# **Development of Technology for Obtaining Protein Hydrolysate from Camel Offal using Enzymatic Hydrolysis**

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Corresponding Author: Department «Technology of Food products», Faculty of Food Production, Almaty Technological University, Almaty, Kazakhstan Email: satayeva.zhuldyz@bk.ru Abstract: The research is devoted to the creation of protein hydrolysate from camel wool offal. Protein deficiency in diets leads to the search for additional sources of protein. In this regard, protein hydrolysates are a promising reserve for enriching food products with proteins, the problems of obtaining which attract the attention of researchers. The quality and properties of protein hydrolysates are determined by the raw material, the method of hydrolysis and subsequent processing of the resulting product. The total protein content in offal can be from 19.5% (camel's legs with a put joint) to 21.2% (pork's legs), fat-from 3.2% (camel's legs with a put joint) to 21.0% (pork's legs). Data analysis shows that the fat content in camel offal is 2.25 times lower than in beef offal and in pork offal- 6.5 times, which will allow obtaining protein hydrolysate with higher storage capacity. It was found that the maximum degree of hydrolysis, the highest yield of protein hydrolysate in dry form was achieved at a temperature of 45°C with a hydrolysis duration of 8 h with the addition of 15% pancreatic suspension. As a result of the conducted research, the possibility of using camel offal in the production of protein hydrolysate is justified, optimal modes of obtaining protein hydrolysate are established and its nutritional value is evaluated.

**Keywords:** Camel Meat, Camel Wool Offal, Protein Hydrolysate, Herodietic Meat Product, Enzymatic Hydrolysis

## Introduction

Modern dietetics has established the dependence of human health on nutrition and therefore, to improve the functioning of all body systems, increase its to various adverse resistance environmental influences, scientists are faced with the task of correcting the composition of food. The creation of food products that meet, on the one hand, physiological needs, on the other hand, perform preventive functions, is the direction of research of many domestic and foreign scientists (Analytical review February 15, 2019; Petrova et al., 2018; Tejano et al., 2019; da Silva, 2018; Dieterich et al., 2014; Antipova et al., 2002). To solve this problem, food products are enriched with certain substances, biologically active additives that can optimize the diet by introducing proteins, amino acids, vitamins, micro - and macronutrients, dietary fiber and other useful substances. Proteins are the main building material and the set of continuously occurring chemical transformations of proteins occupies a leading place in the metabolism of the human body. Its lack can lead to health problems, while protein biosynthesis in the bone marrow is disrupted, muscle mass is reduced, there are significant violations of the skin, hair, nails, the function of the endocrine system is weakened, significant violations occur in the pituitary gland, children have a lag in physical and mental development (Dieterich *et al.*, 2014; Antipova *et al.*, 2002).

The current protein deficit in the diets leads to search additional sources, this contributes to the production of combined meat products based on meat and protein preparations obtained from different raw material sources, subject to the mutual enrichment of their compositions (the total chemical and amino acid), combinations of functional and technological properties, enhance biological value, improve the



organoleptic characteristics of the ready product, reducing its cost. Of particular interest as an additional resource of protein in the human diet, along with meat, is offal, the share of which on average is about 10% of the live weight of slaughtered animals. Protein hydrolysates are a promising reserve for fortification of food products with proteins, the problems of obtaining which attract the attention of researchers. On the basis of protein hydrolysis, various products are obtained that are widely used in practice: In the food industry to compensate for protein deficiency in the diet; in biotechnology as a source of amino acids and peptides for bacterial culture media, etc. The quality and properties of protein hydrolysates intended for various applications are determined by the raw material, the method of hydrolysis and subsequent processing of the resulting product. Works of scientists (Gevorkyan, 2006; Taeva et al., 2016; Kaimbayeva, 2009; Rskeldiev and Baibolova, 1998; Berdutin, 2000; Surnin, 2001; Dudnikova, 2009) they showed the prospects of using collagen-containing raw materials and by-products in the creation of protein hydrolysates.

