The quality and purity of the proteins extracted from the Lathyrus sativus seeds and optimization by the response surface methodology (RSM)

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Abstract- *Lathyrus sativus Linn. (chickling vetch or grass pea) seeds could be valuable sources of protein, Protein constitutes ۲۵.۶-۲۸.٤% of the seed dry weight. It is cultivated widely in every part of Iran but scarce studies about their protein isolated production exist. In this research, a series of statistically designed studies (central composite design (CCD) and response surface methodology (RSM)) were performed to investigate the effect of each of the independent variables (time, ۲۰-۸۰ minutes), (water to solid ratio, ۱:۵-۱:۲۰) and (pH, ۲.۵-۱۰) on protein extraction yield and purity. Results showed that extraction time and pH are two effective factors on quality and purity of the protein isolated (p< ۰.۰۰۱); In the event that quadratic of water to solid ratio is significant at the ۵% The optimum protein purity was found to be ۹۳.٦% at ٤۳.۳۸ min, pH ۲.۵۷ and water: solid ratios of ۹.۹۱. Results of this research showed a good performance of response surface methodology in the optimization of this process.*

Keywords- Lathyrus sativus; optimization; protein purity; response surface methodology

I. INTRODUCTION

The search for alternative or new protein sources became an important research trend over the past decades, not only to face an increasing demand of protein but also to look for alternative crops that may be cultivated in marginal soils or to seek plant species capable of supplying a high-quality protein. Among those, legume seeds have certainly played a major role, not only as animal feed but also for human consumption.

Lathyrus sativus, is a legume (family Fabaceae) commonly grown for human consumption and livestock feed in Asia and East Africa. It is a particularly important crop in areas that are prone to drought and famine, and is thought of as an 'insurance crop' as it produces reliable yields when all other crops fail. It is also known as grass pea, blue sweet pea, chickling vetch, Indian pea, Indian vetch, white vetch, almorta or alverjón (Spain), guixa (Catalonia),chícharos (Portugal), cicerchia (Italy), guaya (Ethiopia), and khesari (India) (Rao et al., ۱۹٦٤).

Lathyrus sativus L. is compatible with tropical and subtropical climate conditions. This plant are grown in Azerbaijan, Kerman, Isfahan and some western provinces; for example, the equivalent of ٤ to ۵ thousand hectares in Hamedan and ۲ to ۳ thousand hectares in Kermanshah are under cultivation (Hojabri ,۱۹۹۷; Mohammadinejad, ۱۹۸۷; Zargari, ۱۹۹٦). Lathyrus sativus has lots of energy and protein and is rich in lysine but the amount of sulfur-containing amino acids (methionine - cysteine - tryptophan) is restrictive (Gatel, ۱۹۹٤; Hanbury et al., ۲۰۰۰; Low et al., ۱۹۹۰). Based on the results of Paredes-Lopez et al. (۱۹۹۱), protein isolated from the non-fat chickpea flour at pH=۸.۵ and ratio of ۱:۱۰ flour to water by deposition at the isoelectric pH on freeze-drying method has ۸٤.۸% protein content. Sanchez-Vioque et al. (۱۹۹۹) have expressed characteristics of the two types of Lathyrus sativus's protein isolates, including isolates of A: (no sodium sulfate acid) and B: (with sodium sulphate and acid). Protein purity of "A" and "B" isolated were ۷۸ and ۸۸.۱%, respectively. This protein was obtained range of digestible in ۹۵.٦ to ۹٦.۱% for "A" isolate due to a high pH. Asli Can Karaca et al. (۲۰۱۱) compared three types of chickpea at pH: ۹.۵, water to solid ratio of ۱:۱۰ and in ٤۰ minutes and then reported purity of between ۹۰ and ۸۰ percent. Wani et al. (۲۰۰٦) examined the watermelon seed protein extraction and reported purification of proteins in ۲.۱% salt, ratio of water to grain ۷۰:۱, extraction time ۱۵ minutes and temperature of ٤۰°C is high amount. Under these optimal conditions, the amount of

protein purity was ٧١.٨٠ percent. The purpose of this study was to achieve optimal conditions for protein purification from Lathyrus sativus seeds using response surface methodology to determine optimal factors were examined.

II. MATERIALS AND METHODS

A. Material

La. sativus Linn. (chickling vetch or grass pea) seeds were obtained from Agricultural Research Institute (Mashhad, Iran) and other chemicals such as sodium hydroxide, hydrochloric acid, sulfuric acid, normal hexane were obtained from Merck Germany.

B. Preparation of protein isolates

The seeds were ground in a household mill to pass through a ٦٠-mesh Sieve. Grass pea meals (٢٠-٢٥g) were added to normal hexane solution at a meal to solvent ratio of ١:٥ (w/v); the mixture was stirred for ٦٠ min at ٢٥ °C). Each extract was separated by centrifugation at ٨٠٠٠g for ١٥ minutes (Gatel, ١٩٩٤; Low, et al., ١٩٩٠). The defatted flour was dried in the ventilator overnight at ٢٠ °C.

