Effect of Enzymatic Hydrolysis on Nutritional Value of Protein Extracted From Tomato Seed

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Abstract

The effect of hydrolysis temperature (40 to 80^{o}C), pH (4 to 8), time (1 to 9 h) and enzyme activity ([E]/[S] values of 1.73%, 3.47%, 5.20%, 8.67%, and 13.8%) on the concentration of glutamic and aspartic acids in seed protein extracted from defatted tomato seed meal (DTSM) was investigated and optimized using the Response Surface Methodology. The optimum conditions to produce maximum concentration of amino acids were the temperature - 40^{o}C; pH - 3; time - 6 h; and enzyme activity - 0.1822 U/g. The corresponding concentrations of glutamic acid and aspartic acid were 727.6 µg/mL and 149.9 µg/mL, respectively. The results showed that the protein extracted from DTSM is rich in amino acids and could be used as an ingredient in food products rich in umami flavors for both vegetarians and non-vegetarians.

Introduction

About 3 to 5% (in weight) of fresh tomato is generated as pomace containing about 60% of seeds and 40% of peels from tomato processing industries. Tomato pomace is currently used as livestock feed and soil amendment or otherwise disposed into landfill that causes environmental problem. Tomato seed contains lysine rich seed protein and has cholesterol-lowering effect, which shows the potential application as protein supplement. Chemical extraction methods used for protein extraction have limitations as they may induce side-reactions, such as hydrolysis and extraction of non-protein components, and denaturation of protein, which affect functional properties of protein. Enzymatic hydrolysis (EH) was used in this study because of milder process condition, easier control of reaction and minimal secondary products formation which helps to improve functional properties of protein. Response surface methodology was used to optimize the EH conditions for maximizing concentration of glutamic and aspartic amino acids in protein extracted from DTSM.

Objectives

1) To study the effect of hydrolysis temperature (T), pH, time (t) and enzyme activity [E]/[S] on the concentration of glutamic and aspartic acids in protein extracted from DTSM.
2) To optimize the hydrolysis conditions to maximize glutamic and aspartic amino acids in protein by using Response Surface Methodology technique.

Materials and Methods

Preparation of DTSM

Tomato pomace of hot break process (The Morning Star Company, Williams, CA) was collected and stored in freezer until use. It was thawed at 4^{o}C and then dried at 50^{o}C in an oven to a moisture content of 5.0 ± 0.2%. The seeds were separated from the dried pomace samples using an aspirator system. 70 g seeds were ground to powder using a mill for 30 s and sieved in a Tyler Sieve Shaker with a 14 mesh sieve. The powder was defatted with the hexane (10mL/g meal) for 4 h. The DTSM was packed in ziplock bags after removing residual hexane.

Papain: Papain for enzymatic hydrolysis was obtained from Carica papaya with 1.937 U/mg (U refers to amount of protease needed to hydrolysis 1 µmol casein in 1 min).

Single Factor Experiments

Effect of enzyme activity: [E]/[S] values were at 1.73%, 3.47%, 5.20%, 8.67%, and 13.8%, while keeping T - 50^{o}C, t - 3h and pH - 5.5 as constant

Effect of pH: pH values were at 4, 5, 6, 7, and 8, while keeping T - 50^{o}C, t - 3 h and [E]/[S] - 5.20% as constant.

Effect of temperature (T): Temperatures were at 40^{o}C, 50^{o}C, 60^{o}C, 70^{o}C, and 80^{o}C, while keeping t - 3h, pH - 5.5 and [E]/[S] - 5.20% as constant.

Effect of time (t): times were at 1 , 3, 5, 7, and 9 h, while keeping T - 50^{o}C , pH - 5.5 and [E]/[S] - 5.20% constant.

Materials and Methods (continued)

Hydrolysis of DTSM

A sample of 2 g of DTSM was extracted with 20 mL of phosphate buffer, composed of 0.2 mol/L Na_2HPO_4 and 0.1 mol/L citric acid at different hydrolysis conditions in a reciprocal water bath shaker (Model R 76, New Brunswick Scientific, Edison, N. J., U.S.A.). At the end of hydrolysis the mixture was heated in boiling water for 20 min to inactivate the protease.

Determination of total free amino acids

The hydrolysate sample was precipitated with 10% sulfosalicyclic acid for 2 h and then centrifuged at 11,000g for 15 min. The pH of supernatant was adjusted to 2.0, and passed through a microfiltration membrane (0.45 µm). After precolumn derivatizing with phthalic dicarboxaldehyde (OPA), the filtrate was subjected RP-HPLC to determine the free amino acid compositions. Triplicate experiments were performed.