For the Republic of Kazakhstan camel meat is one of the most promising resources of non traditional high quality domestic raw materials for meat processing industries (Taeva *et al.*, 2016; Kenenbay *et al.*, 2019; Taeva, 2016; Taeva *et al.*, 2019). Complex processing of camel meat will provide an additional source of protein and reduce the cost of finished meat products.

The aim of the work is to develop a technology for obtaining protein hydrolysate from camel offal to create specialized food products.

To achieve this goal, as a result of the research, the following tasks were solved: Search for information about the biological value of raw materials in the meat processing industry based on generalization of existing information; justification of the choice of proteolytic enzymes of animal origin; selection of mathematical models for designing protein systems of high biological value with the effect of mutual enrichment of components; assessment of the nutritional, biological value and functional properties of protein hydrolysates obtained using enzymes.

## **Materials and Methods**

The objects of research were camel wool offal-legs with a putty joint, suspension of the pancreas of camels and cattle. For the preparation of enzyme preparations, the pancreas of camels and cattle was used in accordance with (GOST 11285-2017).

To conduct a comprehensive assessment of the quality of raw materials and finished products, we used generally accepted, standard and special methods for organoleptic and physico-chemical indicators, food value and safety.

The moisture content of the product is determined by drying the sample to a constant mass in a drying Cabinet at a temperature of 100-105°C (GOST 8756.2-82, 1989 "food Products. Method for determining dry substances or moisture").

*The protein content* is determined by the Kjeldahl method on the kjel-FOSS-16200 device.

This indicator in meat is found by the difference between the amount of total and non-protein nitrogen converted to protein.

Since meat proteins contain about 16% nitrogen, the conversion factor is 6.25. Connective tissue proteins (collagen and elastin) contain about 17.8% nitrogen, so the conversion factor is 5.62, for milk proteins 6.37, etc.

Determination of the total nitrogen content according to Kjeldahl (GOST 25011-81) is the most common universal and arbitration method.

The fat content is determined according to the standard method by the soxlet method, based on the extraction of fat from a dried sample with volatile solvents.

The soxlet method (GOST 23042-85) is the most accurate and arbitration method. It is based on extracting fat with a solvent, then removing the solvent and drying the fat to a constant mass.

*The ash content* was determined by an accelerated method using magnesium acetate.

## pH of Raw Materials

For potentiometric measurement in laboratory and production conditions, the pH meter millivoltmeter of domestic production "pH-150" was used.

## Determination of Proteolytic Activity

Proteolytic Activity (PA) was determined by the modified Anson method according to (GOST 20264.2-88, 1989).

## Determination of Proteolytic Activity

The substrate was a 2% solution of sodium Caseinate, to which 2 cm<sup>3</sup> of the enzyme solution was added and placed in an ultrathermostat at a temperature of  $30^{\circ}$ C.

After hydrolysis, 4 cm<sup>3</sup> of trichloroacetic acid solution was added to the test tube for 10 min. They were kept for another 20 min at a temperature of  $30^{\circ}$ C. Then filtered in dry test tubes.

 $5 \text{ cm}^3$  of 0.5 M sodium carbonate solution was added to 1 cm<sup>3</sup> of the filtrate, mixed and 1 cm<sup>3</sup> of the working Folin solution was added. After 30 min, the optical density of the solution was measured on the KFC-2 FEK at 670 nm in cuvettes with a light-absorbing layer of 10 mm against the control. The amount of the enzyme is taken as a unit of PA, which in 1 min at 30°C catalyzed the transition to non-precipitated trichloroacetic acid products of hydrolysis of sodium Caseinate in an amount corresponding to 1 mmol of tyrosine (1 mmol of tyrosine is 0.181 mg).

Determination of amine nitrogen by formol titration according to GOST 29311-92. The essence of the method for determining amine nitrogen is to bind the amino group of amino acids of the hydrolysate with formaldehyde, which results in the formation of methylene compounds, which are acids, which are further titrated with a solution of NaOH alkali.

