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Optimization of Protein Extraction and Evaluation of Functional Properties of Tomato Waste and Seeds from Tomato Paste Plants

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There is an increasing interest to bio-components obtained from fruit and vegetable wastes. Response surface methodology (RSM) was used optimization of condition for extraction of protein from tomato waste and seeds. So the independent variables, alkaline and acidic pH (10-12 and 3.1-4.3), temperature (10-50°C), time (30-70min) and solvent to powder ratio (1:10-1:50w/v) were used. Also, the functional properties of fat and defatted proteins were evaluated. The results showed that the pH 12.00 for first and 3.73 for the second precipitation phase, temperature 37.73°C, time 60 min, solvent to powder ratio 1:40 were the best conditions of extraction. The responses in this condition, Protein Extraction Yield to Defatted Tomato Waste 86.84%; Defatted Tomato Waste Protein 35.29%; Protein Extraction Yield to Defatted Tomato Seeds 64.15% and Defatted Tomato Seeds Protein 44.65% were measured. Also, the results showed that the lowest of bulk density were for Tomato Waste Protein and Tomato Seeds Protein. The Water Absorption Capacity was increased to 55°C, while, the Oil Absorption Capacity were increased to 75°C. The Emulsification Activity Index and Emulsification Stability Index were increased along with pH, but the Emulsification Stability Index was highest at pH 7. The Foaming Capacity and Foaming Stability had significantly increased same to pH ($p<0.05$).

Keywords: Response surface methodology, tomato, waste, seeds, protein concentrate.

Tomato (*Solanum lycopersicum* L.) is member of family *Solanaceae*. Tomato is one of the most popular a garden crop of much interest, being widely used either fresh or processed such as salads, juice, soup, puree, paste, sauce, ketchup and salsa[1-6]. In 2015, Iran is ranked the world's sixth largest producer of tomato. Iran produces about 6,000,000 tons of fresh tomatoes per year[7]. It well known that, tomato is one of the most consumed vegetable in the world approximately 30% is consumed as processed products. Global processing tomato production this year is expected to increase 6% to reach 42.24 million tons, according to the latest estimate by the World Processing Tomato Council[8]. Besides its economic importance, the nutritional value of its vegetables and fruits, the tomato, one of the main worldwide agricultural and horticultural crops, is rich in a large number of natural antioxidant compounds. The use of tomato products has been related epidemiologically to a lower incidence of gastrointestinal diseases, cardiovascular, epithelial cell cancers and prostate[9]. When tomatoes are cooked or processed do not lose their health benefits. In cooked and processed tomatoes (salsa, paste, sauce, canned tomatoes, etc.), lycopene absorption is more easily than fresh tomatoes. So,

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tomato and its products decrease the risk of heart diseases and cancer of the prostate, lung and stomach due to the existence of antioxidant components, in particular, lycopene and b-carotene, flavonoids and tocopherols and ascorbic acid[2,5,10].

The food wastes contenting of high-quality nutrients that could be extensively used as fertilizer or feed and food. Industrial by products produced from vegetables and fruits processing and cooking represent a major problem for industry concerned. However, they are also valuable source of nutrient compounds which may be used for different purposes in feed or food and other industries[11]. The production units of tomato paste are created 7.0–7.5% solid waste and 71–72% pomace[3, 12, 13]. The wastes are about 3-5 percent of total weight of tomato that have been proposed according to assessments by the World Council[14]. Also, waste of the tomato juice and pulp extraction process are contained lots of seeds, fibrous, skin and the major fundamental of the pomace including of 22-34% protein and 21–30% lipids[3, 12, 13]. In Iran, the major part of tomato waste uses for livestock feed and some extent as fertilizers, but it has not been used for human consumption, although this waste contains valuable antioxidants, carbohydrates and proteins[5, 15].

The seed protein could be extracted to produce protein isolate/concentrate[16].Tomato seed protein is rich of lysine in range of 80 to 100 g/kg N, therefore, could be improve the quality of cereal protein products. The tomato seed protein can be used to improve the physicochemical characteristics such loaf volume, texture and anti-staling[12, 13]. In world, the people use of tomato as a very beneficial vegetable in daily meals. In the USA, about 57 % of daily lycopene absorbs come from cooking and processing tomato products and only 12 % from fresh tomatoes[17].Also, in western countries, the food products based of tomato include 85% of dietary lycopene[18]. Also, tomatoes are cheap and they have lowest levels of anti-nutritional factors in comparison with other vegetables[13]. For several years, the food scientists have done extensive studies in the field of tomato waste reusing and extraction of effective their materials, such as antioxidants, carotenoids and proteins[19].Haddad Khodaparast *et al.*,[20] investigated the production of the protein concentrate from tomato waste. They selected the most appropriate conditions for protein extraction from tomato waste in alkaline pH 8-12, temperature 20-70 °C and acidic precipitation in pH 3.3-5.5. The results showed that the pH of the first phase 12 and for the second phase 3.9 in 25°C were the best condition to produce tomato waste protein. Shao *et al.*[4]evaluated functional properties of two proteins from tomato waste in two product condition (hot and cold break). The results showed that the lower protein extraction yield from 9.1 % to 26.3 % for defatted hot break tomato seed compared to from 25.6 % to 32.6 % for defatted cold break tomato seed under two conditions. Sogi *et al.*[21]evaluated of the functional properties of tomato seed meals and protein. Their results showed that the fat and water absorption for protein were highest. Also the bulk density for the salt-extraction was the highest. Emulsifying capacity values, as well as, water and oil absorption showed that meal had a good wettability and can blend well with oil and water systems. Foaming properties of meal were very poor since foam structure was very weak, and foam capacity and stability at room temperature was also low. The pH values for meals and protein concentrates were neutral and acidic, respectively. The foaming capacity and stability of meals and protein were low. The emulsion capacity of meals and protein was good, while the emulsion stability was excellent except for alkali-extracted. In general, tomato seeds protein isolates produced emulsions with greater globule size as compared to soybean protein isolate (SPI); however, both the concentrates were equally effective in constancy of emulsions.

Response surface methodology (RSM) has been widely used to analyze or to optimize the independent factors which influenced the extraction yield or extract profiles of valuable components of natural materials[22]. The aim of this study was to determine the best extraction conditions of protein from tomato waste and seeds by optimizing extraction parameters. The extraction process was optimized by controlling pH, temperature, time and solvent to solid ratio to maximize protein yield. The second aim was to assess of functional properties of fat and defatted tomato waste protein and tomato seeds protein.

MATERIAL AND METHODS

Material

The chemical reagents and other material that used in study for example NaOH, HCl, Hexane and etc., were purchased from Merck (Merck, Darmstadt, Germany).

