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Proteolysis of sodium caseinate using *Withania coagulans* extract: An optimization study

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Abstract

In this study, sodium caseinate was hydrolyzed with *Withania coagulans* extract and the response surface methodology (RSM) was applied to optimize the effects of hydrolysis conditions including hydrolysis temperature, enzyme concentration and hydrolysis time on the degree of hydrolysis, solubility, and foaming properties. The analysis of variance in RSM showed that the linear effects of enzyme level and hydrolysis time and quadratic effects of hydrolysis temperature were important factors affecting the hydrolysis process remarkably ($P<0.0001$). Results were indicative of the fact that the increase in responses was obtained by an increase in hydrolysis time and enzyme level. The generated quadratic model showed that the optimum conditions for maximizing the responses were when enzyme concentration of 1.75 (%w/w), temperature of 55.43°C and hydrolysis time of 490 min.

Keywords: Sodium caseinate, Hydrolysis, *Withania coagulans* protease, Solubility, Foaming properties.

Introduction

Enzymatic proteolysis and the effect of hydrolysis parameters on the functional properties of proteins such as emulsifying, foaming, viscosity and solubility have been extensively investigated and reported by several researchers (Banach *et al*. 2013; de Castro *et al.* 2015; Guan *et al*. 2007; Miedzianka *et al*. 2014; Pralea *et al*. 2011). Process conditions including pH, time, enzyme to substrate ratio and temperature are influential on the hydrolysate composition and thereby the functional properties (Ovissipour *et al*. 2012).

Caseins are the main proteinaceous component of milk (approximately 80% of the total nitrogen in milk consists of three proteins, α-, β- and γ-caseins). Sodium caseinate is a dairy ingredient that its solubility is poor at acidic conditions near its isoelectric point (Luo *et al.* 2014). Different proteases such as a bacterial protease (Hidalgo *et al.* 2015), papain, pancreatin, trypsin (Luo *et al*. 2014) and chymotrypsin (Pralea *et al.* 2011) have been implemented for the hydrolysis of sodium caseinate and some physical, chemical and biochemical properties of the obtained hydrolysates have been characterized. Slaterry and Fitzgerald (1998) employed commercial Bacillus proteinase complex to hydrolyze sodium caseinate. These hydrolysates have improved the emulsion activity and foam expansion at low degrees of hydrolysis (0.5 and 1.0% DH).

Response surface methodology has been used successfully to model and optimizes the enzymatic hydrolysis of proteins from different sources (Nilsang *et al*. 2005; Ovissipour *et al.* 2012; Surówka *et al*. 2004). It is a valuable tool for investigating complex processes which determine the effects of the multiple variables and their interactions on response variables (Majd *et al*. 2014).

Withania coagulans is belonging to the *Solanaceae* family that is grown commonly in Pakistan, Afghanistan, India and the south of Iran (Sarani *et al.* 2014). Fruits from this plant have been widely used as the main source of milk coagulant (Beigomi *et al*. 2014) for the preparation of traditional cheese in Sistan and Baluchistan province of Iran. Also, the

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purification and characterization of *W. coagulans* enzyme (Beigomi *et al*. 2014; Naz *et al*. 2009) and the production of mozzarella cheese (Nawaz *et al*. 2011), Feta cheese (Pezeshki *et al*. 2011)and tofu (Sarani *et al*. 2014) using *W. coagulans* as a new coagulant has been reported. However, it is noteworthy that no study has been carried out on the other applications of the *W. coagulans* protease.

Withania coagulans is available in natural habitats of Iran. Considering the fact that in the available literature, there has been no report on the use of *Withania coagulans* protease for modifying functional properties of proteins. Hence, the aim of the present research was to optimize the effects of reaction conditions (i.e., enzyme activity, hydrolysis temperature, and hydrolysis time) of *Withania coagulans* protease on the degree of hydrolysis, foaming properties and solubility of sodium caseinate.

Materials and Methods

Dried fruits of *W. coagulans* were collected from wild plants which grow in Nikshahr city (Sistan and Baluchistan, Iran). Fruits were washed with distilled water and dried at ambient temperature. Casein sodium salt from bovine milk, bovine serum albumin (BSA) and O-phthaldialdehyde (OPA) were purchased from Sigma-Aldrich Co. (Steinheim, Germany). Other chemicals and solvents were of analytical grade.