## Determining the Output of Finished Products

The output of the finished product was determined by weighing with an accuracy of 1 g.

## **Results**

Studies of the chemical composition of wool offal: Camel, beef and pork showed that the content of nutrients and moisture depends on the type of animal, the anatomical origin of the offal Table 1. Thus, the total protein content in offal can be from 19.5% (camel's legs with a put joint) to 21.2% (pork's legs), fat-from 3.2% (camel's legs with a put joint) to 21.0% (pork's legs).

The fat content is one of the main indicators of the quality of protein hydrolysates, since the fat content in the hydrolysate of more than 15-20% complicates the drying process, reduces the shelf life and increases its hygroscopicity (Berdutin, 2000).

Table 1 shows a comparative table of the chemical composition and energy value of camel, beef and pork legs.

Analysis of the data in Table 1 shows that the fat content in camel offal is 2.25 times lower than in beef offal and in pork offal- 6.5 times, which will allow obtaining protein hydrolysate with a higher storage capacity.

Protein hydrolysate from camel offal was obtained by the enzymatic method. As an enzyme-containing raw material, we used the pancreas of camels and cattle, which contain hydrolytic enzymes that can break down proteins, lipids, carbohydrates and nucleic acids and differ in stability.

Animal pancreatic cell enzymes have a number of advantages, including the presence of cofactors (thiamine, Riboflavin, nucleotides, etc.), as well as higher resistance to changes in the pH of the medium (Surnin, 2001).

According to the literature data, it is known that the proteolytic activity of animal pancreatic enzymes increases depending on the amount of distilled water in its suspension (Surnin, 2001).

To obtain a suspension of the pancreas, the pancreas of a camel and cattle was crushed on a top with a hole diameter of 2-3 mm, homogenized with distilled water, while changing the amount of water in the samples in the range from 0.25 to 1.0 in increments of 0.25, i.e., in the ratio 1:0,25; 1:0,5; 1:1. To activate the pancreatic enzymes of animals, suspensions were kept at temperatures from 20 to 50°C with an interval of 5°C for 1.5 h. In view of the lability of the enzymes, ethanol was used as a preservative, which was added to the suspension samples in an amount of 2%.

The total proteolytic activity of the suspension of the camel pancreas and bovine pancreas was determined by the modified Anson method according to (GOST 20264.2-88, 1989). "Preparations of the enzyme. Method for the determination of proteolytic activity". A 1% aqueous solution of sodium Caseinate is used as the substrate. The optimal dilution coefficient of SPF was considered to be the one with the highest proteolytic activity of the pancreas.

In contrast to the literature data (Berdutin, 2000; Surnin, 2001), Table 2 shows that the maximum proteolytic activity was observed in the case of dilution of the pancreas with water (hydromodule 0.50). It should be noted that the enzymatic activity of the camel pancreatic suspension is higher than the activity of the bovine pancreatic suspension. Increasing or decreasing the holding temperature, reducing the holding time leads to a decrease in enzymatic activity. The pH of the pancreatic suspension has a pH optimum of 7.0, since the enzymes are in an immobilized state. The proteolytic activity of animal pancreatic suspensions increased at a holding temperature of 45°C and further heating apparently leads to some inactivation of enzymes.

Thus, we note that when using a hydromodule of 0.5 and a holding temperature of 45°C for 1.5 h, a high proteolytic activity of the camel pancreatic suspension is observed.

Prepared suspensions of the camel pancreas were used as an enzyme in the preparation of protein hydrolysate.

Before hydrolysis, the legs with the camel's put joint were cleaned, washed for 5-10 min, crushed with a band saw into discs weighing 50 g, then degreased by prolonged cooking. During the degreasing stage, 200 mL of distilled water was added to 100 g of camel and beef offal (hydromodule 1:2) and heated in a thermostated glass at a temperature of 95-98°C for 50-55 min in a water bath. Then cooled to 3-4°C in the refrigerator, separated the released fat. The fat yield is 2.3-2.8% of the weight of camel offal, 0.2-0.5% -in the supernatant.