Methods: Protein preparation from tomato waste and seeds

Tomato protein concentrate from fat and defatted Tomato Waste Meal (TWM and DTWM), Tomato Seeds Meal (TSM and DTSM) were separated from the pulp, which had been collected from a tomato paste manufacturing plant located at Mashhad (Iran), by a sedimentation technique. Tomato waste and seeds dehydrated at 50 °C for 10 h in a dryer with air condition (Memmert, GmbH, Germany), then crushed by using a laboratory mill (Toos Shekan, Mashhad, Iran) to pass through a 80 mesh screen; for make defatted meal, fat was extracted with hexane in ratio 1:50 w/v, then desolventized with centrifuge at 5000×g (Sigma, Osterode am Harz, Germany) , and powder sieved again with 80 mesh screen (whole meal). Protein concentrates from defatted TW and TS were prepared in five ratio of solvent (distilled water) to TW and TS powder 1:10 to 1:50, in two phase, first, alkaline phase with NaOH 0.1 N at 10 to 50°C for 30 to 70 min and pH 9 to 13 in five point and centrifuge at 2600×g for 10 min and then solution phase were separated, second, acidic phase with HCl 0.1 N to pH 3.1 to 4.3 in 5 point (isoelectric region) and centrifuge at same above condition. Finally, proteins concentrates dried with freeze dryer (Martin Crist, Osterode am Harz, Germany) and hold at -18°C to before use them.

Determination of Protein

A titration method (Kjeldahl method) was used for determination of protein concentrates[23]. In this method, the protein content (%) was calculated as Eq. 1 and 2:

$$\%\text{Nitrogen} = \frac{(V_{s,HCl} - V_{b,HCl}) \times N_{HCl} \times 14}{\text{Sample weight} \times 1000} \times 100 \quad ...(1)$$

$$\%\text{Protein} = \%\text{Nitrogen} \times 6.26 \quad ...(2)$$

Functional Properties

Bulk Density

The bulk density was determined by a scaled plastic centrifuge tube. The samples in six replicate were filled to 25 ml and the tubes were stroked to delete the spaces between the particles. The bulk density was calculated as Eq. 3[24]:

$$\text{Bulk density} = \frac{W_2 - W_1}{V} \quad ...(3)$$

In the above equation W_1 is weight of the tube without sample (g), W_2 weight of the tube with sample (g), V is volume observed (ml).

Water and Oil Absorption Capacity

The WAC and OAC was determined by using of Shao *et al.* [4] method with a little modification. For WAC and OAC test, a sample (1 g) was taken in a test tube and mixed with 10 ml of distilled water for WAC and corn oil for OAC. The tube held in 6 temperature and then they were centrifuged at 4000×g for 10 min. Finally, after removed of the supernatant, the tube with the sediment was weighted. The WAC and OAC were calculated as Eq.4[4, 24]:

$$WAC\ or\ OAC = \frac{W_1 - W_2}{W_s} \quad ...(4)$$

The WAC and OAC were calculated as Eq.4[4, 24]:

In the above equations W_1 is weight of the tube plus the dry sample (g), W_2 weight of the tube plus the sediment (g), W_s is weight of the dry sample (g).

Emulsification Properties

The EAI and ESI of protein samples were determined as described by [25, 26]with some modification. First, made of a 0.5% (w/v) protein solution prepared in distillation water and after stirring for 1min with a magnetic stirrer (Heidolph, Germany), the pH of the solution was adjusted to 4-10 with either 0.1 M HCl or NaOH. Then, 4.5 ml of this solution mixed with 1.5 ml of corn oil were homogenized at 4500×g for 1 min. 250 ìL of this emulsion was picked out from the bottom at two times (at 0 and 10 min) and diluted with 50 mL of 0.1% sodium dodecyl sulfate solution ($NaC_{12}H_{25}SO_4$) and then vortexed for 10 s. Absorbance of this samples were measured at 500 nm using a UV–visible Spectrophotometer (Biochrom, England). ESI (min) and EAI (m^2/g) were calculated using the following Eq.5 and 6:

$$EAI = \frac{2 \times 2.303 \times A_0 \times N}{c \times \varphi \times 10^4} \quad ...(5)$$

$$ESI = \frac{A_0}{A_0 - A_{10}} \times t \quad ...(6)$$

In the above equations A_0 is the

absorbance of the diluted emulsion instantly after homogenization, A_{10} is the absorbance of the diluted emulsion 10 min after homogenization, N is the dilution factor (×150), c is the weight of sample per volume (g/mL), φ is the fat volume fraction of the emulsion and t is the time distance (10 min).

Foaming Properties

The FC and FS was determined according to the method described by[4]with minor modifications, 0.5 g of protein was dispersed in 50 ml of distilled water. The pH of the protein solution was regulated to 4, 5, 6, 7, 8, 9 and 10 with either 0.1 M HCl or 0.1 M NaOH. The solutions were stirred for 3 min with blender at the maximum speed. The stirred protein solution was instantly transferred into a 100 ml cylinder, then volume was observed before (V_b) and after (V_a) stirring. The FC was calculated by the following Eq. 7:

$$FC = \frac{V_a - V_b}{V_b} \times 100 \quad ...(7)$$

In the above equations V_a is volume after stirred, V_b is volume before stirred.

Also, the FS was determined as time (min) required to decline 50 % volume of foam.

Statistical analysis

RSM was used to optimize the protein extraction from tomato waste and tomato seeds. A Box-Behnken design (BBD) was used in the optimization of process variables with five factors at five levels with 50 runs, including 8 central points (condition of the produce: Alkaline pH, x_1; Acidic pH, x_2; Temperature, x_3; Time of extraction, x_4; Ratio of solvent to powder, x_5) (Table 1). The experimental design and statistical analysis were performed using Design-Expert software (version 8.0.7.1, Stat Ease Inc., Minneapolis, MN, USA). The design included 50 experiments, that adopted by adding 8 central points and this used for estimating the experimental error and a measure of lack-of-fit.[27].

The responses function (Y) was partitioned into linear, quadratic and interaction. Experimental data were fitted to the second-order regression Eq. 8:

$$y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ij} X_i^2 + \sum_{i>j}^{n} \sum_{j=1}^{n} \beta_{ij} X_i X_j + \varepsilon \quad ...(8)$$

The model sufficiency were checked in terms of the values of R^2 and adjusted R^2. Analysis of variance (ANOVA) was employed to determine the significance of the models ($p<0.05$). Verification of optimized conditions and predicted values were done in triplicate to confirm the validity of the models.

Also, for functional properties of protein extracted from TW and TS (bulk density, EAI, ESI, WAC, OAC, FC and FS) data were subjected to analysis of variance (ANOVA) and Duncan's by using of IBM SPSS Statistics V.22 (SPSS Inc., USA), in three replication.

As stated above, the data were analyzed by RSM statistical design for the first phase, then the optimized condition of protein extraction selected for making protein concentrate of DTW and DTS. Then for second phase, the functional tests were used by determine the nature of technological of these proteins and compared with the protein extracts from TW, TS, DTW and DTS in central point condition.

