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Obtaining protein hydrolysates with chemical and enzymatic methods

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The goal of our research was to work up a method of hydrolisates protein production using as raw material fresh pork meat-bone tissue after dismantling the process of half carcasses. Actually this raw material was practically all utilized as waste. The results of laboratory research and an industrial test allowed to state that is possible to produce hydrolysates containing 8 – 10% of proteins, and the most advantageous parameters of the chemical process are: the reaction time ~105 min., temperature 120°C, pressure 3.0 bars. With the use of enzymes (Protamex and Flavourzyme), it is possible to obtain a non-gelling protein hydrolysate with a high degree of clarity and light cream colour. The best results were achieved with the following parameters: the meat-bone feedstock to the water ratio from 1:1 to 1:2, the temperature of 40 – 45°C, the time of the process 3 h, pH ~6.

Keywords: meat waste, protein hydrolysate, chemical method, enzymatic method.

INTRODUCTION

At present two principal methods of the production of proteins and their derivatives are proposed for an industrial scale. The first one is based on chemical hydrolysis of the protein feedstock, usually conducted at high temperatures and at an increased pressure. The other one makes use of enzymatic hydrolysis of the feedstock, which most frequently is conducted at the temperatures of 40 – 65°C for the reason of specific properties of enzymes. Both methods use the mechanism of the decomposition of proteins into smaller peptide particles, and in the final phase, into amino acids[1 – 3].

The goal of our research was the development of a method of obtaining protein hydrolysates on the basis of fresh, pork meat-bone tissue from the processing of half carcasses. The research whose aim is to use potential wastes as production feedstock is one of the basic directions applied in the methods of cleaner technologies[1, 4 – 7]. The feedstock used was pork meat-bone tissue (from Duda-Bis Company in Sosnowiec) resulting from the process of the disintegration of pork bones which were left after slaughtering, of the following content [wt %]: water ~40, protein ~18, fat ~20, mineral parts ~22, bulk density 1.1 - 1.2 kg/dm^3, granulation 0.2 - 20 mm[8 – 10].

EXPERIMENTAL

Research on obtaining protein hydrolysates with chemical methods

The method of acidic hydrolysis was selected for the experiments. The product was analyzed from the viewpoint of both the qualitative parameters (colour, clarity) and the quantitative ones (the protein content in hydrolysate).

Laboratory experiments were carried out with Buchi Co., compact autoclave, which is suitable for work at the temperatures up to 250°C and the pressure of 100 bars. Glycerine was used as the heating medium. 80% lactic acid, food grade, was added to the samples in the proportion of 1% to the mass of the meat-bone pulp. The obtained mixture was pre-filtered on a sieve, where the solid parts were separated, and afterwards the solution under-went centrifugation in a laboratory centrifuge, where fat was separated from the hydrolysate solution[1, 3].

The aim of the first laboratory research was to check the influence of temperature and the reaction mixture composition upon the selected properties of the final product, such as colour, clarity, and gelling capacity. The results (Table 1) show that the best parameters proposed for an industrial scale should be: temperature: 130°C, time: 0.5 – 2 h, pulp to water ratio 1:1.

The second stage of the research realised at 130 – 150°C proved that temperature ≥130°C causes a significant de-

Table 1. The results of the first stage of experiments aiming to obtain protein hydrolysates

No.	Ratio (pulp:water)	Temp. [°C]	Time of hydrolysis [h]	Colour	Clarity	Gelling properties	Other (additive) [wt %]
1	1:2	150	2.0	Brown - orange	No	No	1% of active carbon
2	1:1	150	0.5	Light straw - coloured	Yes	No	2% of active carbon
3	1:3	140	2.0	Brown - orange	No	No	No additive
4	1:1	130	2.0	Dark brown	No	No	2% of active carbon
5	1:0.5	110	2.0	Beige	No	Yes	No additive
6	1:1	100	2.5	Brown	No	Yes	No additive
7	1:3	100	2.0	Yellow	Yes	Yes	No additive
8	1:0.5	90	2.0	Yellow	No	Yes	No additive
9	1:0.5	90	2.0	Beige	No	Yes	1% of active carbon
10	1:0.25	90	2.0	Yellow	No	Yes	No additive
11	1:0.5	80	2.0	Beige	Yes	Yes	1% of active carbon
12	1:0.5	70	2.0	Beige	Yes	Yes	1% of diatomaceous earth

composition of protein chains, and the loss of gelling properties. These properties may be preserved if the process is conducted at the temperatures from 70 – 110°C[1].