In order to determine the optimal ratio of the enzyme to the substrate for hydrolysis of substrate proteins, the suspension of the camel pancreas was added in various amounts (10, 15 and 20%).

To carry out enzymatic hydrolysis, 2% ethanol was added to a thermostated glass with skimmed camel offal as a preservative and a suspension of the pancreas was added in the amount of 10, 15, 20%. Temperature control was performed at a temperature of 45°C and pH-7.0 for 5 to 9 h in 1 h increments until the collagen proteins are completely dissolved.

Amine nitrogen is the nitrogen of free amine groups contained in amino acids, polypeptides and proteins. The increase in amine nitrogen was used to determine the rate of protein hydrolysis and the end of hydrolysis.

Table 3 shows the results of dry protein hydrolysate yield, amine nitrogen accumulation and the degree of protein breakdown depending on the temperature and duration of hydrolysis. The results of the studies shown in Table 3 showed that the amount of pancreatic suspension and hydrolysis time significantly affect the yield of dry protein hydrolysate, the accumulation of amine nitrogen and the degree of protein breakdown. However, there is no significant difference in the yield of dry protein hydrolysate when adding 15 or 20% of the pancreatic suspension.

Analyzing the results of Table 3, we believe that the most optimal time for hydrolysis of camel offal is 8 h with the addition of 15% pancreatic suspension, since the yield of dry protein hydrolysate changes slightly with the addition of 20% pancreatic suspension. Optimal hydrolysis modes were selected taking into account the savings in the consumption of pancreatic suspension and time. It was found that the maximum degree of hydrolysis, the highest yield of protein hydrolysate in dry form was achieved at a temperature of  $45^{\circ}$ C with a hydrolysis duration of 8 h with the addition of 15% pancreatic suspension.

## Discussion

In order to inactivate the enzyme complexes and thermocoagulate the residual protein at the end of the hydrolysis process, the resulting substrate-enzyme complexes were cooked at a temperature of  $95\pm2^{\circ}C$  for 30 min. After cooling, the fat was further separated (0.2-0.5% of the original fat mass) and the hydrolysate was filtered through cellulose as an auxiliary filter element.

The mixture was centrifuged at 10000 G on Eppendorf Centrifuge 5810 R for 20 min, the supernatant was frozen to -70°C in the refrigerator and dried on a Telstar Cryodos freeze dryer under vacuum and in parallel on a nanbei spray dryer.

When using various drying methods, the yield of dry hydrolysate was 6.6-6.8% by weight of the enzymesubstrate mixture. The yield of protein hydrolysate varied by 0.2% depending on the low bulk weight (0.1 g/cm<sup>3</sup>), hydrolysates and entrainment of hydrolysate particles into the atmosphere.

The biological activity of the hydrolysate depends on the particle size and solubility. The fractional composition of protein hydrolysates obtained using the camel pancreatic suspension enzyme complex depending on the duration of hydrolysis was determined on the bio-RAD Consort EV265 electrophoregram Fig. 1. the Electrophoregrams of the studied samples were analyzed on a densitometer. The electrophoregram was processed using the BioCapt program (Vilber Lourmat, France).

The results of quantitative determination of protein fractions are shown in Table 4.

#### Note:

Lane 1-Molecular	marker-kDa,	(Kaleidoscope™
prestained prot	tein standards, bi	io-rad)
Lane 2-Sample 1, the	e hydrolysis of 4 l	n
Lane 3-Sample 2, the	e hydrolysis of 5 l	n
Lane 4-Sample 3, hy	drolysis 6 h	
Lane 5-Sample 4, the	e hydrolysis of 7 l	n
Lane 6-Sample 5, the	e hydrolysis of 8 l	n
Lane 7-Sample 6, the	e hydrolysis of 9 l	n
-		

From the comparison of densitograms, it can be concluded that the most digestible low-molecular-weight peptide fractions with molecular weights of 8-25 kDa of 53.4 and 39.6% (samples 6 and 7) were obtained by enzymatic hydrolysis of camel pancreatic suspension for 8-9 h, respectively.

