



DETERMINATION OF QUANTITATIVE PROPERTIES OF CAMEL MILK PROTEINS BY THE ACTION OF PROTEOLYTIC ENZYMES

Yuldasheva M, Ishimov U

Tashkent pharmaceutical institute

Electronic Email: yoldoshevamadina@icloud.com

<https://doi.org/10.5281/zenodo.18401618>

Abstract

In this scientific article, biologically active peptide fractions formed from proteins in camel milk using proteolytic enzymes were analyzed. In the research process, camel milk proteins were separated using physicochemical methods and hydrolyzed using pepsin, papain and bromelain enzymes. The amount of proteins in the obtained hydrolysates and extracts was determined using the Lowry method, and the molecular weights of peptide and protein fractions were estimated by electrophoretic analysis. According to the results, it was determined that the protein content in the sample hydrolyzed with the pepsin enzyme was highly preserved and that biologically active peptides with low molecular weights of up to 20 kDa were present.

Keywords: camel milk, biologically active peptides, pepsin, papain, bromelain, Lowry method, electrophoresis.

Login

In recent years, there has been a growing interest in biologically active peptides obtained from milk proteins by enzymatic hydrolysis. These peptides have antioxidant, antimicrobial, antihypertensive, and immunomodulatory properties, and are of great importance in the development of functional food and pharmaceutical products.

Camel milk is distinguished from other types of milk by its unique biochemical composition. Its proteins are characterized by easy digestion, low allergic reactions, and high biological activity. Therefore, studying the breakdown of camel milk proteins by proteolytic enzymes and analyzing the resulting biologically active peptides is an urgent scientific issue.

The main objective of this study is to obtain biologically active peptide fractions by hydrolyzing camel milk proteins using pepsin, papain, and bromelain enzymes and to evaluate their protein content and molecular properties.

It is worth noting that the composition and quantity of peptides formed during the hydrolysis of proteins by proteolytic enzymes directly depend on the type of enzyme, hydrolysis conditions, and substrate quality. Pepsin is an animal protease with high activity in an acidic environment, while papain and bromelain are enzymes of plant origin and are characterized by wide substrate specificity.





Comparative study of the effects of these enzymes allows for a deeper understanding of the mechanism of formation of biologically active peptides.

In addition, the determination of quantitative and qualitative parameters of camel milk proteins after enzymatic hydrolysis plays an important role in assessing their technological and biological significance. The use of the Lowry method in determining protein content provides high accuracy and reliability, while electrophoretic analysis allows for the assessment of the molecular weights of the resulting peptide fractions.

To date, studies on the enzymatic degradation of camel milk proteins and the quantitative analysis of biologically active peptides formed from them are insufficient. Therefore, scientific research in this area is of not only theoretical but also practical importance, serving as a scientific basis for the creation of new functional products and bioactive additives based on camel milk.

Research object: Camel milk extract and hydrolysate

Materials and methods: A sample of freshly milked camel milk was taken as the object of the study. First, the milk sample was purified using physicochemical methods and the pH value was balanced to 4.6. Then, proteins were separated from the solution by centrifugation.

The resulting protein fractions were subjected to dialysis to remove low molecular weight salts and impurities. The purified samples were then hydrolyzed using proteolytic enzymes. The hydrolysis process was carried out using papain and bromelain enzymes in different doses. Hydrolysis with pepsin enzyme was carried out in an acidic environment. In order to stop the enzymatic reaction, the samples were heat-inactivated at 85 °C for 5 minutes. Then, the sample was centrifuged at 3000 × g for 10 minutes at 25 °C. In the final step, the hydrolysates obtained were dried using a lyophilizer.

Protein quantification was performed using the Lowry (Laurey) method. The molecular weights of the obtained protein and peptide fractions were estimated using electrophoretic analysis (SDS-PAGE).

Results and their Discussion . According to the results obtained based on the Lowry method, the amount of proteins in the hydrolysate in the sample hydrolyzed using the pepsin enzyme was 268 mcg/mg , and the amount of proteins in the extract is 324 mcg/mg . These results indicate that the proteins in camel milk are preserved at a relatively high level even after the enzymatic hydrolysis process. The high protein content indicates the stability of camel milk proteins to proteolytic enzymes and the efficient formation of biologically active





peptides during the hydrolysis process. The enzyme pepsin has high activity in an acidic environment and ensures the breakdown of casein and whey proteins.

