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Research Article

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The effect of extraction and isoelectric pH values on functional and thermal properties of tahini meal protein

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ABSTRACT - Tahini is an important product of peeled sesame. As a byproduct of oil extraction from tahini, tahini meal (TM) is a valuable protein source. However, it is only used to feed livestock. TM can be used as an affordable protein source in human diet. In this research work, the effects of extraction pH (10, 11 and 12) and isoelectric pH (4.5, 5.5 and 6.5) on functional and thermal properties of proteins isolated from TM were studied. Functional properties of extracted proteins including water and oil absorption, foaming capacity and foam stability as well as thermal properties were measured. The results showed that the highest protein extraction efficiency (87%) was obtained in extraction pH at pH≥11 and isoelectric pH 6.5. In addition, the highest levels of water and oil absorption capacity were obtained in proteins precipitated at pH 6.5 (P≤0.05). The highest level of foaming capacity and stability was observed in proteins extracted at pH 11 and precipitated at pH 6.5. On the other hand, thermal properties of TM protein including onset denaturation temperature (T_m), denaturation peak temperature (T_d) and denaturation enthalpy (ΔH_d) were affected by sediment pH and pH 6.5 led to the highest thermal characteristics. Since the optimum characteristics of proteins isolated from TM were obtained at isoelectric pH 6.5 and extraction pH 11, these pH values are recommended for the extraction and precipitation of TM proteins, respectively.

INTRODUCTION

Now a day, plant based proteins play a vital role in human nutrition, especially in developing countries where the mean protein intake does not satisfy the minimum requirements of the body (Kanu et al., 2007). The shortage of animal based protein sources has intensified attempts made to find new protein sources with suitable functional properties and nutritional value (Egorova et al., 2017). On the other hand, it has been reported that consuming plant based proteins is more cost effective compared to animal based proteins (Muhamyankaka et al., 2013). Sesame, *Sesamum Indicum*, Linn, is the seed of an herbaceous plant of the family, Pedaliaceae. In 2019, the total production rate of sesame was 6,549,725 million tons (FAOSTAT, 2019). It ranks 8th in the global ranking in terms of production rate The contribution of Iran to the production of sesame is 29 thousand tons (FAOSTAT, 2019). Sesame is a rich source of protein with a high nutritional value. The protein content of sesame seed is approximately 25% varying from 17% to 31%, depending on the seed source. Protein is mainly stored in sesame seed as globulin (67.3%), albumin (8.6%), prolamin (1.4%) and glutelin (6.9%) (Achouri et al., 2012; Inyang and Iduh, 1996). It is having fat contents of about 80% unsaturated and 20% saturated fatty acids (Onsaard et al., 2018).

Tahini is a processed product of sesame. Traditionally, it is consumed with sweet substances such as honey and grape syrup in the Middle East countries and it is an important ingredient of Halva (one kind of dense, sweet confection with roots in the Middle East, Central Asia, and India). Tahini is actually an oily liquid which is derived from peeled sesame. It constitutes from 24.7% protein, 59% fat, 2.3% fiber and 3% mineral substances (Torlak et al., 2013). It has been reported

that in Tunisia, sesame is useful for manufacturing halaweh, a food product which is produced by mixing dehulled, roasted, and ground white sesame seed (tehineh), root of *Saponaria officinalis*, and caramelized sugar (Elleuch et al., 2012). To produce tahini, the anti-nutritional compounds are removed from sesame by removing the bran. The presence of phytic acid and oxalic acid in the shell of sesame has been reported (Ishii and Takiyama 1994; Kapadia et al., 2002). Tahini oil is increasingly being produced in factory as a product. Consequently, the production of tahini meal (TM) as a byproduct of tahini, is being increased. In last, tahini meal was only used to feed livestock (Jahandideh et al., 2013).

By evaluation the functional properties of TM protein, in addition to improving nutritional value, it can be used in more food formulation. In the bakery products especially gluten free foods, isolated protein was used frequently as gluten matrix imitative (Crockett et al., 2011 and Ribotta et al., 2004). Also lack of protein in pastry product causes improper appearance, lesions, stickiness of the strands to each other and weight loss during cooking (Adegunwa et al., 2012 and Phongthai et al., 2017). In this study to provide TM protein, the sesame isolated protein was prepared through alkaline extraction and acidic sedimentation in three points of isoelectric point. Its functional and thermal properties were evaluated in order to determine suitable industrial applications.