Enzyme extraction

According to the method optimized by Shavandi *et al*. (2015), the dried fruits powder was subjected to the extraction by immersing 10 g of powder in 62 mL sodium phosphate buffer, 0.1 mol L^{-1} (pH 3) for 3 hours at 44.2ºC with agitation. Samples were filtered and then centrifuged (Gallenkamp centrifuge 200, Gallenkamp, UK) at 4,000×rpm for 20 minutes. Ammonium sulfate was added to supernatants (85% saturation) and after 24 h of storage at 4ºC, the sediment was separated by centrifugation (10,000×g for 20 min). The precipitate was re-suspended in phosphate buffer (25mM, pH 7.5) and dialyzed (D9527; Sigma-Aldrich) against the same buffer for 24 h at 4ºC. The resulting extracts have been freeze-dried (freeze dryer Christ Alpha 1-4/LD Plus, Christ, Germany), and stored at -20ºC for further use.

Protease activity

The protease activity of *Withania coagulans* extract was determined according to the previously published protocol of Anson (1938), with some modifications. Aqueous enzyme extracts were prepared by dissolving 0.15 g of freeze-dried extract in 5 mL of extraction buffer (pH=7.5, 10 mM sodium acetate and 5 mM acetate calcium). 1 mL of enzyme extract was added to 5 mL of 0.65% casein as a substrate and incubated at 37 ºC for exactly 10 min. After this time, 5 mL of trichloroacetic acid (110 mM) was added to stop the reaction, which was in turn incubated at 37 ºC for 30 min. Following this, each of the test solutions has been filtered and then 5 mL of sodium carbonate (500 mM) and 1 mL of Folin & Ciocalteu's Phenol Reagent were added to 1 mL of solution and the mixture was incubated at 37 ºC for 30 min. The absorbance of the solution was measured using a spectrophotometer at 660 nm. The protease activity was calculated using a standard curve of L-tyrosine.

Hydrolysis of sodium caseinate

Samples of 0.05 g mL^{-1} of sodium caseinate were dissolved in 25 mM phosphate buffer, pH 7. *Withania coagulans* extract was added according to the experimental runs shown in Tables 1. All reactions have been performed in a shaking incubator (Heidolph incubator 1000, Heidolph, Germany) with constant agitation (300 rpm). After inactivating the enzyme by boiling it for 20 min, the samples were cooled on ice and centrifuged at 5000 rpm for 20 min to remove any insoluble contents. Finally, supernatants were freeze-dried and stored at 20ºC for further use (Luo *et al.* 2014).

The degree of hydrolysis (DH)

The DH of hydrolysates was determined by reacting free amino acids with o-

phthaldialdehyde (OPA), according to the method modified by Luo *et al*. (2014). The fresh preparation procedure of the reagent was carried out by mixing 25 ml of 0.1 M Borate buffer (pH 9.5), 2.5 ml of 20% sodium dodecyl sulphate aqueous solution, 40 mg of OPA in 1 ml ethanol, 100 µl of b-mercaptoethanol and the remainder being distilled water to a total volume of 50 ml (Church et al. 1983). Samples (50 µl) were mixed with 3 ml of OPA reagent and the mixture was allowed to stand for 2 min before measuring the absorbance at 340 nm. To achieve the maximum DH, sodium caseinate was hydrolysed with 6 N HCl for 24 h at 120°C. The DH of the samples was calculated by the following equation:

$$DH(\%)= \frac{A_{sample}-A_{blank}}{A_{total}-A_{blank}} \times 100 \qquad (1)$$

Where A_{total} was the absorbance of the sample with maximum DH and A_{blank} was the absorbance of sample replaced with distilled water to the same procedure of the hydrolyzed samples.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

The SDS-PAGE was carried out by the method of Luo et al (2014) on 15% polyacrylamide gel. The hydrolysates were diluted in an SDS–PAGE sample buffer containing b-mercaptoethanol and heated at 95 °C for 5 min. Samples with 4 mg/ml protein were loaded onto the gel for electrophoresis at 200 V. Protein marker (Fermentas, Biotechnology company), ranging from 20 kDa to 120 kDa, was also loaded to estimate peptide molecular weight. After staining using Coomassie brilliant blue G-250, the gel was scanned.