Thus, based on the results of research, a technology for obtaining protein hydrolysate from camel offal (legs with a putty joint) has been developed and optimal parameters of enzymatic hydrolysis have been established. We have studied the physicochemical and organoleptic parameters of dry protein hydrolysate from camel by-products (Table 5). The physicochemical and organoleptic parameters of dry protein hydrolysate from camel by-products were compared with protein hydrolysates obtained from other types of raw materials of animal origin.

The resulting hydrolysate is a homogeneous fine powder of light beige color, with a weak specific smell, well soluble in water.

The moisture content of protein hydrolysate from camel offal was 7%, the mass fraction of protein was 85% and the mass fraction of fat was 3%, which correlates with the literature data and GOST 33692-2015 "animal connective tissue Proteins".

Thus, the resulting protein hydrolysate containing low-molecular protein fractions and amino acids, which has a high biological and nutritional value, will be used to enrich meat products for the elderly.

Content					
Indicators	Moisture	Fat	Total protein	Ash	Energy value, kcal
camel's legs with a put joint	76,18±2,15	3,20±0,1	19,5±1,0	1,12±0,02	101,42
beef 's legs with a put joint	71,41±2,06	7,2±0,12	20,61±1,2	0,94±0,02	147,85
pork's legs	57,0±1,9	21,0±0,08	21,2±0,85	0,8±0,002	273,8

#### Table 1: Chemical composition and energy value of wool offal: Camel, beef, pork

# Table 2: Proteolytic Activity of Pancreatic Suspensions (APS) of camel and cattle at different temperatures and hydromodule Proteolytic activity $PE/g*10^{-3}$

	Proteolytic activity, PE/g*10 <sup>3</sup>					
	APS of camel		APS of cattle			
	hydromodule (1:	:0,25; 1:0,5; 1:1)				
t, °C	0,25	0,5	1,0	0,25	0,5	1,0
20	2,35±0,11	2,78±0,11	2,13±0,11	2,25±0,11	2,38±0,11	1,95±0,11
25	3,25±0,10	3,45±0,12	2,86±0,11	2,76±0,10	2,85±0,10	$2,25\pm0,10$
30	4,05±0,20	4,68±0,20	3,17±0,19	3,05±0,21	3,35±0,20	$2,75\pm0,10$
35	4,25±0,16	5,57±0,18	3,95±0,19	3,75±0,18	4,05±0,17	$3,28\pm0,11$
40	5,12±0,22	6,25±0,22	4,82±0,22	4,12±0,20	4,72±0,22	3,95±0,12
45	5,93±0,29	6,60±0,32	5,40±0,19	4,48±0,21	5,01±0,20	$4,24\pm0,18$
50	5,05±0,21	5,74±0,29	4,98±0,15	3,92±0,15	4,78±0,16	3,76±0,14

## Table 3: Effect of hydrolysis time and amount of pancreatic suspension on the yield of dry protein hydrolysate

Prototype	°C Temperature,	The time of hydrolysis, hours	Total nitrogen, %	Amine nitrogen, mg%	Yield of dry protein hydrolysate, mg%	Degree of protein breakdown, mg%
Sample 1 (10%	45±1,5	5	576±20,5	125±5,5	11,8±0,25	3,6±0,12
pancreatic suspension)						
		6	593±20,6	136±5,8	14,5±0,30	3,8±0,14
		7	664±22,2	150±6,2	18,3±0,32	4,0±0,10
		8	$744 \pm 25,1$	275±6,5	23,4±0,40	4,4±0,20
		9	840±32,0	310±9,2	23,8±0,38	4,6±0,21
Sample 2 (15% pancreatic suspension)	45±1,0	5	728±25,2	134±5,8	15,0±0,45	4,2±0,20
		6	945±33,6	142±6,6	19,4±0,60	4,5±0,20
		7	995±30,5	153±7,2	$21,7\pm0,80$	5,1±0,23
		8	1143±42,0	362±12,0	31,7±1,0	6,8±0,25
		9	$1145 \pm 38,2$	354±10,0	32,93±1,2	6,8±0,27
Sample 3 (20% pancreatic suspension)	45±1,7	5	936±25,0	140±5,5	17,2±0,4	4,4±0,20
		6	1011±30,0	$148\pm 5,8$	22,5±0,8	4,9±0,24
		7	1072±26,0	161±3,6	27,2±1,0	5,4±0,21
		8	1157±27,0	362±14,5	33,7±1,2	6,7±0,25
		9	1215±36,0	384±12,2	34,5±1,4	6,8±0,24