RESULT AND DISCUSSION

Statistical analysis

In this study, BBD was used for RSM with five process variables (pH, temperature, time and solvent to powder ratio) at five levels on protein extraction of TWM and TSM. Designs using BBD are generally more efficient in terms of the number of required runs and so they are less expensive to run compared to CCD. The points of design fall

Table 1. Independent variables and their levels used for Box-Behnken design

Independent variables	Factors	Levels				
	(X)	-2	-1	0	+1	+2
Alkaline pH	X1	10	10.5	11	11.5	12
Acidic pH	X2	3.1	3.4	3.7	4	4.3
Temperature(°C)	X3	10	20	30	40	50
Time of extraction(min)	X4	30	40	50	60	70
Solvent to powder ratio(ml/g)	X5	1:10	1:20	1:30	1:40	1:50

Table 2. The ANOVA for the experimental variables as a linear, quadratic and interaction terms of each response variable and corresponding coefficients for the predictive models

Source	DF	PEY of DTW (%)			DTWP (%)			PEY of DTS (%)			DTSP (%)		
		Sum of Squares	Mean Square	p-Value	Sum of Squares	Mean Square	p-Value	Sum of Squares	Mean Square	p-Value	Sum of Squares	Mean Square	p-Value
Model	20	6471.259	323.563	< 0.0001	697.867	34.893	< 0.0001	1689.667	84.483	< 0.0001	713.641	35.682	< 0.0001
Linear													
b_1	1	1230.267	1230.267	< 0.0001	158.126	158.126	< 0.0001	372.22	372.22	< 0.0001	188.226	188.226	< 0.0001
b_2	1	39.907	39.907	0.2694 ^{ns}	4.823	4.823	0.2032 ^{ns}	1.631	1.631	0.6792 ^{ns}	0.705	0.705	0.7070 ^{ns}
b_3	1	199.836	199.836	0.0175	29.156	29.156	0.0033	138.238	138.238	0.0006	48.642	48.642	0.0037
b_4	1	367.047	367.047	0.0019	9.811	9.811	0.0735 ^{ns}	0.01	0.01	0.9737 ^{ns}	0.039	0.039	0.9294 ^{ns}
b_5	1	1526.755	1526.755	< 0.0001	236.536	236.536	< 0.0001	629.338	629.338	< 0.0001	220.196	220.196	< 0.0001
b_{11}	1	0.216	0.216	0.9345 ^{ns}	128.537	128.537	< 0.0001	30.006	30.006	0.0836 ^{ns}	59.193	59.193	0.0016
b_{22}	1	1292.619	1292.619	< 0.0001	91.483	91.483	< 0.0001	278.173	278.173	< 0.0001	132.023	132.023	< 0.0001
b_{33}	1	309.428	309.428	0.0039	2.34	2.34	0.3720 ^{ns}	28.304	28.304	0.0924 ^{ns}	0.47	0.47	0.7588 ^{ns}
b_{44}	1	155.966	155.966	0.0339	6.457	6.457	0.1428 ^{ns}	119.813	119.813	0.0012	27.83	27.83	0.0238
b_{55}	1	741.729	741.729	< 0.0001	4.43	4.43	0.2222 ^{ns}	11.783	11.783	0.2707 ^{ns}	26.861	26.861	0.0262
b_{12}	1	0.128	0.128	0.9497 ^{ns}	8.663	8.663	0.0916 ^{ns}	0.446	0.446	0.8286 ^{ns}	0.368	0.368	0.7859 ^{ns}
b_{13}	1	8.439	8.439	0.6085 ^{ns}	1.518	1.518	0.4710 ^{ns}	26.338	26.338	0.1039 ^{ns}	0.608	0.608	0.7270 ^{ns}
b_{14}	1	189.216	189.216	0.0205	0.581	0.581	0.6549 ^{ns}	0.461	0.461	0.8257 ^{ns}	0.002	0.002	0.9855 ^{ns}
b_{15}	1	180.687	180.687	0.0232	2.492	2.492	0.3571 ^{ns}	11.208	11.208	0.2824 ^{ns}	4.212	4.212	0.3611 ^{ns}
b_{23}	1	31.303	31.303	0.3269 ^{ns}	2.959	2.959	0.3164 ^{ns}	2.896	2.896	0.5820 ^{ns}	0.226	0.226	0.8313 ^{ns}
b_{24}	1	5.067	5.067	0.6912 ^{ns}	2.117	2.117	0.3955 ^{ns}	5.195	5.195	0.4619 ^{ns}	0.842	0.842	0.6813 ^{ns}
b_{25}	1	14.743	14.743	0.4991 ^{ns}	1.092	1.092	0.5406 ^{ns}	1.36	1.36	0.7056 ^{ns}	0.23	0.23	0.8300 ^{ns}
b_{34}	1	6.601	6.601	0.6504 ^{ns}	0.43	0.43	0.7003 ^{ns}	0.633	0.633	0.7964 ^{ns}	0.161	0.161	0.8573 ^{ns}
b_{35}	1	0.279	0.279	0.9257 ^{ns}	0.513	0.513	0.6744 ^{ns}	12.83	12.83	0.2508 ^{ns}	2.744	2.744	0.4599 ^{ns}
b_{45}	1	171.025	171.025	0.0269	5.806	5.806	0.1639 ^{ns}	18.781	18.781	0.1669 ^{ns}	0.066	0.066	0.9085 ^{ns}
Residual	29	912.667	31.471		82.533	2.846		270.967	9.344		141.843	4.891	
Lack-of-fit	22	828.15	37.643	0.0635 ^{ns}	64.498	2.932	0.4613 ^{ns}	248.644	11.302	0.0501 ^{ns}	125.076	5.685	0.1215 ^{ns}
Pure error	7	84.517	12.074		18.035	2.576		22.323	3.189		16.767	2.395	
Cor Total		7383.926			780.4			1960.635			855.484		
R^2		0.8764			0.8942			0.8618			0.8342		
Adj-R^2		0.7912			0.8213			0.7665			0.7198		
CV		9.64			3.52			6.05			4.18		

Table 3. Box-Behnken design arrangement and responses for protein extraction yield and percent of protein

