Artículo de investigación

The effect of enzymatic modification on the depth of hydrolysis of modified protein isolates

ВЛИЯНИЕ ФЕРМЕНТАТИВНОЙ МОДИФИКАЦИИ НА ГЛУБИНУ ГИДРОЛИЗА МОДИФИЦИРОВАННЫХ БЕЛКОВЫХ ИЗОЛЯТОВ

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Abstract

This study was aimed at comparing the effect of enzymatic modification of the protein isolates obtained from sunflower cake on their fractional composition. The obtained chromatograms show that electrophoretic fractions of the modified protein isolates are substantially different from the initial protein isolates. The chromatogram of the proteins modified by sunflower sprouts (SS) showed the presence of 11 fractions represented by 17 peaks (with the total area of 39.65 mAU×sec), the chromatogram of a protein isolate modified by whey lactoserum (WLS) — of eight fractions and 19 peaks (with the total area of 28.73 mAU×sec), and the chromatogram of a complex enzymatic modification — of 13 fractions and 21 peaks (with the total area of 24.20 mAU×sec). Thus, a comprehensive modification caused the differentiation of the first fraction from three peaks with the total relative area of 18.7 % to five peaks with the area of 13.6 % from the total relative area of the peaks of

Аннотация

Сравнение влияния ферментативной модификации белковых изолятов, полученных из подсолнечного жмыха, на их фракционный состав. Из полученных хроматограмм установлено, что электрофоретические фракции модифицированных белковых изолятов, существенно отличаются от исходных белковых изолятов. Хроматограмма белков, модифицированных РП, показала наличие 11 фракций, представленных 17 пиками (общей прлощадью 39,65 mAU×сек), хроматограмма белкового изолята, модифицированного ПМС, - 8 фракций и 19 пиков (общей площадью 28,73 mA×Uсек), хроматограмма комплексной ферментативной модификации – 13 фракций и 21 пик (общей площадью 24,20 mAU×сек). Таким образом, комплексная модификация вызвала дифференциацию первой фракции с трех пиков с общей относительной площадью

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fractions of the corresponding protein product. With that, the comprehensive enzymatic modification and modification of SS resulted in increasing the speed of peaks separation by six minutes, compared to the native protein product, and the modification of WLS reduced the duration by 4.5 minutes. Therefore, reducing the molecular weight and changing the fractional composition of proteins is achieved by the action of proteolytic enzymes of vegetative and microbial origin.

Keywords: Protein, enzymatic modification, hydrolysis, vegetative and microbial proteases, molecular weight, fractional composition.

18,7%, до пяти – площадью 13,6%, от общей относительной площади пиков фракций соответствующего белкового продукта. При этом комплексная ферментативная модификация и модификация РП привела к увеличению скорости разделения пиков на 6 минут, по сравнению с нативным белковым продуктом, а модификация ПМС сократила время на 4,5 минуты. Следовательно, уменьшение молекулярной массы и изменение фракционного состава белков достигается действием протеолитических ферментов растительного и микробного происхождения.

Ключевые слова: Белок, ферментативная модификация, гидролиз, растительные и микробные протеазы, молекулярная масса, фракционный состав.

Introduction

Sunflower meal is a byproduct of oil extraction obtained after extraction of oil from the seeds of oleaginous plants. By the amino acid composition and the biochemical value, the proteins in sunflower oil cake surpass those in cereal crops: they contain more lysine, methionine, cystine, and tryptophan (Bezverhaya, Ilchishina, Berdina, 2010; Bezverhaya, Ilchishina, 2011; Berdina, Ilchishina, Bezverhaya, 2008; Voronova, 2014). For target-specific regulation of the functional properties of the proteins and increasing the biological value, various processing methods are used: thermodenaturation, chemical, and enzymatic modification. Currently, biomodification using enzymes is of the greatest interest (Voronova, Ilchishina, Berdina, 2010; Voronova, Ovcharov, 2014).