The following products are obtained in the process of chemical hydrolysis: gelling protein hydrolysate, fat suitable for the food industry, the so-called bone sludge and fine bones. Gelling protein hydrolysate is formed after the separation of protein hydrolysate and fat mixture in the centrifuge. Stored at ~3°C, it forms gel of a texture within the range of 100 – 350 g and an average protein content of 8 – 10%. The durability of the gel form is limited[1, 8]. In reality, the gel stored at 3°C preserves its suitability for 3 – 4 days. The product is available commercially as dry mass (powdered proteins)[11 – 14]. The fat obtained in the process of centrifugation has a high quality, comparable with the quality of the fat used in the food industry[1, 7]. Bone sludge and fine bones formed[1, 8 – 10] have the size of up to 25 mm and contain [wt %]: moisture: ~39, fat: ~3, protein: 12, mineral parts: 46.

The industrial experiments of obtaining protein hydrolysates with the chemical process were realised in pressure reactors heated with injected steam. The solid parts were separated on a sieve and decanter, the fat was separated from hydrolysate in the Alfa Laval centrifuges.

The aim of the experiments was to determine, on an industrial scale, the optimal parameter of the process and the quantities of the products[1, 3]. The industrial experiments were started with the following parameters: temperature ~130°C, time of the process ~3 h, bones to water ratio 2:1, addition of lactic acid – 2% in proportion to the input mass. Afterwards the focus of attention was directed upon the search of the parameters which will guarantee obtaining the maximum amount of protein in hydrolysate and the highest product texture.

Figures 1 – 5 [1, 12] present the amounts of generated half-products depending on the mass ratio of the applied input (raw materials + water).

As a result, the duration of the process was reduced to around 2 h, the composition of hydrolysate (8 – 10% of protein) as well as its gelling properties and light colour were stabilized. The obtained hydrolysates demonstrated the high gelling capacity. At higher temperatures (~130°C), hydrolysates were darker than those obtained at lower temperatures (120°C). When temperatures were

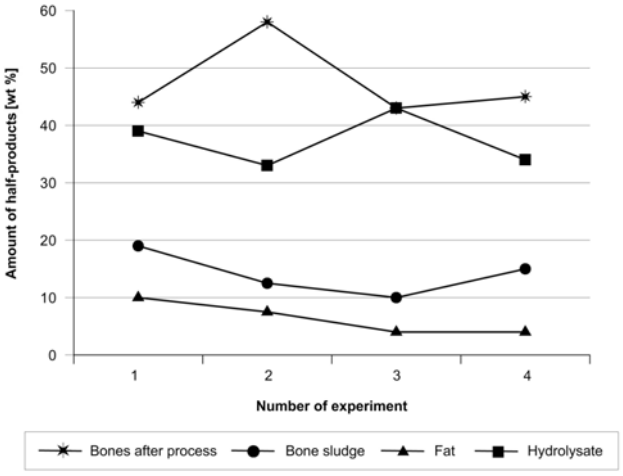

Figure 1. Quantities of the products of chemical hydrolysis at bones to water mass ratio of 1,100:300

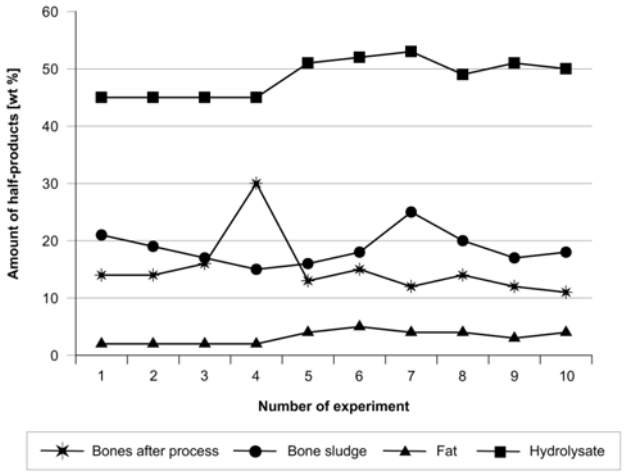

Figure 2. Quantities of the products of chemical hydrolysis at bones to water mass ratio of 1,200:750