MATERIALS AND METHODS

TM sample was prepared in a traditional sesame oil extracting workshop in Ardakan, Yazd, Iran. The required tahini was prepared from Pakistani sesame and its' meal was dried for 3 days under sun in a traditional manner. Then, it was completely turned to pure flour using an industrial hammer grinder (Retsch Co., Germany). All chemicals were prepared from Merk Company (Germany).

Evaluation of Physiochemical Properties of Tahini Meal

Proximate composition of TM including moisture, protein and ash were measured according to approved methods of the American Association of Cereal Chemists (AACC, 2000). Amount of crude fiber content was measured according to the Ranganayaki et al. (2012).

Extraction of TM Protein

A total amount of 50 g TM powder was mixed with liquid alkaline solution (1:18 ratio). pH of the solution was set at alkaline range by using sodium hydroxide, at three pH levels of 10, 11 and 12. Then, 0.1% Na_2SO_3, 4.38 g NaCl and 1.5 g SDS were added to the solution and the solution was stirred for half an hour. The above mentioned compounds were used to separate phenols and phytates. The insoluble portion of the mixture was separated by centrifugation at 2688 *g* for 15 min. In this way, protein was extracted in supernatant and other compounds were separated in subnatant (Kaur and Singh, 2007).

The pH of supernatant, obtained in the previous stage that was containing protein, was adjusted to 4.5, 5.5 and 6.5 by HCl (6 M) to determine the best precipitation pH for the highest protein quality. The pellet was centrifuged at 2688 *g* for 15 min and washed by distilled water 5 times to reach pH 7 (Kaur and Singh, 2007; Cho and Rhee, 2004). The isolated protein of TM was frozen immediately by liquid nitrogen and dried by a freeze dryer (Operon Co., Korea), then kept in capped containers in room temperature (Rhee, 2004).

Measurement of the Functional Properties of the Isolated Protein of TM

Protein Extraction Yield

Protein content was measured by the Kjeldahl method (AOAC, 2002) by applying a conversion factor of 6.25. Also, Protein-extraction yield was calculated according to Formula (1) (Fetzer et al., 2018).

Protein extraction yield [%] =

$$\frac{\text{mass (extract)[g]} \times \text{protein content (extract) [\%dm]}}{\text{mass (sample) [g] protein content (sample) [\%dm]}} \times 100 \qquad (1)$$

Water Absorption

Water absorption was measured using Kaur and Singh's method (Kaur and Singh, 2007). 0.3 g of the isolated protein sample by 0.75% moisture content was added to 25 mL distilled water and the obtained solution was stirred for 5 min in a pre-weighed centrifuge tubes. After 30 min, it was centrifuged again for 25 min, then surface substance was separated, and the remaining sediment was heated for 25 min at 50 ^{o}C. Then, the sample was weighed and the water absorption recorded as g water/g dry sample (Kaur and Singh, 2007).

Oil Absorption

0.5 g of the isolated protein of TM was added to 6 mL corn oil in a pre-weighed centrifuge tube. The solution was stirred for 1 min and left for 30 min. The mixture was centrifuged for 25 min and separated fat removed using a pipette. The centrifuge pipe was repositioned upside down in order to drain the remained fat from the solution and to reweigh the substance. Oil absorption was recorded as g oil/g dry sample (Kaur and Singh, 2007).

Foaming Capacity (FC) and Foam Stability (FS)

3 g sample of protein was added to 100 mL distilled water and half of the solution were blended for 3 min using a magnetic stirrer (Corning PC-420D, USA) at the highest speed, poured into 250 mL graduated cylinders, and the volume of foam were immediately recorded and the volume increase percentage, as an index of foaming capacity, was calculated using the following formula:

Volume increase [%] =

$$\frac{\text{volume of solution after mixing} - \text{initial volume of solution}}{\text{initial volume of solution}} \times 100 \quad (2)$$

To determine foam stability, the solution was transferred to a graduated cylinder and the volume of the remaining foam after one hour at 25°C was recorded. Percentage of this foam volume to the initial volume considered as a foam stability (Kaur and Singh, 2007; Martins, et al., 2006).

