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Optimising conditions for enzymatic extraction of edible gelatin from the cattle bones using response surface methodology

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Summary This work was initiated to optimise the factors affecting the enzymatic extraction of edible gelatin from the cattle bones using response surface methodology. A central composite rotatable design was used to evaluate the effects of the enzyme concentration, time of enzymatic treatment and extraction temperature on the yield of extraction, gel strength, apparent viscosity and absorption at 420 nm. The R^2 values of regression models for all the response variables were higher than 0.9. Data analysis showed that all the process variables significantly ($P < 0.01$) affected the gel strength and apparent viscosity of extracted gelatin, whereas the effect of extraction temperature on both yield of extraction and absorption at 420 nm was not significant ($P > 0.05$). Graphical optimum conditions for the extraction yield, gel strength, apparent viscosity and absorption at 420 nm were determined as 6.1 ppm, 15.6 h, 70 °C; 9.1 ppm, 11.9 h, 70.3 °C; 7.86 ppm, 14.9 h, 77.5 °C and 2.8 ppm, 10 h, 60 °C, respectively. For all the response variables, the experimental values were very close to the predicted values and were not statistically different ($P < 0.05$).

Keywords Edible gelatin, enzymatic extraction, optimisation, response surface methodology.

Introduction

Gelatin is a very important fibrous protein, which has numerous applications, particularly in the pharmaceutic and food industries because of its unique chemical and physical properties among which the ability to form thermally reversible gels. Heat denaturation of collagen produces gelatin. So far, most of the available gelatins have been produced from mammalian resources, either pigskins or cowhides (Simon *et al.*, 2002). Typical collagen materials are including skins, bones, hides and convective tissue of an animal body. The most preferred collagen source for producing high-quality gelatin is cow bone (Rowlands & Burrows, 2000). The gelatin extracted from fish by-products has poor functional properties; therefore, it is modified by chemical and enzymatic methods to improve gelling and melting points (Cho *et al.*, 2004, 2005). The quality of a gelatin for a particular application largely depends on its rheological properties (Gomez-Guillen *et al.*, 2002). Currently, the manufacturing process for obtaining high purity gelatins involves demineralisation of bone, followed by extended alkaline treatment (liming) and finally gelatin extraction with water of increasing temperature. The liming step of this process requires up to 60 days, the longest step in the approximately 3-month process of producing gelatin. In order to eliminate the liming step, a novel process has been proposed for the production of high purity gelatin utilising proteolytic enzymes for extraction of gelatin from osein. In this method, gelatin production with very low colour and high gel strength over a wide range of viscosities is possible. Further, the low temperature enzyme method of manufacturing gelatin results in lower unit manufacturing costs because of increased yield reduced chemical costs, water usage and utility costs. Among the methods of gelatin production, enzymatic extraction is used to produce high purity gelatins (Rowlands & Burrows, 2000).

The basic purpose of response surface methodology (RSM) is the evaluation of the relationship between the predicted values of the dependent variable and the conditions of dependent variables. Cho *et al.* (2005) previously reported that gelatin extraction was optimised from yellowfin tuna skin using RSM. They demonstrated that the RSM was very effective for investigating the optimum extraction conditions for

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gelatin extraction. The aim of this study was to optimise the factors affecting enzymatic extraction of edible gelatin from the cattle bones using RSM. For this purpose, a central composite rotatable design (CCRD) was utilised to fit a second-order polynomial by a least-squares technique.

Materials and methods

Bone preparation and analysis

The cattle bones were obtained from the Senobar Meat Products Company, Isfahan, Iran. Firstly, the meat residues that adhered to bones were cleaned. The cleaned bones were cut to 5–10 cm pieces with a chopper and then ground to 1–3 mm particles. After that, 200 g portions of the ground bones were packed and then sealed in plastic bags, and finally preserved in −20 °C until the gelatin extraction step. Components of ground bones were measured for protein, fat, moisture and ash content (Helrich, 1990; Nasrallah & Ghossi, 1993).

