Mathematical models of enzymatic hydrolysis of amaranth grain, flour and stem

H. Vaskova, K. Kolomaznik

Abstract**—**Amaranth an ancient crop plant rediscovered in food industry in recent years is characteristic for unique composition, exceptionally high content of quality protein. The content of substances essential for nutrition are even several time higher in comparison with other cereals. The current food processing technologies enable intentional separation of some substances or to increase their concentration to reach products with higher targeted effects. Authors of this paper present mathematical models of the enzymatic hydrolysis of amaranth grain, stem and flour. Enzymatic hydrolysis allows separation of quality amaranth protein as well as starch. Mathematical models are based on the transport phenomena in heterogeneous reaction system in the case of grain and stem and on heterogeneous reaction kinetics in the case of amaranth flour. The purpose is to nutritionally enrich amaranth material of valuable component included in crop and to obtain protein hydrolyzates for further applications in food industry, pharmaceutics or cosmetics.

Keywords**—**amaranth, diffusion, enzymatic hydrolysis, mathematical model.

I. Introduction

AMARANTH is a non-traditional pseudo-cereal ancient plant attracting interest in recent years. This interest arises primarily from an extraordinary composition and levels of nutritive substances, in particular in grains and also in leaves and stem of amaranth, which when compared to other cereals are several times greater [1]. Its use in food can solve many health problems, but mainly nutritionally enriched foods for missing substances. In terms of human nutrition it is especially important to have proteins in the diet [2]. One of the most promising dietary supplements of the 3rd Millennium is plant protein. Next to soybeans and other leguminous vegetables amaranth is a very promising source of high-quality vegetable protein. Studies of amaranth grain prove the composition definitely meets the requirements of a healthy diet [3, 4, 5]. In addition to its unique composition and thus the nutritional value a high resistance to extreme weather conditions, especially heat and dry and low demands on the soil are other advantages of amaranth. Amaranth finds for its unique composition application not only in food industry, but to a large extent also in cosmetics, pharmaceutics and medicine.

Contemporary food processing technologies allow intentional separation of some substances or to increase their concentration and thus to obtain products that have higher targeted effects. Therefore, we were looking for ways to increase the share of protein in amaranth raw material, although this material already contains a considerable amount of protein. Amaranth raw material can be enriched by proteinaceous component by converting amaranth starch into a liquid form by the act of specific enzymes. On the other hand protein liquefaction can be performed due to the proteolytic enzymes leading to the enrichment of the solid phase by amaranth starch. Hydrolysis is a reaction causing the cleavage of chemical bonds by the addition of water. It is a chemical tool for the separation of particular compounds. In this case it means the separation of amaranth protein or the starch from the remaining solid phase. Process of hydrolysis is beneficial in many applications [6, 7, 8].

As a feedstock for the enrichment by one of the mentioned substances we used both possible forms of amaranth raw material the flour and also grain. Both materials have their own characteristics, advantages and disadvantages in terms of mathematical modelling of the process of extraction, experimental and industrial implementation. The stem of the amaranth plant was also considered for utilization.

From the engineering point of view the optimization of specific technologies is important particularly in the processing of large amount of raw material i.e. for the industrial processing. However, papers dealing with the separated valuable amaranth compounds quantitatively which use transport process theory and optimization are rarely published.

II. Highlights

The aim of the research is to obtain high-purity and high-quality products of amaranth material that can find application not only in food industry but also in pharmaceutics, cosmetics or medicine. For the enrichment of the solid amaranth is important to be successful in the process of particular substances separation. This process should not significantly affect the cost. Mathematical model based on utilization of the transport process theory and the heterogenic reaction kinetics or the enzymatic hydrolysis of amaranth raw material is presented both for the flour and the grain.

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III. Amaranth

Ancient crop plant Amaranth rediscovered in food industry in recent years is characteristic for unique composition, exceptionally high content of protein and a very good assimilation property.

It was cultivated and grown as a staple food of ancient civilizations high mountainous areas of South America. The earliest mentions of the cultivation of amaranth are almost 6000 years old. This crop plant was venerated and sacred food until the arrival of European conquerors banned its cultivation [9].

In comparison with other common cereals as is demonstrated in Table 1, amaranth has higher proportion of essential amino acid including lysine, which is important for mental development, a significant proportion of high-quality vegetable oils containing high amount of unsaturated fatty acids and squalene. Squalene strengthens the immune system and has antioxidant properties. Squalene is a main component of shark liver oil or corn germs. Shark oil is the one of the best available products containing squalene but only 1-1.5% [12, 13]. Amaranth is another significant source of this very valuable substance. Content of squalene in some oils is stated in Table 2. High amount of dietary fiber and starch in amaranth provides a balanced diet in terms of mineral substances as magnesium, calcium, iron and vitamins B, C, E. The content of essential nutritive qualities in amaranth grain and leaves is shown in Table 3.

Table 1 Comparison of nutritive qualities in amaranth and other cereals [10]

	Amaranth	Wheat	Rice	Oat
Proteins (g /100g)	16	13,3	7,6	14,2
From that lysine (g /100g)	0,89	0,32	0,31	0,43
Fat (g /100g)	7,5	2	0,3	7,4
Carbohydrates (g /100g)	62	71	79,4	68,2
Dietary fiber (g/100g)	4,2	2,3	0,2	1,2
Iron (mg /100g)	15	3,4	0,8	4,5
Calcium (mg /100g)	250	47,4	24	53
Magnesium (mg /100g)	310	110	120	120

Table 2 Content of squalene in some oils [11]

	Content of squalene (%)
Amaranth oil	7,0
Olive oil	0,4
Rice oil	0,3
Corn oil	0,0
Arachis oil	0,0
Sunflower oil	0,0

Table 3 Chemical composition of amaranth [14]

	Grain (g/100g of dry matter)	Leaves (g/100g of dry matter)
Proteins	15,2	24,1
Lipids	7,0	3,8
Dietary fiber	6,2	14,9
Carbohydrates	62,1	42,9

Amaranth does not contain gluten, a protein that causes intestinal damage in celiac disease. Due to the good solubility, digestibility and small size of the starch grains amaranth starch is especially suitable for people suffering from digestive disorders. The nutritional benefits of pseudo cereals including amaranth are suitable not only for celiac patients, but also for the general population [15]. Examples of the target groups are:

- Children - high content of lysine and histidine that are essential for anormal mental development and for growth.
- Sportsmen – food supplement good for its high content of vitamins and a high-quality protein
- For prevention and treatment of increased level of cholesterol and fat metabolism disorders (arteriosclerosis, ischemic heart disease, heart attack, angina pectoris) – positive effect of amaranth pulp and unsaturated fatty acids
- Patients with food allergy - low antigen potential of amaranth protein
- Diabetes patients – content of slowly degradable carbohydrates
- People with alternative ways of nutrition (vegetarians, macrobiotics) for a high-quality source of protein
- Celiac Disease patients for gluten-free and nutritional diet
- Obese patients as a supplement for reduction diet containing high content of carbohydrates, pulp and high-quality protein.

An anticancer activity of amaranth leaves is described in [16].

Amaranth has also been successfully used in cosmetic industry. Especially amaranth oil containing squalene has a very positive effect on sensitive skin. The oil contents active substances that help heal burns, rashes, eczemas and skin treatment after sunlight or radiotherapy. [17]

With respect to the unique composition amaranth is a potential food resource for the third millennium

IV. MATHEMATICAL MODELS

A. Model of amaranth flour enzymatic hydrolysis

Mathematical model is based on heterogeneous reaction kinetics of amaranth protein/starch extraction running on the interface solid - liquid phase. Due to the very small particles of flour can be expected that the hydrolysis process is not slowed down by internal diffusion. Assuming the validity of the 1st order mechanism what means the rate of conversion degree is directly proportional to undecomposed share of protein/starch we can write the following differential equation

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

Where y is degree of conversion
τ is time
k is rate constant of hydrolysis

By separation of variables and subsequent integration (y limits: $y(0)=0$ a $y(\tau)=y$) we obtain

$$-\ln(1-y) = k\tau \tag{2}$$

By plotting the natural logarithm of the unreacted share over time we get a straight line, the directive is equal to the rate constant of hydrolysis of liquefied substance.

B. Diffusion model of enzymatic hydrolysis of amaranth grain

Amaranth has small size, on average 0.7 to 1.5 mm, a small weight (1000 seeds/g) grains as shown in Fig 1. For utility species is preferred light colour. Grain shape is approximately spherical. Just under the rigid shell is in the periphery of the plane of the largest perimeter centrifuged a sprout occupying one third of the grain. Up to 65% amaranth protein is concentrated in this sprout. The internal structure of amaranth grain is displayed in Fig. 2.

Fig. 1Seeds of amaranth

Fig. 2 The internal structure of amaranth grain with the highlighted sprout containing the protein [18]

One plant produces huge quantities (200-500 thousand) of grains. The grain harvest is under climate perhaps three times a year.

In experiments with amaranth grain is, however, necessary to consider a more complex mathematical model because of the diffusion of protein from grains, which decelerate the process. Due to the almost spherical shape of amaranth grains we propose for mathematical description of the process in order to optimize it the use of the 2nd Fick's law for spherical shape bodies.

$$\frac{1}{D}\frac{\partial c(r,\tau)}{\partial \tau} = \frac{\partial^2 c(r,\tau)}{\partial r^2} + \frac{2}{r}\frac{\partial c(r,\tau)}{\partial r}, \tag{3}$$

$$0 < r < a,\ \tau > 0.$$

where: r is radius
c is concentration
τ is time
D is diffusion constatnt
a is radius of the grain,
0 indicates the center of the grains

Equation (3) is valid on the presumption that control step is diffusion of reaction compounds (alkali or enzyme).