C. Protein extraction

In the present study protein isolates derived from tow extraction method include; acidic extraction (Isolate A) and alkaline extraction (Isolate B). The defatted flour was dispersed in deionized water at a ratio between ١:٥ to ١:٢٠ (Mohammadinejad, ١٩٨٧; Gatel, ١٩٩٤), for isolate (B) pH was raised to ١٠.٠ using ١N NaOH solution, and for isolate (A) pH adjusted to ٢.٥ using ١N HCl solution. Because of the high extraction efficiency of protein acidic and alkaline pH, ٢.٥ and ١٠ were followed (Wani et al., ٢٠٠٦). The dispersion was stirred for ٤٠ min with a mechanical stirrer. The resulting protein extract was separated by centrifugation at ٧٠٠٠ × g for ٣٠ min. The extracts were combined and the pH adjusted to ٤.٥ with ١N HCl and ١N NaOH to precipitate the protein. The proteins were recovered by centrifugation at ٧٠٠٠g for ١٥ min, followed by removal of the supernatant by decantation. Protein curd was washed twice with distilled water and centrifuged at ٥٠٠٠g for ٥ min. The washed precipitate was then vacuum oven dried as protein isolate. The protein contents of isolates were determined by the micro-Kjeldhal method (AACC, ٢٠٠٠) (Mizubuti et al., ٢٠٠٠). Nitrogen to protein conversion factor of ٦.٢٥ was used.

D. Experimental design and statistical analysis

Response surface methodology (RSM) was used to estimate the effect of independent variables (Shan-shan, Xu, ٢٠٠٦; Asli Can Karaca, et al., ٢٠١١) (pH, x_1; time, x_2 and water to seed ratio, x_3) on the protein purity (%). A Central Composite Rotatable Design was employed for designing the experimental data. The RSM was applied to the experimental data using a commercial statistical package, Design-Expert version ٦.١.١٧ (Statease Inc., Minneapolis, USA).

Experiments were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors. The experimental design included star points, and six centre points to calculate the repeatability of the method (Montgomery, ٢٠٠١).

Numerical and graphical optimization technique of the Design-Expert software was used for simultaneous optimization of the multiple responses. The desired goals for each variables and response were chosen. All the independents variables were kept within range while the responses were either maximized or minimized (Shan-shan, Xu, ٢٠٠٦; Asli Can Karaca, et al., ٢٠١١).

III. RESULTS AND DISCUSSION

A. Model fitting

The second-order polynomial response surface model was fitted to the response variable (Y). For the corresponding fitting of the explanatory models and the variation of the protein purity, the sum of squares of the sequential model was analyzed. Regression analysis and ANOVA were used for fitting the model and to examine the statistical significance of this term. The estimated regression coefficients of the quadratic polynomial models for the response variable, along with the corresponding coefficient of determination (R^2) are high. In addition and adj-R^2 and coefficient of variation (CV) were calculated to check the model adequacy. The lack of fit is an indication of the failure for a model representing the experimental data at which points were not included in the regression or variations in the models cannot be accounted for random error (Montgomery, ٢٠٠١). If there is a significant lack of fit which could be indicated by a low probability value, the response predictor is discarded. The lack of fit did not result in a significant p-value for this variable, meaning that this model was sufficiently accurate for predicting the relevant response.

Coefficient of determination, R^2, is the proportion of variation in the response attributed to the model rather than to random error and was suggested that for a good fitted model, R^2 should not be less than ٨٠%, When R^2 approaches to the unity, signifies the suitability of fitting empirical model to the actual data. The lower value of R^2 shows the inappropriateness of the model to explain the relation between variables (Little & Hills, ١٩٧٨; Mendenhall, ١٩٧٥).

TABLE I. ANOVA AND REGRESSION COEFFICIENTS OF THE SECOND-ORDER POLYNOMIAL MODEL FOR THE PROTEIN PURITY

Source	Sum of squares	df	Mean squares	f-value	p-value
Model	٤٠٧.٨٧	٩	٤٨.٣٢	١٠٨.٠٤	٠.٠٠٠١
A: time	٤٣. ٨٩	١	٤٣.٨٩	١٠٤.٦٤	٠.٠٠٠١
B: pH	٣١. ١٠	١	٣١.١٠	٧٤.١٣	٠.٠٠٠١
C: ratio	٢.٥٦٥	١	٢.٥٦٥	٦.١١٥	٠.٩٣٩٢
AB	٢٢.٠٤	١	٢٢.٠٤	٥٢.٥٦	٠.٠٠٠١
AC	٦٢.٩٤	١	٦٢.٩٤	١٥٠.٠٦	٠.٠٠٠١
BC	١٠٢.٦٧	١	١٠٢.٦٧	٢٤٤.٧٨	٠.٠٠٠١
A^2	١٠١.٥٥	١	١٠١.٥٥	٢٤٢.١١	٠.٠٠٠١
B^2	١٢٨.٤	١	١٢٨.٤	٣٠٦.١٣	٠.٠٠٠١
C^2	٦. ٥٤	١	٦.٥٤	١٥.٥٩	٠.٠٠٢٧

