Lupine protein hydrolysis using fermented whey

STEPAN HUBALOVSKY, JIRI JELINEK, MILOS JELINEK, KAREL KOLOMAZNIK
Faculty of Science
University of Hradec Kralove
Rokitanskeho 62, Hradec Kralove
CZECH REPUBLIC
stepan.hubalovsky@uhk.cz, jiri.jelinek@centrum.cz,
milos.jelinek@centrum.cz, kolomaznik@fai.utb.cz, http://www.uhk.cz

Abstract: - One of the most important methods in current scientific and technological research is the process of modeling of real experiments as well as the modeling of real systems. As a system approach, modeling and simulation is a discipline with its own theory and research methodology. This paper focuses to the process of modeling of real experiment and real system. This paper will, step by step, show the process of system identification and creation of mathematical model of determination of optimum reaction time of whey acidic for the hydrolysis of proteins of lupine flour.

Key-Words: - Mathematical model, Chemical reaction model, Hydrolysis, Fermentation, Time optimization, Reaction time, Lupine flour.

1 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.

2 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 hydrolysed 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.
3 Mathematical model

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.

3.1 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 monosaccharides 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.

![Figure 1: Schematic description of the fermentation process](image)

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 $\tau$ (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:

$$\begin{align*}
\text{CH}_3\text{[CH}_3\text{NH CO]}_n\text{CH}_3 + \text{CH}_3\text{CHOHCOOH} &= \text{HOOCCH}_2\text{[CH}_3\text{NH CO]}_n \\
\text{CH}_2\text{NH}_2\text{OOC CHOH CH}_3
\end{align*}$$

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

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

$$\begin{align*}
\text{CH}_2\text{[CH}_3\text{NH CO]}_n\text{CH}_3 + m\text{RCOOH} + m\text{H}_2\text{O} &= \text{COOH} \quad \text{NH}_2\text{OOC CHOH CH}_3 \\
+ m\text{HOOCCH}_2\text{NH}_3\text{OOC CHOH CH}_3
\end{align*}$$

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.

3.3 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 ($N_0$):

$$N_S = N_E + N_0$$  \hspace{1cm} (4)

$$\begin{align*}
N_S &= K_E \cdot P \cdot \tau + K_P \cdot (\Delta H)_{\text{Evap}} \cdot m_{\text{H}_2\text{O}} \\
\text{According the (5) is the cost of electricity given by multiplication of power of an electric mixer } P \text{ (kW), reaction time } \tau \text{ (h) and unit price of electrical energy } K_E \text{ (CZK/kW.h).}
\end{align*}$$

Costs associated with the evaporation of water are given by the multiplication of evaporated heat $\Delta H_{\text{Evap}}$ (kJ), the amount $m_{\text{H}_2\text{O}}$ of evaporated water (kg) and thermal energy unit price (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:

$$\frac{dc_o}{d\tau} = \left\{ \frac{c_p}{1 + N_o} - c_o \right\} \cdot k \Rightarrow (c_o - c_r) \cdot k$$  \hspace{1cm} (6)

where $N_o$ indicates the ratio of the volume of liquid lactic acid solution to the volume of dry yeast biomass.

By integrating formula (6) we obtain:

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

hence results for the time:

$$\tau = \frac{1}{K} \cdot \ln \left( \frac{c_o}{c_{ro}} \right)$$  \hspace{1cm} (8)
**Total balance is given by following equation:**

\[ V_{o_1} \cdot \rho_1 = m_{H_2O} + V_{o_2} \cdot \rho_2 \]  

(9)

**Balance of the hydrolysate is given by:**

\[ V_{o_1} \cdot c_o = V_{o_2} \cdot c_k \Rightarrow V_{o_2} = V_{o_1} \cdot \frac{c_o}{c_k} \]  

(10)

Combining the formulas (9) and (10) we calculate the amount of evaporated water \( m_{H_2O} \).

Assuming that the input density \( \rho_1 \) and output density \( \rho_2 \) of solutions equal (this assumption is justified, the density of hydrolysate \( \rho \) is approximately equal to density of water) we get:

\[ m_{H_2O} = V_{o_1} \cdot \left(1 - \frac{c_o}{c_k}\right) \cdot \rho \]  

(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.

\[ N_s = -\frac{K_E \cdot P}{k} \cdot \ln \left(1 - \frac{c_o}{c_k}\right) + K_p \cdot (\Delta H)_{Evap} \cdot N_a \cdot V \cdot \left(1 - \frac{c_o}{c_k}\right) \cdot \rho \]  

(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:

\[ c_{opt} = c_o - \frac{A}{B} \cdot c_k \]  

(13)

where

**4 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.

**4.1 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.

**4.2 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.

**4.3 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.

5 Results
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 $6.25 \times 10^{-3}$ 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 5 and 6.

Fig.5 Fermentation without lupine flour suspension

Assessed rate constant of lactic acid has a value $6.0 \times 10^{-2}$ 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.

6 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
This research was supported by the research project of Technology Agency of the Czech Republic (TACR), No. TA01010737.

References: