Influence of partial hydrolysis on the protein extraction from sunflower meal

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Abstract

Introduction. We investigated the influence of protein hydrolysis degree in the presence of neutral protease from *Bacillus subtilis* on the process of protein extraction from sunflower meal. Correlation between protein hydrolysis degree and affectivity of protein extraction was analyzed.

Materials and methods. The degree of protein hydrolysis was determined as increase of TCA-soluble proteins concentration compared to control samples. Protein concentration in extracts was determined photometrically.

Result and discussion. Determination of protein concentration in obtained extracts have shown that main part of soluble proteins was extracted during first 20 min. The protein concentration in extracts obtained in the presence of protease was approximately twice higher than in control samples. The protein hydrolysis degree was sufficiently low and varied from 3,5 % to 5,2 % at moderate enzyme/substrate ratio. When enzyme/substrate ratio increased the degree of hydrolysis was also rising and it reached 9,0-9,5 % during 40-60 min. Such level of hydrolysis is desirable for improvement of protein functional properties and higher hydrolysis level results in loss of protein functionality. The protein hydrolysis degree was dependent from the enzyme/substrate ratio and the duration of reaction. Nonlinear model was obtained for estimation of the affectivity of protein extraction as function of enzyme/substrate ratio and extraction time. Obtained model explains 92.81% of data variation and approximates well the available data. It was shown also that high correlation (r=0,69) exists between concentration of proteins in extracts and degree of protein hydrolysis.

Conclusion. The partial hydrolysis of sunflower meal proteins by neutral protease resulted in increase of protein concentration in extracts. Obtained model could be used in prediction of protein hydrolysis level during protein extraction from sunflower meal in the presence of proteolitic enzymes and their functional properties.

Introduction

Proteins of sunflower meal have high biological value and functional properties [1, 2]. Usually, the yield of protein isolates is not high upon extraction of proteins in water solution. Functional properties of traditional isolates need to be improved.

Partial enzymatic hydrolysis of proteins improves their functional properties and increases the field of application [3-6]. Protein hydrolysis by proteases results in synthesis of peptides with smaller molecular mass that have high solubility at high extent of hydrolysis, which is a substantially useful characteristic for many food applications. Emulsifying properties and foaming properties can be improved with limited degree of hydrolysis [7,8]. It was shown also that peptides with smaller size have better digestibility than amino acids [9].

Recently accessibility of industrial proteases from microorganisms and fungi causes the widespread hydrolysates production in the world. Most of them are from milk and soy proteins, but another source of protein, such as sunflower meal can be also used. Some authors have investigated the properties of such protein hydrolysates obtained from sunflower protein isolates [4,10].

In our previous work we have used an animal protease trypsin during protein extraction from sunflower meal [11]. We have detected the increase of protein concentration in extracts and yield of protein isolates as well as increase of solubility in wide range of pH, foam and emulsifying capacities.

In this study we have investigated the influence of protein hydrolysis degree in the presence of neutral protease from *Bacillus subtilis* on the process of protein extraction from sunflower meal. We have also analyzed the correlation between degree of protein hydrolysis and affectivity of protein extraction using.

Materials and Methods

Materials

The sunflower meal was collected from a "Melitopol oil extraction plant", Ukraine. The protein content of meal varied from 33 to 39%. Protolad (protease from Bacillus subtilis, 70 units/g, Enzyme, Ukraine) was used for hydrolysis.

Protein extraction and obtaining of protein isolates

Proteins were extracted from sunflower meal by sodium chloride solution (70 g/ dm^3, pH 7.0) under constant stirring and temperature 40-45° C during 20-60 min, meal/solution ratio was 1:10 (w/v). Protein extraction in the presence of a protease was carried out in the same conditions at enzyme/substrate ratio from 0,25:1 to 1,75:1. In the case of enzyme applying the reaction mixture was immediately heated at 80 °C for 15 min to inactivate enzyme activity.

After this, insoluble residues were precipitated by centrifugation. The supernatant (protein extract) was used for isoelectric protein precipitation at pH 4.0-4.5. After protein coagulation, pellet was separated by centrifugation (3 000 x g), protein pellet was collected, washed and dried to 6-8 % fluidity.

Determination of protein concentration

Protein contents of the extracts were determined photometrically at 540 nm according to the Biuret method [12] using bovine serum albumin for calibration. All determinations were performed in triplicate.

