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Electrohydrodynamic (EHD) –assisted extraction of protein from mung bean (*Vigna radiate* L.) sprout: Effect of solid to solvent ratio on the functional properties

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ABSTRACT

Background & Aim: Mung bean knwn as a traditional food which has been used both as nutritional food and herbal medicine over 2000 years. Mung bean sprouts are one of the most commonly used bean sprouts and considered an as appropriate source for the extraction of highly valuable proteins.

Experimental: In this study, the effect of different solid to solvent ratios (1:5, 1:10, 1:15 and 1:20 g/mL in electrohydrodynamic (EHD)-assisted extraction on the extraction yield and functional characteristics of sprouted mung bean protein isolate (SMPI) was evaluated. In addition, the structural and thermal properties of SMPI were investigated using Fourier transform infrared spectroscopy (FTIR), and differential scanning calorimetry (DSC), respectively.

Results: The highest protein extraction yield, protein solubility (PS), oil absorption capacity (OAC), foaming capacity (FC) and foaming stability (FS) were obtained in the solid to solvent ratio of 1:20 g/mL. The results of FTIR showed that in the solid to solvent ratio of 1:20, the α-helix structure in SMPI decreased and transformed to random coil structure, leading to increased protein solubility. According to the DSC analysis, the highest denaturation temperature and protein stability were attributed to the solid-to-solvent ratio of 1:20 due to higher water content.

Recommended applications/industries: The present results indicated that EHD pretreatment with the solid to solvent ratio of 1:20 could improve the functional properties of SMPI and EHD-assisted extracted SMPI could be considered as a potential nutraceutical or ingredient of functional and health-promoting foods.

1. Introduction

The growing world-population and economic aspects of sustainable development have increased the need for the new protein resources. Owing to the greater sustainability and lower production costs, plant proteins are becoming of great interest (Yue *et al.*, 2021). Legumes are enumerated as good plant protein sources which contain a great amount of high quality proteins, carbohydrates, dietary fibers, small amounts of mineral vitamins, bioactive compounds and saturated fats (Pastor-Cavada *et al.*, 2014; Saleh *et al.*, 2019). The

high protein amount of legumes (20-50%) compared to cereals (2 to 4 times higher) as well as the high nutritional value of the legumes protein have led to the current increasing interest in their application. They have been used in the production of different pharmaceutical and nutraceutical food products (Bamdad *et al.*, 2009; Kudre *et al.*, 2013). On the other hand, legumes, play an important role in decreasing the kidney and heart diseases, lowering the sugar intake and showed anti-inflammatory, anti-cancer, anti-acterial and anti-oxidant activities (Du *et al.*, 2014; Zhang *et al.*, 2013). Among legumes, mung bean is consumed in appreciable amounts in Asia due to resistance to heat and drought stress, as well as its high protein content (Ebert *et al.*, 2017). Protein is the most abundant component in the mung bean grain after starch which contains sufficient amounts of all amino acids (Du *et al.*, 2018). A low cost technology to enhance the nutritional and phytochemical characteristics of beans is obtained by germination. The protein, dietary fiber and phenolic compound contents are increased by germination (Paucar-Manacho *et al.*, 2017). Sprouts are functional nutraceutical foods (Ebert *et al.*, 2017). In Europe mung beans are often used as sprouts. The mung bean sprout is also widely consumed in Asia (Du *et al.*, 2018) due to its plentiful and nutritive value (Hoque *et al.*, 2011). Mechanical disintegration and/or application of special organic solvents are common methods for the extraction of components from plant matrices. The yields of such methods are affected by the degree of permeabilization of the cell walls (Sarkis *et al.*, 2015).

Non-thermal emerging technologies such as electrohydrodynamic (EHD) have been applied to overcome the limitations of conventional methods and decrease the processing time, improve the recovery yield, increase the quality of the products, enhance the functionality of the extracts (Rosello-Soto *et al.*, 2014) and reduce the cost, energy consumption and solvent usage. EHD process could increase the release of the intercellular components due to the extensive damage to the cell walls by generating shock waves (Sarkis *et al.*, 2015). The critical parameters which affect the efficiency of EHD extraction process, are voltage or electric field intensity, time, flow rate, solid to solvent ratio, and solvent type (Li *et al.*, 2019). EHD has been recently used by different workers to recover proteins and phenolic components from some plant food materials (Maher *et al.*, 2020; Shahram *et al.*, 2019; Pojic *et al.*, 2018; Barba *et al.*, 2015, Sarkis *et al.*, 2015, Parnaikov *et al.*, 2014). However, to the best of our knowledge the effect of EHD pretreatment in combination with traditional methods (i.e. alkaline extraction and acid precipitation) on the extraction of protein from sprouts, has never been investigated. On the other hand, literature review shows that most of the studies concern the effect of voltage and time of EHD process rather than the other parameters such as solid to solvent ratio. The effect of EHD voltage and time on the functional properties of sprout mung bean protein isolate (SMPI) has been investigated in our other study. Therefore, due to the importance of the optimization of the EHD parameters such as solid to solvent ratio to obtain the highest efficiency and functionality, in this study the effect of solid to solvent ratio on the protein extraction yield and functional properties of SMPI were investigated. Protein solubility (PS), water holding capacity (WHC), oil absorption capacity (OAC) and foaming properties are considered the most important functional characteristics of the SMPI.