Differential Scanning Calorimetry (DSC)

5±0.01 mg of the isolated protein of TM poured into small DSC aluminum containers and their caps fastened tightly. Onset denaturation temperature (T_m), denaturation peak temperature (T_d) and denaturation enthalpy (ΔH_d) measured by heating the sample in DSC containers (model DSC1 Mettler Toledo). The DSC was first calibrated in terms of temperature and enthalpy using Indium (T_m, onset=156.6ºC, ΔH_d=28.45J/g). A small water container (with the same weight) was considered as a test control. The samples were heated from 20°C to 120°C at a heating speed of 5°C/min. Tm as the onset temperature of denaturation and Td as the peak denaturation temperature were determined. ΔH_d calculated by computing the area under protein denaturation curve, which is a heat absorbent reaction, using the STARE system.

Statistical Analysis

Results were reported as averages of each of the three replications. In order to assess significant differences among the samples, a complete randomized design of triplicate analyses of the 9 samples was performed using the MSTATC program (version 1.42). Duncan's new multiple range tests were used to study the statistical differences of means with 95% confidence.

RESULS AND DISCUSSION

Physiochemical Properties of TM

The proximate composition of TM is shown in Table 1. As expected, this product had more than 35% protein and it could be used as a complementary nutrient in carbohydrate foods such as pasta.

Table 1. Physicochemical properties of tahini meal

Properties	Amount (%)
Moisture	9.31 ± 0.02
Ash	6.86 ± 0.05
Crude fiber	7.08 ± 0.02
Protein	35.5 ± 0.15
Oil	16.01 ± 0.09

Functional Properties of the Isolated Protein of TM

Protein Extraction Yield

Fig. 1 shows the effects of extraction pH and sedimentation pH on the protein extraction yield. As shown in Fig. 1, protein extraction increased by increasing pH; but no significant different was observed in extraction pH between pH 11 and pH 12. On the other hand, the highest amount of protein sedimentation was observed at pH 6.5, therefore isoelectric pH of Tahini meal protein was determined to be pH 6.5. The highest amount of protein extraction yield was 87% that obtained in extraction pH 11 or 12 and sedimentation pH 6.5. In this regard, Gupta et al. (2018) investigated effect of extraction temperature on functional properties of rice bran protein concentrates (RBPCs) and revealed the region of least nitrogen solubility was pH 4, which corresponded to the isoelectric point of RBPCs while the maximum nitrogen solubility was observed at pH 10. In this studt, a significant difference was observed in nitrogen solubility as the pH was increased from 2 to 10 (p<0.05). Also, the results of this study are consistent with the findings of Naghizadeh Raeisi et al., (2019).

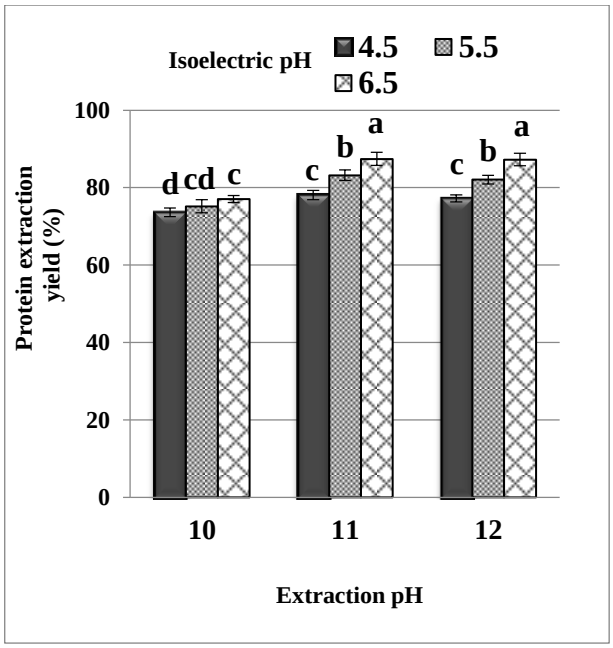

Fig. 1. Protein extraction yield of tahini protein isolate extracted at different isoelectric and extraction pH. Different letters show significant difference (P<0.05). Vertical bar shows Standard Error of the difference (SE).

Water Absorption

Water holding capacity as an index of capacity of protein to absorb and retain moisture is an important index in the manufacture of bakery and meat products, pasta and similar materiala. (Demirhan et al., 2013). Water absorp capacity of isolated protein influenced heavily by isoelectric pH (Fig. 2).The highest and the lowest level of water absorption capacity were obtained at isoelectric pH 6.5 and pH 4.5, respectively. The extraction pHs used in this study had no significant effect on this property of TM (P≤0.05). As it is shown in Fig. 2, water absorption capacity in three levels of extraction pH was almost the same.