The initial and boundary conditions are:

Behaviour on the surface of the grain is given by generalized 1st Fick's law, where:

$$c_0(0) = 0, \tag{4}$$

$$c(r,0) = c_p. \tag{5}$$

Axial symmetry of the concentration field

$$\frac{\partial c}{\partial r}(0,\tau)=0 . \quad (6)$$

Condition of the perfect mixing of the liquid phase ensuring a perfect mass transfer to the system environment

$$c(a,\tau)=\psi c_0(\tau). \quad (7)$$

Mass balance of equality of surface diffusion and flow rate of accumulation of the protein hydrolyzate in an aqueous solution

$$-S_z D\frac{\partial c}{\partial r}(a,\tau)=V_0\frac{\partial c}{\partial \tau}(\tau), \quad (8)$$

where: c_0 is protein concentration in the solvent at the beginning of the reaction,
c_p is concentration on the surface of grain
S_z is the surface of the grain
V_0 is volume of the area outside the grain.

In order to obtain a more general solution we apply dimensionless quantities on the model of diffusion.

$$C=\frac{c}{c_p}, \quad (9)$$

$$R=\frac{r}{a}, \quad (10)$$

$$F_0=\frac{D\tau}{a^2}, \quad (11)$$

$$C_0=\frac{\psi c_0}{c_p} \quad (12)$$

Dimensionless diffusion model is then

$$\frac{\partial C(R,F_0)}{\partial F_0}=\frac{\partial^2 C(R,F_0)}{\partial R^2}+\frac{2\partial C(R,F_0)}{R\partial R} \quad (13)$$

$$0<R<1,\ F_0>0$$

$$C_0(0)=0 \quad (14)$$

$$C(R,0)=1 \quad (15)$$

$$\frac{\partial C}{\partial R}(0,F_0)=0 \quad (16)$$

$$C(1,F_0)=C_0(F_0) \quad (17)$$

$$-\frac{\partial C}{\partial R}(1,F_0)=\frac{Na}{3\psi}\frac{\partial C_0}{\partial F_0}(F_0) \quad (18)$$

Where Na indicates a dimensionless specific consumption of enzyme extraction solution.

Analytical solution of the equations was published for the temperature field of spherical body in [19]. By adjusting the published solutions for concentration field in case of amaranth grain we get equation (19).

For a processes on the surface of the grain, i.e. for R = 1 we get the equation for the protein concentration in the area outside of grain, that is the protein concentration in the obtained hydrolyzate.

$$C_0=\frac{c_0}{c_p}=\frac{1}{\psi+Na}-\frac{6Na}{\psi}\sum_{n=1}^{\infty}\frac{e^{-F_0 q_n^2}}{\frac{Na^2}{\psi^2}q_n^2+9\left(\frac{Na}{\psi}+1\right)}, \quad (20)$$

$$F_0=\frac{D\tau}{a^2}, \quad (21)$$

where q_n are roots of the equation

$$tg(q)=\frac{3q}{3+\frac{Na}{\psi}q^2}. \quad (22)$$

Concentrations fields of amaranth protein in the spherical grain for different dimensionless consumption of enzyme solution that were visualized in Matlab are shown in Fig. 3 – Fig. 5.

$$C=\frac{c}{c_p}=\frac{\psi}{\psi+Na}-\frac{2Na}{3\psi R}\sum_{n=1}^{\infty}\frac{\left[\frac{Na^2}{\psi^2}q_n^4+3\left(\frac{2Na}{\psi}+3\right)q_n^2+9\right]\sin(Rq_n)\sin(q_n)}{\frac{Na^2}{\psi^2}q_n^4+9\left(\frac{Na}{\psi}+1\right)q_n^2}e^{-F_0 q_n^2} \quad (19)$$