Our results showed that the R^2 values for these response variables were higher than ۰.۸۰, indicating the regression models were suitable to explain the behavior. The R^2 values for protein purity were found to be ۰.۹۸۹۸. It should be noted that adding a variable to the model will always increase R^2, regardless of whether the additional variable is statistically significant or not. Thus, a large value of R^2 does not always imply the adequacy of the model. For this reason, it is more appropriate to use an adj-R^2 of over ۹۰% to evaluate the model adequacy. With the exception of protein content (adj-R^2 =۰.۹۸۰۷), the adj-R^2 values were found to be higher than ۰.۹۸ for this response. Higher adj-R^2 indicated that non significant terms have not been included in the model.

As shown in the figure ۱, with increasing pH from ۲.۵ to ۱۰ pure protein isolates decrease. This phenomenon is due to greater extraction of non-protein material in high pH (Farhoosh, ۱۹۹۵). The presence of phytic acid in protein isolates from rapeseed/canola due to the formation of insoluble phytate complexes-proteins that facilitate protein precipitation. In a study by Gillberg & Tornell (۱۹۷۶), found that attaining Medium purity and yield of protein extraction could be related to the presence of low phytic acid, because very little solubility of phytic acid in the high pH has been reported. Also the presence of phytic acid in the protein extracted effected strongly on the purity and yield of the protein precipitation on using pH range for isolation. Also interaction between the protein and phytic acid is related to the gravity of the charge.

According to the figure ۱, by increasing the time from ۲۰ to ٤۰ minutes due to the withdrawal of protein compounds protein purity have increased; and from ٤۰ to ۸۰ minutes due to the withdrawal of non-protein compounds, the purity have decreased. The results of this research are in agreement with Ragab & Babiker (۲۰۰٤) on cowpea and Kaur & Singh (۲۰۰۷). Washing of the precipitate increases the purity of the isolate and improve the quality of the final product, because this operation eliminates flatulent carbohydrates, pigments and other non-protein materials (Farhoosh, ۱۹۹۵).

TABLE II. THE INDEPENDENT VARIABLES AND THEIR RESPONSES

The independent variables	*symbol*	*relevant level*		
	X	+۱	۰	-۱
ratio	x_1	۲۰	۱۲.۵	۵
time (min)	x_2	۸۰	۵۰	۲۰
pH	x_3	۱۰	٦.۲۵	۲.۵

B. Optimization of protein purification from Lathyrus sativus seeds

In this study, the objective of the optimization was to maximize protein purity; therefore the optimum conditions for protein purification from *Lathyrus sativus* seeds was calculated ۹۳.۵۹ (%) at pH ۲.۵۷, extraction time ۳۸.٤۸ minutes and ratio of water to flour, ۹.۹۱, respectively.

IV. CONCLUSION

The results of this study showed that the response surface methodology is useful performance for optimization of Lathyrus sativus purified protein. Extraction time and pH are two effective factors on the purity of the isolated protein at ۱% and the effect of the ratio was less. The results can be claimed that Lathyrus sativus purified protein isolate has a high purity and useful for the formulation of protein-based food ingredients.

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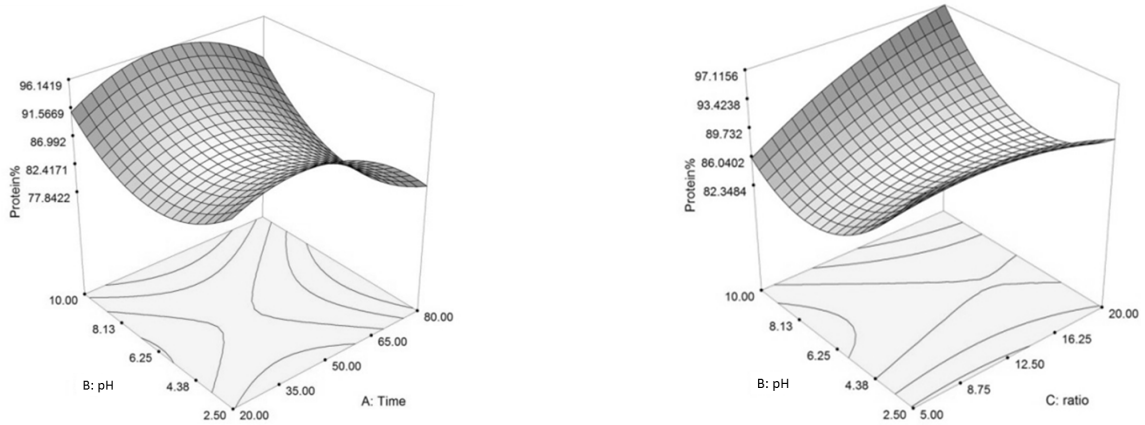

Figure ١. Response surface for the effect of extraction time and pH (A), and water to seed ratio and pH (B) on the protein purity of Lathyrus sativus protein isolate.