Run	Coded[a]					Uncoded					PEY of DTW	DTWP (%)	PEY of DTW	DTWP (%)	Run	Coded					Uncoded					PEY of DTW	DTWP (%)	PEY of DTS	DTSP (%)
Std	X_1	X_2	X_3	X_4	X_5	X_1	X_2	X_3	X_4	X_5					Std	X_1	X_2	X_3	X_4	X_5	X_1	X_2	X_3	X_4	X_5				
1	-1	-1	-1	-1	-1	10	3.2	20	40	1:20	39.4	21.27	46.9	31.14	26	1	-1	-1	1	1	12	3.2	20	60	1:40	65.2	34.09	56.4	43.01
2	1	-1	-1	-1	-1	12	3.2	20	40	1:20	49.4	28.34	49	36.2	27	-1	1	-1	1	1	10	4.2	20	60	1:40	51	29.87	46.6	36.78
3	-1	1	-1	-1	-1	10	4.2	20	40	1:20	40.8	21.73	41.7	32.12	28	1	1	-1	1	1	12	4.2	20	60	1:40	85.1	33.51	56.3	42.14
4	1	1	-1	-1	-1	12	4.2	20	40	1:20	50.5	28.3	49.6	38.4	29	-1	-1	1	1	1	10	3.2	40	60	1:40	52.3	29.98	50.6	37.89
5	-1	-1	1	-1	-1	10	3.2	40	40	1:20	46.7	25.71	44.3	34.24	30	1	-1	1	1	1	12	3.2	40	60	1:40	88	35.34	62	43.68
6	1	-1	1	-1	-1	12	3.2	40	40	1:20	54.4	29.88	55.4	41.22	31	-1	1	1	1	1	10	4.2	40	60	1:40	54.4	30.92	53.6	40.16
7	-1	1	1	-1	-1	10	4.2	40	40	1:20	45.1	23.16	43.3	33.21	32	1	1	1	1	1	12	4.2	40	60	1:40	80.1	34.21	63.7	43.7
8	1	1	1	-1	-1	12	4.2	40	40	1:20	51.8	27.51	54.7	41.27	33	-2	0	0	0	0	9	3.7	30	50	1:30	71.2	33.11	54.7	41.35
9	-1	-1	-1	1	-1	10	3.2	20	60	1:20	42.2	21.31	41.3	33.24	34	2	0	0	0	0	13	3.7	30	50	1:30	77.8	35.21	53.1	42.2
10	1	-1	-1	1	-1	12	3.2	20	60	1:20	50.6	26.35	46.8	37.83	35	0	-2	0	0	0	11	2.7	30	50	1:30	46.7	17.34	38.2	27.41
11	-1	1	-1	1	-1	10	4.2	20	60	1:20	45.3	23.36	46.5	32.21	36	0	2	0	0	0	11	4.7	30	50	1:30	50.8	21.42	38.4	29.01
12	1	1	-1	1	-1	12	4.2	20	60	1:20	51.5	28.18	47	37.21	37	0	0	-2	0	0	11	3.7	10	50	1:30	58.4	25.11	50.1	33.75
13	-1	-1	1	1	-1	10	3.2	40	60	1:20	47.4	23.48	43.5	34.87	38	0	0	2	0	0	11	3.7	50	50	1:30	65	29.34	57.5	37.95
14	1	-1	1	1	-1	12	3.2	40	60	1:20	56.8	29.98	51.2	41.03	39	0	0	0	-2	0	11	3.7	30	30	1:30	64.8	26.77	57.8	41.21
15	-1	1	1	1	-1	10	4.2	40	60	1:20	49.3	25.19	44.5	34.42	40	0	0	0	2	0	11	3.7	30	70	1:30	65.9	29.11	57.8	38.92
16	1	1	1	1	-1	12	4.2	40	60	1:20	57.7	28.8	51.7	40.63	41	0	0	0	0	-2	11	3.7	30	50	1:10	37.1	20.83	34.1	26.15
17	-1	-1	-1	-1	1	10	3.2	20	40	1:40	46.8	26.79	46.9	37.14	42	0	0	0	0	2	11	3.7	30	50	1:50	72.7	28.48	61.2	39.19
18	1	-1	-1	-1	1	12	3.2	20	40	1:40	55.3	31.8	55.4	41.98	43	0	0	0	0	0	11	3.7	30	50	1:30	72.9	29.37	52.4	38.21
19	-1	1	-1	-1	1	10	4.2	20	40	1:40	51.1	28.91	48.1	37.6	44	0	0	0	0	0	11	3.7	30	50	1:30	71.1	28.87	51.3	39.4
20	1	1	-1	-1	1	12	4.2	20	40	1:40	54.9	30.35	53.6	41.03	45	0	0	0	0	0	11	3.7	30	50	1:30	71.8	27.13	52	41.83
21	-1	-1	1	-1	1	10	3.2	40	40	1:40	48.3	27.27	49.4	38.15	46	0	0	0	0	0	11	3.7	30	50	1:30	71.3	27.54	47.6	37.92
22	1	-1	1	-1	1	12	3.2	40	40	1:40	58.5	32.55	59	43.21	47	0	0	0	0	0	11	3.7	30	50	1:30	67.4	27.8	50.4	39.32
23	-1	1	1	-1	1	10	4.2	40	40	1:40	52.7	30.39	52	40.18	48	0	0	0	0	0	11	3.7	30	50	1:30	67.2	26.17	48	36.71
24	1	1	1	-1	1	12	4.2	40	40	1:40	61.5	32.01	60.6	43.12	49	0	0	0	0	0	11	3.7	30	50	1:30	76.7	29.63	49.5	39.15
25	-1	-1	-1	1	1	10	3.2	20	60	1:40	49.7	26.53	47.8	37.24	50	0	0	0	0	0	11	3.7	30	50	1:30	66.4	24.97	51.3	40.23

[a] X_1: Alkaline pH , X_2: Acidic pH, X_3: Temperature, X_4: Time of Extract, X_5: Solvent to Powder Ratio

within a safe operating restrict, within the substantival low and high levels, as BBD does not consist any points at the vertices of the cubic region. This could be useful when the factor-level compositions are expensive or impossible to test because of the physical process restrictions[27]. According to the information that presented above, the variation of each response (Y) was

Table 4. Chemical composition of tomato waste and seeds meal

Samples (%)	TWM	DTWM	TSM	DTSM
Protein	22.83±1.08	27.16±1.23	34.42±0.94	39.81±1.11
Fat	9.22±0.51	-	27.65±0.82	-
Ash	3.46±0.04	4.88±0.08	4.91±0.05	5.52±0.04
Fiber	26.12±0.97	29.52±0.86	31.77±1.05	34.68±0.82

Table 5(a). The Water Absorbance Capacity (ml H_2O/g) of TWP, DTWP, Opt of DTWP, TSP, DTSP and Opt of DTSP under different temperature. (n=3)

Temperature(°C)	25	35	45	55	65	75
TWP	4.10±0.361^{a}	4.73±0.208^{b}	5.63±0.153^{c}	6.17±0.252^{c}	5.73±0.306^{c}	5.67±0.379^{c}
DTWP	5.10±0.100^{a}	5.47±0.252^{b}	6.07±0.252^{c}	6.50±0.173^{d}	6.20±0.100^{cd}	6.00±0.100^{c}
Opt of DTWP	5.33±0.058^{a}	5.53±0.153^{a}	6.23±0.153^{a}	6.20±0.100^{a}	6.30±0.100^{a}	5.90±0.100^{a}
TSP	3.10±0.100^{a}	3.67±0.153^{b}	4.57±0.153^{d}	4.93±0.153^{e}	4.37±0.153^{cd}	4.27±0.153^{c}
DTSP	3.50±0.100^{a}	4.80±0.100^{b}	5.27±0.153^{c}	5.77±0.115^{d}	5.40±0.100^{c}	5.20±0.100^{c}
Opt of DTSP	3.50±0.200^{a}	4.73±0.115^{b}	5.47±0.153^{cd}	5.70±0.265^{d}	5.47±0.252^{cd}	5.27±0.153^{c}

Mean ± S.D. values superscripted with dissimilar letters in rows are significantly different ($p< 0.05$)

Table 5(b). The Oil Absorbance Capacity (ml oil/g) of TWP, DTWP, Opt of DTWP, TSP, DTSP and Opt of DTSP under different temperature. (n=3)