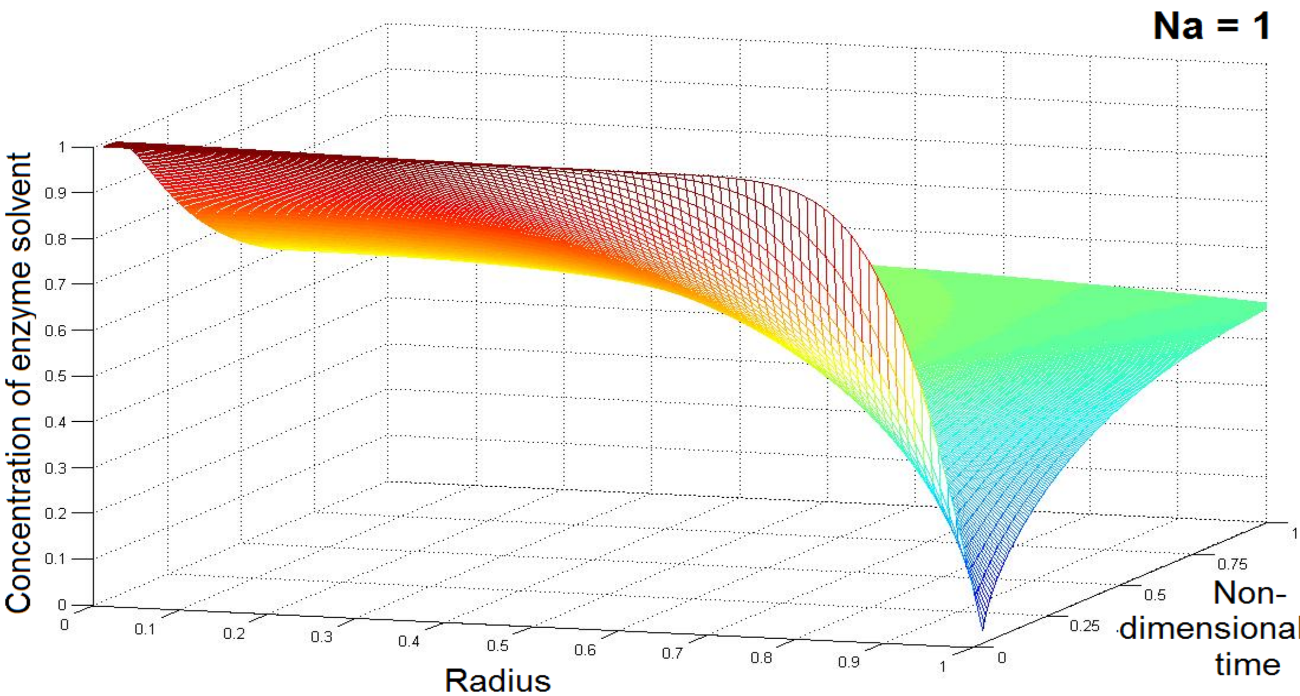

Fig. 1 Concentration field for the dimensionless consumption of enzyme solution Na = 1 for model of amaranth grain

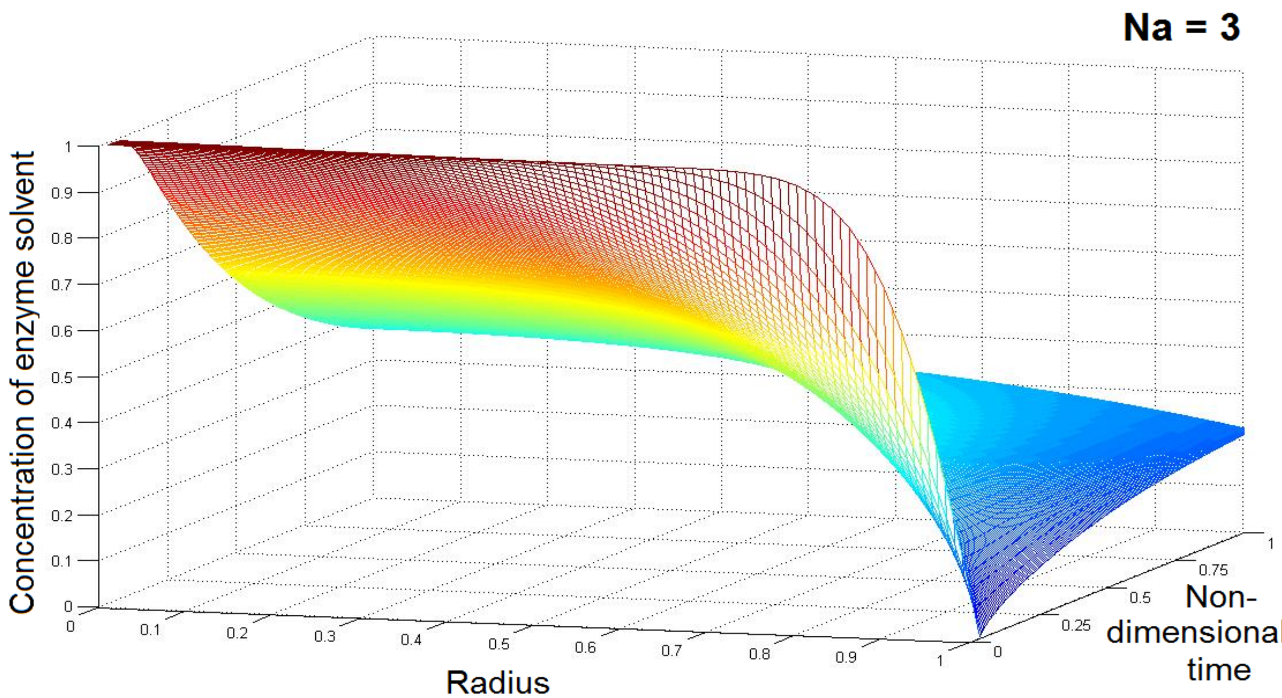

Fig. 2 Concentration field for the dimensionless consumption of enzyme solution Na = 3 for model of amaranth grain