Solubility measurement

Hydrolysates were dispersed in acetate buffer (pH 5, 25 mM) at 10 mg/ml. After incubation for 30 min at room temperature, samples were centrifuged at 10,000g for 10 min (Refrigerate Centrifuge SIGMA 3-30K). The protein content in the supernatant was measured by the biuret method (Layne 1957). The solubility was calculated as (Banach et al. 2013):

Solubility (%)= (protein in supernatant/protein in dispersion) × 100 (2)

Foaming capacity and stability determination

Foaming properties were measured according to the method described by Guan *et al* (2007) with minor modifications. Portions of 20 ml sample solutions (2 mg/ml) at pH 5 were homogenized for 1.5 min at the speed of 10,000 rpm. Foaming capacity (FC) was determined as the percentage increase in the volume of the sample solutions upon mixing. Foam stability (FS) was evaluated as the percent of the foam remaining after 30 min.

Experimental design for optimization

One of the main experimental designs which have been widely used in the second order response surface models is the central composite design (CCD) (Chabeaud et al. 2009; de Castro *et al*. 2015). In this investigation, the effects of three process variables, namely the enzyme loading, hydrolysis time and hydrolysis temperature as well as optimization of the hydrolysis conditions were studied using a full-factorial rotatable CCD. The dependent variables (responses) were the degree of hydrolysis, foaming properties, and solubility. The factors and their levels have been determined based on the preliminary experiments and previously published studies (Shavandi *et al*. 2015). The levels of three variables were: hydrolysis time (30, 145, 260, 375 and 490), hydrolysis temperature (30, 40, 50, 60 and 70 °C), and enzyme loading (1%, 2%, 3%, 4% and 5%). The level of alpha was 2.

A three-factor CCD design with a total of 20 runs, including 8 factorial points, 6 axial points and 6 replicates at the center points was employed to determine the response pattern and then to establish a model. Design-Expert 7 software was used to analyze the observed data. The design of the experiments and response variable values are given in Table 1. The responses could be related to the

independent variables by a second-order polynomial as follows:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{1 \le i \le j} \beta_{ij} X_i X_j \quad (2)$$

Where Y is the predicted response, k is the number of the factors, x_i and x_j are the independent variables, β_0 is a constant term, β_i, β_{ii}, β_{ij} are the regression coefficients for the linear, squared and cross-product terms, respectively. The optimum extraction conditions of different independent variables were determined based on the highest value of responses including DH, solubility, FC, and FS by using 'Numerical Optimization' of the Design Expert 7 software.

Table 1. Central Composite Design (CCD) with the experimental values for the response variables (degree of hydrolysis, solubility and foaming capacity, and stability) applied for hydrolyzing sodium caseinate by *Withania coagulans* extract

Run	X_1	X_2	X_3	DH	S	FC	FS
1	5	50	260	18.15	62.20	128.57	35.55
2	2	40	375	13.89	39.55	142.86	66.49
3	4	40	375	17.32	61.34	150	38.22
4	2	60	145	10.42	35.02	110.91	71.22
5	3	70	260	9.74	27.03	92.86	68
6	2	60	375	14.91	48.9	142.86	72.59
7	4	60	375	15	46.45	92.86	48.48
8	3	50	260	15.28	52.28	117.86	62.59
9	3	50	260	14.03	56.16	135.7	55.16
10	1	50	260	12.66	24.88	121.43	83.22
11	4	60	145	12.04	38.47	103.57	66.67
12	4	40	145	14.23	39.98	142.86	51.72
13	3	50	260	14.20	60.91	125	53.54
14	2	40	145	9.45	24.66	117.24	67.55
15	3	50	260	13.43	57.89	125	47.22
16	3	50	490	16.83	68.14	132.14	37.5
17	3	50	30	11.87	22.72	114.29	73.72
18	3	30	260	8.04	34.58	135.71	52.02
19	3	50	260	15.33	57.67	128.57	51.75
20	3	50	260	14.05	57.02	114.29	58.02