Pre-treatments, enzymatic extraction and gelatin purification

Defatting of ground bone was carried out by high-pressure water as previously described (Jones, 1970). Demineralisation of bone was carried out by adding 1428 mL of hydrochloric acid (50 g L^{-1}) to 195 g of the degreased bone and mixing them at 8 °C for 2 h to dissolve inorganic materials (mainly calcium phosphate). After 2 h, osein was separated from the soluble phase of the mixture and then was washed with 6.2-L distilled water for 7 min. The washed osein then was used for the extraction process (Moy & Takerkart, 1995; Nicolas-Somonnot *et al*., 1997). During the enzymatic extraction stage, the pH of osein was firstly adjusted to 9 by adding saturated calcium hydroxide solution, then the osein was treated with 2, 6 and 10 ppm Neutrase solution, an alkaline protease obtained from Novo Nordisk Co., (Bagsvaerd, Denmark) for 8, 12 and 16 h at 50 °C (50 °C and pH 9 were the optimum conditions for Neutrase activity). The Neutrase solution weight was twice as much of osein weight. Temperature and pH of the enzymatic treatment were controlled using stirrer hot plate and adding the saturated calcium hydroxide solution, respectively. After enzymatic treatment, the Neutrase was deactivated by elevating the mixture temperature to 100 °C, for a 1 min. Then, pH of the mixture was adjusted to 7. Finally, gelatin extraction was carried out at 60, 70 and 80 °C for 3 h (Petersen & Yates, 1977; Rowlands & Burrows, 2000). Solubilised gelatin was separated from residual bone fragments using centrifugation at 900 × *g* for 30 min at 30 °C. The supernatant was vacuum-filtered with a filter paper (Whatman No. 4). Purification of the gelatin solution was carried out by adding calcium hydroxide solution (6 °Bé) to the filtrate to achieve pH 9, followed by adding aluminium sulphate (25%) to reduce the pH to 7.5 and addition of phosphoric acid (10%) to further reduce the pH to 5.5. A floating floc was formed by applying polyacrylamide polymer (10% w/v) to the gelatin solution and the floc was removed by centrifugation (Rowlands & Burrows, 2000). The clear gelatin solution was decolourised with 0.6 g active carbon, well blended and then filtered (Petersen & Yates, 1977). The gelatin solution was demineralised using Purolite cation and anion exchange resins. The resins were removed after 16 h using filtration through a 250-mesh nylon filter (Holzer, 1996). During the treatment with ion exchange resins, the pH in the gelatin solutions was increased from 5.5 to 6.8 because of net removal of anions. After filtration and ion exchange treatment, the extracted gelatins were colourless and free from any odour. To investigate the quantitative and qualitative characteristics of the purified gelatin solution, the extraction yield, gel strength, apparent viscosity and absorbance at 420 nm were determined. The extracted and purified gelatin was analysed to determine pH, melting point and ash content. Furthermore, the Cu, Fe and Pb contents of purified gelatin were measured (results were average of three replications) (Helrich, 1990; Choi & Regenstein, 2000; Francis, 2000).

Determination of yield, gel strength, apparent viscosity and colour of gelatin

After the extraction time, 5 mL sample was taken and centrifuged, and then the supernatant was analysed by refractometer to determine the gelatin content and extraction yield. The Abbe table refractometer (Carl Zeiss model, Oberkochen, Germany) was used to measure the Brix (%), and the relationship used to calculate the gelatin concentration was as follows (Nicolas-Somonnot *et al*., 1997):

Concentration of gelatin (g L^{-1}) = 6.81 × (% from refractometry)

Gel strength was determined using an Instron model 1140 (Instron, Norwood, MA, USA) food tester with 5–50 kg load cell, a crosshead speed of 10 cm min^{-1} and chart speed of 40 cm min^{-1}. Gelatin was dissolved in distilled water (6.67%, w/v) at 62 °C for 15 min until completely dispersed and then kept at 10 ± 0.1 °C for 17 h. After cool maturation, the gel strength (expressed in Bloom value, g) was determined by forcing a plunger (12.7 mm diameter) to a 4-mm depth into a 6.67% gel in a Bloom jar (Helrich, 1990; Poppe, 1997; Francis, 2000). Apparent viscosity was measured by a Brookfield DV-II+ programmable viscometer (JD Instruments, Inc., Houston, TX, USA) equipped with a No. 1 spindle. Gelatin solutions (6.67% w/v) were prepared by dis-

solving dry powder in distilled water and heating to 60 °C and then apparent viscosity was measured at 60 rpm and at the temperature 60 °C in terms of cP (Helrich, 1990; Francis, 2000). The colour of gelatin was investigated by determining the absorbance at 420 nm of the 1% gelatin solution (Rowlands & Burrows, 2000).

