled the analysis, edited the paper, and revised the paper. **Farshad Sohbatzadeh:** As the advisor, reviewed the research and edited the paper.

Founding Source

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بررسی خواص فیزیکوشیمیایی، عملکردی و رئولوژیکی ایزوله‌های پروتئین سویا تهیه شده از واریته‌های مختلف سویای ایرانی

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چکیده

علی‌رغم کشت گسترده واریته‌های سویا در مناطق مختلف ایران، تاکنون ویژگی‌های عملکردی این واریته‌ها مورد بررسی قرار نگرفته‌اند. در این پژوهش، ویژگی‌های فیزیکوشیمیایی، عملکردی و رئولوژیکی ایزوله‌های پروتئینی چهار واریته سویا شامل کتول، سحر، تِلار و ساری، که بیشترین سطح زیر کشت در ایران را دارند، مورد بررسی قرار گرفت. آنالیز آماری نتایج نشان داد بین واریته‌های مختلف تفاوت معنی‌داری ($p<0.05$) از نظر رطوبت، خاکستر، پروتئین و چربی وجود دارد، به‌طوری‌که ایزوله حاصل از واریته کتول بالاترین میزان پروتئین (۹۰/۷۵٪) و کمترین میزان چربی (۳/۶۷٪) را دارا بودند. آب‌گریزی سطحی به‌طور قابل توجهی بین واریته‌های مختلف متفاوت بود و سحر بیشترین مقدار (۳۶۰/۳۰a.u.) را نشان داد. حلالیت پروتئین در ایزوله‌های کتول (۶۹/۴۳٪) بیشترین مقدار را داشت که بر ویژگی‌های عملکردی مانند امولسیون‌کنندگی و کف‌کنندگی تأثیر می‌گذارد. ظرفیت جذب آب (WAC) و ظرفیت جذب روغن (OAC) تفاوت‌های قابل توجهی داشتند، به‌طوری‌که تِلار بالاترین ظرفیت جذب روغن ۲/۴۲ g/mL را نشان داد. خواص امولسیون‌سازی، از جمله پایداری امولسیون (ES) و ظرفیت امولسیون‌کنندگی (EC)، در ایزوله‌های پروتئینی ساری و کتول بیشترین بود. خواص کف‌کنندگی نیز تفاوت‌های قابل توجهی داشتند و کتول بالاترین ظرفیت کف‌کنندگی (۱۸۰/۵۰٪) و پایداری کف را به‌دلیل محتوای بالای پروتئین خود نشان داد. آنالیزهای رئولوژیکی نشان داد که واریته کتول دارای بالاترین شاخص قوام (K) و خواص شل شوندگی با برش است، در حالی‌که واریته سحر رفتار جریانی نزدیک به سیال نیوتنی را نشان می‌دهد. مطالعات ژل‌سازی نیز نشان داد که کتول با کمترین غلظت ژله‌ای شدن (۱۰٪) به‌عنوان کارآمدترین واریته ظاهر شد. این یافته‌ها تأثیر واریته سویا را بر ویژگی‌های عملکردی ایزوله‌های پروتئینی نشان می‌دهند و کاربردهای بالقوه‌ای را در محصولات غذایی مختلف، بسته به ویژگی‌های عملکردی موردنظر، پیشنهاد می‌کنند.

واژه‌های کلیدی: پروتئین گیاهی، سویا، واریته، ویژگی‌های عملکردی

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