Mathematical modeling and computer simulation of optimal reaction time of the Lupine protein hydrolysis using fermented whey

S. Hubalovsky, J. Jelinek and J. Sedivy

***Abstract*—**The paper focuses to the process of modeling and computer simulation of the real experiment. Step by step there is shown system identification and creation of mathematical model of determination of optimum reaction time of whey acidic for the hydrolysis of proteins of lupine flour. Computer simulation model created in MS Excel spreadsheet and visualized in MS Excel *XYZ* surface chart is used to validate mathematical model.

***Keywords*—**Computer simulation model, chemical reaction model, lupine flour, mathematical model, time optimization.

I. Introduction

Whey is a valuable by-product of cheese production and the raw material for a wide range of applications. We explored the possibility of using the whey acidic for the hydrolysis of proteins. As a source of protein was used lupine flour. It contains over 30% protein. Lupine protein belongs among the most valuable proteins. Analyses of the nutrient content report clearly a very high content of valuable amino acids.

Mentioned values are determined in g / 1 kg in 100% of dry matter of grain lupine. Out of these can be named for example [1]:

- arginine - 36.33 to 41.04,
- leucine - 29.62,
- lysine - 19.25 to 21.33,
- isoleucine - 17.53 to 36.48,
- phenylalanine – 14.91 to 15.49,
- valine - 12.51 to 15.19, etc.

From these indicators it is obvious that the lupine contents of amino acids are very close to the animal proteins. Furthermore the use of lupine flour and products from Lupine protein and hydrolysates in the production of a wide range of so-called gluten-free products cannot be omitted- Lupine does not contain gluten.

II. Hydrolysis of proteins

For the hydrolysis of proteins are basically used three types of fission. Fission of strong acids, the most common of which is hydrochloric acid [2]. Another way is alkaline fission [3]. The big disadvantage of these acid or alkaline hydrolysis is the fact that the finished hydrolysates have a high content of inorganic salts formed after the hydrolytic reaction by neutralizing the acid or alkali.

These disadvantages are eliminated by enzymatic hydrolysis, which represents much milder conditions than acidic and alkaline hydrolysis. Enzymatic hydrolysis is usually carried out at 40 °C to 60 °C and *pH* from 6 to 8 [4].

To enzyme technology can be included the preparation of protein hydrolysates using reaction mixtures of whey fermentation product, when is the optimum *pH* of the reaction mixture controlled by yeast milk which is the subject of our report. The resulting lactic acid is neutralized by free amino groups of yeast biomass and the reaction is much faster than ordinary milk fermentation. In addition, there is a better use of the lactose content (milk sugar), which is part of the whey.

After the fermentation the reaction mixture contains 2% wt. of free lactic acid, which performs in the next step hydrolysis of yeast biomass at a temperature of 120 °C while it is released. The resulting hydrolyzate is concentrated and then dried in a spray drier and contains milk salt of the free amino groups of hydrolyzed protein from both yeast and proteins, contained in the original whey.

Currently there have been carried out the clinical tests of resulting products, such as supportive medication for cancer patients and results so far are very promising.

III. Mathematical modeling

Modeling is a method that is often used in professional and scientific practice in many fields of human activity.

The main goal of modeling is to describe the content, structure and behavior of the real system representing a part of reality.

Modeling is also becoming one of the academic programs of choice for students in all disciplines – see e.g. in [5], [6], [7], [8]. Modeling is a discipline with its own body of knowledge, theory, and research methodology. The ability to define a system, to build up a mathematical model develops logical thinking skills and imagination and is an inseparable part of a student's study skills.

The models are always only approaching the reality, because the real systems are usually more complex than the models are. The system homomorphism is applied in the process of modeling, which means that each element and interaction between the elements of the model corresponds to one element and interaction of the modeled real system, but the reverse is not true. The model is always to be understood as a simplification of the original.

The first step in the process of computer simulation is the creation of a mathematical model of the studied real system. The model can be obtained either theoretically, based on basic physical properties of the system, or numerically by means of the measured values. The determination of parameters of a theoretical model developed from empirical data is called system identification.