Environment pH can effectively influence the water absorption capacity of proteins due to changes to the charged groups on protein surface (Ragab et al., 2004).

Due to the low presence of non-protein compound in the isolated protein in samples by high protein extraction yield, the polar amino groups of protein molecules become the main water-protein interaction locations. Therefore, the rate of polar amino acids which absorb more water has been reported to be higher in the isolated protein (Yadav et al., 2018). When pH is below the isoelectric point, particles repel each other by repulsive force because the protein charge is positive. When pH is above isoelectric, protein particles are influenced by repulsive force, repel each other, and remain in solution form and less settled because of the negative charge of protein. On this basis, in this study, the amount of protein at pH 6.5 was higher in comparing to those at the other two pH levels.

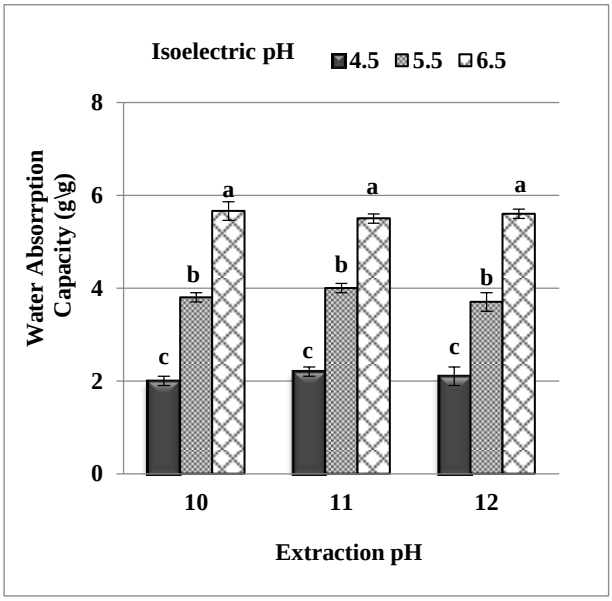

Fig. 2. Water absorption capacity (WAC) of tahini protein isolate extracted at different isoelectric and extraction pH. Different letters show significant difference (P<0.05). Vertical bar shows Standard Error of the difference (SE).

Oil Absorption

Oil absorption is one of the most important properties of isolated protein of TM. This trait of isolated protein was affected by isoelectric and extraction pH in this research. Maximum oil absorption of isolated protein was achieved at isoelectric pH 6.5 and extraction pH 11 and 12, whereas minimum of it was seen in isolated protein obtained at isoelectric pH 4.5 and extraction pH 10 (Fig. 3). The higher oil absorption capacity of the isolated protein at high isoelectric and extraction pH levels may be rooted in the presence of more non-polar amino acids in the isolated proteins. Therefore, the non-polar chains may be bonded to the hydrocarbon chains of oils. It has been reported that this, in turn, increases oil absorption (Jahandideh et al., 2013; Kaur and Singh, 2007). On the other hand, according to Kuar and Singh's (2007), oil absorption depends on the number of non-polar side chains over the protein, that bound to the hydrocarbon chains along fatty acids. Oil absorption is an important property from an industrial point of view because it has a direct relationship with emulsifying capacity, which is an ideal and important property in some products such as mayonnaises. The high oil absorption capacity of proteins is necessary in some foods such as breakfast cereals, meat replacements and extenders, sweets, soups and bakery products.

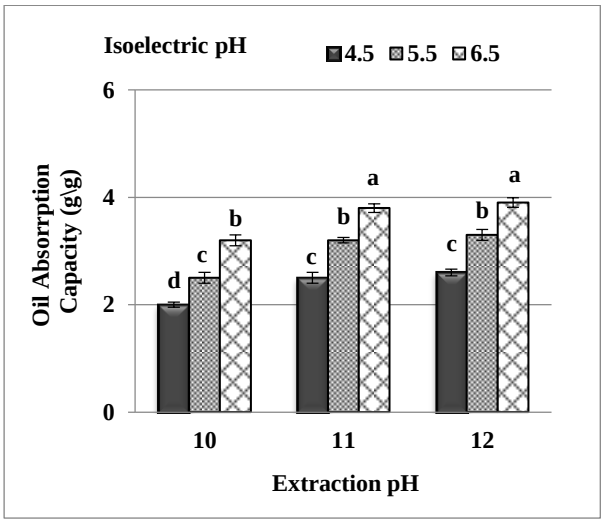

Fig. 3. Oil absorption capacity (OAC) of tahini protein isolate extracted at different isoelectric and extraction pH. Different letters show significant difference (P<0.05). Vertical bar shows Standard Error of the difference (SE).