Determination of the Degree of Hydrolysis

The degree of protein hydrolysis (DH) was determined according to Popovic et al. [13] in some modification. To a 0.5-mL aliquot of the supernatant obtained after hydrolysis, an equal volume of 0.5 mol/dm^3 3-chloro-acetic acid (TCA) was added. The mixture was incubated for 30 min at 4 °C. Thereafter, the mixture was centrifuged at 7 000 rpm for 10 min. The TCA-soluble fraction and the reaction mixture were analyzed to determine the protein content by the method of Lowry et al. [14]. The DH value was calculated as the increase of TCA-soluble protein concentration in the presence of protease ($C_{protease}$) to protein content in control samples ($C_{control}$), expressed as a percentage:

$$DH = \frac{C_{protease} - C_{control}}{C_{control}} \times 100 .$$

Statistical Analysis

Data were expressed as means ± standard deviation for triplicate determinations. A least significant difference test with a confidence interval of 95 % was used to compare the means. The main results of modeling process obtained with STATISTICA program, are shown below (Fig. 1, 2, 3).

	Model: Prot=exp(b0+b1*R**2+b2*T) (matr_1.sta in FoodJournal.stw) Dep. var: Prot Loss: (OBS-PRED)**2 Final loss: 12,538345879 R= ,96338 Variance explained: 92,810% Exclude cases: 11		
N=11	b0	b1	b2
Estimate	4,024242	0,039936	0,002308

Fig.1. Result of nonlinear estimation in STATISTICA

	Regression Summary for Dependent Variable: LN-V1 (matr_1.sta in FoodJournal.stw) R= ,96016152 R?= ,92191015 Adjusted R?= ,90238768 F(2,8)=47,223 p<,00004 Std.Error of estimate: ,01965 Exclude cases: 11					
N=11	Beta	Std.Err. of Beta	B	Std.Err. of B	t(8)	p-level
Intercept			4,029706	0,016876	238,7841	0,000000
T	0,580556	0,098833	0,002197	0,000374	5,8741	0,000372
V3**2	0,780127	0,098833	0,039188	0,004965	7,8934	0,000048

Fig. 2. Model (2) after dependent variable transformation.

	Correlations (matr_1.sta in FoodJournal.stw) Marked correlations are significant at p < ,05000 N=11 (Casewise deletion of missing data) Exclude cases: 11	
Variable	Prot	DH
Prot	1,00	0,69
DH	0,69	1,00

Fig. 3. Correlation between two variables.

Result and Discussion

The degree of protein hydrolysis depends from the duration of reaction and enzyme/substrate ratio (Fig. 4). The protein hydrolysis degree was sufficiently low and varied from 3,5 % to 5,2 % at moderate enzyme/substrate ratio ((0,25-0,75):100). The increase of hydrolysis degree with time was statistically insufficient. As enzyme/substrate ratio increased to (1,25-1,75):100 the degree of hydrolysis was also rising and it reached 9,0-9,5 % during 40-60 min. It was shown that mainly such level of hydrolysis was desirable for improvement of protein functional properties and that higher hydrolysis level resulted in loss of protein functionality [4].

Determination of protein concentration in obtained extracts has shown that main part of soluble proteins was extracted during first 20 min (Fig. 5). The protein concentration in extracts obtained in the presence of proteases was approximately twice higher than in control samples. Only negligible part of proteins was extracted during next time of reaction at every enzyme/substrate ratio, except control samples and samples with enzyme/substrate ratio 1,75:100. The protein concentration increased by 11,6 % relatively to 20 min when reaction was carried out during 60 min at last ratio.

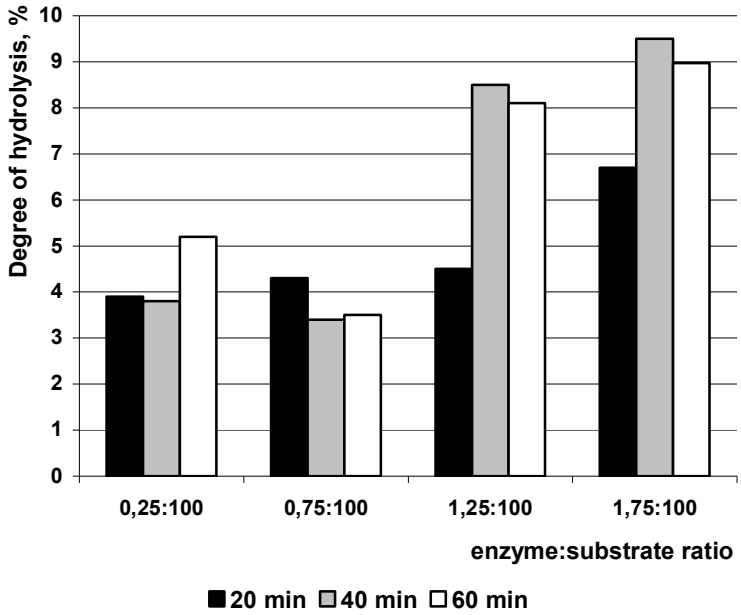

Fig. 4. The degree of protein hydrolysis dependence from the time of reaction and protease/substrate.