The technological effects on the proteins in obtaining isolates from various natural objects (mostly secondary resources of food production) change their structure, amino acid and fractional composition, functional properties, and biological value (Lobanov, Shazzo, Shcherbakov, 2002; Melnikova et al., 2018). For target-specific regulation of the functional properties of the proteins, various processing methods are used: thermodenaturation, chemical, and enzymatic modification. Currently, biomodification with the use of vegetative and microbial proteases is of the greatest interest. Enzymes allow influencing the initial substrates in mild process conditions and with the use of natural agents, thus obtaining optimal results at a minimum cost (compared to the thermal and chemical effects) (Cherkasov, 2006; Shcherbakov, 1991; Karasev et al., 2018a; Karasev et al., 2018b).

Various functional behaviors upon modification of hydrolysates are explained by changes in their thermal behavior, hydrophobicity of the compounds surface, and the molecular weight distribution (Molina Ortiz, Wagnera, 2002).

Proteins are widely used in many food compositions due to their excellent nutritional and functional properties, i.e., solubility and emulsifying, film-forming, and foaming properties (Zhang et al., 2015).

However, their mechanical and barrier properties are to be improved to comply with the functional products (Zink et al., 2016).

This study was aimed at comparing the effect of enzymatic modification of the protein isolates obtained from sunflower cake on their fractional composition.

Materials and Methods

The object of the study was the protein isolate obtained using the modified method (Shcherbakov, 1991) from sunflower cake that had been in turn obtained at a small-scale oil pressing enterprise in the Krasnodar region. The enzyme preparations of vegetative and microbial nature were extracts of enzymes of sprouted

sunflower seeds (SS) and whey lactoserum (WLS). As a result of the preliminary experiments, the optimal conditions for modification were determined: temperature of 25 – 35°C, and the exposure time of 45 – 60 minutes.

The protein isolate was modified by exoproteases in three variants: by the enzyme extract from the germinated sunflower seeds (SS); by microbial enzymes of WLS (including serum rennet enzymes), and by the joint use of vegetative and microbial enzymes (VME).

The distribution of protein isolates electrophoretic fractions was quantitatively assessed by capillary electrophoresis on the Kapel-103P analyzer (Lumix, Saint-Petersburg) (Voronova, Ilchishina, Berdina, 2010). The method of capillary electrophoresis is a complex combination of processes of various nature and properties that occur in the capillary while an electric field is applied. Upon applying an electric field that is directed along the capillary, carriers of the electrical charge (including ions) start moving in the capillary in opposite directions. Since some excessive concentration of cations exists in the diffuse part of the double electrical layer, their movement drags the entire mass of the fluid in the capillary due to the molecular adhesion and friction. The so-called electro-osmotic flow (EOF) occurs, which is directed toward the cathode, which ensures the passive transfer of the solution inside the capillary. Along with this movement, electrical mobility of ions, and the electrophoretic mobility of other charged particles occur in the capillary under the influence of the electric field.

The cationic components of the sample move toward the cathode, outracing the EOF. Their speed is the sum of the EOF speed and the speed of electric migration; therefore, at the output of the capillary, the cationic components are the first to appear; the higher the electric mobility of the ion is, the earlier they appear.

Neutral components of the sample move only under the action of the EOF and appear at the output when they reach the zone of the sample. Drifting to the anode, anionic components move with the speeds that are lower than the speed of the EOF. Some of them, slowly migrating in the field, appear at the output after the appearance of the EOF, and those in which the speed of electric migration in absolute values exceeds the speed of the EOF exit the capillary into the anode space.

While the duration of sample passage through the capillary (which is controlled by the thickness of the capillary, the speed of the EOF, or, to a lesser extent, by the voltage) varies, zones of distribution with individual components of the sample are observed at the output of the capillary near the cathode. Thus, the initial mixture is separated.

By registering the changes in the concentrations of components at the outlet of the capillary using a photometric detector, an electrophoretogram is obtained, which is a set of consecutive peaks above the baseline and the basis for qualitative and quantitative analysis of the mixture. The heaviest protein fractions exit the first.

The samples were prepared for analysis as follows: a suspension of 0.05 g was filled with 0.25 ml of aqueous solution 6 M of urea, stirred and left for 12 hours. Then it was centrifuged, diluted with water two times, and placed into the instrument for analysis.

The fractional composition of the protein complex of isolates was determined using the Osborne's method. The proteins were fractionated by sequential extraction of respective groups of proteins (albumins, globulins, or glutelins) from the tested material with distilled water, a 10 % solution of NaCl, and 0.2 % solution of NaOH.