2. Materials and Methods

2.1. Materials

Mung bean seeds (*Vigna radiate* L.) were purchased from a local market in Isfahan, Iran. Bradfoard reagent, sodium hydroxide, sodium hypochlorite, hydrochloric acid, and bovine serum albumin were supplied by Merck Chemical Company (Darmstadt, Germany). Ethanol with a purity of 96 % was purchased from Sina Fariman Company (Khorasan Razavi, Iran).

2.2. Seed germination

Mung bean seeds were sterilized with 70% ethanol for 2.5 min and 2.5% sodium hypochlorite for 15 min, followed by washing four times with distilled water to remove the chemicals. Afterwards the seeds were allowed to absorb water in the seed to water ratio of 1:5 (w/v) for 17 h. at 18 ºC. After drainining the soaking water, the hydrated seeds were placed in Petri dishes containing wet Whatman No. 2 filter paper and allowed to germinate in the dark at room temperature (25 ± 2°C) for 3 days. During the germination the filter paper was kept humid by spraying distilled water 3 times a day (Cevallos-Casals *et al.*, 2010). The mung bean sprouts were then dried in a convective drier designed in the Department of Food Engineering, Islamic Azad University of Shahreza (Shahreza, Iran) at 30 °C for 2

h. Finally, the dried sprouts were grounded with a domestic mill (Sanyo Industrial Blender, Japan) and the powder was passed through a 150-mesh sieve. The fine powder was then packed in polyethylene zipper bags and stored in a freezer (Model REFST170, Pars, Iran) at the temperature of -18 °C until further analysis.

2.3. Protein extraction

2.3.1. EHD pretreatment

The EHD system consisted of a grounded plate and 5 copper wires with length of 19.2 cm and external diameter of 1.2 mm as discharge electrodes. The wire and electrode gaps between the grounded plate and the discharge electrodes were 4 and 5 cm, respectively. A DC high voltage power supply (Model HV50P OC, Fanavaran Nano-Meghyas, Tehran, Iran) with the output, voltages of 0 to 60 kV; was used to produce corona wind. The suspensions of dried mung bean sprouts powder in water at different solid to solvent ratios (1:5, 1:10, 1:15 and 1:20 w/v) were prepared at ambient temperature (25± 2 °C) and poured into to an aluminum plate (16 cm × 16.5 cm × 2 cm height) which was then placed on the grounded plate electrode and the EHD pretreatment was performed at voltage of 20 kV for 10 min.

2.3.2. Alkaline extraction and acid precipitation process

The SMPI was prepared according to the method described by Du *et al.* (2018) with some modifications. Briefly, the pH of the EHD pretreated suspensions was adjusted to 9.0 using sodium hydroxide (0.1 M) at 25± 2 °C. The solution was then stirred using a magnetic stirrer (Model Hp-840, Alfa, Iran) for 1 h and centrifuged at 4000×g, for 10 min. Next, the supernatant was separated and centrifuged (4000×g, 15 min) after adjusting the pH to 4.5 using hydrochloric acid (0.1 M). The obtained precipitate was dried in an oven (Model E. O 155, Shimifann Co., Iran) at 40 °C for 12 h. Finally, the dried protein was grounded and the soft powder was stored in a refrigerator at 4 °C until further analysis. The schematic representation of EHD pre-treatment followed by alkaline extraction/acid precipitation process is shown in Fig. 1.