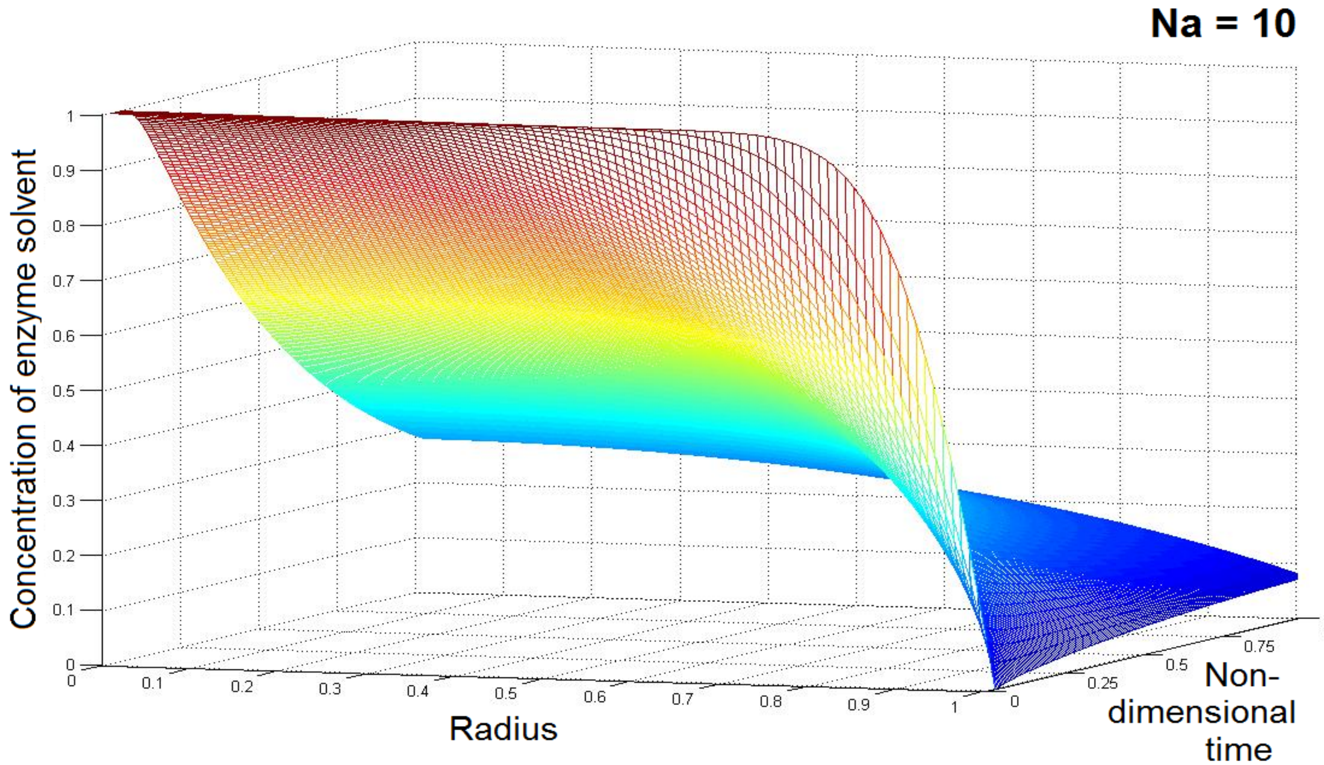

Fig. 3 Concentration field for the dimensionless consumption of enzyme solution Na = 10 for model of amaranth grain

C. *Model of amaranth stem enzymatic hydrolysis*

Amaranth stem contains also a considerable amount of protein form a certain developing period of the plant. Considering the fact the above-ground part of the plant reaches a large mass and thus a rather high content of protein, there offers another diffusion model. Taking into account the length of the stem compared to the diameter of the stem an approximation of infinite cylinder is used. Extraction of proteins from amaranth stem is described on the basis on the 2nd Fick's law, this time in the form of cylindrical coordinates, considering a change in gradient flow only with radius r. 2nd Fick's law in cylindrical coordinates is:

$$\frac{1}{D}\frac{\partial c(r,\tau)}{\partial \tau} = \frac{\partial^2 c(r,\tau)}{\partial r^2} + \frac{1}{r}\frac{\partial c(r,\tau)}{\partial r}, \qquad (23)$$