X1: Enzyme level (%w/w); X_2: Hydrolysis temperature (°C); X_3: Hydrolysis time (min); DH, the degree of hydrolysis; S, Solubility; FC, Foam capacity; FS, Foam stability

Results and discussion

Enzyme activity and SDS-PAGE

Withania coagulans extract displayed a proteolytic activity of 0.323 UmL^{-1}(0.141 U mg $protein^{-1}$) according to the casein method (Anson 1938). The resulting extract was run in SDS-PAGE in combination with protein marker for correct size identification of the partially purified protease. Proteins obtained from the extract were observed in an electrophoretogram with the protein band of approximately 60 kDa with a range of Mw values from 20 to 35 kDa (Fig. 1). These bands had Mw values which were similar to the reported Mw for *W. coagulans* protease by Beigomi *et al*. (2014) and Naz *et al*. (2009).

The degree of hydrolysis (DH) and SDS-PAGE characterization

To evaluate the influences of the three contributing factors, namely the enzyme loading, hydrolysis temperature and hydrolysis time on DH, the design matrix of experimental conditions with the corresponding response values in Table 1 was fitted to a polynomial model. A quadratic model was proposed, based on the results of the sequential model, sum of squares and the calculated statistics for all model terms. The mathematical equation suggested for this response in terms of coded values is shown in Table 3. The coefficient of determination (R^2) of the model could be applied for checking the experimental data variability. R^2 for DH indicated that the model could explain 94.15% of the variability in the response and only 5.85% of the total variation could not be attributed to the variables. In addition, lack of fit test was insignificant (Table 1) that indicates the model is careful to predict the degree of hydrolysis.

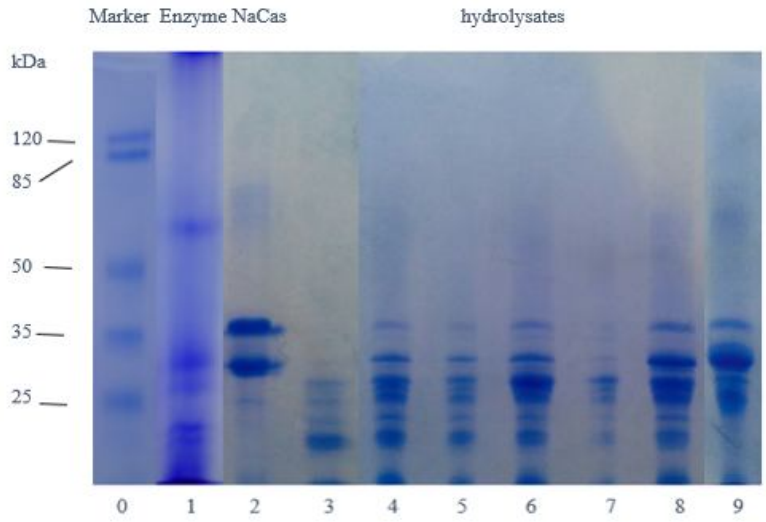

Fig. 1. SDS-PAGE of the enzyme (lane 1), sodium caseinate (NaCas, lane 2) and its hydrolysates. Lanes 3, 4, 5, 6, 7, 8 and 9 represent samples 3, 12, 2, 4, 11, 14 and 5, respectively. Lane 0 shows protein markers.

Among the independent variables, linear effects of the enzyme level and hydrolysis time and quadratic effects of hydrolysis temperature had a very prominent effect ($p<0.0001$). Furthermore, the effect of the interaction between the enzyme loading and hydrolysis temperature on the DH was notable ($p<0.01$).

Figure 2(A) depicts the response surface plots of the effects of the three variables, the enzyme loading, hydrolysis time and hydrolysis temperature on DH. The temperature demonstrated quadratic effects on the response; hence DH increased up to approximately 50 ^{o}C followed by a decline with its further increase. However, hydrolysis time and enzyme loading caused a linear increase in the response. Considering that the DH is defined as the percentage of peptide bonds cleaved by protease (Adler-Nissen 1979), the hydrolysate with high DH is believed to contain more low-molecular-weight peptides in comparison with the hydrolysate with low DH. Similar trends of DH growth with the enhancement of hydrolysis time has been reported by Nilsang *et al.* (2005), and with the rise of enzyme loading by Ovissipour et al. (2012). The reduction of the degree of hydrolysis with an increase in the temperature (above 50^{o}C) is probably due to the decreased enzyme activity at high temperatures.