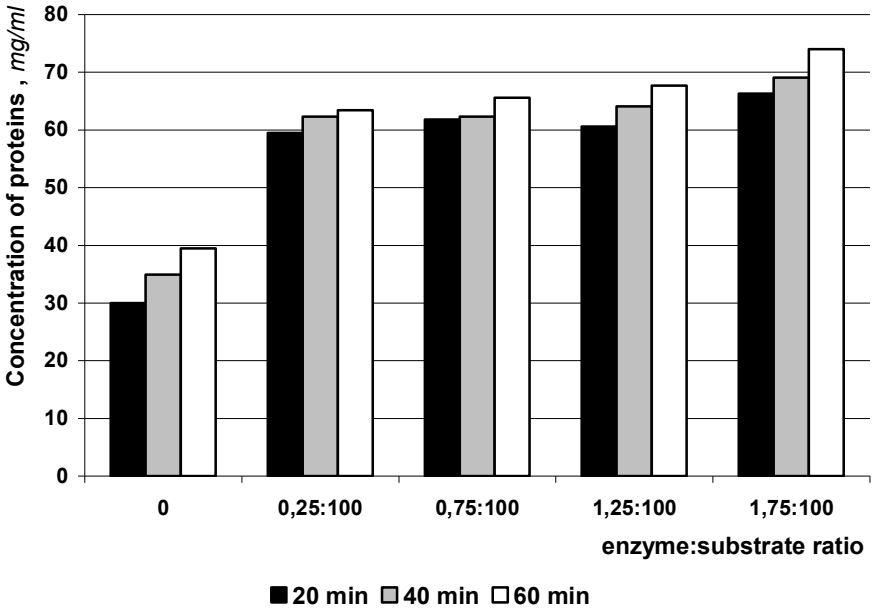

Fig. 5. Dependence the protein concentration in extracts from sunflower meal from protease/substrate and time

We have supposed that there was some relation between these two investigated parameters the degree of protein hydrolysis and protein extraction affectivity.

The experimental data for the regression model are presented in Table 1.

Table 1

Experimental data for analysis in STATISTICA (v.7)

Prot – protein concentration (random variable), mg/ml; DH – degree of hydrolysis (random variable), %; R – ratio enzyme/substrate (nonrandom variable); T – time (nonrandom variable), min.

№	**Prot,** protein concentration	**DH,** degree of hydrolysis	**R,** ratio enzyme/substrate	**T,** time
1	59.5	5,9	0,25	20
2	61,8	4,3	0,75	20
3	60,6	1,5	1,25	20
4	66,3	6,7	1,75	20
5	62,3	3,8	0,25	40
6	62,3	3,4	0,75	40
7	64,1	8,5	1,25	40
8	69,1	9,5	1,75	40
9	63,4	5,2	0,25	60
10	65,6	3,5	0,75	60
11	57,7	8,1	1,25	60
12	74,0	8,9	1.75	60

Unsatisfactory results were obtained using Multiple Regression module, but case number 11 was excluded from the analysis as outlier.

Thus, we used the Nonlinear Estimation module. Technically speaking, Nonlinear Estimation is a general fitting procedure that will estimate any kind of relationship between a dependent, and a list of independent variables. In general, all regression models can be stated as: $y = f(x_1, x_2, \ldots, x_n)$. In most general terms, we are interested how a dependent variable is related to a list of independent variables. Generalized Linear/Nonlinear Models (GLZ) module includes efficient algorithms for fitting. We can write any type of regression equation, which STATISTICA will then fit to our data (User-Specified Regression, Least Squares and User-Specified Regression, Custom Loss).

Simplex and quasi-Newton method was used for estimation of the model parameters. Nonlinear model

$$\text{Prot} = \exp\{b_0 + b_1 R^2 + b_2 T\} \quad (1)$$

shown on Fig. 1 explains 92.81% of data variation. Conclusion: the model approximates well the available data.

Thus, we have the nonlinear model

$$\text{Prot} = \exp\{4.0242 + 0.0399 R^2 + 0.0023 T\} \quad (2)$$

We can see, that residuals of nonlinear model (2) are normally distributed (Fig. 6). The points on the graph (Fig. 7) are located along the bisector, thus the ratio of the actual data to the model predicted is close to 1.

Thus, obtained nonlinear model (2) is an estimation of the Prot variable – the affectivity of protein extraction. Fluctuations of model residuals (Fig. 8) around zero proves estimation unbiasedness.