Experimental design and statistical analysis

A CCRD with the three variables was followed to examine the response pattern and also to determine the optimum synergy of variables (Cochran & Cox, 1957). The factors and their optimised ranges that were chosen for independent variables were enzyme concentration (2–10 ppm), time of enzymatic treatment (8–16 h) and extraction temperature (60–80 °C), each at three levels, namely −1, 0 and 1. The range and centre point values of three independent variables were based on the results of preliminary experiments (Table 1). Parameters in gelatin extraction that were measured as dependent variables were the extraction yield, gel strength, apparent viscosity and absorption at 420 nm. The CCRD in the experimental design consists of twelve factorial points and three replicates of the central point. Three replicates at the centre of the design were used to estimate the error sum of squares. Experiments were randomised in order to minimise the effects of unexplained variability in the observed responses because of extraneous factors. The CCRD and responses of dependent variables for enzymatic gelatin extraction from the cattle bones are shown in Table 2. Although the exact mathematical model for any response (dependent variables) usually is unknown, it can be estimated accurately by a second-order polynomial expression that accounts for variations caused by linear and quadratic order effects as well as by interaction (Cochran & Cox, 1957; Floros & Chinnan, 1988; Mudahar *et al*., 1990; Hu, 1999). The data were analysed using the response surface regression (RSREG) procedure of the STATISTICAL ANALYSIS SYSTEM software (Version 6.12®, SAS 1996; SAS Institute, Inc., Cary, NC, USA) to fit the following quadratic polynomial equation:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \sum \beta_{ij} X_i X_j \quad (1)$$

where Y is the dependent variable (extraction yield, gel strength, apparent viscosity and absorption in 420 nm); β_0 is constant; β_i, β_{ii} and β_{ij} are regression coefficients and X_i and X_j are levels of the independent variables. To visualize the relationship between the response and the experimental levels of each factor, the fitted polynomial regression equations were expressed graphically using the SURFER ACCESS SYSTEM (Version 7, 2001; Golden Software, Inc., Golden, CO, USA) software package. Equation (1) fit to the experimental data (Table 2) and four models were obtained. The adequacy and fitness of these models was tested by analysis of variance (ANOVA). When the optimum conditions of enzymatic gelatin extraction were deter-

Table 1 Coded and uncoded levels of variables used in enzymatic process for the gelatin extraction

		Coded levels of variables		
Independent variables	**Symbol**	**−1**	**0**	**1**
Enzyme concentration (ppm)	X_1	2	6	10
Time of enzymatic treatment (h)	X_2	8	12	16
Extraction temperature (°C)	X_3	60	70	80

Table 2 The CCRD and the responses of dependent variables for enzymatic gelatin extraction from the cattle bones

				Response variables			
Run	X_1	X_2	X_3	**Yield (%) Y_1**	**Gel strength (g) Y_2**	**Apparent viscosity (cP) Y_3**	**Absorbance at 420 nm Y_4**
1	1	1	0	12.35	220.1	4.53	0.067
2	1	−1	0	9.96	238.4	3.23	0.047
3	−1	1	0	8.35	135	4.51	0.040
4	−1	−1	0	7.89	96	3.03	0.033
5	1	0	1	12.16	230.7	4.12	0.072
6	1	0	−1	11.1	223.8	3.98	0.065
7	−1	0	1	8.28	119	4.63	0.039
8	−1	0	−1	8.07	101	3.12	0.037
9	0	1	1	14.92	194.6	5.16	0.077
10	0	1	−1	13.6	190.5	4.25	0.055
11	0	−1	1	10.86	177.9	3.68	0.051
12	0	−1	−1	9.9	167.4	4.63	0.069
13	0	0	0	12.73	217.6	4.63	0.069
14	0	0	0	12.65	219	4.59	0.059
15	0	0	0	12.56	217.9	4.76	0.065

Table 3 Estimated coefficients of regression models for dependent variables in the enzymatic gelatin extraction