Results of SDS-PAGE presented in Fig. 1 generally agreed with DH. The hydrolysis of sodium caseinate resulted in hydrolysates with smaller molecular weights, as observed in SDS-PAGE (Fig. 1). Among the SDS- PAGE samples, lower molecular weights were observed in sample 3 (Lane 3) with the highest DH.

Solubility

A second-order polynomial regression model was selected to predict the solubility response. The simplified model is shown in Table 3. The model was found significant for solubility and the lack-of-fit test indicated no significance with p-values <0.0001 for the model (Table 2).

This indicates that the fitted model is quite appropriate for predicting and determining the values for solubility within the design space (Jain & Anal. 2016). Regression coefficients have indicated that the solubility of the hydrolysate was firstly dependent on hydrolysis time, secondly on enzyme level, and thirdly on hydrolysis temperature in their linear and quadratic forms.

Table 2. ANOVA table for the degree of hydrolysis, solubility and foaming properties of sodium caseinate hydrolyzed by *Withania coagulans* extract

Regression	Sum of squares	df [a]	Mean square	F-value	p-value
Degree of hydrolysis					
Model	127.27	5	25.45	45.04	< 0.0001
X_1	27.30	1	27.30	48.31	< 0.0001
X_3	38.78	1	38.78	68.63	< 0.0001
$X_2 X_1$	5.27	1	5.27	9.33	0.0086
X_2^2	48.92	1	48.92	86.56	< 0.0001
X_1^2	1.88	1	1.88	3.32	0.0899
Lack of Fit	5.03	9	0.56	0.97	0.5451
Solubility					
Model	3658.48	6	609.75	27.35	< 0.0001
X_1	794.69	1	794.69	35.64	< 0.0001
X_3	1386.74	1	1386.74	62.19	< 0.0001
$X_2 X_1$	162.96	1	162.96	7.31	0.0181
X_2^2	1149.73	1	1149.73	51.56	< 0.0001
X_1^2	322.23	1	322.23	14.45	0.0022
X_3^2	242.74	1	242.74	10.89	0.0058
Lack of Fit	250.35	8	31.29	3.96	0.0731
Foam capacity					
Model	4347.36	5	869.47	20.78	< 0.0001
X_2	2220.17	1	2220.17	53.07	< 0.0001
X_3	502.96	1	502.96	12.02	0.0038
$X_2 X_1$	1014.68	1	1014.68	24.25	0.0002
$X_1 X_3$	467.20	1	467.20	11.17	0.0048
X_2^2	142.36	1	142.36	3.40	0.0863
Lack of Fit	294.47	9	32.72	0.56	0.7870
Foam stability					
Model	2847.66	4	711.91	23.89	< 0.0001
X_2	280.11	1	280.11	9.40	0.0078
X_1	1765.96	1	1765.96	59.25	< 0.0001
X_3	673.61	1	673.61	22.60	0.0003
$X_1 X_3$	127.99	1	127.99	4.29	0.0559
Lack of Fit	307.57	10	30.76	1.10	0.4866

[a] df = degree of freedom; X1: Enzyme level (%w/w); X_2: Hydrolysis temperature (°C); X_3: Hydrolysis time (min)

Response surface plots were created from the polynomial model to demonstrate the effect of each pair of independent variables on the solubility (Fig. 2(B)). An increase in solubility was achieved by an increase in hydrolysis time and enzyme level. Results presented in Fig. 2(B) depict that the solubility with increasing temperature up to an average value increased and then began to fall.

A remarkable rise in protein solubility during the enzymatic hydrolysis has been reported by Luo *et al* (2014). The low molecular weight of hydrolysates and the corresponding increase in the number of the exposed ionizable amino and carboxyl groups accounted for the high solubility of hydrolysates (Panyam & Kilara. 1996).

