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Extended Abstract

Optimization of Gelatin Extraction Process, from Sheep Skin Waste Using Alcalase Enzyme by Response Surface Method

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Abstract:
This study determined the optimal conditions for enzyme-assisted gelatin extraction from sheep skin waste using the effects of three variables: enzyme concentration (0.5-2.5%), hydrolysis time (60-240 min), and extraction temperature (60-80 °C). The impact of these three variables was studied on extraction, yield, gel strength, viscosity, degree of hydrolysis, melting point and SDS-PAGE, and the results were analyzed by using the central composite design (CCD) and response surface methodology (RSM). Also the analysis of variance table showed that the lack of fit was not significant for all response surface models at 95%. The results showed that enzyme concentration 0.5%, hydrolysis time of 166 min, and extraction temperature 60 °C are the desirable condition for optimal extraction (11.03%) and optimal degree of hydrolysis (23.00%). In addition, the effects of these three variables on gel strength, viscosity and melting point were significant, and in optimal conditions, gel strength was measured as 175.33 g, viscosity as 3.26 Mpa/sec, and melting point as 25.39 °C.

Keywords: Enzymatic Extraction, Gelatin, Response Surface, Sheep Skin Waste

Introduction

Gelatin is one of the most commonly used proteins which can be obtained from partial hydrolysis of animals' collagen-based raw materials (Mad-Ali, Benjakul, Prodpran, & Maqsood, 2016c; Mulyani, Setyabudi, Pranoto, & Santoso, 2017). Alcalase is an alkaline enzyme obtained from *Bacillus Licheniformis*. It can act in a pH range of 8-10 and temperature range of 50 °C that can reduce the probability of microbial contamination during the production process (Salwanee, Mustapha, Mamot, Maskat, & Ibrahim, 2013; See, Hoo, & Babji, 2011). The reports suggest that in our leather industry about 20 million of sheep skins are made into leather products each year and 20% of these skins are wasted through the process. It is possible to get 12-15% gelatin from this amount of waste (Tehran Chamber of Commerce, Industries, Mines and Agriculture, 2017). And therefore the leather industry waste can be used as a suitable source for gelatin production. In a research about gelatin extraction from fish skin using enzyme, alkaline pre-treatment and acetone (skin degrease), it has been reported that increasing the extraction time will lead to an increase in product yield and molecular weight distribution (Xu *et al*., 2017). Some researchers also extracted gelatin from Buffalo skin using alkaline and acidic treatment and thermal hydrolysis which met 14.67-29.17% of product yield, 293.41-239.44 g of gel strength range and 16.37- 22.17 Pa/s of viscosity (Mulyani *et al*., 2017).

The purpose of this study was to investigate the optimization of enzymatic gelatin extraction process from sheep skin waste using response surface methodology. With this operation, properties such as: gel strength, viscosity, extraction yield, degree of hydrolysis and electrophoresis pattern of the produced gelatin were investigated.

Material and methods

Materials

The required materials for this research including ship skin waste by Arya Leather Corporation, the Alcalase Protease enzyme by Novo Company and German Merck firm respectively.

Method

Gelatin extraction in this study was carried out according to method (Lassoued *et al*., 2014) with a few changes.

Physicochemical Properties of Skin

The protein, fat, ash and moisture content of the skin samples were measured according to method (Iranian National Standardization Organization [ISIRI], 1994).

Extraction Yield

The extraction yield of each produced sample was calculated according to method (Hosseiniparvar, Keramat, Kadivar, Khanipour, & Milani, 2006).

Gel Strength Determination

Gel Strength Determination was measured according to method (British Standards Institution, 1975).

Viscosity

Viscosity was measured according to method (BSI, 1975).

Degree of Hydrolysis (DH) Determination

For determining the degree of hydrolysis method (AOAC, 2000) was used.

Determination of Melting Point

The melting point of the obtained gelatin sample was determined using method (BSI, 1975).

Sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE)

The SDS-PAGE gelatin test was done based on method (Laemmli, 1970).

Statistical Analysis

In this study, to optimize the gelatin extraction process we used central composite design with six replications in the central region, for three variables and at three levels. The results of the research were analyzed using Design Expert (version 7) and response level methodology (RMS), and each of the response variables (extraction yield, Gelatin strength, and degree of hydrolysis) was shown in the form of the following linear regression model and each was counted as a function of the Independent variables (enzyme concentration, time of hydrolysis and extraction temperature):

$$y=\beta 0+\Sigma\beta X \quad (1)$$

And the other response variables (viscosity and melting point) were provided through a quadratic regression.