Foaming Capacity and Stability

Foaming capacity and stability of protein isolated from TM at different extraction and isoelectric pH were measured in three replications and mean of them showed that maximum foaming capacity of protein was appeared in the isolate extracted at pH 11 and then sedimented at pH 6.5 (Table 2). It means that suitable pH for extracting and sedimentation of the protein isolated from TM are 11 and 6.5, respectively. These results are consistent with the observations of Akintayo et al. (1999), Gupta *et al*. (2018) and Kaur and Singh (2007).

Foaming stability of TM protein 60 min post-extraction was also affected by extraction and isoelectric pH as foaming capacity (Table 2). Maximum foaming stability of protein, i. e. 64.7%, was observed at pH 11 and pH 6.5 of the extraction and isoelectric, respectively. Minimum stability, 50.4%, was appeared in protein isolated at pH 10 of the extraction and pH 4.5 of the isoelectric. Presence of fat destabilizes the thin layers of protein. Therefore, foaming capacity and stability significantly increases in isolated proteins due to the elimination of a large amount of fats. Increasing of solubility of protein at alkaline pH may help protein flexibility and inhibits hydrophobe-hydrophobe interactions between protein molecules (Tan et al., 2014).

Thermal Properties

Protein denaturation causes structural or conformational changes to the initial structure of protein without any change in the sequence of amino acids (Xu et al., 2012). The data of these thermal properties including onset denaturation temperature (T_m), denaturation peak temperature (T_d), and area under protein denaturation curve, ΔH_d of the protein isolated out of TM in various pH of extraction and isoelectric are shown in Table 3. There was no significant difference among three levels of protein extraction pH based on T_m, T_d and ΔH_d at the same isoelectric pH. But proteins isolated at different isoelectric pH had significant differences in all thermal properties (T_m, T_d and ΔH_d). Maximum and minimum ranges of T_m, T_d and ΔH_d were observed in protein isolated at sedimentation pH of 6.5 and of 4.5, respectively. It has been shown that the differences among protein structures as well as heat temperatures in the different stages of preparing meal out of tahini may make some changes to the thermal stability of protein (Scopes, 1994).

Denaturation changes of a protein are heat absorbent reactions represented as a peak point in DSC thermo gram (Parniakov et al., 2018). DSC is a valuble method for testing the thermal properties including T_m and T_d. T_m and T_d are measures of protein denaturation influenced by temperature degree and protein concentration (Escamilla-Silva et al., 2003; Saini et al., 2018). The thermal properties of many proteins including barley globulin (Harwalkar and MA 1987) and red bean globulin (Meng and Ma, 2001) were studied using the DSC method.

CONCLUSIONS

Tahini meal is a byproduct of oil extraction from peeled sesame that has high nutritional value while being used only as a food. In this research, the effect of different protein extraction conditions on functional and thermal properties of tahini meal protein were investigated. The results showed that protein extraction yield increased up to 87% by regulating extraction pH and isoelectric pH. It was also observed that the protein isolate that extracted at pH 11 and sedimented at pH 6.5 had favorable functional properties such as water and oil absorption capacity, foaming capacity and stability and acceptable thermal characteristics (T_m, T_d and ΔH_d). So, it can be said that the protein extracted from Tahini meal as a low-cost protein source can be used in food formulations to increase the nutritional value and improve the technological properties of the final product.

Table 2. Foaming capacity and stability of TM protein isolated in different conditions.[a]

Extraction pH	Isoelectric pH	Foaming capacity (%)	Foaming Stability (%)
10	4.5	22.3 ± 0.3^{d}	50.4 ± 0.5^{e}
	5.5	27.1 ± 0.2^{cd}	53.3 ± 0.7^{d}
	6.5	30.3 ± 0.4^{c}	58.4 ± 1.1^{c}
11	4.5	29.4 ± 0.7^{c}	58.1 ± 0.8^{c}
	5.5	35.1 ± 0.8^{b}	61.3 ± 0.6^{b}
	6.5	40.6 ± 1.0^{a}	64.7 ± 0.3^{a}
12	4.5	27.4 ± 0.6^{cd}	55.1 ± 0.6^{d}
	5.5	33.2 ± 0.8^{bc}	57.7 ± 0.7^{c}
	6.5	35.5 ± 0.6^{b}	61.3 ± 1.0^{b}