Response Surface experimental design

Based on the single factor tests, a series of experiments were conducted using the Response Surface Methodology (RSM) to optimize the hydrolysis conditions. The non-coded values of four independent variables by Central Composite (Uniform Precision) Rotatable design are shown in Table 1,

Results

Figure 1(a) shows the particle size distribution of DTSM determined through sieve analysis. Large proportion of DTSM were in the particle size range of 0.85 mm ~ 0.43 mm followed by 0.43 mm ~ 0.25 mm size. Figure 1(b) shows the effect of particle size on the protein extraction ratio of DTSM. The DTSM with particle size of less than 0.25 mm did not have significant effect on the extraction ratio of protein. However, the extraction ratio of protein significantly decreased with the increase in particle size when DTSM with particle sizes of > 0.25 mm was hydrolysed.

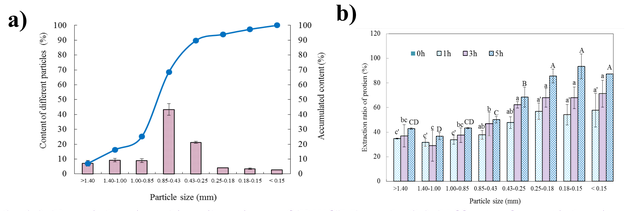


Fig.1. (a) Particle size distribution of DTSM and (b) Effect of particle size on protein extraction of DTSM

The effect of hydrolysis conditions ([E]/[S], pH, T and t) on the concentrations of glutamic and aspartic amino acids in protein extracted from DTSM is shown in Figure 2. From the results of single factor experiments, ([E]/[S] values between 5.20%~13.88%; pH values of 4, 5 and 6; temperatures of 40^{o}C , 50^{o}C and 60^{o}C; and time period of 3 h, 4 h and 5 h were selected for optimization using RSM technique.

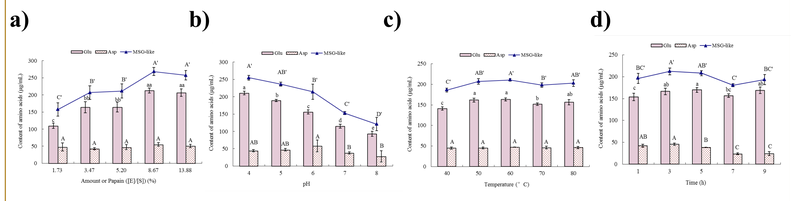


Fig.2. Effect of hydrolysis conditions a) enzyme activity ([E]/[S], b) pH, c) temperature (T) and d) time (t) on concentrations of glutamic and aspartic amino acids in protein extracted from DTSM

Results (continued)

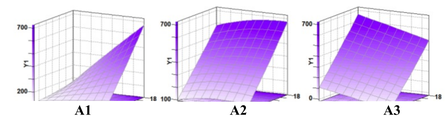
Table 1. Central composite rotatable experimental design and results

RUN	X1: Enzyme activity (U/g)	X2: pH	X3:Temperature (°C)	X4: Time (h)	Y1: CGA	Y2: CAA
1	5.20	4	50	3	196.96±9.96	46.00±1.64
2	5.20	4	50	5	238.89±14.89	53.99±8.42
3	5.20	4	70	3	213.06±12.11	47.79±0.68
4	5.20	4	70	5	232.26±14.02	62.89±5.27
5	5.20	6	50	3	154.60±10.06	44.79±4.79
6	5.20	6	50	5	152.88±8.79	29.16±1.64
7	5.20	6	70	3	143.61±14.11	42.11±3.26
8	5.20	6	70	5	141.87±7.85	40.34±4.40
9	13.88	4	50	3	375.79±8.50	80.56±2.32
10	13.88	4	50	5	474.68±4.24	96.30±24.79
11	13.88	4	70	3	405.71±22.52	80.16±8.95
12	13.88	4	70	5	398.16±14.10	76.96±2.83
13	13.88	6	50	3	204.21±3.12	49.40±4.12
14	13.88	6	50	5	210.52±7.85	37.62±1.38
15	13.88	6	70	3	178.65±7.34	43.38±12.31
16	13.88	6	70	5	180.76±9.11	39.47±1.77
17	0.86	5	60	4	120.84±6.82	33.91±2.10
18	18.22	5	60	4	375.42±10.06	69.77±1.01
19	9.54	3	60	4	312.81±1.83	62.05±5.68
20	9.54	7	60	4	146.84±18.14	38.11±0.24
21	9.54	5	40	4	218.93±2.10	43.13±1.53
22	9.54	5	80	4	189.08±13.93	43.60±2.76
23	9.54	5	60	2	235.67±1.46	48.12±1.09
24	9.54	5	60	6	255.06±43.31	46.64±8.00
25	9.54	5	60	4	231.31±20.80	64.69±7.68
26	9.54	5	60	4	270.48±16.52	54.35±4.16
27	9.54	5	60	4	238.08±7.49	45.12±1.89
28	9.54	5	60	4	238.74±4.94	49.69±1.58
29	9.54	5	60	4	253.71±7.56	48.71±2.62
30	9.54	5	60	4	238.78±3.35	43.48±1.24
31	9.54	5	60	4	265.30±15.52	51.98±4.51