Results and discussion

The sheep skin used in this research contains 23.54±1.43 Protein, 18.90±0.27 Fat, 27.91± 0.35 moisture and 28.93±0.10 ash.

Extraction yield

In this research hydrolysis time, extraction temperature and enzyme concentration were shown as significant variables. It was indicated that the effect of hydrolysis time is more than the effects of enzyme concentration &extraction temperature and also the effect of enzyme concentration is more than that of the extraction temperature (Fig. 1).

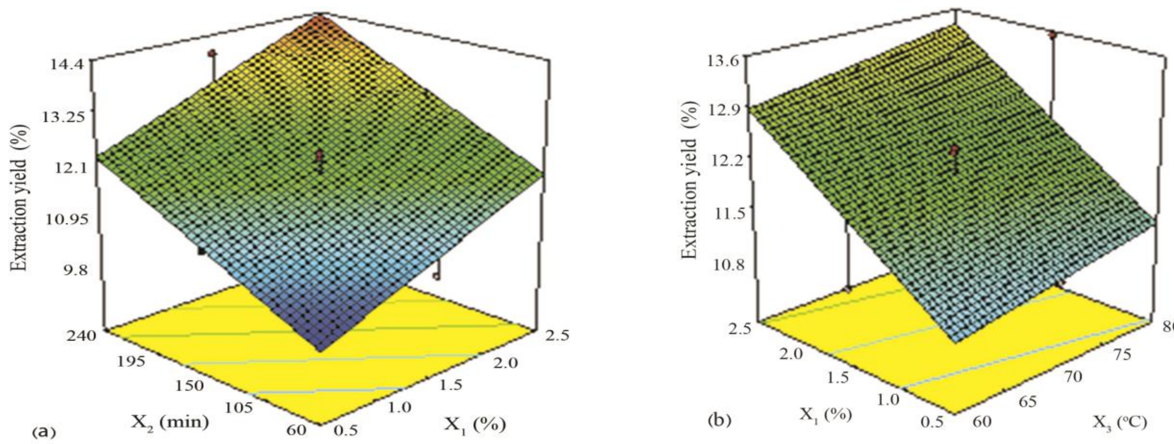

Fig. 1. The simultaneous effect of enzyme concentration(x_1), extraction time(x_2) and extraction temperature (x_3) on gelatin extraction yield

Gel Strength (Bloom)

The results of the variance analysis showed that only the linear effects of enzyme concentration and hydrolysis time had significant impacts (Fig. 2).

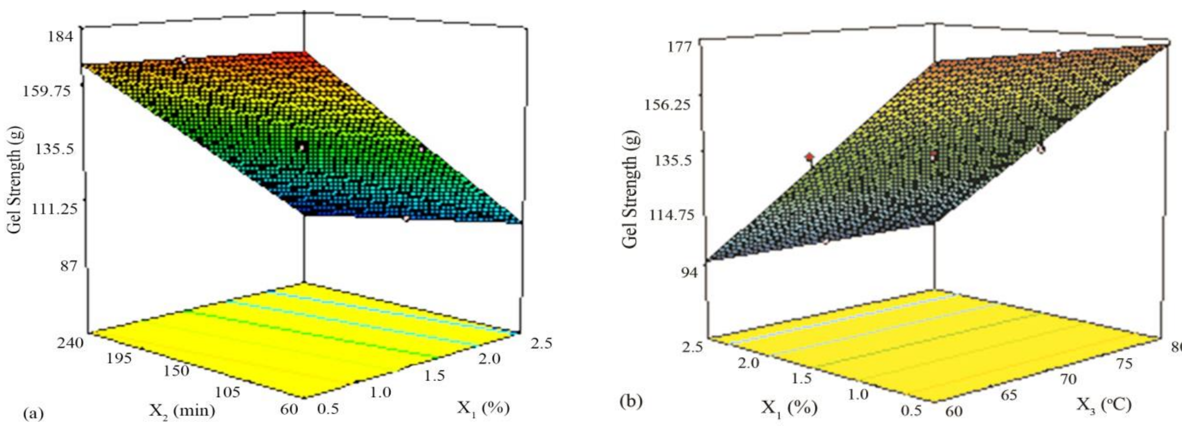

Fig. 2. The simultaneous effect of enzyme concentration(x_1), extraction time(x_2) and extraction temperature (x_3) on gel strength

Viscosity

According to the results, enzyme extraction and hydrolysis time are both two important factors which affect viscosity. And also it was indicated that the effect of enzyme concentration on viscosity is more than that of the hydrolysis time & extraction temperature (Fig. 3).