	Yield (%) Y_1		Gel strength (g) Y_2		Apparent viscosity (cP) Y_3		Absorbance at 420 nm Y_4	
Coefficient	**Coefficient estimate**	**SE (±)**	**Coefficient estimate**	**SE (±)**	**Coefficient estimate**	**SE (±)**	**Coefficient estimate**	**SE (±)**
β_0	12.647**	0.4650	218.167**	2.099	4.6600**	0.0468	0.06433**	0.00354
β_1	1.6225**	0.2848	57.750**	1.285	0.0712	0.0287	0.01275**	0.00217
β_2	1.3262**	0.2848	7.562**	1.285	0.6650**	0.0287	0.00837*	0.00217
β_3	0.4437	0.2848	4.937*	1.285	0.3812**	0.0287	0.00512	0.00217
β_{11}	−2.7133**	0.4192	−29.883**	1.892	−0.4712**	0.0422	−0.01017*	0.00319
β_{21}	0.4825	0.4027	−14.325**	1.818	−0.0450	0.0406	0.00325	0.00306
β_{22}	−0.29583	0.4192	−15.908**	1.892	−0.3637**	0.0422	−0.00742	0.00319
β_{31}	0.2125	0.4027	−2.775	1.818	−0.3425**	0.0406	0.00125	0.00306
β_{32}	0.09	0.4027	−1.6	1.818	0.1050*	0.0406	0.00300	0.00306
β_{33}	−0.03083	0.4192	−19.658**	1.892	−0.22625**	0.0422	−0.00092	0.00319
R^2 values	0.9526		0.998		0.995		0.935	

*Significant ($P < 0.05$), **Highly significant ($P < 0.01$).

mined by the models derived using RSM, gelatin extraction was carried out under these conditions and experimental data were compared with the values predicted by regression models.

Results and discussion

Chemical composition

Components of the bone in this research were determined as 23.5 ± 1.25% protein, 16.93 ± 0.3% fat, 25.54 ± 0.27% moisture and 34.03 ± 1.43% ash. Characteristics of extracted and purified gelatin in the optimum conditions were measured as pH 5.78, melting point 29.6 °C and ash content 1.76%. Furthermore, the Cu, Fe and Pb contents were measured as 7.22, 20.9 and <1.05 ppm, respectively. These characteristics were comparable with the features reported for the commercial gelatins from the cattle bone (Poppe, 1997).

Analysis of experimental data

The coefficients of second-order regression models for response variables in gelatin extraction are presented in Table 3. The results indicated that none of the models possessed significant ($P > 0.05$) lack of fit and all models were significant with satisfactory values of R^2. Therefore, all developed models were good predictors of dependent variables (Table 4). The overall effects of the process variables on the responses were further analysed and the results showed that all the process variables significantly ($P < 0.01$) influenced the gel strength and the apparent viscosity (Table 5). However, as the enzyme concentration and time of enzymatic treatment had only significantly influence on extraction yield and absorbance at 420 nm.

Extraction yield (Response Y_1)

The results of ANOVA for the model equation of extraction yield are summarised in Tables 3 and 4. The

Table 4 Analysis of variance of the second-order regression models showing the effects of independent variables as a linear, quadratic and cross-product term on the response variables

		Yield (%) Y_1				Gel strength (g) Y_2				Apparent viscosity (cP) Y_3				Absorbance at 420 nm Y4			
Source	**DF**	**SS**	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	***F*-value**	***P*-value**
Regression																	
Linear	3	36.71	12.24	18.86	0.0037	27333	9111	689.5	0.0000	4.74	1.58	240	0.0000	0.00210	0.0007	18.375	0.0039
Quadratic	3	27.34	9.11	14.05	0.0072	4987.8	1662.6	125.8	0.0000	1.32	0.44	66.92	0.0002	0.00055	0.00018	4.839	0.0611
Cross-product	3	1.14	0.38	0.59	0.6489	861.86	287.29	21.74	0.0027	0.52	0.17	26.39	0.0017	0.00008	0.000027	0.749	0.5677
Model	9	65.19	7.24	11.16	0.0081	33183	11061	279	0.0000	6.58	2.19	111.1	0.0000	0.00270	0.0009	7.988	0.0170
Residual																	
Lack of fit	3	3.23	1.08	15.43	0.067	64.99	21.66	39.87	0.0246	0.017	0.0057	0.723	0.6249	0.00014	0.000046	1.806	0.3758
Pure error	2	0.14	0.07	–	–	1.09	0.54	–	–	0.016	0.0079	–	–	0.00005	0.000025	—	—
Total error	5	3.37	0.67	–	–	66.07	13.21	–	–	0.033	0.0066	–	–	0.00019	0.000038	—	—
Total	14	68.56	4.90	–	–	33249.1	2374.93	–	–	6.613	0.472	–	–	0.00289	0.000206	—	—

DF, degrees of freedom; SS, sum of square; MS, mean square.