The mathematical model must adequately describe the dependency system outputs on its inputs. Models of physical systems are usually established as a system of mathematical equations as will be shown in the following paragraphs of this paper.

The mathematical models and chemism of lactic fermentation of whey and for yeast biomass hydrolysis with lactic acid is the scope of the following subsections.

A. Fermentation of whey

Lactic fermentation converts the milk sugar (lactose) into lactic acid. In the first step is lactose, a disaccharide, fission caused by the action of enzymes produced by lactic acid bacteria to a mixture of galactose and glucose. In the following second step, the two monosacharides convert to lactic acid. However, the resulting lactic acid is acting as an inhibitor, so the whole process stops after the lactic acids have reached the concentration of about 2% wt.

To utilize all the lactose, it is necessary to neutralize the produced lactic acid and yet keep its free concentration in the optimum *pH* and altogether to avoid wild, butter fermentation, which would damage the reaction mixture. In quantitative terms it is possible to describe the fermentation process in the mechanism shown on the figure 1.

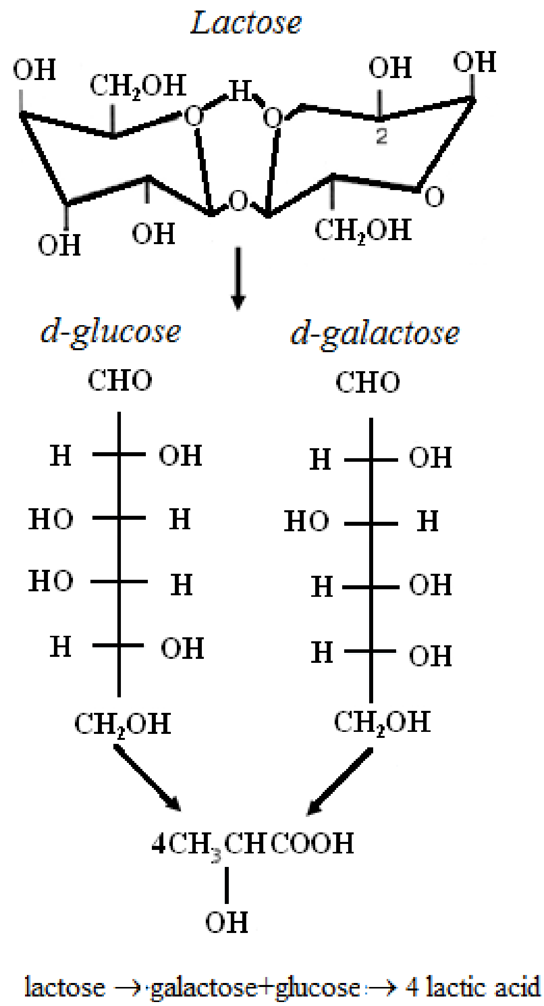

Fig. 1 Schematic description of the fermentation process

If lactic acid is neutralized, its inhibitory effect can be neglected and the whole process from the perspective of the slowest step describes milk production caused by salt first order mechanism. Control thus the slowest step, assuming formation of lactic acid:

$$\frac{dx}{d\tau} = k(1-x) \tag{1}$$

The solution of (1) is:

$$x = 1 - e^{-k\tau} \tag{2}$$

or

$$k\tau = \ln(1-x) \tag{3}$$

where x is the degree of lactose conversion to lactic acid.

By plotting the natural logarithm $\ln(1-x)$ versus τ (time) we obtain a straight line from which we calculate the value of rate constant k.