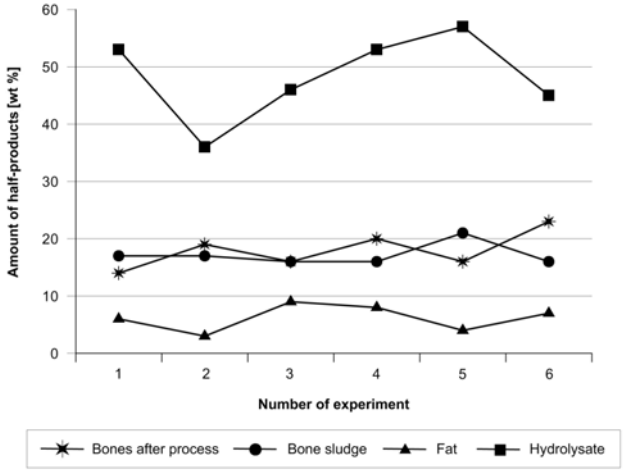

Figure 3. Quantities of the products of chemical hydrolysis at bones to water mass ratio of 1,250:700

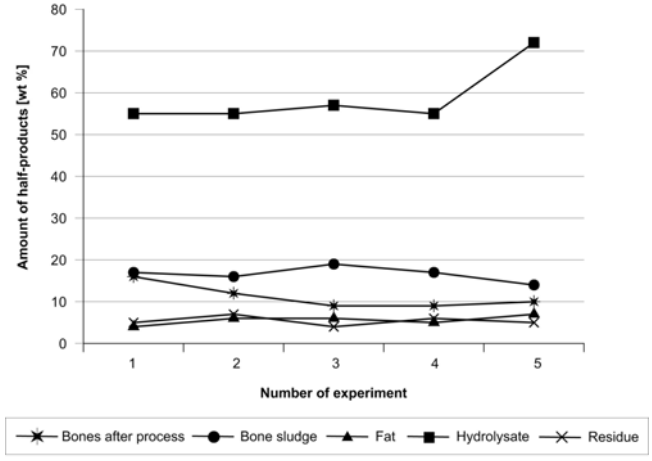

Figure 4. Quantities of the products of chemical hydrolysis at bones to water mass ratio of 2:1

too low (<110°C), the process generated large amounts of waste, which resulted from an incomplete hydrolysis. The feedstock for the process was stored for no longer than 24 hours in a cool environment of 0 – 7°C. The application of the feedstock which had been stored longer than recommended led to a decrease in pH in the produced hydrolysate.

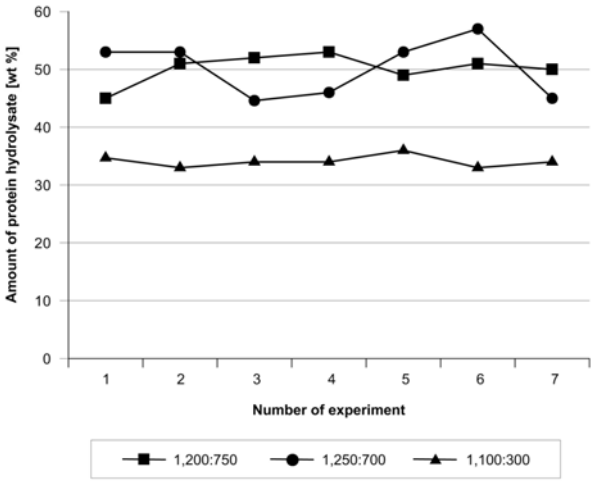

Figure 5. The yields of hydrolysate production depending on the feedstock's ratio

On the basis of the research on an industrial scale, it may be concluded that the beneficial parameters of the chemical process are: the temperature of 120°C, pressure of 3.0 bar, the meat-bone tissue: water ratio 1,200 – 1,250:700 – 750, the time of the process of around 105 min[1]. The content of the protein in the hydrolysate obtained under such conditions amounts to 7.98 – 10.64% on average. The hydrolysate is cream-coloured, demonstrates a homogeneous character, but is not clear.

Research on obtaining protein hydrolysates in enzymatic process

The goal of the experiments was obtaining clear, light-coloured solutions with high protein content. Additionally, the experiments were conducted on obtaining non-gelling hydrolysates using gelling hydrolysate produced with a chemical method.