Table 2. ANOVA of response surface experimental results

Master	ANOVA for Y1(CGA)				ANOVA for Y1 (CAA)			
Source	DF	MS	F	Pr> F	DF	MS	F	Pr> F
X1	1	89244.23	137.9945	0.0001	1	1811.344	28.62853	0.0001
X2	1	93793.76	145.0292	0.0001	1	2953.933	46.6873	0.0001
X3	1	1263.676	1.953967	0.18124	1	0.59535	0.00941	0.923929
X4	1	1016.732	1.572128	0.227909	1	0.00735	0.000116	0.991534
X1*X1	1	171.4396	0.26509	0.613684	1	42.41874	0.670434	0.424929
X1*X2	1	21903.26	33.86806	0.0001	1	754.0516	11.91789	0.003278
X1*X3	1	499.4108	0.772217	0.392538	1	116.1006	1.834986	0.194361
X1*X4	1	110.723	0.171206	0.684535	1	4.8841	0.077194	0.784695
X2*X2	1	129.4588	0.200177	0.660576	1	17.30616	0.273526	0.608143
X2*X3	1	100.9523	0.156098	0.697992	1	11.18903	0.176844	0.679693
X2*X4	1	1359.95	2.102831	0.166347	1	295.1524	4.664923	0.046306
X3*X3	1	2106.452	3.257115	0.089868	1	23.20393	0.366741	0.55328
X3*X4	1	1112.056	1.719523	0.208262	1	6.125625	0.096816	0.759701
X4*X4	1	15.77449	0.024391	0.877847	1	0.303057	0.00479	0.945681
Model	14	212907.1	23.51493	0.0001	14	6042.819	6.821969	0.000239
(Linear)	4	185318.4	71.63744	0.0001	4	4765.879	18.83134	0.0001
(Quadratic)	4	2502.344	0.967317	0.452259	4	89.43648	0.353389	0.837891
(Cross Product)	6	25086.35	6.464989	0.001307	6	1187.503	3.128108	0.031795
Error	16	10347.57			16	1012.329		
(Lack of fit)	10	8962.632	3.882896	0.055242	10	714.794	1.44143	0.339159
(Pure Error)	6	1384.94			6	297.5354		
Total	30	223254.7			30	7055.148		
R^2	0.9638				0.8565			

Y1)



Y2)

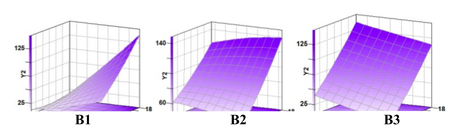


Fig.3. Response surface of the optimization of protein hydrolysis from DTSM

X1: Enzyme activity (%) , X2: pH, X3: Temperature (^{o}C), X4: Time (h),
Y1: Concentration of glutamic acid , Y2: Concentration of aspartic acid

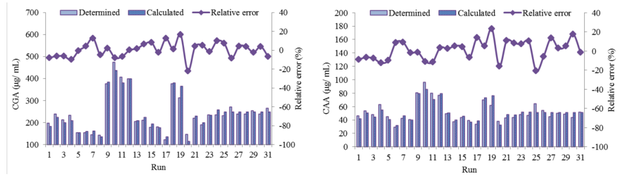


Fig.4 Verification of fittingness of model for CGA and CAA

CGA: concentration of glutamic acid, CAA: concentration of aspartic acid.
Determined values are from Table 2, Calculated values are obtained using the fitting model.

Conclusion

The results showed that the particle sizes of the DTSM were distributed in a wide range (0.15 to 1.4 mm) with majority of particles in the range of 0.85 mm ~ 0.43 mm, followed by 0.43 mm ~ 0.25 mm. The maximum protein extraction ratio of 85.64% was obtained when the DTSM with particle sizes of ≤ 0.25 mm was hydrolyzed by papain for 5 h. The results of the response surface optimization showed that the optimal hydrolysis condition for preparing the protein with highest concentration of glutamic acid and aspartic acid were enzyme activity of 0.1822 U/g; pH of 3; hydrolysis temperature of 40^{o}C; and hydrolysis time of 6 h. The corresponding concentrations of the glutamic acid and aspartic acid obtained were 727.6 µg/mL and 149.9 µg/mL, respectively.

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