$0 < r < R',\ \tau > 0,$

where: R' is radius of the stem
0 is the centre of the stem

The initial and boundary conditions are:

$$c_0(0) = 0, \qquad (24)$$

$$c(R',0) = c_p, \qquad (25)$$

$$\frac{\partial c}{\partial r}(0,\tau) = 0, \qquad (26)$$

$$c(R',\tau) = \psi c_0(\tau), \qquad (27)$$

$$-S_z D\frac{\partial c}{\partial r}(R',\tau) = V_0\frac{\partial c}{\partial \tau}(\tau) \qquad (28)$$

In order to obtain a more general solution again we apply dimensionless quantities on the model of diffusion.

$$C = \frac{c}{c_p}, \qquad (29)$$

$$R = \frac{r}{R'}, \qquad (30)$$

$$F_0 = \frac{D\tau}{R'^2} \qquad (31)$$

$$C_0 = \frac{\psi c_0}{c_p} \tag{32}$$

Dimensionless diffusion model is then

$$\frac{\partial C(R,F_0)}{\partial F_0} = \frac{\partial^2 C(R,F_0)}{\partial R^2} + \frac{\partial C(R,F_0)}{R \partial R} \tag{33}$$

$$0 < R < 1,\ F_0 > 0$$

$$C_0(\tau = 0) = 0 \tag{34}$$

$$C(R,0) = 1 \tag{35}$$

$$\frac{\partial C}{\partial R}(1, F_0) = 0 \tag{36}$$

$$C(1, F_0) = C_0(F_0) \tag{37}$$

$$-\frac{\partial C}{\partial R}(1, F_0) = \frac{Na}{2\psi}\frac{\partial C_0}{\partial F_0}(F_0) \tag{38}$$

Solving the equations we obtain

$$C_{(R,F_0)} = \frac{\psi}{\psi + Na} - 2Na\sum_{n=1}^{\infty}\frac{J_0(Rq_n)e^{-F_0 q_n^2}}{2\psi J_0(q_n) - \frac{4\psi J_1(q_n)}{q_n} - q_n Na J_1(q_n)} \tag{39}$$

$$C_0(F_0) = \frac{\psi}{\psi + Na} - 2Na\sum_{n=1}^{\infty}\frac{e^{-F_0 q_n^2}}{2\psi - 4q_n Na + q_n^2 \frac{Na^2}{\psi}} \tag{40}$$

Where q_n are roots of the equation

$$\frac{J_1(q)}{J_0(q)} = \frac{qNa}{2\psi} \tag{41}$$

Concentrations fields of amaranth protein in the cylindrical shaped stem for different dimensionless consumption of enzyme solution that were visualized in Matlab are shown in Fig. 6 – Fig. 8.