Table 3. Simplified second-order polynomial equations of the four responses studied

Responses	Simplified polynomial model (coded factors)	CV (%)	R^2	Adj R^2	PRESS
DH	$Y_1 = 14.42 + 1.31X_1 + 1.56X_3 - 0.81X_1X_2 - 1.36X_2^2 + 0.27X_1^2$	5.55	0.9415	0.9206	16.10
S	$Y_2 = 56.55 + 7.05X_1 + 9.31X_3 - 4.51X_1X_2 - 6.76X_2^2 - 3.58X_1^2 - 3.11X_3^2$	10.31	0.9266	0.8927	1248.11
FC	$Y_3 = 125.56 - 0.64\times X_1 - 11.78\times X_2 + 5.61\times X_3 - 11.26\times X_1X_2 - 7.64\times X_1X_3 - 2.29\times X_2^2$	5.23	0.8813	0.8389	1270.02
FS	$Y_4 = 58.06 + 4.18X_2 - 10.51X_1 - 6.49X_3 - 4X_1X_3$	9.4	0.8643	0.8281	781.69

CV, Coefficient of variation; R^2, Coefficient of multiple determination; Adj R^2, Adjust R^2; PRESS, Predicted Residual Sum of Squares; X1: Enzyme level (%w/w); X_2: Hydrolysis temperature (°C); X_3: Hydrolysis time (min); DH, degree of hydrolysis; S, Solubility; FC, Foam capacity; FS, Foam stability

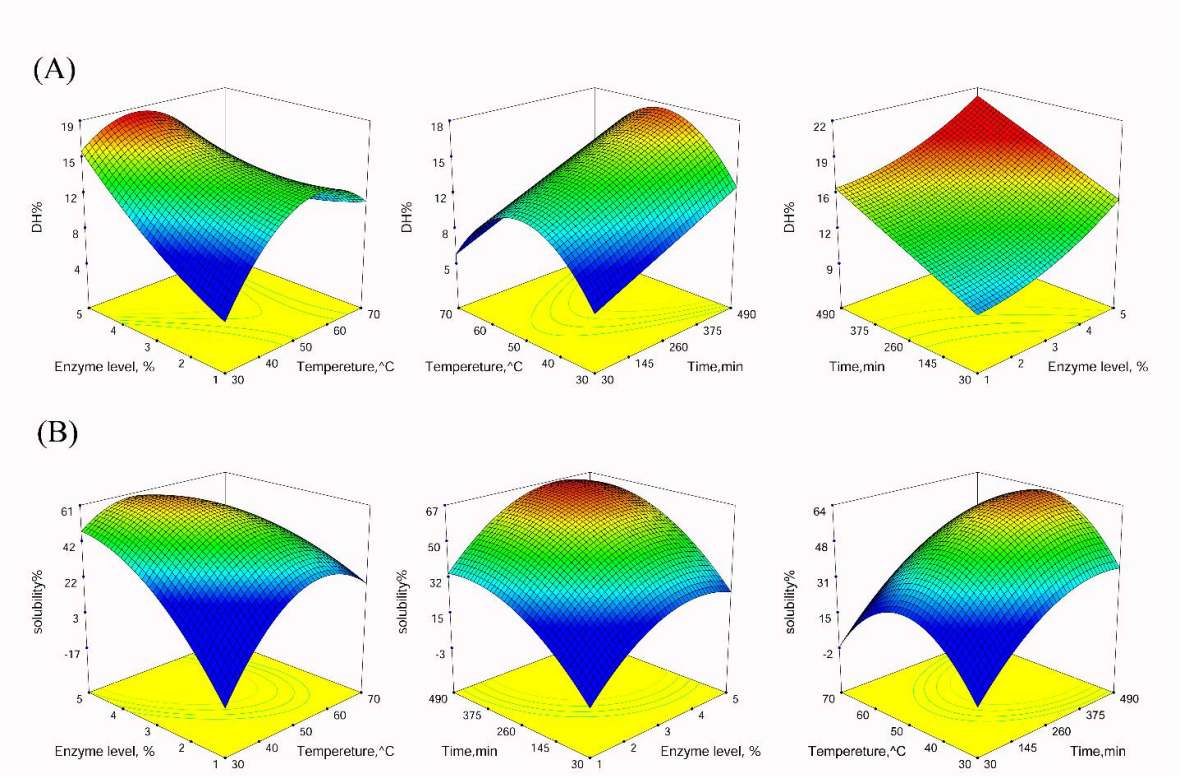

Fig. 2. Response surfaces for the degree of hydrolysis (DH) (A) and solubility (B) as a function of hydrolysis time, hydrolysis temperature, and enzyme level. The missing independent variable in each plot was kept at the center of levels.