The qualitative assessment of protein fractions before and after enzymatic modifications was performed by the method of capillary electrophoresis, by evaluating the area of peaks in the chromatograms.

Results and discussion

The electrophoretic spectra of the protein before the modification are characterized by seven fractions with similar areas — the areas of their peaks range from 0.1129 to 0.6771 mAU×sec (Figure 1). The fraction with the peak appearing on the chromatogram in the 17th minute with the area of 32.4 % of the absolute area of all fractions of the sample is predominant. The first faction, which is represented by three peaks with the total area of 18.7 %, appears in the chromatogram in the 10th minute. The remaining minor fractions are present in small amounts and are presented by the peaks of the same type that appear in the chromatogram in the 11th, the 20th, and the 22nd minute that have the relative areas of 12.09 %, 14.11 %, and 11.34 %, respectively.

On the 24th minute, the final fraction appears which occupies 9.01 % of the total area of the chromatogram. The total area of the peaks in the reference sample is 2.089 mAU×sec.

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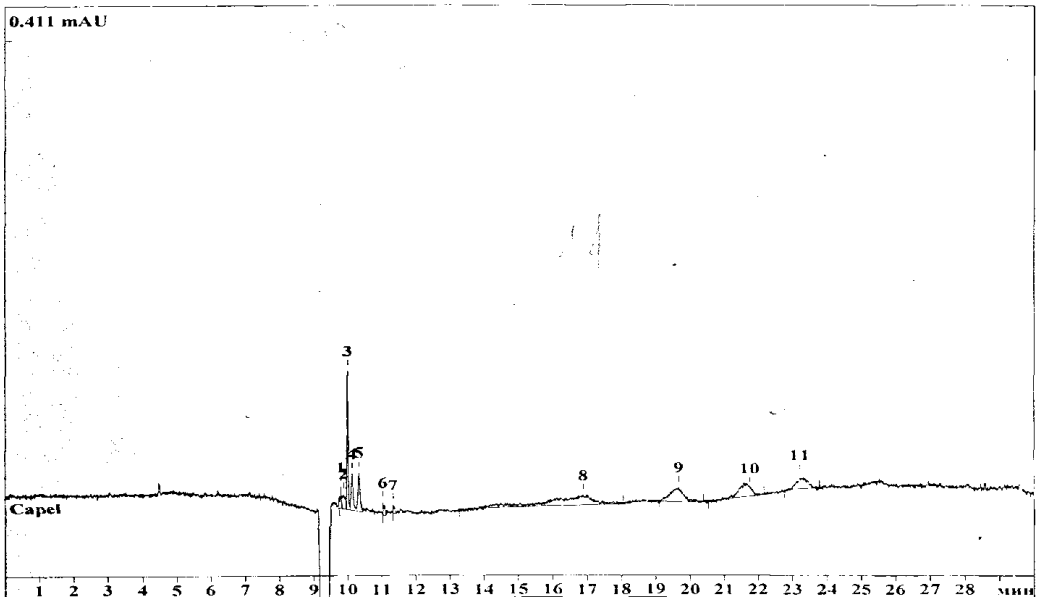

Figure 1. An electrophoretogram of the distribution of the protein isolate electrophoretic fractions before modification.

As it follows from the obtained chromatograms of the electrophoretic fractions, the modified protein isolates are substantially different from the initial protein isolates. For instance, the number of peaks increases from 11, with the area of 2.089 mAU×sec for unmodified protein isolate, up to 21, with the area of 24.203 mAU×sec for protein isolate modified by VME. The chromatogram of the proteins modified by SS showed the presence of 11 fractions represented by 17 peaks (with the total area of 39.65 mAU×sec) (Fig. 2), while the chromatogram of the protein isolate modified by WLS (Fig. 3) showed eight fractions and 19 peaks (with the total area of 28.73 mAU×sec), the chromatogram of a complex enzymatic modification showed the presence of of 13 fractions and 21 peaks (with the total area of 24.20 mAU×sec).