We have a good model due to the analysis of the data, which was built on the basis of intuitive knowledge about the nature of the possible behaviour of biological data. But we still do not know whether received model evaluation coefficients are significant. Therefore, we provided more research. With corresponding transformations of the dependent and independent variables we lead our model to the linear type (3) and used our facilities to build a multi-linear regression. Obtained results are shown on Fig. 2. Estimates of the coefficients are in column B, and all of them are significant. LN-V1 is ln(Prot); T is Time;V3** 2 is R^2.

$$\ln(\text{Prot}) = b_0 + b_1 R^2 + b_2 T \quad (3)$$

The estimation of correlation [15] between two variables – protein concentration and degree of hydrolysis is presented on the Fig. 3. Sample correlation coefficient (Pearson's coefficient) is equal to 0.69. This indicates that high correlation exists between two functions: concentration of proteins in extracts and degree of protein hydrolysis. In addition, we found that the result is statistically significant ($p < 0.05$).

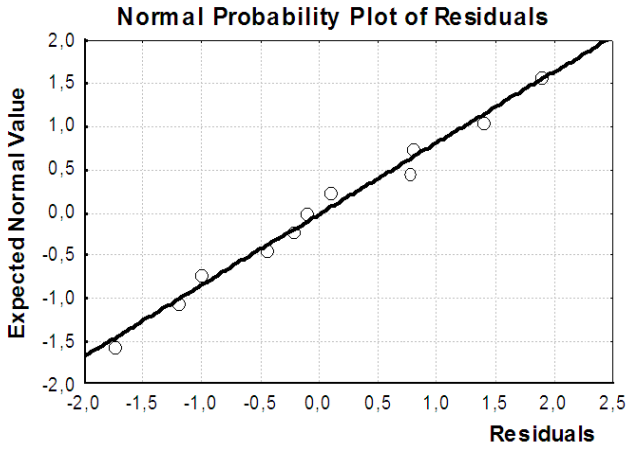

Fig.6. Residuals of nonlinear model (2)

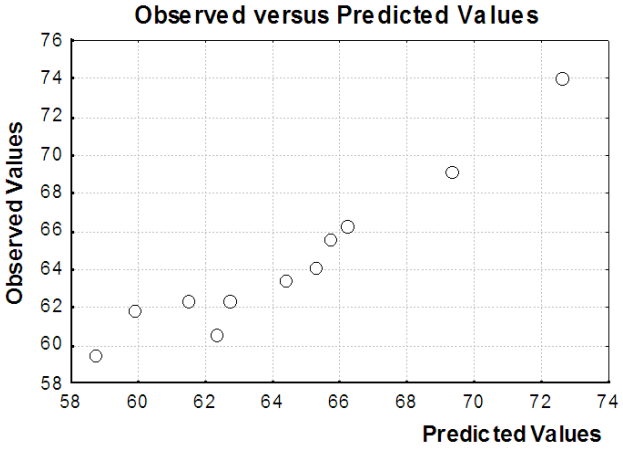

Fig. 7. Observ. vs. Predict. val. for mod. (2)

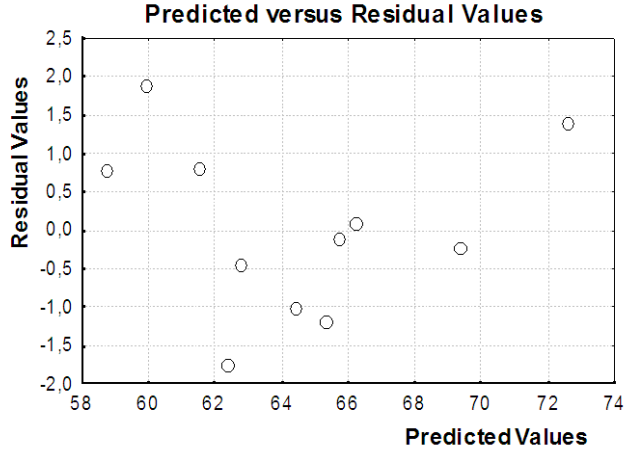

Fig. 8. Predict. vs. Resid val. for mod. (2)

Conclusions

1. Obtained experimental results have shown that protein extraction from sunflower meal is accelerated in the present of proteolitic enzyme as a result of partial protein hydrolysis. The degree of protein hydrolysis was not very high and depended from enzyme/substrate ratio and extraction time.
2. Nonlinear model was obtained for estimation of the affectivity of protein extraction as function of enzyme/substrate ratio and extraction time.
3. It was shown that high correlation (r=0,69) exists between two functions concentration of protein in extracts and degree of protein hydrolysis. Linear dependence was obtained for estimation of protein hydrolysis degree using protein concentration.

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