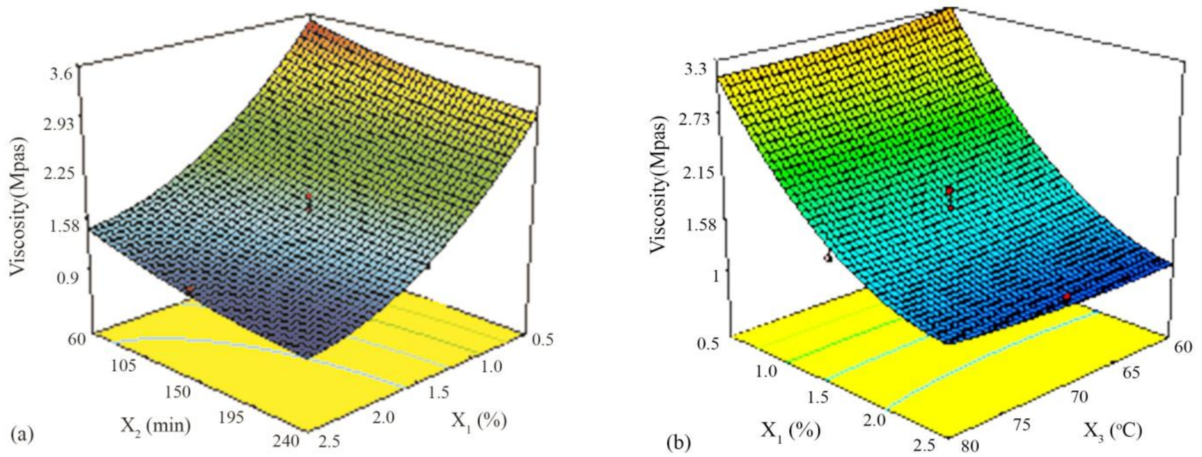

Fig. 3. The simultaneous effect of enzyme concentration(x_1), extraction time(x_2) and extraction temperature (x_3) on viscosity

Degree of Hydrolysis (DH)

Based on variance analysis, the effect of enzyme concentration and hydrolysis time on the degree of hydrolysis of the produced gelatin was proved to be significant. Moreover, the effect of enzyme concentration on degree of hydrolysis was reported to be higher than the other two (Fig. 4).

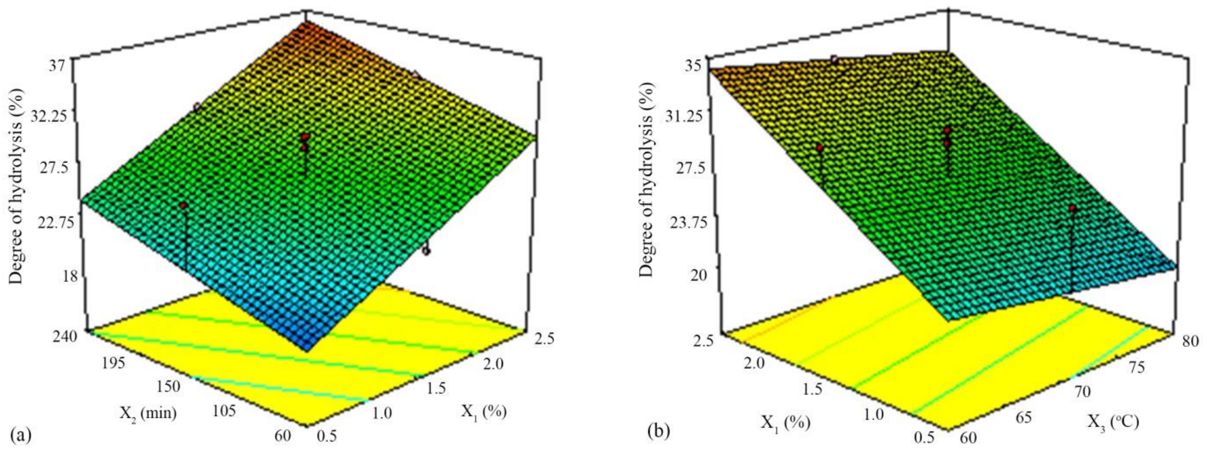

Fig. 4. The simultaneous effect of enzyme concentration(x_1), extraction time(x_2) and extraction temperature (x_3) on degree of hydrolysis

Melting Point

The results indicated that enzyme concentration had a significant effect on gelatin melting point. Also it was concluded that the effect of enzyme concentration on melting point is more significant than the effect of hydrolysis time. The melting point for the produced gelatin was measured as 15-27 °C (Fig. 5).