Table 3. Thermal properties of different treatments of tahini meal protein isolate

Extraction pH	Isoelectric pH	DSC properties		
		Tm (°C)	Td (°C)	ΔHd
10	4.5	89.18 ± 1.14^{c}	97.00 ± 1.28^{c}	0.28 ± 0.14^{c}
	5.5	95.45 ± 0.98^{b}	106.3 ± 1.33^{b}	2.64 ± 0.15^{b}
	6.5	100.9 ± 0.65^{a}	110.2 ± 1.04^{a}	4.96 ± 0.08^{a}
11	4.5	88.87 ± 1.08^{c}	97.16 ± 1.08^{c}	0.32 ± 0.12^{c}
	5.5	96.12 ± 0.74^{b}	107.8 ± 1.11^{b}	2.72 ± 0.11^{b}
	6.5	101.2 ± 0.76^{a}	111.5 ± 1.21^{a}	5.11 ± 0.10^{a}
12	4.5	89.09 ± 0.826^{c}	96.60 ± 0.96^{c}	0.25 ± 0.09^{c}
	5.5	95.73 ± 1.11^{b}	107.1 ± 1.01^{b}	2.50 ± 0.12^{b}
	6.5	101.5 ± 0.90^{a}	102.0 ± 0.88^{a}	4.84 ± 0.08^{a}

Values are mean ± SD of three replicates. Different letters in the same parameter are significantly different (P<0.05)
T_m: Onset denaturation temperature. T_d: Denaturation peak temperature, DSC: Differential Scanning Calorimetry
ΔHd: denaturation enthalpy

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بررسی تأثیر pH استخراج و ایزوالکتریک بر خصوصیات عملکردی و حرارتی پروتئین کنجاله ارده

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واژه‌های کلیدی:
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بازده استخراج پروتئین کنجاله ارده
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چکیده- ارده یکی از محصولات مهم به‌دست آمده از دانه کنجد پوست‌گیری شده می‌باشد. کنجاله ارده بعنوان یک محصول جانبی در استخراج روغن از کنجد تولید می شود و یک منبع پروتئینی ارزشمند می باشد. با وجود این، در حال حاضر فقط به مصرف دام می‌رسد، در حالی که می تواند بعنوان یک منبع پروتئینی با ارزش و قابل دسترس در رژیم غذایی انسان استفاده شود. در پژوهش حاضر، تأثیر pH استخراج (۱۰، ۱۱ و ۱۲) و pH ایزوالکتریک (۴/۵، ۵/۵ و ۶/۵) بر خصوصیات عملکردی و حرارتی ایزوله پروتئینی کنجاله ارده مورد مطالعه قرار گرفت. خصوصیات عملکردی پروتئین استخراج شده نظیر جذب آب و روغن، قدرت تشکیل کف و پایداری آن و ویژگی‌های حرارتی ارزیابی شد .نتایج نشان داد که بیشترین میزان بازده استخراج پروتئین (۸۷ درصد) در pH استخراج ۱۱ و ۱۲ و pH ایزوالکتریک ۶/۵ بدست آمد. بیشترین میزان جذب آب و روغن از کنجاله ارده در پروتئین ترسیب شده در pH ۶/۵ گزارش شد ($P\leq0.05$). بالاترین میزان کف و پایداری آن نیز در pH استخراج ۱۱ و pH ایزوالکتریک ۶/۵ مشاهده گردید. از سوی دیگر بالاترین میزان خصوصیات حرارتی پروتئین کنجاله ارده نظیر دمای اولیه دناتوراسیون (T_m)، نقطه اوج دمای دناتوراسیون (T_d) و آنتالپی دناتوره شدن (ΔH_d) در pH ایزوالکتریک ۶/۵ ملاحظه شد. از آنجایی که بهترین خصوصیات ایزوله پروتئینی کنجاله ارده در pH ایزوالکتریک ۶/۵ و pH استخراج ۱۱ مشاهده گردید، در نتیجه این شرایط به منظور استخراج و ترسیب پروتئین موجود در کنجاله ارده پیشنهاد می‌گردد.