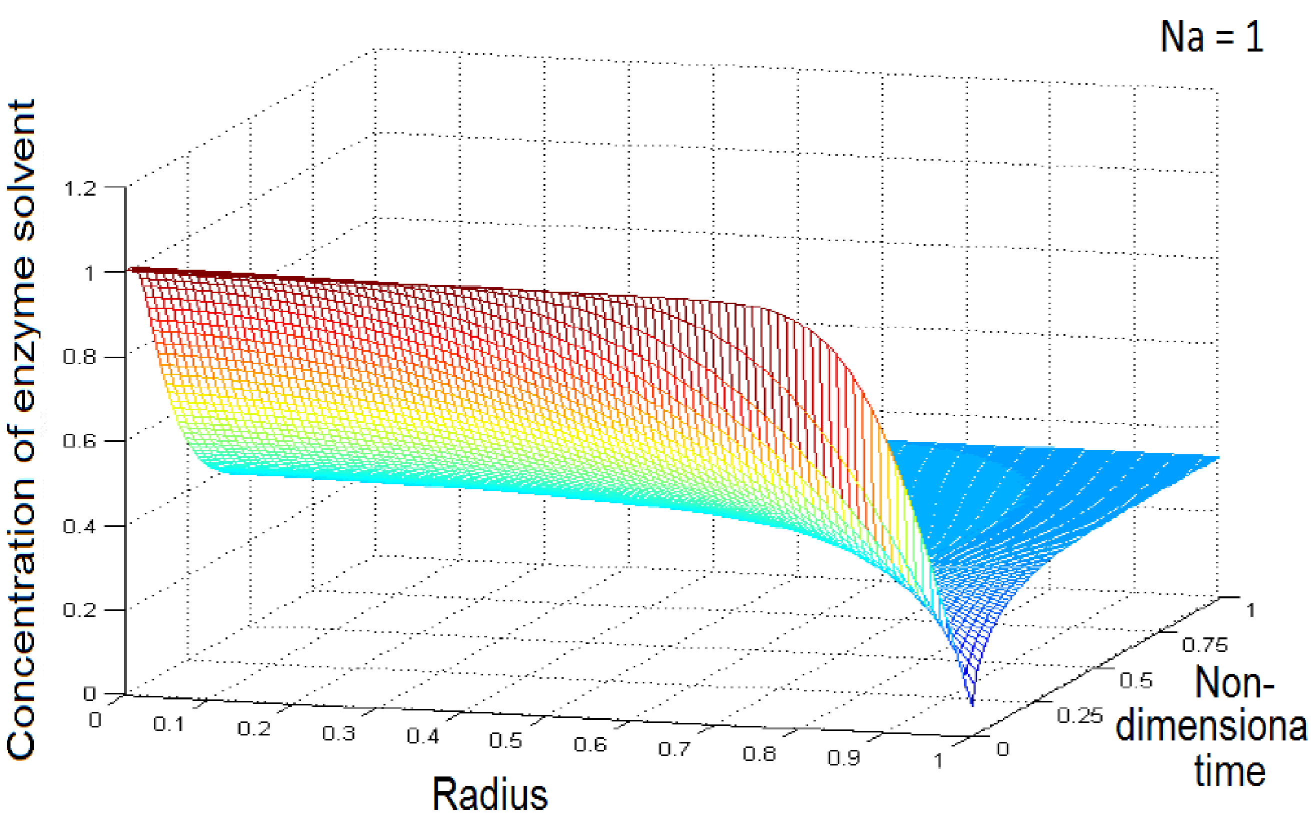

Fig. 6 Concentration field for the dimensionless consumption of enzyme solution Na = 1 for model of amaranth stem

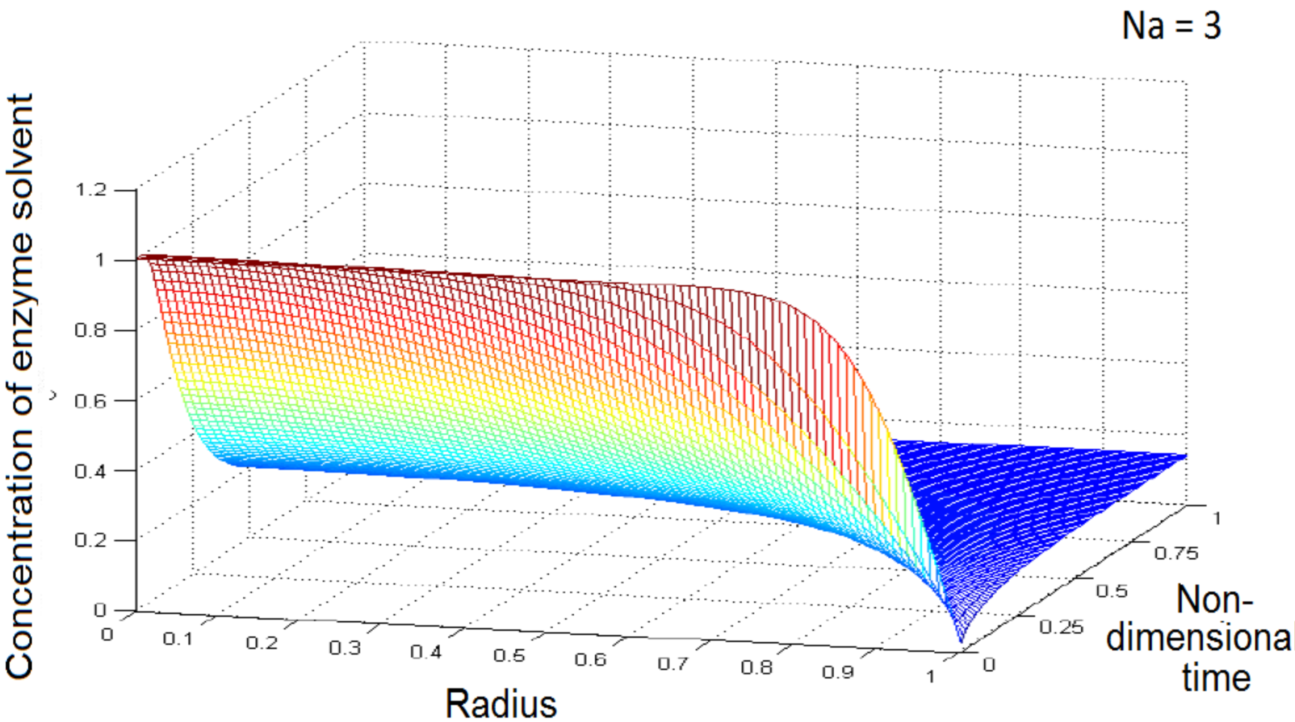

Fig. 7 Concentration field for the dimensionless consumption of enzyme solution Na = 3 for model of amaranth stem