Table 5 Analysis of variance for the overall effect of the independent variables on response variables

Independent variables	DF	Yield (%) Y_1				Gel strength (g) Y_2				Apparent viscosity (cP) Y3				Absorbance at 420 nm Y_4			
		SS	MS	*F*-value	*P*-value	SS	MS	*F*-value	*P*-value	SS	MS	*F*-value	*P*-value	SS	MS	*F*-value	*P*-value
X_1	4	49.35	12.34	19.02	0.0032	30829	7707.35	583.2	0.0000	1.34	0.33	50.79	0.0003	0.00173	0.00043	11.51	0.0098
X_2	4	15.36	3.84	5.92	0.0389	2223.02	555.76	42.05	0.0005	4.08	1.02	154.8	0.0000	0.00084	0.00021	5.60	0.0432
X_3	4	1.79	o.48	0.69	0.6293	1662.97	415.74	31.46	0.0010	1.86	0.47	70.810	0.0001	0.00025	0.00006	1.70	0.2854

DF, degrees of freedom; SS, Sum of square; MS, mean square.

cross-product terms β_{21}, β_{31} and β_{32}, the linear term β_3 and the quadratic terms β_{22} and β_{33} were not significant ($P > 0.05$) and were removed from the model, but the linear terms β_1 and β_2 and the quadratic term β_{11} were used because of significant effects at the confidence level of more than 99%. As the regression model is determined with coded values of the variables, the size of each coefficient gives a direct measurement of the importance of each effect (Psomas *et al*., 2007). The magnitude of coefficients showed that the enzyme concentration had more positive (linear) influence on the yield extraction than the time of enzymatic treatment. In contrast, the enzyme concentration had only a negative quadratic effect on the yield (Table 3).

Gel strength (Response Y_2)

Analysis of variance for the model equation of gel strength (Tables 3 and 4) showed that the cross-product terms β_{31} and β_{32} were not significant ($P > 0.05$), and were removed from the model, but the linear terms β_1, β_2 and β_3, the cross-product term β_{21} and the quadratic terms β_{11}, β_{22} and β_{33} were used because of their significant effects at the confidence level of more than 99%, except for the linear term β_3, which was significant at $P < 0.05$. Similarly, for the gel strength, the magnitude of coefficients showed that the enzyme concentration had more positive linear influence on gel strength than the time of enzymatic treatment and extraction temperature (about seven times more than the time of enzymatic treatment effect and eleven times more than extraction temperature effect). Conversely, the enzyme concentration had more negative quadratic effect than the time of enzymatic treatment and extraction temperature (the magnitude of enzyme concentration's effect was two times more than the time of enzymatic treatment's effect and 1.5 times more than extraction temperature's effect in absolute values). From these results, it can be concluded that the interaction effect of enzyme concentration/time of enzymatic treatment was negative.

Apparent viscosity (Response Y_3)

For the model equation of apparent viscosity, ANOVA (Tables 3 and 4) showed that the linear term β_1 and the cross-product term β_{21} were not significant ($P > 0.05$), thus they were removed from the model, but the linear terms β_2 and β_3, the quadratic terms β_{11}, β_{22} and β_{33} and the cross-product terms β_{31} and β_{32} were included in the model (confidence level of all significant terms was more than 99%, except for the cross-product term β_{32}, which was significant at $P < 0.05$). The magnitude of coefficients showed that the time of enzymatic treatment had more positive linear influence (about two times) on apparent viscosity than the extraction temperature. On the contrast, the enzyme concentration had more negative quadratic effect than the time of enzymatic treatment and extraction temperature (the magnitude of enzyme concentration's effect was about 1.3 times more than the time of enzymatic treatment's effect and two times more than extraction temperature's effect in absolute values). Furthermore, the interaction effect of enzyme concentration/extraction temperature was negative, whereas interaction effect of time of enzymatic treatment/extraction temperature was positive (the magnitude of the former interaction effect was three times more than the latter one in absolute values).

Absorption in 420 nm (Response Y_4)

Based upon an ANOVA for the model equation of absorption in 420 nm (Tables 3 and 4) that the linear term β_3, the quadratic terms β_{22} and β_{33} and all cross-product terms were not included in the model because of their insignificant effects ($P > 0.05$), but the linear terms β_1 and β_2 and the quadratic term β_{11} had significant effects on the model (confidence level of β_1 was more than 99%, but it was more than 95% for β_2 and β_{11}). As it is shown in Table 3, the time of enzymatic treatment had more positive linear influence (about 1.5 times) on the absorption in 420 nm than the time of enzymatic treatment. However, the enzyme concentration showed a negative quadratic effect.