Chemism of lactic acid neutralization with yeast biomass is possible to represent in the scheme shown on figure 2:

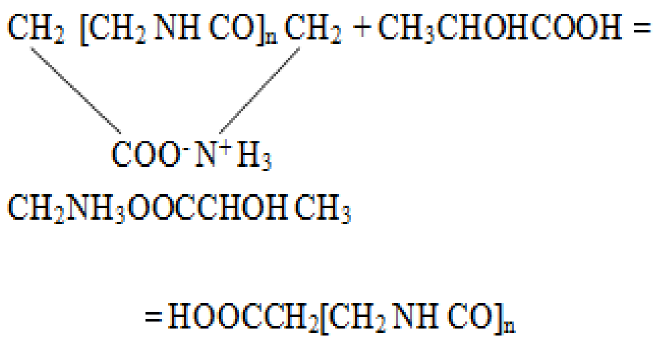

Fig. 2 Schematic description of the lactic acid neutralization with yeast biomass

B. Hydrolysis

Chemism of yeast biomass hydrolysis with lactic acid can again be expressed in following scheme:

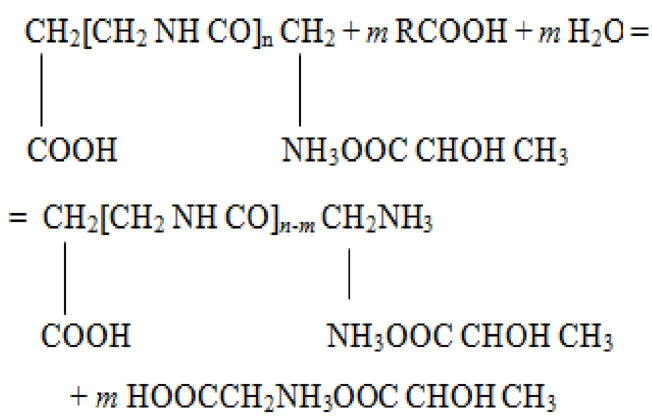

Fig. 3 Schematic description of the yeast biomass hydrolysis with lactic acid

Here the polymer yeast biomass as a polycondensate of glycine (Acetic acid) is schematically illustrated.

C. Determination of optimum reaction time of hydrolysis

The main part of the operating costs (N_S) for the preparation of hydrolysate consist of the cost of energy (N_E) required to drive the mixer of hydrolysis reactor and costs to achieve the desired concentration of hydrolysate (N_0):

$$N_S = N_E + N_0 \tag{4}$$

$$N_S = K_E \cdot P \cdot \tau + K_P \cdot (\Delta H)_{Evap} \cdot m_{H_2O} \tag{5}$$

According the (5) is the cost of electricity given by multiplication of power of an electric mixer P (kW), reaction time τ (h) and unit price of electrical energy K_E (CZK/kW.h).

Costs associated with the evaporation of water are given by the multiplication of evaporated heat ΔH_{Evap} (J/kg), the amount m_{H2O} of evaporated water (kg) and thermal energy unit price K_P (CZK/J).

Energy costs are rising with time, while the cost of the desired final concentration of the product decreases as the concentration increases in time. The result is that the main part of the operating costs depending on the reaction time shows a minimum. The purpose of optimization is to find a minimum i.e., the optimal reaction time at which the total energy costs and the required concentration are minimal.

To determine optimal reaction times we start again from the mechanism of hydrolysis of the first order:

$$\frac{dc_o}{d\tau} = \left(\frac{c_p}{1+N_a} - c_o\right) \cdot k = \left(c_{ro} - c_o\right) \cdot k \tag{6}$$

where N_a indicates the ratio of the volume of liquid lactic acid solution to the volume of dry yeast biomass, c_o is concentration of hydrolyzed yeast biomass of lactic acid, c_p is initial concentration of yeast biomass, k is time rate constant of whey fermentation of yeast biomass hydrolysis of lactic acid and $c_{ro} = c_p/(1+N_a)$.

By integrating formula (6) we obtain:

$$c_o = c_{ro} \cdot \left(1 - e^{-k\tau}\right) \tag{7}$$

hence results for the time:

$$\tau = -\frac{1}{k} \cdot \ln\left(1 - \frac{c_o}{c_{ro}}\right) \tag{8}$$

To determine the cost to the desired concentration of hydrolysate (N_0) it is necessary to know the equivalent weight of evaporated water which we find out by the balance of the evaporator – see figure 4.