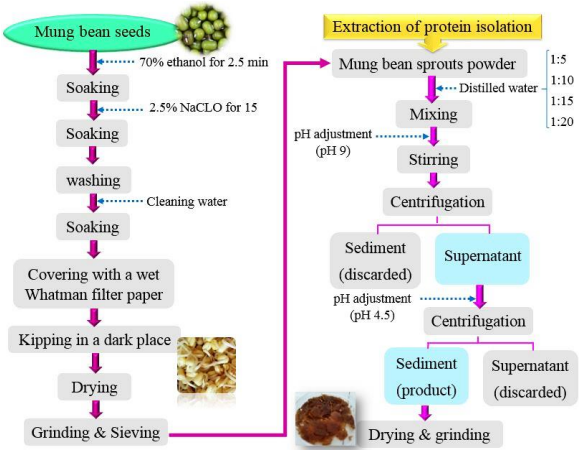


Fig. 1. Schematic process diagram of the overall experimental design.

2.4. Protein extraction yield

The extraction yield of SMPI was determined using equation 1:

where m_p and m_t are the weights of SMPI (g) and mung bean sprout powder (g), respectively (Wali *et al.*, 2019).

$$Extraction\ yield\ (\%) = M_p/M_t \quad (1)$$

2.5. Physicochemical properties

2.5.1. Protein solubility (PS)

For PS determination, first, 0.5 g of SMPI was added to 100 mL of distilled water, and the pH of the mixture was adjusted to 7.3 using sodium hydroxide (0.1 N). The mixture was then centrifuged (Model CE 148, Shimifan Company, Tehran, Iran) at 4000×g for 10 min (Tsumura *et al.*, 2005). Next, according to the Bradford method, 0.2 mL of the supernatant and 1.8 mL of 3 times diluted Bradford reagent were mixed. The sample was kept for 5 min in a dark place at room temperature (25± 2 °C) and the absorbance was measured at the wavelength of 595 nm (Barba *et al.*, 2015) by a UV-Vis spectrophotometer (Model UV Vis Cary 60, Agilent, USA). Bovine serum albumin was used as the standard solution.

2.5.2. Water holding capacity (WHC)

WHC of SMPI was measured according to the method of Du *et al.* (2018). In detail, 10 mL (V_1) of distilled water and 0.5 g ($W_{initial}$) of protein were poured into a 100 ml glass graduated cylinder. Next, the mixture was kept for 80 min at room temperature and then centrifuged for 10 min at 4000×g. Finally, the volume of the supernatant (V_2) in the graduated glass cylinder was recorded. The WHC was calculated using equation 2:

$$WHC\ (g/ml) = (V_1 - V_2)/(W_{initial}) \qquad (2)$$

2.5.3. Oil absorption capacity (OAC)

OAC of SMPI was measured using the method reported by Du *et al.* (2018). In detail, 0.5 g of SMPI ($M_{initial}$) was mixed with 5 g of soybean oil (M_1) in a 50 mL beaker and the mixture was stirred using a magnetic stirrer at average speed for 30 min at room temperature. The mixture was then centrifuged at 4000×g for 20 min by mixing every 5 min. Finally, the supernatant was separated and weighed (M_2). The OAC was estimated using equation 3:

$$OAC(g/g) = (M_1 - M_2)/(M_{initial}) \qquad (3)$$

2.5.4. Foaming properties

The foaming properties of the extracted protein were measured based on the procedure reported by Kaushik *et al.* (2016) with slight modifications. Briefly, 50 mL of the protein isolate solution (20 mg/mL) were poured into a 100 mL graduated glass cylinder and the volume of the mixture was recorded as V_1. Afterwards, the mixture was homogenized for 1 min using a homogenizer (Model T18 basic, KA-Werke GmbH & Co., Staufen, Germany) at 7000 rpm and the new volume of the mixture was recorded as V_2. The prepared mixture was then kept at room temperature for 10 min and the volume of the mixture was recorded as V_3. The percentages of foaming capacity (FC) and the foaming stability (FS) were calculated using equations (4) and (5), respectively.

$$FC(\%) = (V_2 - V_1)/(V_1) \times 100 \qquad (4)$$

$$FS(\%) = (V_3 - V_2)/(V_2) \times 100 \qquad (5)$$

2.6. Fourier transform infrared spectroscopy (FTIR) analysis

FTIR spectroscopy is an important tool to provide information about the structural composition of proteins (Du *et al.*, 2018). FTIR spectroscopy shows the possible degradation and changes in the structure of SMPI caused by the EHD pre-treatment. To perform FTIR analysis, 2 mg of the SMPI powder and 100 mg of potassium bromide (KBr) were mixed and pressed at room temperature (25± 2 °C) to form a pellet. Next, FTIR spectra of pellets were recorded in the wave number range of 450–4000 cm^{-1} at the resolution of 4 cm^{-1} at the room temperature (25± 2 °C) using an FTIR spectrophotometer (Model Spectrum 65, Norwalk, Connecticut, PerkinElmer, USA). Spectrum software (Perkin Elmer, version 10, USA) was used for the analysis of the spectra. The spectrum of the pure KBr pellet was used as the background FTIR spectrum before the acquisition of each spectrum (Shahram *et al.*, 2019).