Temperature(°C)	25	35	45	55	65	75
TWP	1.42±0.015^{a}	1.47±0.010^{b}	1.50±0.015^{b}	1.54±0.015^{c}	1.48±0.010^{b}	1.60±0.015^{d}
DTWP	1.45±0.015^{a}	1.48±0.015^{ab}	1.56±0.010^{c}	1.58±0.025^{cd}	1.51±0.010^{b}	1.62±0.025^{d}
Opt of DTWP	1.46±0.025^{a}	1.50±0.015^{ab}	1.59±0.015^{c}	1.59±0.010^{c}	1.53±0.015^{b}	1.58±0.010^{c}
TSP	1.05±0.050^{a}	1.12±0.015^{b}	1.23±0.036^{c}	1.27±0.015^{c}	1.21±0.010^{c}	1.27±0.032^{c}
DTSP	1.26±0.010^{a}	1.31±0.017^{ab}	1.34±0.031^{b}	1.36±0.015^{b}	1.34±0.055^{b}	1.46±0.032^{c}
Opt of DTSP	1.28±0.006^{a}	1.33±0.021^{ab}	1.36±0.020^{bc}	1.40±0.015^{c}	1.36±0.051^{bc}	1.47±0.021^{d}

Mean ± S.D. values superscripted with dissimilar letters in rows are significantly different ($p< 0.05$)

Table 6(a). The Emulsifier Activity Index of TWP, DTWP, Opt of DTWP, TSP, DTSP and Opt of DTSP under different pH. (n=3)

pH	4	5	6	7	8	9	10
TWP	4.28±0.046^{a}	5.88±0.070^{b}	7.14±0.092^{c}	9.14±0.116^{d}	12.97±0.148^{e}	22.82±0.116^{f}	26.67±0.092^{g}
DTWP	3.82±0.046^{a}	5.24±0.070^{b}	6.48±0.116^{c}	8.74±0.148^{d}	12.56±0.070^{e}	21.25±0.70^{f}	24.04±0.092^{g}
Opt of DTWP	3.78±0.046^{a}	5.13±0.070^{b}	6.17±0.122^{c}	8.55±0.116^{d}	11.99±0.116^{e}	22.17±0.053^{f}	25.39±0.070^{g}
TSP	3.68±0.046^{a}	4.31±0.070^{b}	5.96±0.116^{c}	8.71±0.092^{d}	15.38±0.092^{e}	28.94±0.070^{f}	32.67±0.070^{g}
DTSP	3.24±0.096^{a}	3.84±0.116^{b}	5.53±0.046^{c}	7.97±0.046^{d}	14.55±0.092^{e}	24.66±0.070^{f}	29.63±0.096^{g}
Opt of DTSP	3.10±0.096^{a}	3.50±0.092^{b}	5.39±0.046^{c}	7.74±0.166^{d}	14.42±0.122^{e}	24.43±0.116^{f}	30.98±0.096^{g}

Mean ± S.D. values superscripted with dissimilar letters in rows are significantly different ($p< 0.05$)

evaluated as a function of linear, quadratic and interaction effect of alkaline pH (X_1), acidic pH (X_2), temperature (X_3), time of extraction (X_4) and solvent to powder ratio (X_5). The results of variance (ANOVA) and coefficients of the models for the responses, along with the corresponding coefficients of determination (R^2), adj-R^2 and coefficient of variation (CV) are given in Table 2. Multiple linear regression analysis of the experimental data produced second-order polynomial equations for PEY of DTW (%), DTWP (%), PEY of DTS (%) and DTSP (%) as postulated before. The statistical analysis demonstrated that the proposed model was enough, showing no significant lack-of-fit ($p>0.05$) with satisfactory values of R^2 for all responses.

Two different tests, videlicet, sequential model sum of squares and model summary statistic were accomplished to check the sufficiency of the models generated from the obtained data and the

Table 6(b). The Emulsifier Stability Index of TWP, DTWP, Opt of DTWP, TSP, DTSP and Opt of DTSP under different pH. (n=3)

pH	4	5	6	7	8	9	10
TWP	3.10±0.141^{a}	3.03±0.152^{a}	10.76±0.580^{b}	26.96±0.630^{e}	24.63±1.555^{d}	24.00±0.709^{d}	22.65±0.225^{c}
DTWP	3.99±0.085^{a}	4.64±0.147^{a}	13.19±0.538^{b}	32.78±3.283^{d}	21.10±0.724^{c}	18.77±0.034^{c}	20.47±0.460^{c}
Opt of DTWP	4.06±0.570^{a}	5.25±0.341^{a}	15.44±1.536^{b}	34.21±2.191^{e}	27.37±0.748^{d}	19.65±0.313^{c}	20.35±0.226^{c}
TSP	6.00±0.451^{a}	5.97±0.208^{a}	18.96±0.493^{c}	31.69±3.925^{e}	25.53±1.141^{d}	16.22±0.177^{b}	19.11±0.186^{c}
DTSP	5.63±0.542^{a}	7.48±0.784^{b}	23.33±0.831^{d}	40.88±1.204^{f}	26.46±0.650^{e}	21.99±0.418^{c}	21.80±0.379^{c}
Opt of DTSP	5.54±0.604^{a}	8.54±0.418^{a}	27.74±0.896^{c}	46.63±5.282^{e}	32.30±0.651^{d}	22.08±0.453^{b}	19.55±0.124^{b}

Mean ± S.D. values superscripted with dissimilar letters in rows are significantly different ($p< 0.05$)

Table 7(a). The Foaming Capacity of TWP, DTWP, Opt of DTWP, TSP, DTSP and Opt of DTSP under different pH. (n=3)

pH	4	5	6	7	8	9	10
TWP	7.69±1.923^{a}	10.90±1.110^{b}	14.74±1.110^{c}	17.95±1.110^{d}	25.00±1.923^{e}	30.77±1.923^{f}	42.31±1.923^{g}
DTWP	9.62±1.923^{a}	16.67±2.938^{b}	25.00±1.923^{c}	32.69±1.923^{d}	42.31±1.923^{e}	53.85±1.923^{f}	68.59±2.938^{g}
Opt of DTWP	13.46±1.923^{a}	22.44±1.110^{b}	28.85±1.923^{c}	39.74±1.110^{d}	48.08±1.923^{e}	62.82±4.441^{f}	73.08±1.923^{g}
TSP	16.67±1.110^{a}	20.51±1.110^{a}	30.13±1.110^{b}	37.82±2.938^{c}	48.72±2.938^{d}	58.33±2.9238^{e}	73.08±5.088^{f}
DTSP	19.23±1.923^{a}	23.72±2.221^{ab}	32.05±1.110^{b}	42.95±2.221^{c}	58.33±11.268^{d}	69.87±5.875^{e}	82.69±3.846^{f}
Opt of DTSP	22.44±1.110^{a}	35.26±2.938^{b}	44.23±1.923^{c}	57.05±2.938^{d}	67.31±1.923^{e}	79.49±2.938^{f}	87.82±1.110^{g}