Response surface and contour plotting

The profile of the process responses against some of process variables is shown as the response surface and contour plots in Figs 1 and 2. As it is shown in Fig. 1a, increasing the enzyme concentration to 6 ppm led to increasing the extraction yield at the lowest time of enzymatic treatment, but it was almost constant for

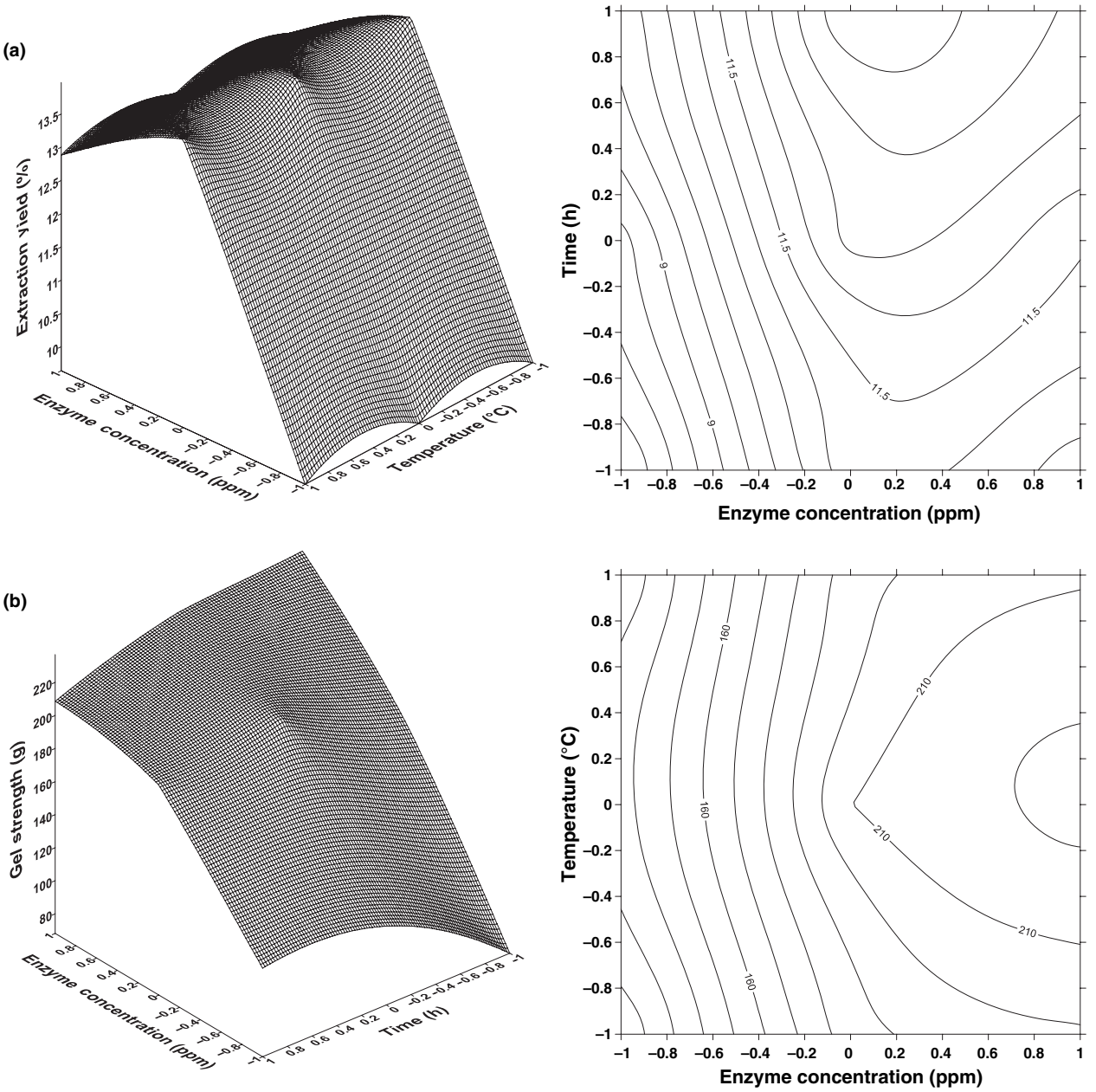

Figure 1 Response surface and contour plots showing effects of process variables on response variables. (a) Effects of enzyme concentration and extraction temperature (left), enzyme concentration and time of enzymatic treatment (right) on the extraction yield. (b) Effects of enzyme concentration and time of enzymatic treatment (left), enzyme concentration and extraction temperature (right) on the gel strength.