2.7. Differential scanning calorimetry (DSC)

The thermal properties of SMPI were determined using a DSC instrument (Model STA 449 F3 Jupiter, NETZSCH, Germany). Four mg of SMPI were placed in hermetically sealed aluminum pans, which were then heated under nitrogen from 20-200 °C at a rate of 10 °C/min. The temperature of denaturation (T_d) and denaturation enthalpy (ΔH; the area underneath the peak from the thermal curve in J/g of protein isolate) were computed from the thermograms using the Proteus analysis software (Netzsch, Germany) (Feyzi *et al.*, 2018).

2.8. Protein determination

The total protein content of the samples at 1:5 and 1:20 ratios was measured by the micro-Kjeldahl method as descried in AOAC (1995) with a protein conversion factor of 6.25.

2.9. Statistical analysis

In the present study, Complete Randomized Design (CRD) was used to statistically analysis of the effects of solid to solvent ratio in the EHD step on the dependent parameters including extraction yield, PS, WHC, OAC and foaming properties. The data were analyzed by SPSS software (Version 25, Chicago, USA) using Duncan's multiple range test ($p \leq 0.05$) and

were expressed as mean ± standard error (SE). All treatments were replicated at least twice.

3. Results and discussion

3.1. Physicochemical properties

3.1.1. Protein extraction yield

The effect of solid to solvent ratio in the EHD pretreatment on the protein extraction yield, PS, WHC, OAC, FC, and FS of SMPI were investigated and the results are shown in Fig. 2a-e, respectively. Protein extraction yield is affected by the extraction conditions (Feyzi *et al.*, 2018) such as solid to solvent ratio which is one of the important factors in EHD extraction (Li *et al.*, 2019).

As observed in Fig. 2a, the yield of the protein extraction increased from 16.77 ± 1.65 % to 33.01 ± 1.16 % by decreasing the solid to solvent ratio from 1:5 to 1:20 g/mL.