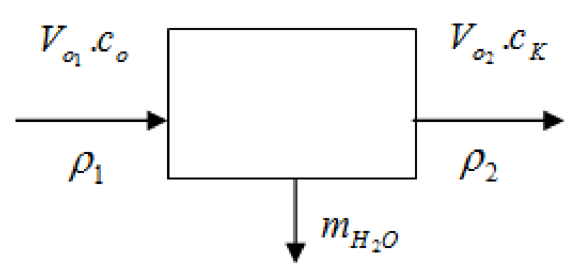

Fig. 4 Balance of the evaporator

Total balance is given by following equation:

$$V_{o_1} \cdot \rho_1 = m_{H_2O} + V_{o_2} \cdot \rho_2 \tag{9}$$

Balance of the hydrolysate is given by:

$$V_{o_1} \cdot c_o = V_{o_2} \cdot c_K \Rightarrow V_{o_2} = V_{o_1} \cdot \frac{c_o}{c_K} \tag{10}$$

where c_k is final concentration of yeast biomass.

Combining the formulas (9) and (10) we calculate the amount of evaporated water m_{H2O}. Assuming that the input density ρ_1 and output density ρ_2 of solutions equal (this assumption is justified, the density of hydrolysate ρ is approximately equal to density of water ρ) we get:

$$m_{H_2O} = V_{o_1} \cdot \left(1 - \frac{c_o}{c_K}\right) \cdot \rho \tag{11}$$

where $V_{o_1} = N_a \cdot V$.

When substituting relation (11) into equation (5) using the relation (8) we obtain the final link of operating costs depending on the concentration of hydrolysate.

$$N_S = -\frac{K_E \cdot P}{k} \cdot \ln\left(1 - \frac{c_o}{c_{r_o}}\right) + K_p \cdot (\Delta H)_{Evap} \cdot Na \cdot V \cdot \left(1 - \frac{c_o}{c_K}\right) \cdot \rho \tag{12}$$

We obtain optimum (minimum cost) when derivating formula (12) according to c_0 and by putting the result equal to zero, then it is implicated the optimal concentration at an optimal time:

$$c_{opt} = c_{r_o} - \frac{A}{B} \cdot c_K, \tag{13}$$

where

$$A = \frac{K_E P}{k}, \quad B = K_p (\Delta H)_{Evap} N_a \rho, \tag{14}$$

The optimal time can be calculated by substituting c_0 for c_{opt} in (8):

$$\tau_{opt}(c_p, c_k) = -\frac{1}{k} \cdot \ln\left(1 - \frac{\frac{c_p}{1+N_a} - \frac{K_E P\, c_K}{K_p (\Delta H)_{Evap} N_a \rho k}}{\frac{c_p}{1+N_a}}\right) \tag{15}$$

Equation (15) represents mathematical model of the optimal reaction time τ_{opt} as function of c_p and c_K of hydrolysis of lupine proteins using fermented whey.

The optimal reaction time for given initial value of c_p and c_K can be calculated either analytically either it can be simulated in computer simulation model.

In the following part of the paper there is described computer simulation model built up based on mathematical model (15). The computer simulation model allows visualization and validation of the mathematical model.

IV. Computer Simulation

The process of modeling is closely related to the simulation. Simulation can be understood as process of executing the model. Simulation enables representation of the modeled real system or real process and its behavior in real time by means of computer. The simulation enables also visualization and editing of the model.

A typical simulation model can be written both through specialized programming languages that were designed specifically for the requirements of simulations, or the simulation model can be created in standard programming languages and spreadsheets (MS Excel).

From the above considerations, it is clear that simulation is a process that runs on the computer. In some publications, therefore, can be found the term ”computer simulation“. It generally is valid that computer simulation is a computer-implemented method used for exploring, testing and analysis of properties of the conceptual (mathematical or process) models that describe the behavior of the real systems or real process which cannot be solved using standard analytical tools, se e.g. [8].

The simulation models represented by executable computer program have to be isomorphic with the conceptual model that is a representation. It means that the mathematical model and simulation model have to represent the real system, its elements, internal interactions and external interaction with the environment in the same way.