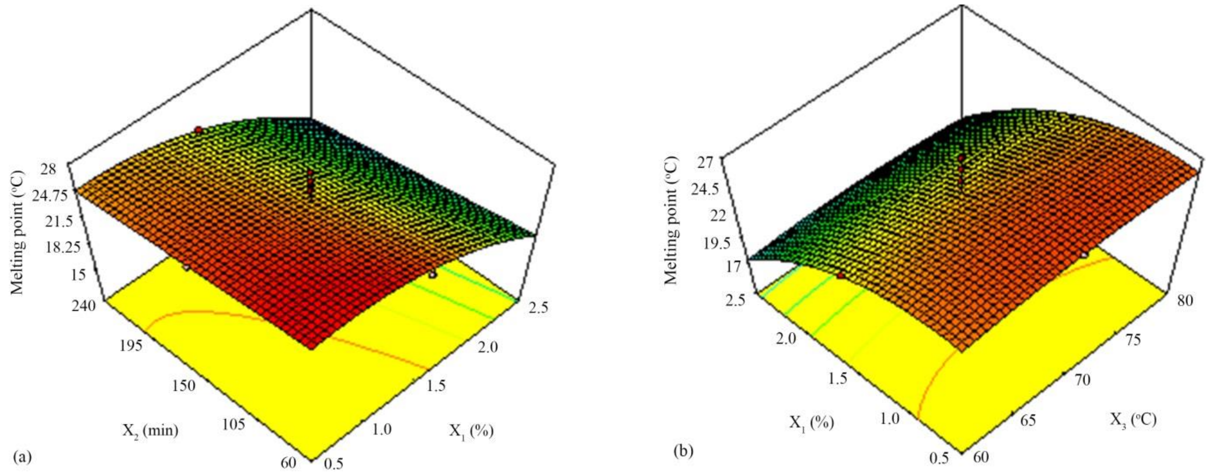

Fig. 5. The simultaneous effect of enzyme concentration(x_1), extraction time(x_2) and extraction temperature (x_3) on melting point

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE profiles suggest that some of the gelatin samples extracted from sheep skin waste contain the Beta chain main components and Alpha chain protein bands. The highest molecular weight were seen in low enzyme concentration-regardless of hydrolysis time (Fig. 6).

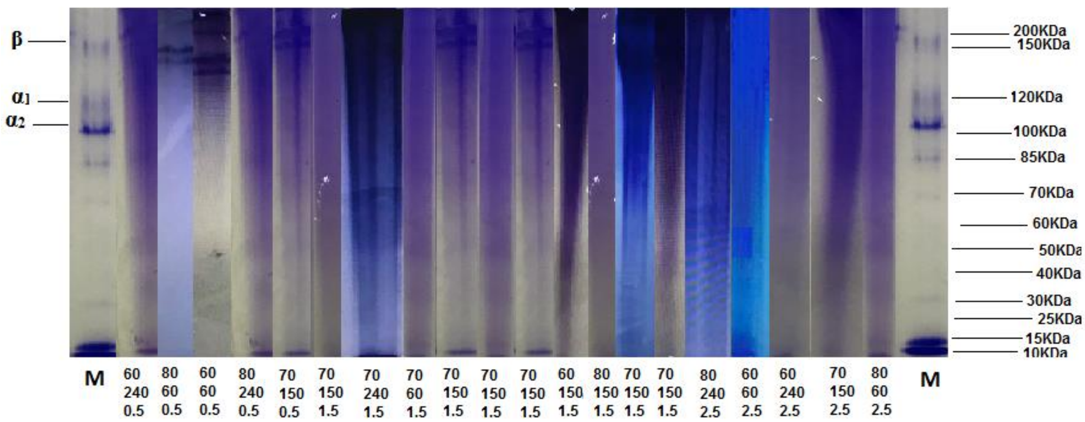

Fig. 6. profiles SDS- derived from sheep skin lesions, M indicate the molecular weight and the numbers in each column from top to , indicate the extraction temperature(℃) , hydrolysis time (min), enzyme concentration(%).

Conclusion

This research showed that by optimizing the response level method, it is possible to simulate (model) the process of enzymatic extraction from sheep skin waste. According to the tests, 60 °C of extraction temperature, enzyme concentration of 5% and 142 min of hydrolysis time was considered as the optimum point.

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