Mean ± S.D. values superscripted with dissimilar letters in rows are significantly different ($p< 0.05$)

Table 7(b). The Foaming Stability of TWP, DTWP, Opt of DTWP, TSP, DTSP and Opt of DTSP under different pH. (n=3)

pH	4	5	6	7	8	9	10
TWP	2.33±0.577^{a}	4.67±0.577^{b}	11.33±1.528^{c}	15.67±0.577^{d}	19.00±0.000^{e}	21.67±0.577^{f}	25.00±1.000^{g}
DTWP	3.33±0.577^{a}	6.33±0.577^{b}	13.00±1.000^{c}	21.33±1.528^{d}	25.33±1.155^{e}	29.33±1.528^{f}	33.33±1.528^{g}
Opt of DTWP	4.33±0.577^{a}	7.33±0.577^{b}	13.67±1.155^{c}	24.00±2.000^{d}	27.67±1.528^{e}	32.00±2.000^{f}	35.67±2.082^{g}
TSP	3.00±0.000^{a}	5.33±0.577^{b}	12.67±0.577^{c}	16.00±1.000^{d}	20.00±0.000^{e}	24.00±1.000^{f}	26.67±1.528^{g}
DTSP	4.67±0.577^{a}	7.33±0.577^{b}	15.33±0.577^{c}	25.00±1.000^{d}	30.00±1.000^{e}	34.33±1.528^{f}	37.67±1.528^{g}
Opt of DTSP	5.67±0.577^{a}	9.33±0.577^{b}	17.00±1.000^{c}	28.00±1.000^{d}	32.33±1.528^{e}	36.67±2.082^{f}	42.00±3.000^{g}

Mean ± S.D. values superscripted with dissimilar letters in rows are significantly different ($p< 0.05$)

results are given in Table 2. Model summary statistics output (Table 2) showed that, for protein extraction of TWM and TSM the values for the R^2 and adjusted R^2 were the highest compared the other models while the cubic model was disregarded as it is aliased. For quadratic versus 2FI, the *P* value obtained was less than 0.0001which shows strength of significance. The addition of the quadratic term to the mean, linear, and the 2FI terms would only strengthen the model. With the leaving aside of the cubic model, the BBD has sufficient data to interpret the results of the present system[28]. The R^2 values were 0.8764, 0.8942, 0.8618 and 0.8342 for PEY of DTW, DTWP, PEY of DTS and DTSP, respectively; this showed that a high percentage of response variations were described by the response surface models.

Adjusted R^2 is a modification of R^2 that adjusts for the number of expository terms in a model. Vice versa R^2, the adjusted R^2 increases only if the new term improves the model more than would be envisaged by chance. Thus, it is recommended using an adj-R^2 to evaluate the model adequacy[29]. In this study, the values of adj-R^2 coefficient were rather enough, advocating the significance of the model. The coefficient of variation (CV), which indicates the extent to which data were dispersed, were found to be 9.64%, 3.52%, 6.05% and 4.18% for PEY of DTW, DTWP, PEY of DTS and DTSP, respectively (Table 2). Thus, it can be concluded that the selected model adequately displayed the data for all the responses obtained.

Fig. 1 shows that the polynomial regression model was in agreement with the experimental results. In this figure, each of the observed values is compared to the predicted value calculated from the model.

Determination and Optimization of Protein

According Table 3, the ranges of protein content of DTW, DTS, PEY of DTW and PEY of DTS were 17.34-35.34%, 26.15-43.70%, 37.1-88% and 34.1-63.7%, respectively.

The all over results of the tests done on protein extraction of DTW, DTS and PEY of DTW, PEY of DTS showed that this extraction process was similar to other seeds protein, especially the protein of other tomato variety, soy and chickpea protein[4, 20, 30, 31].Then, formulation optimized protein extraction; for the maximum of yield and protein content by the Design Expert 7.0.0 software, were accomplished.

Fig. 2 and Fig. 3 showed the effect of alkaline pH (10-12), temperature (10-50°C), time of extraction (30-70 min) and solvent to powder ratio (1:10-1:50 W/V) in the first phase and acidic pH

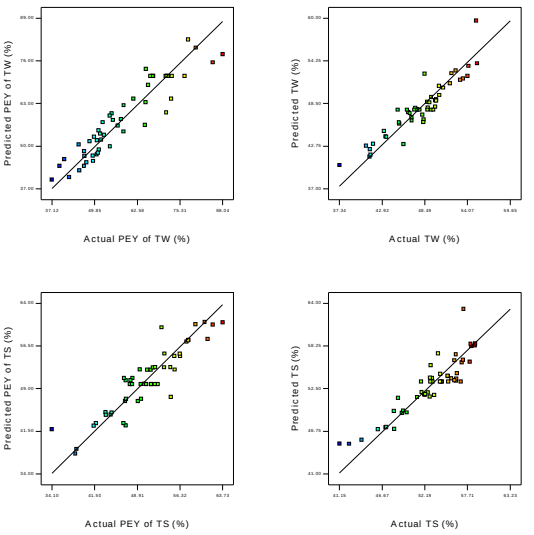

Fig. 1. Comparison between predicted and actual values of PEY of TW, TWP, PEY of TS and TSP

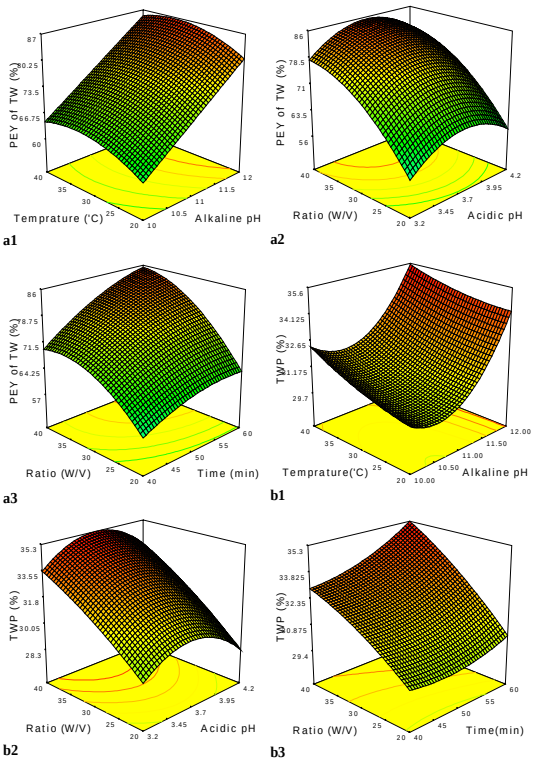

Fig. 2. The 3d response surface graph for PEY of DTW (a1-3) and DTWP (b1-3) responses

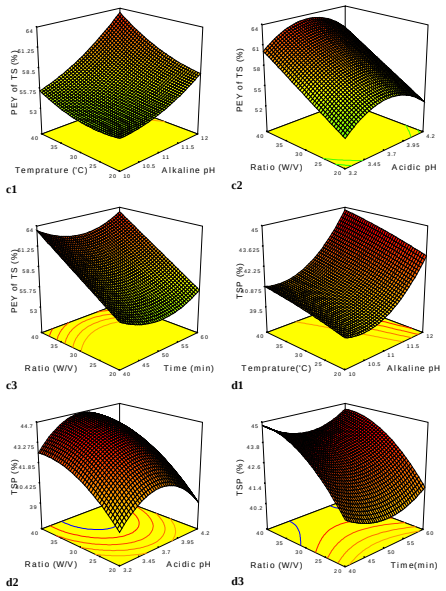

Fig 3. The 3d response surface graph for PEY of DTS (c1-3) and DTSP (d1-3) responses

(3.1-4.3) in the second phase on PEY of DTW %, DTWP %, PEY of DTSP % and DTSP %. In this figures, the interaction between of parameters is viewable on the responses.