enzyme concentration higher than 6 ppm. Whereas, in enzyme concentration values higher than 6 ppm, because of increasing the time of enzymatic treatment, rising it was led to diminishing the extraction yield. It can be also found that the effects of extraction time and time of enzymatic treatment on rising the gel strength were not significant at the lowest enzyme concentration. However, rising the enzyme concentration was led to significant effects of extraction time and time of enzymatic treatment on increasing the gel strength. As it is shown in Fig. 1b, effects of the extraction temperature and time of enzymatic treatment on gel strength were not significant in the lowest enzyme concentration. Whereas, effects of the two mentioned variables on gel strength were significant at higher enzyme concentrations. On the contrast, a definite increase in the time of

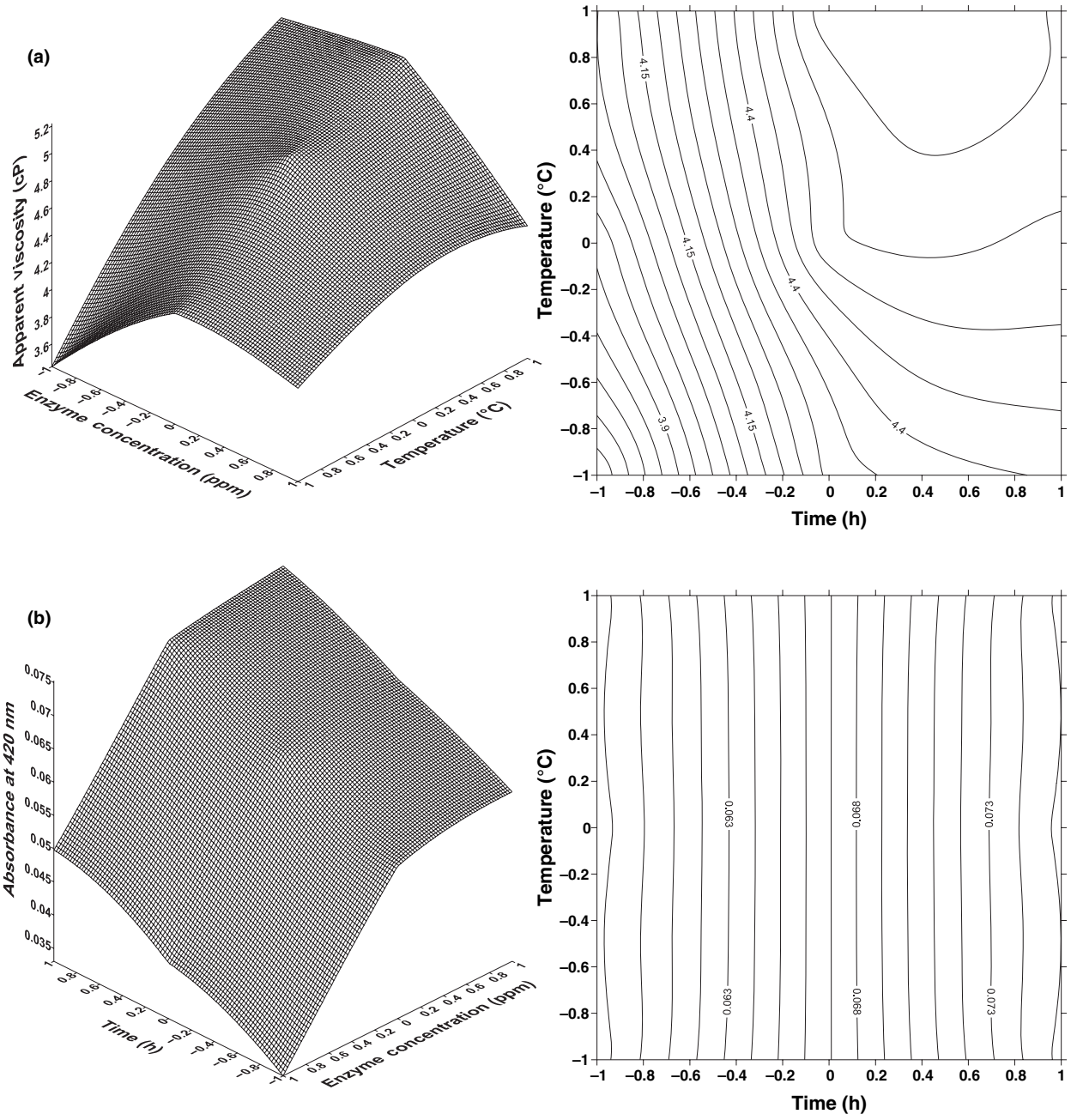

Figure 2 Response surface and contour plots showing effects of process variables on response variables. (a) Effects of enzyme concentration and extraction temperature (left), extraction temperature and time of enzymatic treatment (right) on the apparent viscosity. (b) Effects of enzyme concentration and time of enzymatic treatment (left), extraction temperature and time of enzymatic treatment (right) on the absorbance at 420 nm.