The laboratory experiments were conducted with the use of two enzyme preparations produced by Novozymes Co. The first one is Protamex, which is a complex of proteinases produced by a strain of the *Bacillus* bacteria[1, 15]. The deactivation of the Protamex prepara-tion is achieved by maintaining 85°C for 10 minutes at pH ~8. The other preparation whose effect was checked was Flavourzyme, which is a complex of ptoteinases and mould pepti-dases, produced with the help of deep fermentation by the strain of *Aspergillus oryzae*. The liquid form of Flavourzyme 500 L[15] was applied in the tests. Flavourzyme may be deactivated within 10 minutes at a temperature of ≥ 75°C[1, 15].

There were two variants of the conducted experiments. In the first one, only the Protamex enzyme was applied in the process, whereas in the second one, a mixture of Protamex and Flavourzyme was used for hydrolysis. In both cases lactic acid suitable for the food industry was used to correct the pH.

In the experiments with the use of Protamex, the meat-bone pulp was mixed with water in 1:1 or 1:2 ratios. After heating to 40 – 55°C, lactic acid was applied to adjust the pH of the mixture to 5.5 – 7.5. 0.17% of Protamex in proportion to meat-bone pulp mass was added. The mixture was maintained at a given temperature during the whole time of hydrolysis, which lasted 4 hours. After the process completion, the mixture was heated to 95°C and kept at this temperature for >15 minutes, in order to deactivate the enzyme. After the enzyme deactivation, the product was separated from fat and solid parts. The results have been compiled in Table 2.

The experiments with the use of Protamex and Flavourzyme were carried out in the same way as with Protamex only. 0.1% of Protamex in proportion to the mass of meat-bone pulp and 0.4% of Flavourzyme in proportion to meat-bone pulp mass were added to the mix-ture prepared in this way. The results of the experiments have been compiled in Table 3.

The experiments confirmed the possibilities of obtaining light, clear protein solutions. In the case when the Protamex enzyme was used, the best results were achieved with the meat-bone tissue to water ratio of 1:2,the temperature of 40°C, the time of the process 3 h, pH <6. However, in the case of the Protamex and Flavourzyme mixture, the best results were obtained with the meat - bone pulp to water ratio of 1:1, the temperature of 45°C, the time of the process 3 h, pH ~6,3.

The usefulness of the Protamex for obtaining the non-gelling protein hydrolysate was tested, too. The gelling hydrolysate applied was obtained with the chemical method at 120°C in 2 h. The material was stored at 10°C for ~20 h. The gelled hydrolysate was heated until it reached the liquid phase, and ~300 ml of it was taken for each of the experiments and heated to 50°C in a water bath for 2 h. 0.1 – 0.5% of the Protamex was added to four samples, and one of the samples was left with no additive. Then the samples were heated to 85°C and left for 15 minutes in order to deactivate the enzyme. The results are presented in Table 4.

It can be concluded that the smallest of the applied doses of the Protamex preparation causes complete hydrolysis to the non-gelling hydrolysates. However, each of the experiments with the addition of enzyme resulted in an unfavourable change in the solution colour with a simultaneous increase in the clarity of each of these solutions. None of them, however, was completely transparent. Hydrolysates produced with chemical and enzymatic method should be treated for the improvement of colour and odour. Precipitation of cations for the reduction of

Table 2. The results of the experiments with obtaining protein hydrolysate with the use of the Protamex preparation

No.	Ratio (bones: water)	Protein [wt %]	Temp. [°C]	Additive [wt %]	pH	Hydrolysate colour
1	1:1	6.96	55	0.17% Protamex	7.30	Cream - coloured, clear
2	1:1	8.62	40	0.17% Protamex, Lactic acid	5.88	Cream - coloured, clear
3	1:2	4.79	55	0.17% Protamex	7.50	Light cream - coloured, clear
4	1:2	5.04	55	0.17% Protamex, Lactic acid	6.29	Very light cream - coloured, clear
5	1:2	12.78	40	0.17% Protamex, Lactic acid	5.89	Cream - coloured, clear

Table 3. The results of obtaining protein hydrolysate with the use of Protamex and Flavourzyme preparations