Foaming properties

The analysis of variance (ANOVA) indicated that the P-values for foaming properties (FC and FS) were less than 0.01, suggesting that the proposed models showed high significance at a 99.99% confidence level (Table 2). In addition, the R^2 values indicated the models were able to explain 88.13% (FC) and 86.43% (FS) of the experimental data variability. Moreover, the models show statistically insignificant lack of fit, as is evident from the P value of 0.787 and 0.4866 for FC and FS, respectively (Tables 2 & 3). This shows that the models are sufficiently accurate for predicting the foaming properties for any combination of experimental independent variables.

As shown in Fig. 3, the foaming capacity increased with the rise in the enzyme concentration, hydrolysis time and hydrolysis temperature and decreased with higher levels of these factors. It should be mentioned that the foam stability decreased with the increase in the enzyme concentration and hydrolysis time. The aforementioned results demonstrate that a limited amount of hydrolysis is favorable to increase foam capacity, but foam stability is highly decreased as a result of such hydrolysis. Hydrolysis produces smaller peptides which are probably owing to an initial growth in the polypeptide content and allows more air to be incorporated. However, the polypeptides do not have the ability to stabilize the air cells. Foaming capacity has been reported to experience improvements in enzyme-modified food proteins (Ma 1985; Puski 1975). Some larger protein components were required to make a stable foam (Turner 1969). In this study, the breakdown of larger protein components due to enzyme treatment could cause the loss of the foam stability. In addition, an increase in charge density because

of the hydrolysis leads to the reduction of the foam stability since foam stability is developed when the electrostatic repulsion of proteins is at its minimum amount (İbanoğlu & İbanoğlu. 1999).

