12th International Research/Expert Conference
"Trends in the Development of Machinery and Associated Technology"
TMT 2008, Istanbul, Turkey, 26-30 August, 2008

MODELING AND KINETICS OF PROTEIN ENZYMATIC HYDROLYSIS FROM AMARANTH FLOUR AND WHOLE GRAIN

Klára Kodriková, Karel Kolomazník **Milan Adámek**
Dep. of Automation and Control **Dep. of Electronics and Measurment**
Faculty of Applied Informatics
Tomas Bata University in Zlín
Czech Republic

ABSTRACT
The topic of the article is oriented at modeling of amaranth protein enzymatic hydrolysis. The purpose of the hydrolysis is to enrich the solid phase with very valuable amaranth protein which could be used not only in food industry to produce new products rich in high-quality protein, but also in pharmaceutics, medicine and other branches. Regarding to diverse character of chosen materials (amaranth flour and seed) various hydrolysis course is expected, therefore a different model is built for each of the stuff. The designed mathematical models are verified by experimental measurement.
Keywords: Modeling, amaranth, enzymatic extraction

1. INTRODUCTION
Amaranth, a plant falling into the group of pseudo-cereals, starts to be a goal of the research for its unique specific properties presently. Study of this crop leads to ascertainment of remarkable content of quality protein with high portion of essential amino-acids and other considerable substances, like squalene and flavonoids. Amaranth produces many thousands of tiny seeds having very valuable contents; on the other hand the leaves have also high nutritional value. High-quality vegetable oil is extracted from the amaranth seed, which is used not only in food industry. [1]
Most of the so far published publications deal with amaranth as a food supplement for healthy population or as a possible compound in special diets of diabetic patients or people with protein allergy; new amaranth products are conducive against civilization diseases. On the other hand, amaranth starts to be used also in different areas -medicine, pharmaceutics and cosmetics.
Further research is in progress, for example in amaranth biomass treatment. The rest of the plant after conversion of basic parts contains still high percent of protein, depending on growing season. Therefore amaranth biomass could be successfully used in foodstuff industry or for production of very pure biogas.
Hardly ever are published papers which solve production of separate valuable amaranth compounds and optimalization of particular technologies from the process engineering point of view. It is surprising, that most of technologies are not described quantitatively in terms of the exploitation of the transport process theory and the heterogenic reactions kinetics. This is important especially in the processing of high amount of raw material that means for industry requirements.
The success of amaranth products application for normal utilization direct depends on possibility of particular parts separation. The aim is to obtain products of high purity, abreast with process conditions which do not raise the price of. However, papers dealing with separation are published only rarely as well as optimalization of single technology process using advantage of process engineering is seldom solved.
It is known, that the separation of particular compounds is possible by hydrolysis. The purpose of our present experiments is to enrich the solid phase with the vegetable protein and opposite – enrich the solid phase with valuable starch. The kinetics of these reactions and modeling of concentration fields are presented in our paper.

2. MATHEMATICAL MODELS OF PROTEIN ENZYMATIC EXTRACTION

2.1. The modeling of protein enzymatic extraction from the amaranth flour

As mentioned above, the purpose of the hydrolysis is to enrich the solid phase with required compound – protein or starch. With regard to very small size of amaranth flour particles, is eventual that hydrolysis reaction is not inhibited by diffusion.

On this condition we can apply the first order mechanism in the relationship of non-reacted part of protein for the description of hydrolysis kinetics. On the assumption that the conversion (y) direct depends on irreducible starch (protein) fraction, it is possible to write following equation**:**

$$\frac{dy}{d\tau} = k(1-y) \tag{1}$$

By integration (1) we obtain

$$y = 1 - B^{-k\tau} \tag{2}$$

B is equal 1 on starting condition y(0)=0. For the determination of speed constant *k* we use (3)

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

By setting out the natural logarithm of non-reacted portion –ln(1-y) to the time (τ) we acquire a straight line whose outline equals the speed constant of hydrolyzed starch (protein).

2.2. The modeling of protein enzymatic extraction from the amaranth grain

During the enzymatic hydrolysis of amaranth flour the final heterogeneous mixture contains an aqueous solution of hydrolysed protein and a starch mud containing fixed protein. The separation of the heterogeneous reaction compound is possible only by using a centrifuge, because simple filtration proceeds very slowly.

By this reason we were engaged in hydrolysis experiments of the amaranth grain on condition of its permanent preservation. This technique enables a relative easy separation of the heterogeneous mixture by the pure filtration after finishing the reaction. For the description of the diffusion protein extraction from the amaranth seed we used following model:

$$\frac{1}{D}\frac{\partial c(r,\tau)}{\partial \tau} = \frac{\partial^2 c(r,\tau)}{\partial r^2} + \frac{2}{r}\frac{\partial c(r,\tau)}{\partial r} \qquad 0 < r < a, \quad \tau < 0 \tag{4}$$

$$\frac{\partial c}{\partial r}(0,\tau) = 0 \tag{5}$$

$$-SD\frac{\partial c}{\partial r}(a,\tau) = V_0\frac{\partial c_0}{\partial \tau}(\tau) \tag{6}$$

$$c(r,0) = c_p \tag{7}$$

$$c(a,\tau) = \varepsilon c_0(\tau) \tag{8}$$

$$c_0(0) = 0 \tag{9}$$

The equation (4) describes the concentration field inside the amaranth grain during the extraction process, (5) presents the axis symmetry of the concentration field, (6) responds the solid-state balance equality of the surface diffusion flow with the accumulation speed of the hydrolyzed protein in the aqueous solution. (7) is a complete mixing condition of the aqueous phase and (8), (9) are starting conditions.