In our paper the real process is simulated in Visual Basic for Application and visualized in MS Excel spreadsheet.

A. Significant function of the simulation

Simulation has from the scientific point of view several functions – see e.g. [8].

We will focus in this paper two of them and they are:

- replacing the real process;
- development of educational process;
- multidisciplinary approach.

1) Replacement of the real process

This is an important and indispensable feature of simulations and simulation model because it allows realize a situation of the process that cannot be investigated conventionally. The main advantage of simulations is that simulations model allows providing rather big number of the process steps in relatively short time, changing of input parameters and its visualization and optimization of the process.

2) Development educational process

The simulation is very useful from educational point of view. Using the simulation model and visualization of simulation results on the screen, students can better understand the basic features of the processes and systems and develop their intuition. It is also essential that the teaching by means of simulation is much cheaper and faster than the teaching carried by real experiment. In some cases providing the real experiment cannot be feasible.

3) Simulation and multidisciplinary approach

Another important benefit associated with the modeling and simulation of real processes is a multidisciplinary approach, without which the identification of the real processes using conceptual and simulation model and cannot be realized. This is also emphasized in this paper.

Multidisciplinary approach generally means that specialized disciplines are applied in a study of real process. These disciplines provide partial analysis of the process. These mono-disciplinary analyses are integrated to overall solution by integrating the solver who has basic multi-disciplines knowledge.

In our case study the multidisciplinary approach is applied in creation of computer simulation for determination of optimal reaction time τ_{opt} as function of c_p and c_K of hydrolysis of lupine proteins using fermented whey as function.

V. Model verification and validation

Verification and validation are important aspects of the process modeling and simulation. They are essential prerequisites to the credible and reliable use of a model and its results [9].

A. Verification

In modeling and simulation, verification is typically defined as the process of determining if executable simulation model is consistent with its specification – e.g. conceptual model. Verification is also concerned with whether the model as designed will satisfy the requirements of the intended application. Verification is concerned with transformational accuracy, i.e., it takes into account simplifying assumptions executable simulation model. Typical questions to be answered during verification are:

- Does the program code of the executable simulation model correctly implement the mathematical model?
- Does the simulation model satisfy the intended uses of the model?
- Does the executable model produce results when it is needed and in the required format?

B. Validation

In modeling and simulation, validation is the process of determining the degree to which the model is an accurate representation of the real system / real process. Validation is concerned with representational accuracy, i.e., that of representing the real system / real process in the conceptual model and the results produced by the executable simulation model. The process of validation assesses the accuracy of the models. The accuracy needed should be considered with respect to its intended uses, and differing degrees of required accuracy may be reflected in the methods used for validation. Typical questions to be answered during validation are:

- Is the mathematical model a correct representation of the real system?
- How close are the results produced by the simulation executable model to the behavior of the real system?
- Under what range of inputs are the model's results credible and useful?

Validation and verification are both ultimately activities that compare one thing to another. Validation compares real system / real process and conceptual model. Verification compares conceptual model and executable simulation model. Sometimes validation and verification are done simultaneously in one process.

The whole process of transformation from a real system, the simulation model and its visualization is shown in Fig. 1.

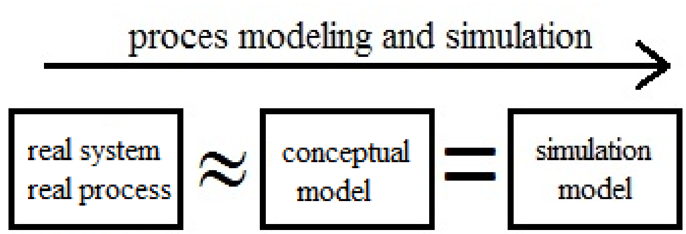

Fig. 5 Process modeling and simulation

Here again let us summarize that the mathematical model that reflects the real system / real process has some limitations and simplifying assumptions (the real system / process and conceptual model are in homomorphic relation).

In contrast, the simulation model is only the computer expression of the conceptual model (the conceptual model and simulation model are in isomorphic relationship).