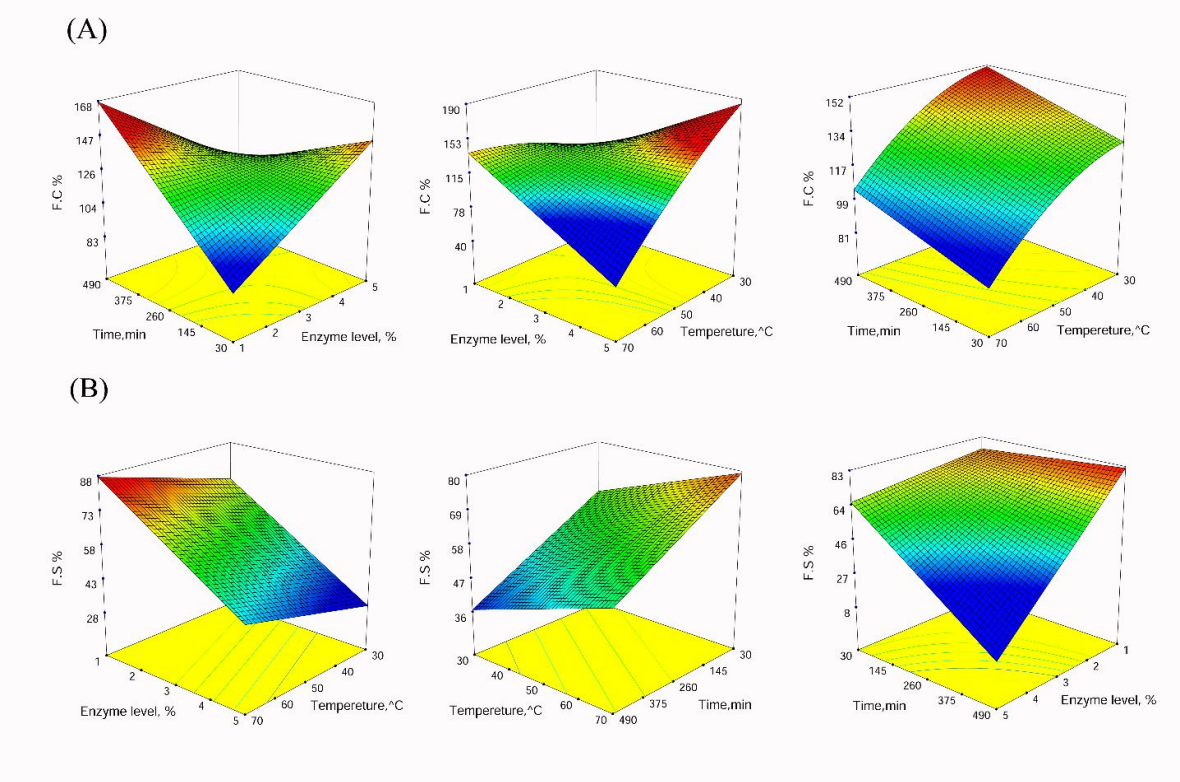

Fig. 3. Response surfaces for foam capacity (A) and foam stability (B) as a function of hydrolysis time, hydrolysis temperature, and enzyme level. The missing independent variable in each plot was kept at the center of levels.

Optimization

Numerical optimization was used to determine the optimal extraction condition. Optimization was done based on the highest values of DH, solubility, FC, and FS as responses. The suitability of the models for producing peptides with the highest functional properties was tested under the conditions: enzyme concentration of 1.75 (%w/w), temperature of 55.43 °C and hydrolysis time of 490 min. Under these conditions, the predicted. DH, solubility, FC, and FS of sodium caseinate hydrolysates were 16.47%, 49.42%, 156.43% and 70.47%, respectively.

A controlled hydrolysis is desirable to increase functional properties of the sodium caseinate. Therefore, the application of *W. coagulans* in the food industry to produce the hydrolyzed proteins with desired properties is highly recommended. Considering the fact that the *W. coagulans* has been employed for the local and medicinal purposes in the human diet, the safety of this plant for industrial applications is approved. The results of the current research present new perspectives on the use of this medicinal plant as an alternative to commercial enzymes for the modification of proteins. Further researches regarding the determination of the other functional properties of sodium caseinate and the use of different proteins are necessary to be done.

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پروتئولیز کازئینات سدیم با استفاده از عصاره *ویتانیا کوآگولانس*: یک مطالعه بهینه‌یابی

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چکیده

میوه ویتانیا کوآگولانس یک منبع پروتئاز گیاهی جدید برای استفاده در صنایع غذایی به‌عنوان جایگزین آنزیم‌های تجاری است. بنـابراین مطالعـه کاربردهای متفاوت این پروتئاز گیاهی در دسترس ضروری است. هدف این مطالعه تولید هیدرولیزات کازئینات سدیم بر اساس خصوصیات کف‌کنندگی، حلالیت و درجه هیدرولیز بهینه، از طریق ترکیب عوامل هیدرولیز مانند زمان، درجه حرارت و میزان آنزیم است. یک طرح مرکب مرکزی برای آزمون‌ها و یک چند جمله‌ای درجه دو برای مدل‌سازی اثرات زمان، درجه حرارت و آنزیم بر روی خصوصیات عملکردی استفاده شد. نتایج نشان داد که افـزایش زمان هیدرولیز و میزان آنزیم سبب افزایش پاسخ‌ها شد. مدل درجه دو ایجاد شده نشان داد که شرایط بهینه برای ماکزیمم پاسخ‌ها غلظت آنزیم 1/75% (وزنی/ وزنی)، درجه حرارت 55/43 درجه سانتی‌گراد و زمان هیدرولیز 490 دقیقه بود. خصوصیات عملکردی هیدرولیزات کازئینات سدیم به‌وسیله روش سطح پاسخ بهینه‌یابی شد و نتایج نشان داد که هیدرولیز کنترل شده برای افزایش خصوصیات عملکردی کازئینات سدیم مطلوب است.

واژه‌های کلیدی: کازئینات سدیم، هیدرولیز، پروتئاز ویتانیا کوآگولانس، خصوصیات عملکردی، بهینه‌یابی

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