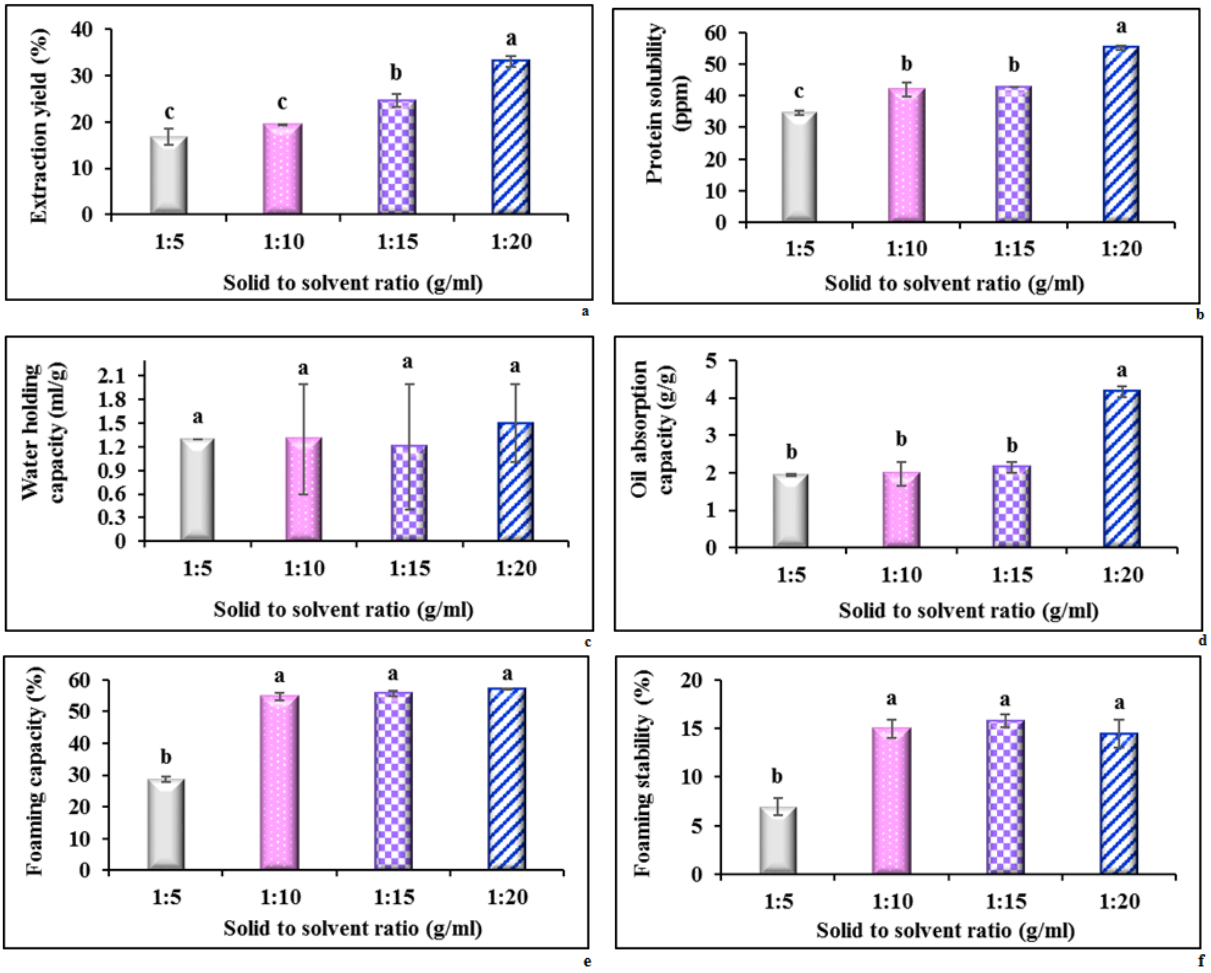


Fig. 2 Effect of solid to solvent ratio in EHD pre-treatment on the protein extraction yield (A), solubility (B), water holding capacity (C), oil absorption capacity (D), foaming capacity (E) and foaming stability (F) of SMPI. Data are resented as mean ± SE. Different letters on bars indicate significant differences ($p \leq 0.05$).

A possible explanation is that a larger volume of the solvent (in the solid to solvent ratio of 1:20 g/mL) could dissolve a larger amount of proteins, which results in a higher extraction yield of the protein isolate (Xi *et al.*, 2017). Furthermore, smaller solid to solvent ratio helps improve the diffusion process because large amounts of solvent can diffuse into the matrix and extract more bioactive compounds, giving rise to a higher yield. High concentration gradient due to low solid solvent ratio enhances the diffusion rate and thus improves protein extraction by the solvent, based on the mass transfer principle, (Li *et al.*, 2019). Therefore, lower solid to solvent ratios result in higher yields of SMPI. However, larger liquid to solid ratio are associated with the problems of handling the exess solvent (Qiayun, 2017). Li *et al.* (2019) reported

similar results during EHD process. They proclaimed that higher extraction yields of isothiocyanates (from rapeseed press cake), proteins and polyphenols (from raw rapeseed) were obtained using lower solid to solvent ratio (1:20 g/g) compared to solid to solvent ratio of 1:5 g/g. Similarly, Xi *et al.* (2017) showed that the extraction yield of phenolic compounds from pomegranate peel was increased with decreasing the solid to solvent ratio from 1:20 to 1:30 g/mL at the fixed voltage of 9 kV. However, further decreasing the solid to solvent ratio to reach the ratio of 1:50 g/mL reduced the extraction yield.

3.1.2. Protein solubility (PS)

PS can be defined as the equilibrium between the protein-protein and protein-solvent interactions (Lam *et al.*, 2016) and has an effective role on flavor, texture and nutritional value of the protein (Du *et al.*, 2018). Fig. 2b shows that the solid to solvent ratio has a significant effect on PS ($P \leq 0.05$). Decreasing the ratio from 1:5 to 1:20 g/mL increase the PS was from 34.63 ± 0.74 ppm to 55.22 ± 0.74 ppm. Upon increasing the water amount, protein-water interactions replace protein-protein interactions when the protein is dissolved in water. Thus, the electrostatic forces and hydrogen bonds in the polar parts of the protein may be damaged by water (Hou *et al.*, 2017). Larger water volumes further damage the electrostatic forces and hydrogen bonds and the destruction of more hydrogen bond leads to more protein solubility (Feyzi *et al.*, 2018). In addition, the mechanism of the protein extraction obeys the dissolution and diffusion kinetics are governed by the driving force related to the concentration gradient of the intended component between the solid and solvent phases (Kain *et al.*, 2009). Thus with increasing the amount of the solvent, the concentration gradient of the intended component (protein) between two phases will be higher and the soluble proteins are extracted more, accordingly. Our results are similar to those of Yue *et al.* (2021), who reported that the presence of the higher amounts of water as solvent greatly improved the solubility of the extracted oat protein.