By applying the desirability function method, according to formulation with desirability 0.992 to condition of protein extraction with properties mentioned, 12.00 of alkaline pH, 3.73 of acidic pH, 37.73°C of temperature, 60 min of time, 1:40 of ratio were determined; in conditions, PEY of DTW 86.84%; DTWP 35.29%; PEY of DTS 64.15% and DTSP 44.65% were measured.

Liadakis *et al.*[32] were used of RSM with a central composite design for extraction of tomato seed proteins. In this study optimum condition were temperature: 50°C, pH: 11.5, time of mixing: 20 min and water/solid ratio: 1:30 w/v. With above condition protein extract yield 66.1%, protein content of product 72.0% and total protein yield 43.6% were calculated. Ma *et al.*[33] were produced the peanut protein concentrate from defatted peanut flour by ethanol precipitation and separation with centrifuge, then for the extraction of protein they were used of RSM. The optimization condition of protein extract with ratio of liquid-to-solid of 1:11.79 w/v, ethanol of 85 mL/100 mL and temperature of 36.35°C for having highest of protein content were determined. Firatligil-Durmus and Evranuz[34] were used of RSM for extraction of protein from red pepper seed meal. The maximum of yield protein was obtained 96.7% when temperature, pH, mixing time and solvent/meal ratio were 31°C, 8.8, 20 min, 1:21 w/v, respectively. Wani *et al.*[35] were used RSM in extraction conditions for maximum protein recovery of watermelon seeds. The extraction of protein yield between 72.03 and 81.52 g/100 g. Also, the optimum of protein extraction was obtained when: 0.12 g/L NaOH, 15 min extraction time and 1:70 w/v solvent/powder ratio at 50°C.

Functional Properties

Functional properties of protein concentrate not only dictate its usage but also the level of its concatenation into different food products. It might either improve or destroy the quality of food product as well as the storage period. Various functional properties of the meals and concentrates provide sufficient knowledge to predict their optimal utilization. The result of chemical composition analysis of TWM, DTWM, TSM and DTSM are given in Table 4. In continue were evaluated of functional properties for them.

Bulk Density

Bulk density (Fig 4) of different tomato protein concentrates from fat to defatted TWP, TSP and optimum of each DTWP and DTSP was 0.408-0.432, 0.444-0.484, 0.460-0.472, 0.416-0.432, 0.448-0.464 and 0.444-0.456 g/mL for TWP, DTWP, Opt of DTWP TSP, DTSP and Opt of DTSP, respectively. The protein concentrate prepared from Tomato of Mashhad variety had the highest value in antioxidant, pectin and protein in industrial waste after extraction process for making tomato paste[20]. The differences in bulk density were statistically just significant between fat and defatted protein ($P<0.05$). It is an important property as it determines the behavior of a material (especially powders) in dry mixes as well as volume occupied while packaging. Kramer and Kwee[15]were extracted protein from industrial tomato waste and after that, they were analyzed functional properties. They observed that tomato waste was bulkier than other samples. Sogi *et al.*[21]evaluated functional properties of tomato seed meals (whole and defatted) and its protein concentrate. They observed that the salt-extracted protein was the highest. Also, the defatted meal had lower bulk density significantly compared with whole meal

that might be due to the finer particle size of the former. Shao *et al.*[4] were separated the seeds from tomato pomace and protein isolated from tomato seeds in two industrial methods (cold and hot break), then they were evaluated their functional properties. They observed that the bulk density of hot break tomato seeds (0.73 g/ml) was significant but defatted hot break tomato seeds (DHTS) and defatted cold break tomato seeds (DCTS)(0.62 and 0.61 g/ml, respectively) were not significant, also, which were significantly higher than that of soybean protein (SP) (0.50 g/ml) ($P<0.05$). Also, Liadakis *et al.*[36]who reported 0.27g/ml. The difference in these values might be as a result of reasons like variation in raw material, processing, and analytical procedures.

Water and Oil Absorption Capacity

The WAC in term is the volume of water (H_2O)/weight of protein for different products from tomato waste and seed indicated that the protein in native form can bind more water. The different treatments in the preparation of other products changed the protein functionality resulting in significantly lower water absorption power. WAC and OAC are important of physical parameters and affecting on some of textural and flavor properties in foods[37].Table 5a is presented the ability of different tomato protein concentrates of fat or defatted TWP, TSP and optimum of each DTWP and DTSP to bind water at range of temperature from 25 to 75°C. Protein concentrates from tomato waste and seeds showed significant differences in WAC ($P<0.05$). With increase of temperature from 25 to 55°C, the WAC of all samples were increased which could be ascribed to the denaturation of protein during the processes of crushing and extraction. However, the changes in WAC is due to differences in hydrophilic groups among tomato waste or seeds protein concentrates[20]. It could be ascribed to the fact that as the pH approaches the isoelectric point, the WAC of protein is minimized. Also, the OAC of different tomato waste and seeds protein concentrates increased significantly from 1.05±0.05 to 1.62±0.025 mL/g with temperature increasing from 25 to 75°C. According to table 5b, the results showed that with increasing process temperature, the OAC had significant increasing ($P<0.05$).Generally, the increase of process temperature could be lead to denaturation of proteins, but the low increasing of temperature don't affect WAC significantly. Shao *et al.*[4] observed that all tomato waste protein in compared with soybean protein had significant differences in WAC and OAC. They observed that WAC values of tomato samples were significantly higher than SP (~2.40 g/g) and also, SP had the highest OAC value (~2.80 g/g) followed by DHTS and DCTS (2.36 and 2.37 g/g), which were significantly bigger than that of HTS (~1.80 g/g) ($P<0.05$). WAC of the protein especially connected to condition and associated ingredients of protein, for example, amino acids, surface hydrophobicity, lipids, carbohydrate, protein conformation, temperature and pH[38]. Sogi *et al.*[21] reported a water absorption value of the tomato protein concentrates from alkali, water, and salt extraction procedures that were 2.15, 2.02, and 2.12 mL/g, respectively. They observed that WAC of the protein concentrates was significantly lower than that of meals, of course the difference could be attributed to the variety of tomatoes used and oil extraction procedures and also, reported the whole and defatted meals exhibited fat absorption values of 2.63 and 2.37 mL/g, while those for protein concentrates extraction were 1.87, 2.17 and 2.03 mL/g for alkali, water, and salt, respectively. The values obtained in this study for similar parameters are slightly lower. These deflection could be due to variations in processing conditions during protein concentrate preparation; however, the difference in values was not significant and the less values for concentrate from water extraction as compared to salt extraction complementarity in their findings. The lower OAC for defatted as compared to that for fat meal might be due to denaturation of protein effect as a result of temperature rise during grinding as well as the seed meal water extraction. Between the concentrates, the water extracted (alkali) showed the lowest OAC, which could be attributed to protein denaturation resulting in decreased binding points for the fat molecules. The results of the present study agree with those of Rahma *et al.*[39] who reported similar values for defatted meal of tomato seeds. However, Liadakis *et al.*[36] reported of OAC for water and salt extracted concentrates from tomato seeds to be 3.17 and 4.04 ml oil/g. The OAC in terms is the volume of oil/weight of protein for different products from tomato waste and seed showed the highest value for the whole meal indicating that the protein in

native can bind more fat. Further treatments in the preparation of defatted and concentrates significantly lowered the OAC.