According to the results of electrophoretic analysis, the hydrolysate contains 20 kDa until was low molecular weight protein and peptide. The presence of fractions was determined. This is explained by the fact that large protein molecules are broken down into small biologically active peptides as a result of enzymatic hydrolysis. Such low molecular weight peptides are characterized by high biological activity.

Protein degradation was also observed during hydrolysis with papain and bromelain enzymes, but the degradation rate was relatively lower than that with pepsin. This is due to the substrate specificity and activity conditions of the enzymes.

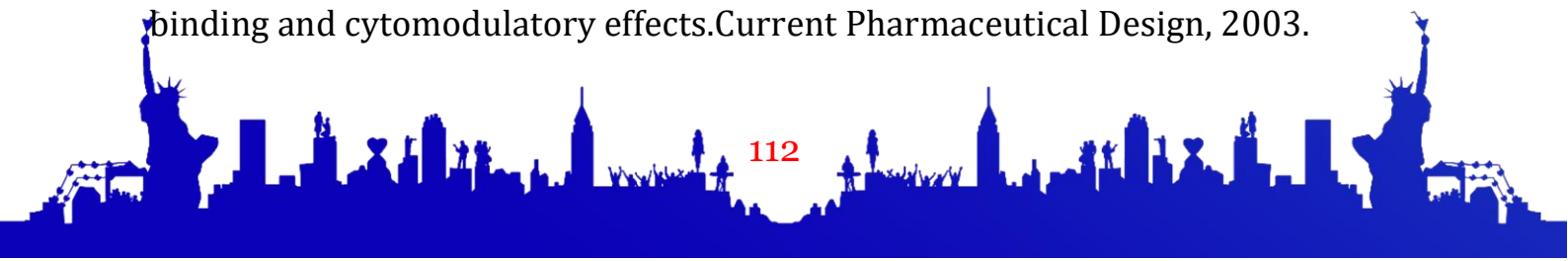
Conclusion: Based on the results of the research, the following conclusions were drawn:

1. Camel milk proteins are a promising raw material for the production of biologically active peptides under the action of proteolytic enzymes.
2. Hydrolysis using the enzyme pepsin ensured high protein preservation and efficient breakdown.
3. According to the results of the Lowry method, it was found that the hydrolysates and extracts had high protein content.
4. Electrophoretic analysis confirmed the presence of biologically active peptide fractions up to 20 kDa.

The results of this study serve as a scientific basis for the development of functional food products and bioactive preparations based on camel milk.

Used literature

1. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ Protein measurement with the Folin phenol reagent . Journal of Biological Chemistry , 1951 .
2. Walstra P., Wouters JTM, Geurts TJ Dairy Science and Technology. CRC Press, Boca Raton, 2006.
3. Fox PF, McSweeney PLH Advanced Dairy Chemistry. Volume 1: Proteins. Springer, New York, 2015.
4. El-Agamy E.I. Bioactive components in camel milk. Food Chemistry, 2009.
5. Konuspayeva G., Faye B., Loiseau G. The composition of camel milk: A meta-analysis. Journal of Food Composition and Analysis, 2009.
6. Meisel H., FitzGerald R.J. Biofunctional peptides from milk proteins: Mineral binding and cytomodulatory effects. Current Pharmaceutical Design, 2003.





7. Nielsen P.M., Petersen D., Dambmann C. Improved method for determining food protein degree of hydrolysis. *Journal of Food Science*, 2001.
8. Damodaran S., Parkin K.L., Fennema O.R. *Fennema's Food Chemistry*. CRC Press, 2017.
9. Laemmli U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 1970.
10. Pihlanto A. Antioxidative peptides derived from milk proteins. *International Dairy Journal*, 2006.
11. Abd El-Salam M.H., El-Shibiny S. Bioactive peptides of buffalo, camel, goat, sheep, mare, and yak milks and milk products. *Food Reviews International*, 2013.
12. Murray R.K., Bender D.A., Botham K.M. *Harper's Illustrated Biochemistry*. McGraw-Hill Education, 2018.