3.1.3 Water absorbtion capacity

WHC is defined as the water amount which could be absorbed per gram of the protein (Lam *et al.*, 2016). The higher WHC, the more usefulness in viscous food systems such as soups, bakery and confectionary products such as cakes and breads (Du *et al.*, 2018). Fig. 2c shows that the solid to solvent ratio has no significant effect on the WHC of the extracted proteins ($P>0.05$). The WHC of the SMPI varied from 1.2 ± 0.8 to 1.5 ± 0.5 g/g for mung bean protein isolate in alkaline extraction, which was less than the results reported by Du *et al.* (2018). In agreement with our results, He *et al.* (2018) reported that the liquid to solid ratio slightly affected the WHC of the perilla protein isolate.

3.1.4 Oil absorbtion capacity

OAC is defined as the amount of oil absorbed per gram of the protein (Lam *et al.*, 2016). OAC may determine whether the protein isolate will perform well as meat extenders or analogs (Udensi *et al.*, 2006). The results of ANOVA analysis showed that the solid to solvent ratio significantly affected OAC (Fig. 2d). With decreasing the solid to solvent ratio from 1:5 to 1:20 g/mL, the OAC increased from 1.95 ± 0.04 to 4.16 ± 0.14 g/g. The oil absorption mechanism of proteins consists of the binding of the oil with the protein components and is associated with the inclination of the non-polar protein chains to bind to the fat (Ravaghi *et al.*, 2010). More hydrophobic proteins cause extraordinary binding of lipids, as reported by Feranzen *et al.* (1976). The amount of protein has also a direct influence on OAC (Du *et al.*, 2014). Therefore, with increasing the protein amount by decreasing the solid to solvent ratio (as discussed in Fig. 2a) the OAC value would increase. Hydrophobic bonds and electrostatic forces may not be destroyed by water and these parts would increase OAC value (Hou *et al.*, 2017). OAC values obtained in this work are similar to the result of Chen *et al.* (2017), who reported OAC of 4.83 g/g for mung bean protein isolate in alkaline extraction.

3.1.5 Foaming properties

The foaming properties including FC and FS are the important functional properties of proteins. A foam is a gas dispersion in a liquid which can create appropriate textural and sensorial attributes to food products such as toppings and whipped desserts (Xiong *et al.*, 2018). Fig. 2e shows that by decreasing the solid to solvent ratio FC has increased from 28.70 ± 0.93 to 57.03 ± 0.1 g/g. FC is the amount of interfacial surface which can be formed by proteins and is dependent on the rate of the production and stabilization of the newly formed air bubbles to that of their destruction. The increase in the

FC of the extracted protein with decreasing the solid to solvent ratio can be related to the increase in PS. Upon unfolding and orienting their hydrophobic and hydrophilic regions to the gas and liquid phases, respectively, a cohesive layer is generated around the gas bubbles and stable bubbles are formed. At the same time, soluble proteins may reduce the collapse of the bubbles *via* reduction of the surface tension. Consequently, the formation and stabilization rates of the bubbles will be greater than that of their collapse, which enhances the foam capacity (Franzen and Kinsella 1976; Lam *et al.*, 2016). Furthermore, the foaming ability is affected by the amount of extracted protein. Given the highest protein content in the solid to solvent ratio of 1:20, more protein can move to air-water interface, ensuring enhanced FC (Yue *et al.*, 2021). Our results are in disagreement with those reported by He *et al.* (2018) who claimed that the liquid to solid ratio slightly affected on the foaming ability. FC value in the present study was higher than that of the mung bean protein isolate (6.25%) in alkaline extraction in the study of Chen *et al.* (2017).

According to Fig. 2f the solid to solvent ratio significantly affected FS ($P \leq 0.01$). With decreasing the solid to solvent ratio from 1:5 to 1:15, the FS increased from 6.94 ± 0.88% to 15.76 ± 0.64%. There were not any significant differences between the FS in the solid to solvent ratio of 1:10, 1:15 and 1:20. Based on the description of foam stability increasing PS with decreasing the solid to solvent ratio from 1:5 to 1:20 increases the stability of the foam. FS is the protein ability to produce a stable foam against stresses. Stable foams tend to be resistant to gas penetration, drainage and thinning of lamella fluid and mechanical shock. Stable protein foams ought to have interfacial films, which are cohesive *via* hydrogen bond formation and electrostatic and hydrophobic interactions (Lam *et al.*, 2018). As described for FC, increasing the PS results in the generation of a cohesive layer around the gas bubbles and the formation of stable bubbles. Thus, FS is expected to increase by decreasing the solid to solvent ratio due to increment of PS. The FS value of 3 days germinated lentil protein extracted by alkaline extraction was in the range of FS value in this study (Bamdad *et al.*, 2009). However, the values of FS in our study were smaller than those reported by Chen *et al.* (2017) for mung bean protein isolate (57.5%).