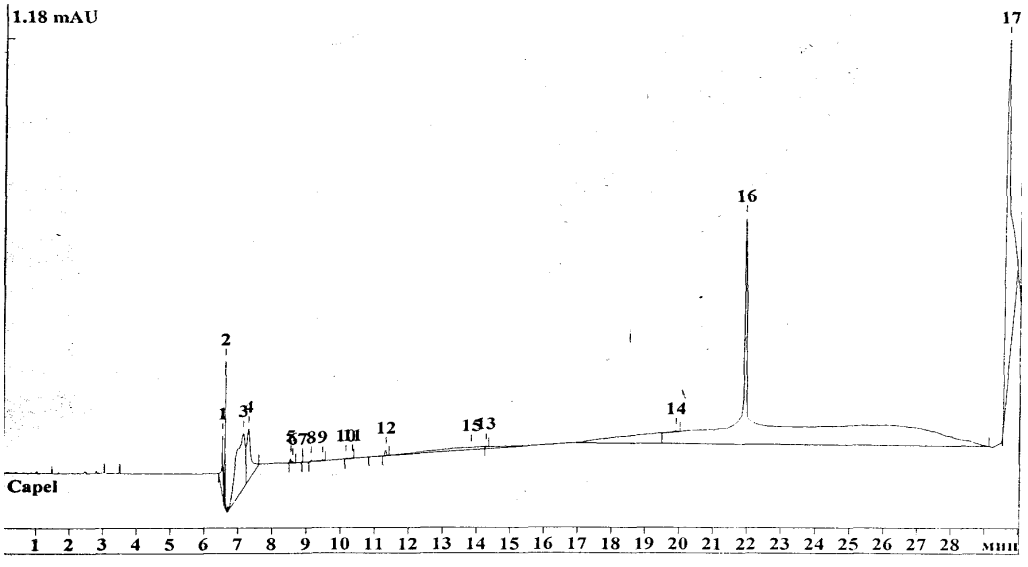

Figure 2. An electrophoretogram of the distribution of the protein isolate electrophoretic fractions modified by SS.

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For the proteins modified by SS, the retention time of the three peaks in the first high molecular fraction decreased by three minutes, and in case of comprehensive modifications — that of five peaks – by 4.5 minutes.

The chromatogram of the protein product modified separately by WLS is characterized by fewer fractions (3 fractions) and shorter peak keeping time (by 6 minutes) with a greater number of them, compared to the modification of protein isolate by SS. With that, it should be noted that the protein isolate modified by SS is represented in the electrophoretic spectra by the largest faction on peak 16 with the area of 25.9058 mAU×sec, and the final fraction with the area of 8.3365 mAU×sec that appears in the 29th minute. The remaining minor fractions are present in minimal quantities – the area of their peaks varies from 0.0048 mAU×sec to 2.5118 mAU×sec.

The chromatogram of the protein isolate modified by WLS (Figure 3) is characterized by several peaks of the same type and configuration and the absolute areas of the peaks, which differ from the chromatograms of the proteins modified by SS by a larger area in the range between 0.0440 mAU×sec and 5.5930 mAU×sec.

The final fraction, which appears in the 19th minute, has the relative surface area of 0.15 %, and for all other described chromatograms, the peak area of the final fraction is higher and reaches 21 %.