enzymatic treatment and extraction time was associated with an increase in gel strength, but more increasing in the mentioned variables was resulted in decreasing the gel strength. These phenomena were observed probably because of increasing and decreasing in the ratio of alpha form of gelatin molecules, respectively (Poppe, 1997; Francis, 2000). The results also showed that the effects of variables, time of enzymatic treatment and extraction temperature on the apparent viscosity were highly significant in the lowest enzyme concentration (2 ppm). However, the enzyme concentrations higher than 6.8 ppm, these variables had diminishing effect on

Table 6 Canonical analysis of response surfaces and graphical optimum of the process variables

	Predicted levels of optimum response				Graphical optimum			
Process variables	Y_1	Y_2	Y_3	Y_4	Y_1	Y_2	Y_3	Y_4
Enzyme concentration (X_1)	1.9674	0.5403	−0.530	1.5123	0.025	0.775	0.465	−0.8
Time of enzymatic treatment (X_2)	7.676	−0.422	1.1654	2.4959	0.9	−0.025	0.725	−0.5
Extraction temperature (X_3)	25.178	−2.546	1.514	7.911	0	0.03	0.75	−1
Morphology	Max.	Max.	Max.	Max.				

apparent viscosity (Fig. 2a). This was because of high enzyme activity and gelatin hydrolysis, consequently decreasing the high molecular weight (HMW) gelatins. Extraction temperature had also insignificant effect on absorbance in 420 nm (Fig. 2b) (Francis, 2000; Rowlands & Burrows, 2000).

Optimum conditions for responses

While some of the stationary points were outside of the range of the experiment, graphical optimisation was adopted to determine the optimum conditions for this operation (Floros & Chinnan, 1988). In order to find out the graphical optimum conditions of enzymatic gelatin extraction for all responses, the contour plots were drawn and the contours of any process response were then conformed. Determination of the optimising conditions was depended on target parameter(s), which could be one or more of dependent variables. Graphical optimum conditions for the extraction yield, gel strength, apparent viscosity and absorption in 420 nm were determined as 6.1 ppm, 15.6 h, 70 °C; 9.1 ppm, 11.9 h, 70.3 °C; 7.86 ppm, 14.9 h, 77.5 °C and 2.8 ppm, 10 h, 60 °C, respectively.

Verification of results

Adequacy of the regression models for predicting the optimum response values were tested using optimum levels of process variables (Table 6), which determined by the RSM optimisation procedure. The predicted and experimental values for the response variables are given in Table 7. It can be seen that for all the response variables, the experimental values were very close to the predicted values and were not statistically different at the 5% significance level. Hence, the models were good predictors for response variables. In this research, the yield of gelatin extraction was obtained lower than the values reported by Rowlands & Burrows (2000). This was probably because of different composition of the raw materials that were used.

Table 7 Predicted and experimental values of the responses at optimum conditions

		Experimental value[a]	
Response	**Predicted value**	**Mean**	**Range**
Yield (%)	13.879	13.901	13.888–13.932
Gel strength (g)	244.92	243.22	242.32–244.38
Apparent viscosity (cP)	4.945	4.915	4.885–4.978
Absorbance at 420 nm	0.0434	0.0439	0.0433–0.0440

[a]Results are of five replications.

Conclusion

The quality of extracted gelatin and the optimum extraction conditions can be predicted using the obtained models in this study and based on the research goals, such as high extraction yield, high viscosity, etc. Because of different bond breakage during enzymatic gelatin extraction that is function of pH, enzyme concentration, time of enzymatic treatment and extraction temperature, extracted gelatin is composed of a distribution of proteins of varying lengths. Molecular weight distribution of extracted gelatin affects its functional properties. Therefore, modelling of functional properties and molecular weight distribution as a function of extraction conditions can be useful for prediction of the precise characteristic of extracted gelatin in enzymatic method.

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