In conclusion, the solid to solvent ratio of 1:20 in the EHD pretreatment resulted in the highest protein extraction yield, PS, OAC, FC and FS. Therefore, the solid to solvent ratio of 1:20 was considered as the optimal value.

3.2. FTIR analysis

Fig. 3 shows the FTIR spectra of SMPI in the solid to solvent ratios of 1:5 and 1:20. The bands at 3302 cm^{-1} and 3416 cm^{-1} are related to amide A, (Hou *et al.*, 2017). The peaks at 2928 cm^{-1} can be attributed to the $–CH_2$ asymmetric vibration {Formatting Citation}. $–CH_2$ groups are found in the aliphatic chain of proteins and lipids (Rodsamran *et al.*, 2017).

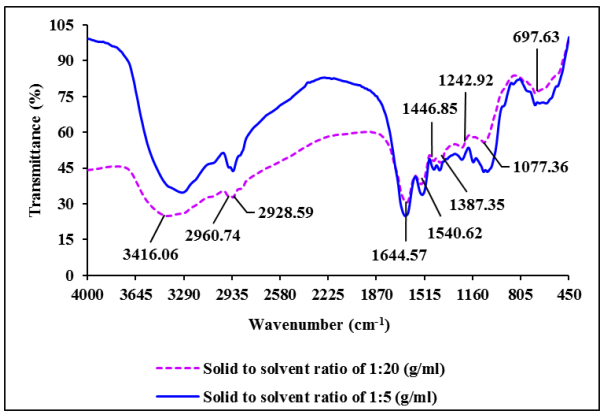


Fig. 3. FTIR spectra of SMPI in the solid to solvent ratio of 1:5 (g/mL) and 1:20 (g/mL).

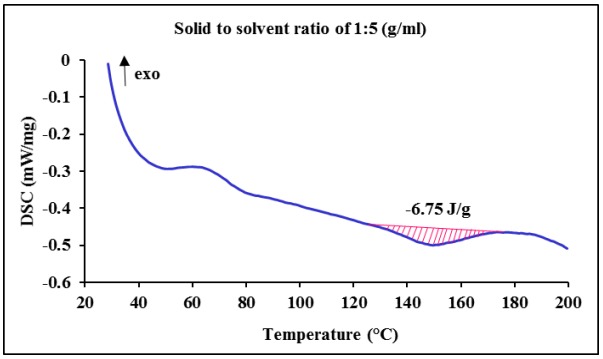
The bands at 1657 cm^{-1} and 1644 cm^{-1} are associated with the stretching vibrations of the C=O and C–N groups, which are usually assigned to amide I (Su *et al.* 2010; Zeng *et al.*, 2011). Amide I band in the secondary structure of proteins showed that the peaks in the range of 1638-1645 cm^{-1} and 1645-1662 cm^{-1} correspond to the random coil and α-helix structures, respectively (Hou *et al.*, 2017). Therefore, with decreasing the amount of solid to solvent ratio from 1:5 to 1:20, the α-helix structure was transformed to the random coil structure, which means that lower solid to solvent ratios break hydrogen bands resulting in the greater solubility in the solid to solvent ratio of 1:20 (Feyzi *et al.*, 2018) as discussed in section 3.1. The results are similar to those of Yue *et al.* (2021) who reported that with increasing the amount of water as the solvent for extraction of oat protein, the content of α-helix structure in the extracted oat proteins decreased. The major bands at 1532 cm^{-1} and 1540 cm^{-1} are ascribed to the in-plane N–H bending, C–N stretching vibrations, and C–C stretching vibrations, which represent the region of amide II (Zeng *et al.*, 2011).