VI. Computer simulation model

In this section the mathematic models expressed by equitation (15) of the optimal reaction time of hydrolysis of lupine proteins using fermented whey as function of initial and final concentration of yeast biomass $\tau_{opt}(c_p, c_k)$ will be transformed to the computer simulation model. The computer model is to be realized in MS Excel spreadsheet. Visualization is to be done by drawing the appropriate characteristic dependency in MS Excel *XYZ*-surface chart.

The basic time unit of the simulation models is taken as 1 hour. The output value τ_{opt} of the simulation model are calculated from input values c_p and c_k based on numerical calculations directly in MS Excel cells in the range B5:K14. The input vales c_p are entered in the row 4 and the inputs values c_k are entered in the column A.

Realization of the computer simulation model is shown on the figure 6 and visualization of the mathematical model is shown on the figure 7.

	A	B	C	D	E	F	G	H	I	J	K	L
1	N_a	k	K_E	K_p	ΔH	ρ	P					
2	0,7	2,5	4	4	454000	1000	2					
3												
4	τ [hod]	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9	1,0	c_p
5	0,1	7,71	7,99	8,15	8,26	8,35	8,42	8,49	8,54	8,59	8,63	
6	0,2	7,43	7,71	7,87	7,99	8,07	8,15	8,21	8,26	8,31	8,35	
7	0,3	7,27	7,55	7,71	7,82	7,91	7,99	8,05	8,10	8,15	8,19	
8	0,4	7,15	7,43	7,59	7,71	7,80	7,87	7,93	7,99	8,03	8,07	
9	0,5	7,06	7,34	7,50	7,62	7,71	7,78	7,84	7,90	7,94	7,99	
10	0,6	6,99	7,27	7,43	7,55	7,63	7,71	7,77	7,82	7,87	7,91	
11	0,7	6,93	7,21	7,37	7,48	7,57	7,65	7,71	7,76	7,81	7,85	
12	0,8	6,88	7,15	7,32	7,43	7,52	7,59	7,65	7,71	7,75	7,80	
13	0,9	6,83	7,11	7,27	7,38	7,47	7,55	7,61	7,66	7,71	7,75	
14	1,0	6,79	7,06	7,23	7,34	7,43	7,50	7,57	7,62	7,67	7,71	
15	c_k											

Fig. 6 Simulation model

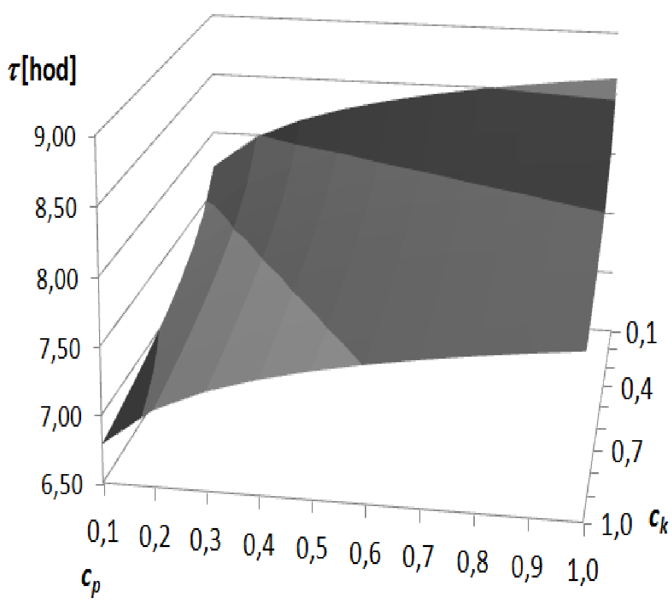

Fig. 7 Visualization of the mathematical model

VII. Experimental Methodologies

Experiments involved both analytical methods to characterize the input ingredients - whey and suspension of Lupine flour and also a description of the kinetics of whey fermentation when the *pH* was guided by addition of lupine flour suspension and also particular hydrolysis of Lupine flour with lactic acid as a whey fermentation product.