Emulsification properties

Table 6a and b, showed that the emulsifying properties (EAI and ESI) of all concentrate (0.5%, w/v; pH 4 to 10) with corn oil were investigated and also, the effects of pH on EAI and ESI were analyzed for protein concentration in above condition. The EAI is an ability to the protein concentrate to emulsion formation. The EAI preparing an approximation of the interfacial area fixated per unit weight of protein based on the turbidity of an emulsion[25, 26].The results showed that the EAI was increased with the growth of pH and had significant differences between pH 4 to 10. Also, the results showed that between the defatted and fat tomato protein in EAI were slight differences ($p<0.05$) (Table 6a). The results of Shao *et al.*[4] were showed that with increased of pH same to present study, EAI were increased. They expressed that the EAI values of SP were the highest (0.539-0.755 AV/mm), and for other test cases, DCTS (0.080-0.951 AV/mm), DHTS and HTS (0.061-0.705 and 0.053-0.527 AV/mm, respectively) were determined. Boye *et al.*[30] were observed that the EAI for the pulse protein concentrate of 4.6 m^2/g for YP-UF (lowest) and 5.7 m^2/g for the DC and KC-IEP (highest), however, no significant differences were observed of pulse varieties.

The ESI is the stability of emulsion in a time of stationary period. The results in present study were showed that the ES value increased the pH of 4 to 7 and pH 7 was highest for all of proteins, Opt of DTSP in pH 7 to 46.63 min and TWP to 26.96 min were highest and lowest, respectively (Table 5b). At pH of 3 to 5, which were around protein isoelectric region[32], the EAI and ESI values of samples were the lowest. This was because most of proteins are slightly soluble at their isoelectric pH, weakly hydrated, and absence electrostatic repellent forces[4]. At the outside of this region[4-5], both EAI and ESI increased significantly because of the increase in solubility of protein at high pH, however, enhances in ESI after pH 7 were not so significant. In general decrease and increase in EAI and ESI should be congruous with the pH-dependent of protein solubility and also, EAI depends upon the lipophilic-hydrophilic balance that was affected by changing of pH. Therefore, EAI and ESI were pH-dependent because the alkaline pH improved the emulsion capacity more than acidic pH[40].

Foaming (Whipping) properties

The FC (Table 7a) and FS (Table 7b) shows the values of TWP, DTWP, Opt of DTWP, TSP, DTSP and Opt of DTSP samples. The FC and FS are used as indices of the whipping properties of protein concentrates. Proteins foam when stirred because of their surface active properties. Also, FS is equally important since food products are generally stored under ambient or refrigeration conditions until consumed[41]. Foam formation is important in food productions such as some beverages and cakes[31]. In present study observed that when the pH increased, the FC and FS had significant increases. In general, in the studied pH 4 to 10, FC and FS showed similar trends ($p<0.05$). For the creating a good foaming agent from a protein concentrate, this protein should be has ability to adsorb swiftly at the air–water joint during the bubbling and the ability to undergo conformational changes and rearrangement at the interface with decrease of surface tension which might be due to extraction of globulin which has a higher FC under neutral pH conditions[42]. Deep Singh *et al.*[43] reported the ability of chickpea protein concentrates to produce foams in two pH 7 and 4.5 and conditions with and without the addition of 10g/L NaCl or 100g/L sucrose on two variety of chickpea (Desi and Kabuli). At pH 7, Desi cultivar PDG-4 showed the highest FC and FS, followed by Kabuli cultivar L-551, while Desi cultivars PBG-1 and GPF-2 showed the lowest FC, though their FS was comparable to that of the other cultivars. Obatolu *et al.*[44] reported FC ranging from 1.98% to 40.2% for processed (boiled) and not processed (raw) yam bean respectively. Also, they observed that the large difference in FC between the boiled and raw bean varieties shows that processing treatments used in the study decreased FC significantly ($P<0.05$). FS of the raw flour was, however, less than that of the processed bean flours. Shao *et al.* [4] reported the FC to 25.13–66.33 % and FS to 36.67–92.00 min for SP that were significantly higher than those of DCTS (5.47–39.87 % and 0.50–61.00 min, respectively), followed by DHTS (4.00–20.53 % and 0.50–30.17 min, respectively) and then HTS (0– 14.50 % and 0–20 min, respectively) ($P<0.05$).

CONCLUSION

Response surface methodology technique demonstrated to be a useful tool in organizing optimum conditions for extracting tomato waste and seed protein. Protein was extracted from fat and defatted tomato waste and seeds powder with 50 selected combinations of temperature, pH (alkaline and acidic phase), extraction time and solvent to powder ratio. The experimental value of protein content for DTW 37.34-55.34%, DTS 41.15-58.70% and protein extraction yield of DTW 37.12-88.04% and DTS 34.1-63.7% were determined. The second order model developed for PEY of DTW, DTWP, PEY of DTS and DTSP represented a non-significant value for lack of fit and semi high value for the coefficient of determination (R^2). The variables with the largest effect were the alkaline pH, temperature and solvent to powder ratio. The optimum condition for extraction of DTW and DTS could be achieved in alkaline pH 12, acidic pH 3.73, temperature 37.73°C, time of extraction 60 min and solvent to powder ratio 1:40. These conditions resulted in PEY of DTW 86.84%; DTWP 35.29%; PEY of DTS 64.15% and DTSP 44.63%.

Also, the present study shows that the functional properties of tomato waste and seed protein concentrates were evaluated to assess their effective use in food systems. However, the functional properties (Bulk density, WAC and OAC, EAI and ESI, FC and FS) on TWP, DTWP, Opt of DTWP, TSP, DTSP and Opt of DTSP were determined. The TWP and TSP were lowest between the other samples in Bulk density. In WAC test, all of the samples were increased to 55°C and after a little decreased. For OAC test, all of the samples with increases to temperature were increased. The emulsifier properties (EAI and ESI) with increases in pH were increased but the ESI value was highest at pH 7 for all samples. Also, the foaming properties (FC and FS) had significantly increased with the growth of pH ($p<0.05$).

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