Similar band regions were observed in the FTIR spectra of amides I and II, (1630-1660 cm^{-1}) and (1520-1550 cm^{-1}), respectively, in most plant proteins such as pennycress press cake protein, lotus seed protein and coconut milk cake protein concentrate (Rodsamran *et al.*, 2017). The bands at 1240 cm^{-1} and 1242 cm^{-1} are ascribed to the in-phase state coupled of N–H bending and the C–N tensile vibration with small contributions from C–O in-plane bending, respectively, and the absorption peak of amide III is assigned to C–C stretching vibration (Barth, 2007). Thus both samples have main (amides I, II and III) and subsidiary (amide A) structures of proteins. The absorption bands at 1079 cm^{-1}, 1051 cm^{-1} and 1077 cm^{-1} are due to the vibrations of the OH, CC, CO, CCH, COH and POC bonds of lipids and protein, respectively (Kudre *et al.*, 2013) and could be attributed to the symmetric stretching modes of PO_2^- (phosphodiester groups in nucleic acids) (Palaniappan *et al.*, 2015). Higher OAC in the solid to solvent ratio of 1:20 is due to the presence of two bands at 1051 cm^{-1} and 1077 cm^{-1} which show more lipid availability for this sample.

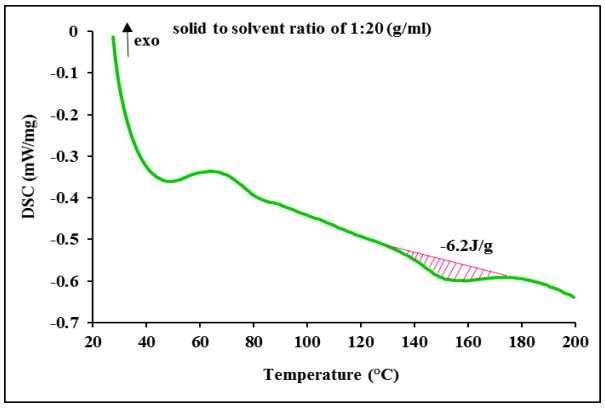
3.3. Differential scanning calorimetry (DSC)

The thermal stability of a protein, which shows its resistance to aggregation upon heating, is demonstrated by denaturation temperature (T_d) and denaturation enthalpy (ΔH). T_d represents the temperature at which denaturation occurs whereas ΔH is the amount of heat required to induce denaturation (Feyzi *et al.*, 2018). T_d and ΔH of SMPI samples extracted using different solid to solvent ratios (1:5 and 1:20 g/mL) by EHD pretreatment are shown in Fig. 4a and b, respectively.

There were no drastic differences between the ΔH values of the samples, but the T_d of the sample extracted at the ratio of 1:20 was 160°C which was higher than that of the sample extracted at the ratio of 1:5 (149.7°C). This means that the increase of water as a solvent drastically affected T_d. Therefore, the sample extracted using the solid to solvent ratio of 1:20 was more thermostable than that extracted using 1:5 ratio. The difference in the T_d of SMPI was determined by the transition between the broken bonds during thermal processing. Increasing of water may change the polarity of the solution, which may alter the structure and thermal stability of the sprouted mung bean protein (Yue *et al.*, 2021). Increased water content enhances the contacts between hydrophilic residues in protein design and may give rise to more stable protein. Water was found to cause loose binding by acting as a buffer, which reduces the undesired polar interactions (Levy *et al.*, 2004).



a



b

Fig. 4. DSC thermograms of SMPI obtained from two treatments of **(a)** 1:5 solid to solvent ratio and **(b)** 1:20 solid to solvent ratio.

Chen *et al.* (2017) reported that the values of ΔH and T_d for mung bean protein isolate were 59.70 j/g and 73.75 °C, respectively. The smaller value of ΔH in our study indicates that more denaturation has probably occurred due to EHD pretreatment.

4. Conclusion

In this research the effects of solid to solvent ratio (at 4 levels of 1:5, 1:10, 1:15 and 1:20 g/ml) on protein extraction yield, protein solubility (PS), water holding capacity (WHC), oil absorption capacity (OAC) and foaming properties of sprouted mung bean protein isolate SMPI were examined. Water plays a significant part in changing the protein conformations and

considerably affects the physicochemical properties of proteins. The Results showed that the solid to solvent ratio of 1:20 led to the highest protein extraction yield, PS, OAC and foaming properties. According to the results of Fourier transform infrared spectroscopy, the samples have the main protein structure. α-helix structure transformed to random coil structure in solid to solvent ratio of 1:20 and caused more PS under these conditions. The results of differential scanning calorimetry showed that the highest thermal stability occurred at the solid to solvent ratio of 1:20. Thus, this study confirms the hypothesis of improving the extraction of protein from mung bean sprouts by the application of electrohydrodynamic process using solid to solvent ratio of 1:20.

5. References

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