A. Determination of total nitrogen TKN

The principle of determining consists in mineralization of organic material with concentrated sulfuric acid at boiling point when the organically bound nitrogen is converted to ammonium sulphate, which is then displaced by caustic soda and released ammonia sorbs boric acid, the excess of which is based on the acid-base titration.

B. Determination of whey fermentation kinetics

Time dependence of the production of lactic acid was monitored by potentiometric titration with 0.1 *N* sodium hydroxide. In the case when the resulting lactic acid was partially neutralized by the yeast milk titration curve has two points of equivalence, when the first item at a lower *pH* corresponds to free lactic acid, and the second, at higher *pH* corresponds to acid bound to the yeast biomass.

C. Experimental material

As starting (raw) materials were used sweet whey and aqueous suspension of Lupine flour (Lupine's milk) in the following composition:

Whey

- Free moisture base: 7.0%
- Lactose: 75.0%
- Nitrogen (TKN): 1.6%
- Ash: 5.0%

Percentages are related to the free moisture base.

Lupine milk

- Free moisture base: 15.0%
- Nitrogen (TKN): 30.0%
- Fat (petroleum ether extract): 6.0%
- Ash: 4.7%

The content of free amino groups 1 mg/g

Percentages are related to the free moisture base.

D. Validation of the mathematical model

Validation of the mathematical model is done by comparison of the dependency $\tau_{opt}(c_p)$ with the experimental measured dependency at $c_k = 0.5$ and $c_k = 0.8$.

The comparison is shown on the figure 8.

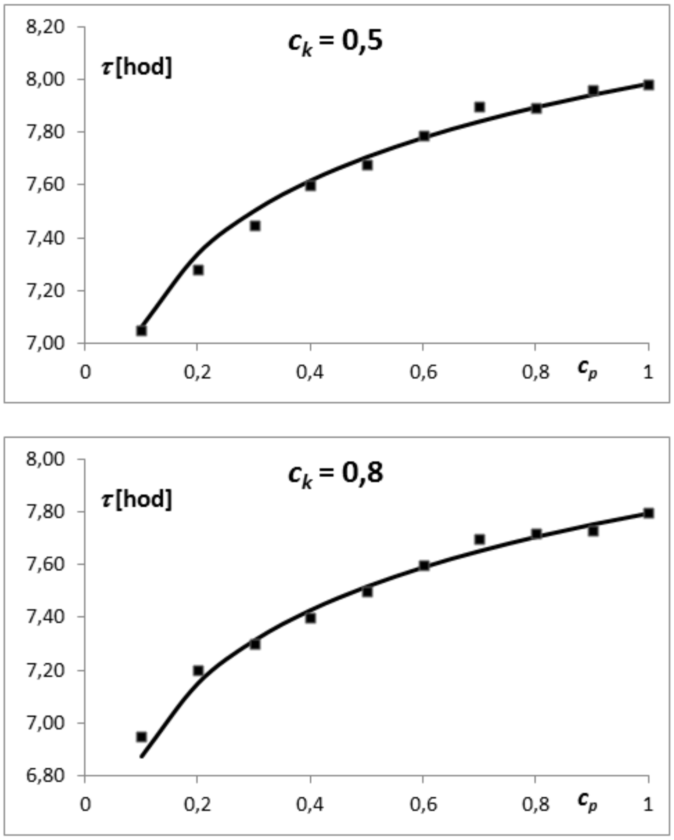

Fig. 8 Validation of the mathematical model

VIII. Other results of the experiments

Experimentally, we monitored the production of lactic acid both with fermentation of pure whey i.e. without additives and with the use of lupine flour suspension. The fermentation process was carried out in both examples, at the average temperature of 20°C, rate constant of lactic acid production was evaluated by linear regression and its value is 6.25×10^{-3} h^{-1}. In the same experiment, under identical conditions, we monitored the time dependence of the total concentration of lactic acid in the presence of lupine flour suspension. The results of this experiment are shown in figures 9 and 10.