#### Table 4: Molecular weight of protein fractions of hydrolysates, kDa

M.W. Values	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
Band 1	250.000	31.259	30.710	30.527	30.527	45.324	30.163
Band 2	150.000	29.436	28.354	27.995	27.817	32.177	27.638
Band 3	100.000	25.521	25.000	24.490	23.981	30.345	23.981
Band 4	75.000	22.015	21.400	20.957	20.814	27.638	21.103
Band 5	50.000	18.159	17.810	15.660	15.601	24.150	17.486
Band 6	37.000	16.075	15.955	14.486	14.486	20.814	15.601
Band 7	25.000	14.683	14.683	13.585	13.657	15.483	14.811
Band 8	20.000	14.285	13.729	10.404	10.485	14.552	13.441
Band 9	15.000	10.727	10.647			13.513	10.324
Band 10	10.000					10.404	

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	Protein hydrolysate from camel	* Protein hydrolysate	Animal proteins connective	
Name of the indicator	offal (legs with a putty joint)	from pork legs	tissue GOST 33692-2015	
Mass fraction of moisture, %	7,0±0,09	3,0	No more than 10	
Mass fraction of protein, %	85,0±2,7	90,0	Not less than 80.0	
Mass fraction of fat, %	3,0±0,15	5,0	No more than 16.0	
Mass fraction of ash, %	5,0±0,06	2,0	Not rated	
Appearance and color	Dry product of uniform			
	consistency in the form of			
	loose powder, hygroscopic	Dry product of uniform	A dry product of uniform	
		consistency in the form of	consistency in the form of	
		loose powder, hygroscopic	a fibrous mass or a loose	
			fine powder, or a loose	
			powder containing single or	
			agglomerated particles. For powders, it is allowed to have	
			larger lumps that crumble with	
			light mechanical pressure	
Color	Light beige	Light yellow	From white to light brown	
Smell	Characteristic of the raw	Characteristic of the raw	Characteristic of raw materials.	
	material from which it is	material from which it	without post-root smell	
	made, without foreign smell	is made, without foreign smell		
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**Table 5:** Physico-chemical and organoleptic parameters of dry protein hydrolysate from camel offal

Fig. 1: Electrophoregram of hydrolysate separation on 12% polyacrylamide gel

## Conclusion

In old age, the human body has a lack of connective tissue proteins of collagen and elastin and diseases such as arthritis and arthrosis develop.

The resulting protein hydrolysate from the legs with the putty joint of a camel will be used in further scientific research in the production of meat products for specialized purposes.

The optimal hydromodule for diluting the suspension of the camel pancreas with distilled water (0.5) was established.

The possibility of using camel offal in the production of protein hydrolysates is shown for the first time.

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This study was conducted in laboratories and meat processing centers of ATU and KATU.

## **Author's Contributions**

Aigul Tayeva and Zhuldyz Satayeva: Conceived and planned the study.

Lyazzat Baibolova and Assel Bulambayeva: Conducted lab work and drafted the manuscript.

Gaukhar Kuzembayeva: Did statistical analysis of data.

Zhuldyz Satayeva: Revised the manuscript.

## **Ethics**

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and there are no ethical issues involved.

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