No.	Ratio (bones: water)	Protein [wt %]	Time [h]	Temp. [°C]	Additive [wt %]	pH	Hydrolysate colour
1	1:1	8.91	3	55	0.1% Protamex, 0.4% Flvourzyme	7.79	Light cream - coloured, clear
2	1:1	7.20	3	55	0.1% Protamex,0.4% Flavourzyme, Lactic acid	5.50	Light cream - coloured, clear
3	1:1	7.91	3	55	0.1% Protamex, 0.4% Flavourzyme	7.46	Flesh - coloured, turbid
4	1:1	7.96	3	45	0.1% Protamex, 0.4% Flavourzyme, Lactic acid	6.04	Cream - coloured, clear
5	1:1	7.18	3	45	0.1% Protamex, 0.4% Flavourzyme, Lactic acid	5.92	Cream - coloured, clear
6	1:1	7.55	4	40	0.1% Protamex, 0.4% Flavourzyme Lactic acid	5.96	Cream - coloured, clear
7	1:2	4.30	3	55	0.1% Protamex, 0.4% Flavourzyme	7.80	Light cream - coloured, clear
8	1:2	4.61	3	55	0.1% Protamex, 0.4% Flavourzyme	7.45	Light cream - coloured, clear
9	1:2	4.68	2,5	55	0.1% Protamex, 0.4% Flavourzyme Lactic acid	6.50	Light cream - coloured, clear
10	1:2	3.93	2	55	0.1% Protamex, 0.4% Flavourzyme	7.60	Cream - coloured, clear

Table 4. Obtaining the non-gelling hydrolysates with enzymatic hydrolysis from the solutions of gelling hydrolysate using Protamex preparation

Sample	Process duration [h]	Temperature [°C]	Protamex [wt %]	Content of protein [wt %]	Texture [g]	Colour
1	4	50	0.0	7.50	29.82	Cream - coloured
2	1	50	0.1	-	0.00	Light orange
3	3	50	0.1	-	0.00	Light orange
4	4	50	0.1	7.94	0.00	Light orange
5	1	50	0.0	7.50	27.06	Light yellow
6	1	50	0.2	-	0.00	Light orange
7	1	50	0.5	8.10	0.00	Light orange

Table 5. The results of the industrial experiments of obtaining protein hydrolysate with the use of Protamex

No.	Ratio (bones: water)	Protein [wt %]	Time [h]	Temp. [°C]	Additive [wt %]	pH	Hydrolysate colour
1	1:1	6.79	4	55	0.17% Protamex	7.15	Cream - coloured, clear
2	1:1	8.72	4	40	0.17% Protamex, Lactic acid	5.91	Cream - coloured, clear
3	1:2	4.87	4	55	0.17% Protamex	7.43	Light cream - coloured, clear
4	1:2	5.13	4	55	0.17% Protamex, Lactic acid	6.27	Very light cream - coloured, clear
5	1:2	12.58	4	40	0.17% Protamex, Lactic acid	5.64	Cream - coloured, clear

the ash content separation of bitter substances by adsorption and fractionation of hydrolysates with membranes are used for these purposes[16]. Colourless hydrolysate could be obtained, too using hydrogen peroxide as an oxidising agent[11].

The industrial experiments on the production of protein hydrolysates in the enzymatic process were conducted in the same way as on the laboratory scale using a reactor equipped with a stir bar and the heating jacket. The results have been compiled in Tables 5 and 6.

Figure 6 presents the flow-sheet of obtaining protein hydrolysate with the enzymatic method.

CONCLUSION

By using as raw material the fresh pork meat-bone tissue after the dismantling process of half carcasses it is possible to produce protein hydrolysates and the beneficial parameters of the chemical process are: the time ~105 min., the temperature of 120°C, the pressure of 3.0 bar, the meat - bone tissue: the water ratio 1,200 – 1,250:700 – 750. The content of protein in the hydrolysate obtained the amounts to 7.98 – 10.64% on average. The hydrolysate is cream-coloured, demonstrates a homogeneous character, but is not clear.

With the use of enzymes, it is possible to obtain a non-gelling protein hydrolysate with a high degree of clarity and light cream colour. During the experiments with the use of the Protamex enzyme, the best results were achieved with the following parameters: the meat - bone feedstock to water ratio 1:2, the temperature of 40°C, the time of the process 3 h, pH <6. In the case when the Protamex and Flavourzyme mixture is applied, the best parameters are: the meat-bone pulp to water ratio 1:1, the temperature of

Table 6. The results of the experiments of obtaining protein hydrolysates with Protamex and Flavourzyme mix-ture