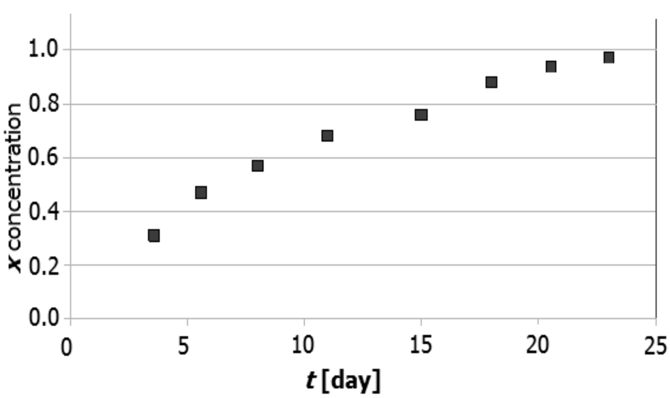

Fig. 9 Fermentation without lupine flour suspension

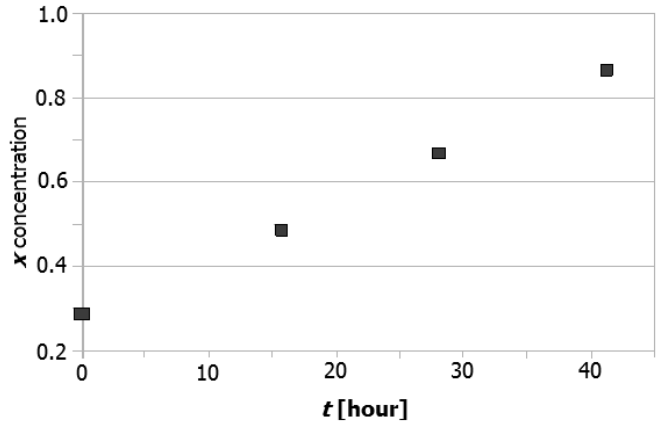

Fig. 10 Fermentation with lupine flour suspension

Assessed rate constant of lactic acid has a value $6.0\times10^{-2}h^{-1}$ and is nearly 10 times greater than in the case of lactic acid without the presence of lupine flour suspension.

In order to prepare large quantities of lupine hydrolysate using lactic acid produced by fermentation of whey in the presence of lupine flour suspension the fermentation was initially performed in a pilot pressure reactor of a total volume of one cubic meter. After 24 hours when we assumed that 73% of lactose converted to acid lactic the content of the reactor were heated to 120°C for time of 1 hour. The hot mixture was filtered after decompression and it was discovered by balance of dry, that there was a 90% suspension liquefaction of lupine flour.

IX. Conclusion

Use of lactic acid whey products, mainly lactic acid and protein lactate seems very promising for the preparation of food supplements, functional foods and supportive drugs, mostly for cancer patients. Lupine protein hydrolysates derived from whey fermentation products, increase their nutritional value, combining highly valuable ingredients contained in both input materials.

Acknowledgements

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Stepan Hubalovsky was born in 1970 in Czech Republic. He obtained master degree in education of mathematics, physics and computer science in 1995 and doctor degree in theory of education in physics in 1998 both in Faculty of Mathematics and Physics, Charles university in Prague, Czech Republic. He works as assistant professor on University of Hradec Kralove His scientific activities are system approach, modeling and simulation.

Jiri Jelinek was born in 1977 in Czech Republic. He obtained doctor degree science engineering in 2007 both in Faculty of Mechatronics, Informatics and Interdisciplinary Studies, Technical University of Liberec, Czech Republic. He works as assistant professor (senior lecturer) on University of Hradec Kralove. His scientific activities are Measurement Technique, Measurement Electric and Non-Electric quantities, Measurement Physical-chemical Quantities, Measuring Software.

Josef Sedivy was born in 1963 in Czech Republic. He obtained master degree in education of physics and technical teaching science in 1987 and computer science in 2002 on University of Hradec Kralove and doctor degree in theory of technical education in 2008 in University of Hradec Kralove, Faculty of Education, Czech Republic. He works as assistant professor on University of Hradec Kralove. His scientific activities are statistics tools for modeling and simulation.