Through the application of dimensionless quantities we get:

$$C = \frac{c}{c_p}, \quad R = \frac{r}{a}, \quad Fo = \frac{D\tau}{a^2}, \quad C = \frac{c_0}{c_p} \tag{10}$$

Finally, we obtain dimensionless model of the diffusion extraction:

$$\frac{\partial C(R,Fo)}{\partial Fo} = \frac{\partial^2 C(R,Fo)}{\partial R^2} + \frac{2\partial C(R,Fo)}{R\partial R} \qquad 0 < \mathrm{R} < 1, \ \mathrm{Fo} > 0 \tag{11}$$

$$\frac{\partial C(0,Fo)}{\partial R} = 0 \tag{12}$$

$$-\frac{\partial C(1,Fo)}{\partial R} = \frac{Na}{3\varepsilon}\frac{\partial C_0(Fo)}{\partial Fo} \tag{13}$$

$$C(R,0) = 1 \tag{14}$$

$$C(1,Fo) = C_0(Fo) \tag{15}$$

$$C_0(0) = 0 \tag{16}$$

The analytical solution of the equations (10) to (12) was published for the temperature field of spherical body [2]. By the modification of the published solution for concentration field in the amaranth grain we acquire:

$$C = \frac{c}{c_p} = \frac{\varepsilon}{Na + \varepsilon} - \frac{2Na}{3\varepsilon R} \sum_{n=1}^{\infty} \frac{\left[\frac{Na^2}{\varepsilon^2} q_n^4 + 3\left(2\frac{Na}{\varepsilon} + 3 \right) q_n^2 + 9 \right] \sin(Rq_n) \sin(q_n)}{\frac{Na^2}{\varepsilon^2} q_n^4 + 9\left(\frac{Na}{\varepsilon} + 1 \right) q_n^2} e^{-Foq_n^2} \tag{17}$$

$$C_0 = \frac{c_0}{c_p} = \frac{1}{Na + \varepsilon} - \frac{6Na}{\varepsilon} \sum_{n=1}^{\infty} \frac{e^{-Foq_n^2}}{\frac{Na^2}{\varepsilon^2} q_n^2 + 9\left(\frac{Na}{\varepsilon} + 1 \right)}$$

$$tg(q) = \frac{3q}{3 + \frac{Na}{\varepsilon} q^2} \tag{19}$$

$$Fo = \frac{D\tau}{a^2} \tag{18}$$

where qn are the equation roots.

The protein concentration fields in the seed of spherical shape are shown in the following pictures Fig.1 to Fig. 2. Axis x represents the radius of a seed (R), axis y shows the concentration (c) of the enzyme solvent and finally, axis z presents non-dimensional time Fo. The differences between varied dimensionless consumption of the enzyme solution are visualized in interactive modeling environment Matlab.

In the first picture, you can see the concentration field inside the amaranth grain by the elected dimensionless solvent consumption 1. In the second case in figure 2 is well seen, how the concentration field changes with high-introduced consumption of enzyme – Na equals to 10.

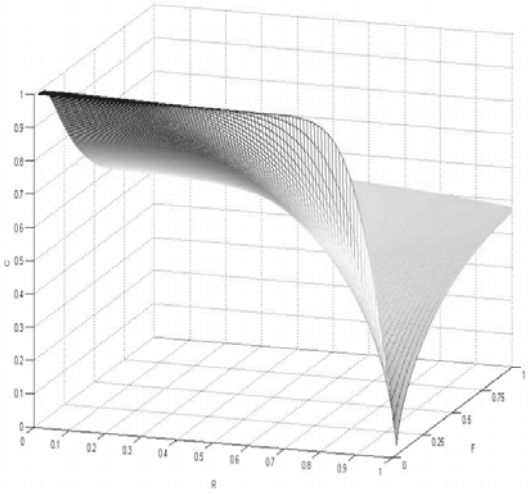

Figure 1. Concentration field for the dimensionless dimensionless solvent consumption Na = 1