No.	Ratio (bones: water)	Protein [wt %]	Time [h]	Temp. [°C]	Additive [wt %]	pH	Hydrolysate colour
1	1:2	4.24	3	55	0.1% Protamex, 0.4% Flvourzyme	7.49	Very light cream - coloured, clear
2	1:2	4.61	3	55	0.1% Protamex, 0.4% Flvourzyme	7.45	Light cream - coloured, clear
3	1:2	4.69	2.5	55	0.1% Protamex, 0.4% Flvourzyme, Lactic acid	6.50	Light cream - coloured, clear
4	1:2	4.14	3	50	0.1% Protamex, 0.4% Flvourzyme, Lactic acid	6.66	Light cream - coloured, clear
5	1:1	8.92	3	55	0.1% Protamex, 0.4% Flvourzyme	7.80	Light cream - coloured, clear
6	1:1	7.92	3	55	0,1% Protamex, 0,4% Flvourzyme	7.46	Flesh - coloured, turbid
7	1:2	3.94	2	55	0.1% Protamex, 0.4% Flvourzyme	7.60	Cream - coloured, clear
8	1:1	7.21	3	55	0.1% Protamex, 0.4% Flvourzyme, Lactic acid,	5.50	Light cream - coloured, clear
9	1:1	8.07	3	45	0.1% Protamex, 0.4% Flvourzyme, Lactic acid	6.35	Cream - coloured, clear
10	1:1	8.62	4	40	0.17% Protamex, Lactic acid	5.88	Cream - coloured, clear

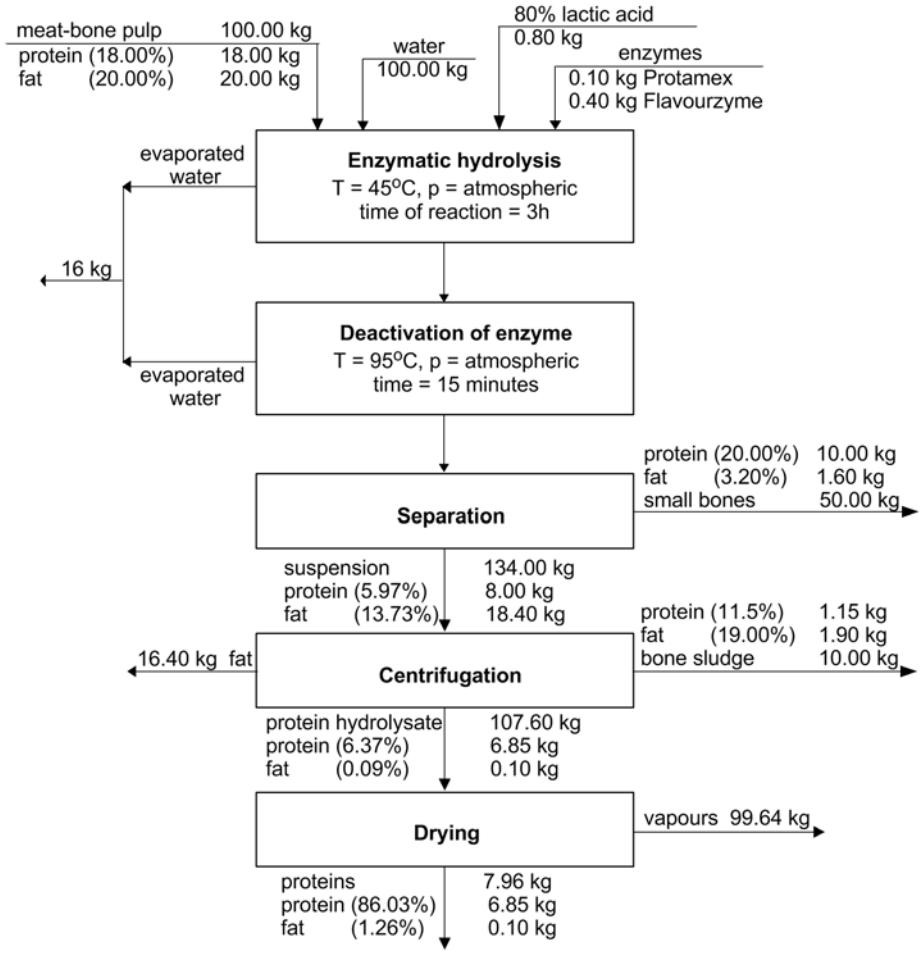

Figure 6. The flow-sheet of protein powder production with the use of Protamex and Flavourzyme enzymes; quantities of the raw materials, the semi-product and the product per 100 kg of the pork meat-bone pulp tissue used

45°C, the time of the process 3 hours, pH ~6.4. An increase in the amount of the added enzymes does not influence an increase in the protein content in the solution.

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