INVESTIGATION INTO BIOLOGICALLY ACTIVE INGREDIENTS FROM PIGS' FEET

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***Abstract* – the diets, possessing nutrient adequacy to the specifics of metabolism of old people with the consideration of pathological characteristics creates real conditions for prolongation of the period of their active life. In the prophylaxis of diseases of muscular-skeleton system, being widely spread among old people, calcium preparations and hydrolysates of connective-tissue proteins play an important role.**
The investigations carried out have shown a high level of connective tissue proteins (12.57%) in a meaty part and calcium salts (25.3%) in bone parts of pigs' feet.
A biotechnological method of obtaining of protein-mineral fortifier, being a mixture of a protein hydrolysate, containing up to 70% of low-molecular peptide fractions with a molecular mass of 16-30kDa and bone powder with the size of particles 50-150 microns has been developed.
In the experiments on laboratory animals a reliably higher effect of calcium accumulation was marked, when using in animals' diets the experimental samples of gerodietetic sausages, containing a protein-mineral fortifier, as compared to usual sausage without it and containing phosphates.

Index terms – bone, connective tissue protein, gerodietetic sausage products, hydrolysate,

I. INTRODUCTION

The requirements of rational nutrition of old people pose the tasks of creation of a range of products possessing nutrient adequacy to specifics of metabolic processes of their organism with the consideration of the most widely spread pathologies. This creates real conditions for prolongation of the working ability and active life period with minimum losses from diseases inherent to old people. The main problem of this group of people is the diseases of muscular-skeleton system, and first of all osteoporosis, which is a systematic disease of skeleton and characterized with the loss of total bone mass. The diseases of bones and joints are increasing in the age group older than 50 years. The research workers forecast an increase of a number of old people on the planet by 2020 twofold. The preparations of calcium and connective-tissue proteins, based on collagen, have an important role in gerodietetic nutrition for the cases of muscular-skeleton systems diseases. Hydrolysates are sources of "building material" for tendons, ligaments and cartilage.

Meat industry has large sources of collagen-containing raw materials with high content of connective tissue proteins. Their sources and those of mineral substances can be pigs' feet which haven't found yet complete and rational production use.

II. NATERIALS AND METHODS

The objective was to investigate food value of meaty and bone part of pigs' feet, develop a biotechnological method of obtaining of biologically active ingredients for meat products, designed for old people, pre-disposed to osteoporosis.

The objects of investigations were pigs' feet, a protein hydrolysate, obtained by enzymatic hydrolysis of pigs' feet, bones, gerodietetic cooked sausages.

In the experiments a mass share of moisture, protein, fat, ash was determined according to generally accepted methods. Amino acid composition – by the method of ion-exchange chromatography on amino acid analyzer "Bekman", a mineral composition – on atom-absorption spectrophotometer "Perkin Elmer", measurements of protein fractions - by electrophoresis in 10% polyacrylamide gel in the presence of SDS according to Laemmli.

Biological investigations were carried out on white male rats at 4 months age, with the weight 366.8 ± 2.5 g during 25 days followed by the determination of clinical and biochemical blood indices.

III. RESULTS AND DISCUSSION

Analysis of food value (Table 1) and amino acid composition (Table 2) of pigs' feet has shown that these low value meat-bone raw materials are abundant in connective tissue protein and a number of amino acids, found in collagen. Mineral composition of bone part of pigs' feet is presented in Table 3.

Table 1.

Food value of meaty part of pigs' feet

Index	Mass share, g/100 g	
	M	S
Moisture	57.0	2.42
Protein, including	21.2	0.96
Connective tissue	12.75	0.53
Fat	21.0	1.38
Ash	0.8	0.03

Table 2

Amino acid composition of protein of meaty part of pigs' feet

№	Amino acids	Mass share, g/100 g of protein		
		Meaty part of feet		*longissimus dorsi* of pig
		M	S	
1	Aspartic acid	9.5	0.41	10.3
2	Threonine	4.4	0.23	3.9
3	Serin	5.2	0.21	2.0
4	Glutamic acid	16.8	0.73	19.8
5	Proline	8.7	0.39	2.4
6	Glycin	10.5	0.41	2.7
7	Alanine	9.9	0.46	4.2
8	Valine	2.0	0.08	4.7
9	Methionine	1.1	0.03	2.8
10	Isoleucin	2.3	0.09	4.5
11	Leucin	6.3	0.29	8.3
12	Tyrosine	2.4	0.09	3.7
13	Phenylalanine	3.4	0.11	5.6
14	Histidin	1.4	0.06	3.8
15	Lysin	6.4	0.31	8.6
16	Arginine	8.7	0.39	8.9

Table 3. Mineral composition of bone part of pigs' feet

Name of salts	Mass share of salts, g/100 g of bones	
	M	S
Potassium	0.30	0.13
Calcium	25.0	1.41
Magnesium	1.05	0.05
Phosphorus	10.8	0.49
Sodium	2.62	0.12
Cobalt	0.0013	0.0005
Manganese	0.00057	0.000026
Copper	0.0012	0.00063
Iron	0.0155	0.0072

Taking into account physiological functionality and a sufficient availability of raw materials a biotechnology of production of complex protein-mineral additive from pigs' feet was developed.

The proteinic part of the additive was obtained by fermentative hydrolysis of pigs' feet by pigs' pancreas suspension. The investigations for finding of rational parameters of hydrolysis have shown that hydrolysis by the suspension of pancreas of pigs (SPZhS) is most effective and provided the yield of free amino acids at the level 80% during 8 hours. The level of yield of amino acids with hydrolysis by collagenase enzyme was lower with hydrolysis time 12 hours. Comparing molecular-mass distributions of the main fractions of protein hydrolysates, one can make a conclusion that the hydrolysate obtained with the use of SPZhS contains about 70% of low molecular peptide fractions, giving molecular mass from 16 to 30 kDa, and with the use of collagenase about 45% fractions in the range of 450 to 480 kDa, and not a high content (about 33%) of the most digestible fractions from 16 to 30 kDa. After filtration hydrolysate was subjected to drying and mixed with bone powder. The bone powder was obtained by treatment of the bone residue after hydrolysis at 120-130^{0}C, drying, and subsequent grinding to particle size 50-150 micron. The obtained protein-mineral fortifier was used in recipes of cooked sausage products for gerodietetic nutrition. Using a method of computer design a recipe of gerodietetic product was developed for old people, suffering osteoporosis, with a protein-mineral fortifier. The ingredient composition of the product is presented in table 4.

Table 4.

The ingredient composition of the product for gerodietetic nutrition

Product	Ingredients
Cooked sausage for old people, suffering from muscular-skeletal disease	Beef of first category, beef (meat of shank, knuckles), pork (meat of shank), egg albumin, isolated animal protein, complex protein-mineral fortifier, inulin, food fibers Vitacel, soy oil, linseed oil, ascorbic acid, phosphates, vitamins (A, D, B_1, B_2, B_5, B_9, B_{12}), cooking salt, spices

Using the results of investigations of amino acid composition the amino acid balance of the recipe composition has been calculated, suggesting about sufficiently high values of the

coefficient of utility (rationality) of amino acid composition, being in the range 0.7 – 0.8, and a minimum score, being in the range 0.9 – 1.0.
To study the influence of the experimental samples of sausages on the organism of laboratory animals compared to usual sausage without addition of protein-mineral fortifier and containing phosphates, clinical and biochemical blood indices were determined, presented in Table 5.

Table 5.
Results of blood analysis of laboratory animals after intake of gerodietetic sausages

Indices	Samples	
	experiment	control
erythrocytes,$\times 10^6$/mcl	6.25±0.25	6.87±0.27
leucocytes,$\times 10^3$/mcl	11.18±0.47	15.72±0.63
hemoglobin, g/dl	15.83±0.63	16.27±0.65
protein, г/л	69.9±2.78	70.9±2.84
albumin, g/l	39.0±1.56	39.0±1.56
glucose, mmol/l	6.1±0.24	6.0±0.24
calcium, mg%	2.6±0.10	2.1±0.08
phosphorus, mg%	1.5±0.06	2.6±0.10
cholesterol, mmol/l	0.97±0.04	1.3±0.05
APh, Ed/l	150.7±6.03	111.7±4.47

The higher is the activity of alkaline phosphatase (APh), the higher is the activity of osteoblasts responsible for bone formations, i.e., the higher is the level of APh, the more active is the growth of bones. Low hemoglobin promotes reduction of activity of alkaline phosphatase. The lower activity of APh is in the control, where calcium is not added, and the product contains phosphates. The higher effect of calcium accumulation is also marked in the experimental sample. The content of cholesterol in blood serum is reduced as compared to the control.

Thus, the addition of protein-mineral fortifier increases activity of APh and calcium accumulation, and phosphates reduce them.

IV. CONCLUSION

1. A high level of connective-tissue proteins (12.57%) in meaty part and of calcium salts (25.3%) in bone part of pork feet was determined.
2. A biotechnological method of production of protein-mineral fortifier, being a mixture of protein hydrolysate, containing up to 70% of low-molecular peptide fractions with molecular mass 16-30 kDa and bone powder with particles size 50-150 micron was developed.
3. In the experiments on animals a reliably higher effect of calcium accumulation was marked in the case of use in animals' diets of gerodietetic sausages experimental samples containing a protein-mineral fortifier, as compared to usual sausage (without its addition) and containing phosphates.