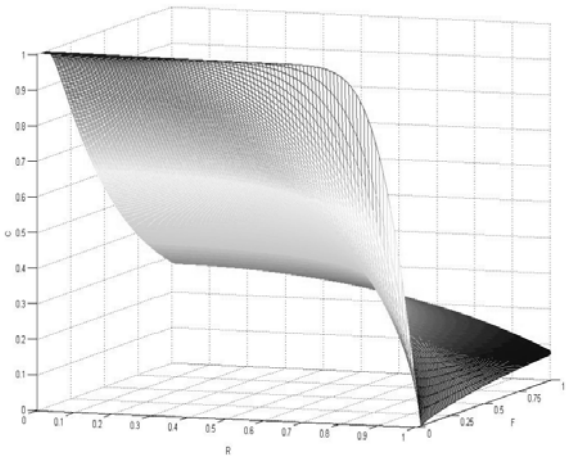

Figure 2. Concentration field for the dimensionless solvent consumption Na = 10

3. EXPERIMENTAL SET-UP

3.1. Protein enzymatic hydrolysis of the amaranth technical flour

The experiments were focused on the hydrolyze effectivity of amaranth flour protein with usage of several readily available enzymes in the market. Protein liquefaction of the technical flour runs under slight reaction conditions (temperature, neutral pH) which were proposed:

- rate flour/water = 1/20
- speed of reaction mixture warming 2 °C. min^{-1}
- enzyme doze: 0,1% to the total flour solids – in laboratory conditions 5 mg of enzyme.5 g^{-1} of the total flour residue

Tab.1 Composition of the amaranth technical flour

Parameter	Value (%)
Total residue	***86,91***
Ash in the total solids	***3,57***
Nitrogen (TKN)	***2,82***
Proteins (N x 5,70) in total solids	***16,07***
Lipid in total residue	***9,81***
Starch in total solids	***67,79***

The experiments proceed in variable hydrolyze times (minimum 1 hour, maximum 5 hours) and variable temperatures (30°C to 50°C). Into the boiling flask was 5 g of the flour weighted out, after that 100ml of distilled water was added and the mixture was stirred in the water bath and simultaneously warmed up. After achieving of the specified temperature 500µl of enzyme solution was tacked with a pipette. Enzyme hydrolysis was running for required time and by rated temperatures. After it the reaction alloy was centrifuged with 6000 rpm. Liquid and solid phase were analyzed for the total residue content and finally screened using TKN method. Examples of the experiments results are presented in the table 1. and showed in the graphs above.

Out of the experiments results it is evident, that the highest efficiency by the enzyme liquefaction of the amaranth technical flour enzyme Alcalase proved. By the five hours hydrolysis and 50°C got almost 37% of protein liquid.

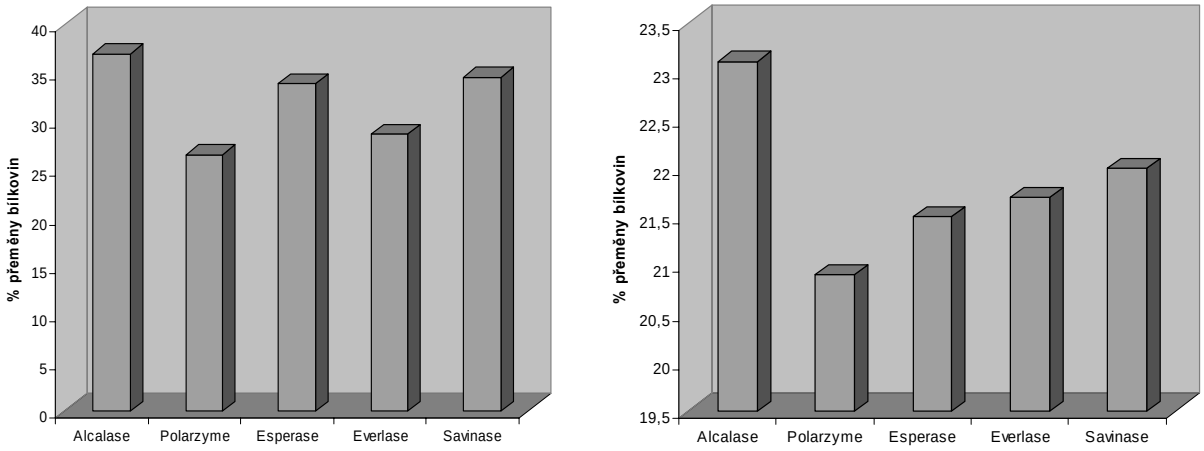

Fig. 4, 5: Percentage of protein conversion using several enzymes by the 1h hydrolysis and 30°C temperature, resp. 5 h hydrolysis and 50°C temperature

4. CONCLUSION

In this paper, the modeling of amaranth raw material (flour and seed) is solved using Matlab computer environment. Amaranth flour particles have very small size so that the enzyme hydrolysis is not broken by diffusion; hence the mathematical model is simpler. On the other hand, amaranth seed has a diffusion bar presenting by its skin. The grains mathematical model was built in spherical coordinates. The models were verified by experimental measurements, which acknowledged the advantages of the whole grain hydrolysis. Fulfilling the condition of seed permanent preservation after protein extraction we considerably simplify the process of mixture separation which leads to an economical profit when industry used.

5. ACKNOWLEDGEMENTS

The work has been supported by the grant VZ MSM 7088352102 the Ministry of Education of the Czech Republic. This support is very gratefully.

6. REFERENCES

[1] PAREDES-LOPEZ, O., Amaranth – biology, chemistry and technology, Library of Congress Cataloging in Publication Data, 1994,
[2] CARLSLAW, H.S. and JAEGER, J.C., Conduction of heat in solids, Oxford at the Clarendon Press, 1959.