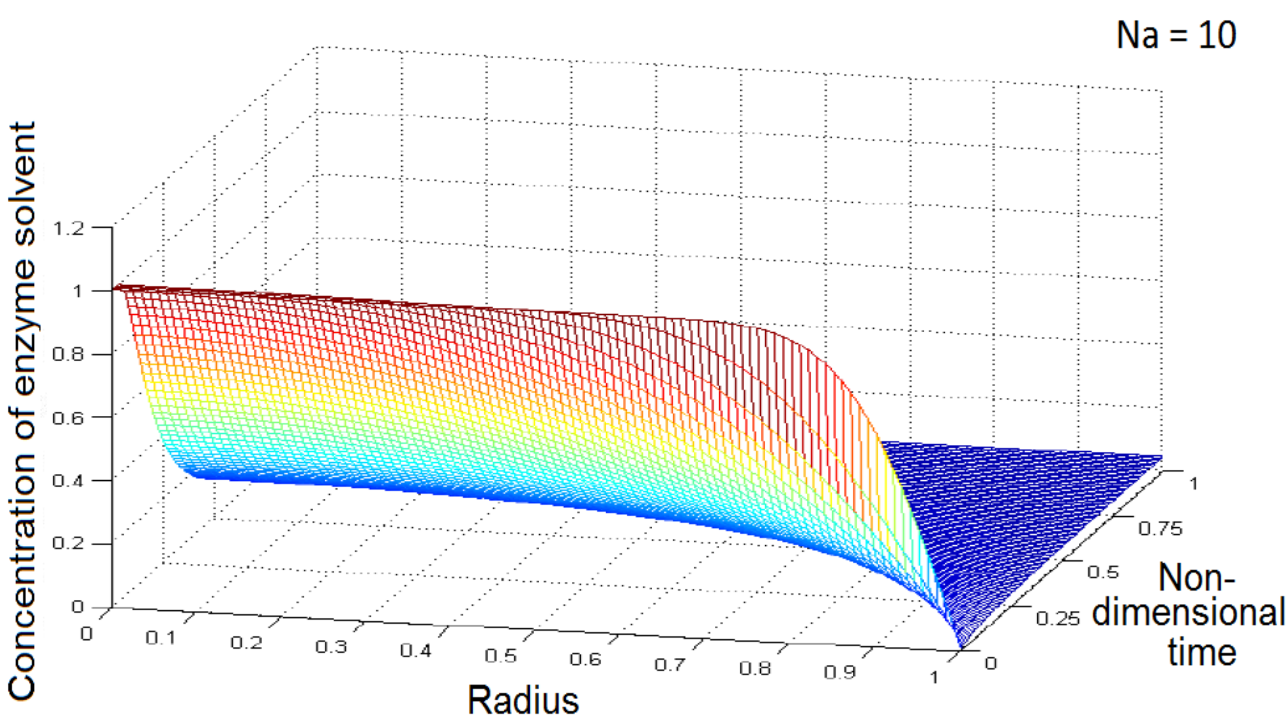

Fig. 8 Concentration field for the dimensionless consumption of enzyme solution Na = 10 for model of amaranth stem

V. Experimental part

Both amaranth protein and starch liquefaction were performed by the enzymatic hydrolysis under laboratory conditions. The aim was to obtain kinetic curves, which are essential for the optimization of a specific manufacturing process. Experiments were carried out in double-walled reactor with a radial shaft stirrer in temperature range 50°C to 80°C in various times from 10 minutes to 5 hours under the previously optimized conditions (pH, rev/min, Na, etc.) using 4 different enzymes [21]. Hydrolyzates were assessed by the UV-VIS spectrophotometric method.

In particular, the extraction of proteins from the flour causing excessive swelling mass, which complicate the separation of liquid protein hydrolyzate from the solid residue. In laboratory conditions, although not too serious complication that resolved using centrifuges instead of simple filtration. However, with regard to the separating the protein solution from the solid phase in the industrial processing this aspect is significant and not negligible. Easier due to the separation of soluble protein from the solid phase appears to be the use of grain as feedstock.

Furthermore, it appears meaningful to the keep the hydrolysis at the maximum temperature still guaranteeing the stability of the enzyme. This saves electricity, as in practice is envisaged stirred reactors. An important benefit is the increase in hourly production. The exact description of the experiments and the measuring apparatus including obtained rate constants and activation energies is the subject of another article.

VI. Conclusion

Amaranth as a non-traditional crop was used because of its unique composition and amounts of quality substances with high nutritional value. Due to enzymatic hydrolysis the separation of the high-quality amaranth protein as well as starch is possible. Experimental results show that amaranth grain is preferable in terms of separation of hydrolyzates and demands on processing facilities. For the description and optimizing these processes mathematical models based on heterogeneous reaction kinetics and the transport processes of diffusion for a spherical body shape are presented.

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