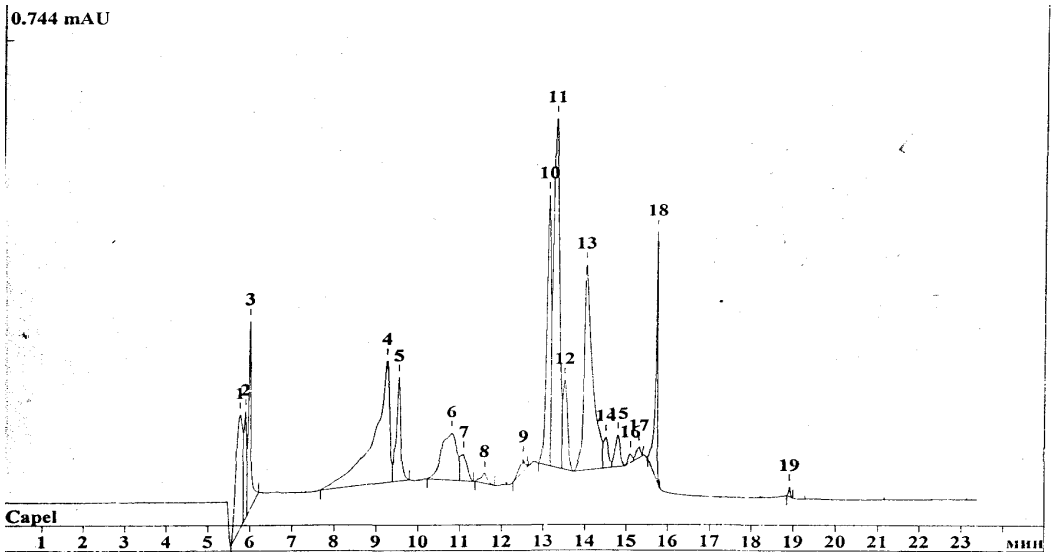

Figure 3. An electrophoretogram of the distribution of the protein isolate electrophoretic fractions modified by WLS.

On the chromatogram of the protein isolate modified consequently by VME (Figure 4) one can see the greatest number of peaks and fractions with a simultaneous decrease in the area of the major fractions and reduction of the peak maintaining duration, which indicates the deepening of the hydrolytic processes. Three peaks of the high-molecular fraction of the native protein product in comprehensive modifications are broken into five peaks, which closely follow each other, with reducing the time by three minutes. Initially, representative are the peaks with a flat, smooth profile of the mid-molecular fraction of the native protein product after a comprehensive modification by vegetative and microbial proteases, representatives are elongated peaks that appear as early as in the 10th minute vs. the 13th minute for the corresponding peaks of the native protein product.

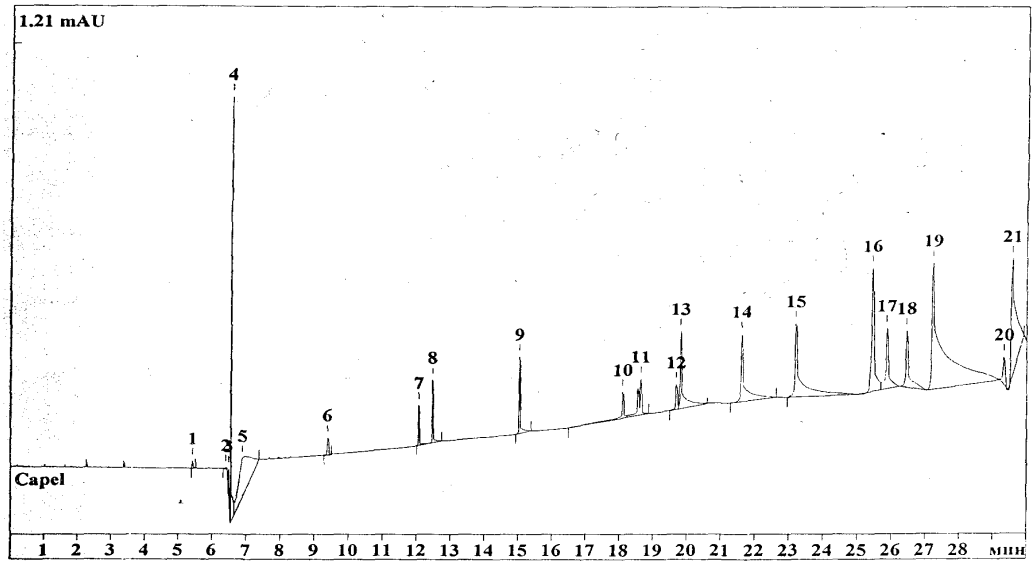

Figure 4. An electrophoretogram of the distribution of the protein isolate electrophoretic fractions modified by VME.

Thus, the comprehensive modification caused the differentiation of the first fraction from three peaks with the total relative area of 18.7 % to five peaks with the area of 13.6 % from the total relative area of the peaks of fractions of the corresponding protein product. With that, it has been found that the comprehensive enzymatic modification and modification by SS resulted in increasing the speed of peaks separation by six minutes, compared to the native protein product, and the modification by WLS reduced the duration by 4.5 minutes. Therefore, enzymes allow influencing the initial substrates in mild process conditions and with the use of natural agents, thus obtaining optimal results at a minimum cost (compared to the thermal and chemical effects).

Conclusion

Thus, under the influence of a set of proteases of various origins, a considerable number of deeply hydrolyzed protein fractions appear in the protein product, which are different from the initial values by the lower molecular weight and the modified fractional composition.

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