Chickpea seeds germination rational parameters optimization

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Abstract. The paper presents the influence of chickpea seeds bioactivation parameters on their enzymatic activity experimental results. Optimal bioactivation process modes were obtained by regression-factor analysis: process temperature - 13.6 °C, process duration - 71.5 h. It was found that in the germination process, the proteolytic, amylolytic and lipolytic enzymes activity increased, and the urease enzyme activity is reduced. The dependences of enzyme activity on chickpea seeds germination conditions were obtained by mathematical processing of experimental data. The calculated data are in good agreement with the experimental ones. This confirms the optimization efficiency based on experiments mathematical planning in order to determine the enzymatic activity of chickpea seeds germination optimal parameters of bioactivated seeds.

1. Introduction

The use of legume seeds can increase the biological value of food products. The chemical composition of these seeds is balanced in protein and carbohydrates. However, they contain anti-nutritive substances, which is accompanied by a vegetable protein. Therefore, in the fortified foods production add no more than 10% recycled natural legume seeds. Different methods to reduce and remove unwanted substances from legume seeds are used. These include soaking, autoclaving, cooking, roasting, chemical processing, germination, irradiation [1]. In order to achieve the maximum possible quality indicators and increased products biological value of enriched with plant protein it is necessary to select the non-traditional production processing modes [2]. Then the optimal modes of seed treatment are determined.

Germination was chosen as a pretreatment method of legume seeds [3, 4]. This is due to the fact that during seed germination, the seed enzymes activation occurs, which affect the high molecular weight substances seed decay to low molecular weight substances. Hydrolysis of starch produces glucose, maltose and dextrins; proteins – amino acids and peptides; lipids - free fatty acids. Low molecular weight substances move to the embryo zone and are used as a building material for complex organic substances under the certain enzymes influence [5, 6]. The main indicator of deep biochemical changes in seed germination is the enzymes amylolytic and proteolytic action increase, and the decrease of a seed anti-nutrient substances proportion.

The article contains the results of influence of different seed germination conditions (temperature and duration) on the seed enzymes activity, followed by germination regimes optimization.

2. Materials and methods

The chickpea seeds that have been grown in Voronezh region in the period of 2015-2016 was the
object of the experimental study. The chickpea seeds meet the GOST 8758-76 requirements.

General proteolytic activity in germinated seeds was determined according to GOST R 53974-2010; starch-splitting activity - according to GOST R 54330-2011; the activity of the enzyme urease - according to GOST 13979.9-69. Lipoygenase activity was determined by the spectrophotometric method [7].

The chickpea seed germination optimal parameters were determined using the regression-factor analysis method. The determination factor allows one to estimate the obtained solution adequacy. This coefficient determines the degree of compliance of calculated optimal parameters with the actual process conditions.

3. The enzymatic activity study of germinated chickpea seeds depending on the process parameters

Chickpea seeds soaked at a temperature of 18 °C for 18 hours to a moisture content of 36 - 38 % and germinated for 72 hours at a temperature of 12 - 15 °C.

In the first germination 12 hours, there was a significant increase in the proteolytic enzymes activity (increased by 3.5 times compared to the original). Further, with the acidity increasing the medium proteolytic activity began to decline, but by the end of germination it was still higher than the initial seeds activity (0.235 units/g (figure 1, a, b)).

The amylolytic enzymes (β-amylase) activity increased from the germination beginning and continuing for 24 hours. Further, its growth was insignificant. The LOX activity increased 1.25 times in the first 24 hours, for the next 36 hours and remained at a steady value (figure 1, c, d). The urease enzyme, belonging to the hydrolytic enzymes group, is an undesirable enzyme for the human body. Its

![Figure 1. Change in the enzyme activity germination: a - amylolytic (β - amylase); b - proteolytic (protease); c – lipoygenase; d - urease.](image-url)
stomach entering may trigger the development of the gastric mucosa peptic ulcer disease [4]. It was experimentally determined that the chickpea seeds germination during 72 hours can reduce the urease enzyme activity 2 times. Experimentally it was found that the chickpea seeds enzymes activity change depends on 2 parameters: temperature and the germination duration.

Formalized mathematical models that express the analytical form of the functional relationships, and constraints arising from the physical sense of the problem, allow us to find optimal solutions for the design of various processes [8, 9].

To obtain an adequate model, it is necessary to associate the output parameters of the system (the activity of seed enzymes during germination) with the input parameters (temperature and duration of the process) [10].

The model characterizing the bioactivation process was built. The following symbols were introduced: $x_1$ – process duration, hours; $x_2$ – temperature, °C. The selected factors and uncontrolled according compatible among themselves.

Then the enzymes activity is a functional dependence of the $Y_i(x_1, x_2)$ type. It is necessary to carry out the model structural synthesis of dependence of proteolytic activity ($Y_2$), amylolytic ($Y_1$) and lipolytic ($Y_3$) enzymes, and also bioactivated chickpea seeds urease ($Y_4$) on the listed parameters.

As a mathematical model of the bioactivation process of chickpea seeds, the second degree polynomial was considered: $Y(x_1, x_2) = \sum a_k \cdot x_1^{i_1} \cdot x_2^{i_2}$, where the input parameters degrees satisfy condition $0 \leq i_1 + i_2 \leq 2$; $a_k$ – the polynomial coefficients calculated by the least squares method [11, 12].

The following functional dependencies were obtained for the polynomials under consideration:

1. $Y_1 = -4.3 \cdot 10^3 + 63218.08 \cdot x_2 - 2325 \cdot x_1^2 - 39.08 \cdot x_1 + 2.87 \cdot x_1 \cdot x_2 - 0.1 \cdot 10^{-2} \cdot x_1^2$  
2. $Y_2 = -9.3 \cdot 10^3 + 1.37 \cdot 10^3 \cdot x_2 - 5049.99 \cdot x_2^2 - 107.57 \cdot x_1 + 7.92 \cdot x_1 \cdot x_2 - 0.003 \cdot x_1^2$  
3. $Y_3 = -8.3 \cdot 10^3 + 1.28 \cdot 10^3 \cdot x_2 - 451.25 \cdot x_2^2 - 7.62 \cdot x_1 + 0.56 \cdot x_1 \cdot x_2 - 0.0002 \cdot x_1^2$  
4. $Y_4 = 1.15 \cdot 10^3 - 1698.57 \cdot x_2 + 62.5 \cdot x_2^2 + 1.41 \cdot x_1 - 0.1 \cdot x_1 \cdot x_2 - 0.5 \cdot 10^{-4} \cdot x_1^2$

Analyzing the obtained equation, the authors come to the conclusion that the enzymatic activity of the most influenced by the temperature of germination, several times smaller value to change the values of enzyme activity is the length of the process. It should be noted that with an increase in temperature values and a reduction in the duration of germination, the activity values of amylolytic, proteolytic and lipolytic enzymes will increase, while the activity of the urease enzyme with an increase in temperature - decreases. The coefficients of the equation in the product ($x_1 \cdot x_2$) have lower values than the coefficients in $x_1$ and $x_2$, which indicates that the joint effect of the input factors on the value of the output parameter is lower. And, despite the fact that the equations (1) - (4) are quadratic polynomials, the coefficients of its terms in the second degree are insignificant, which does not significantly affect the value of the output parameter [13].

The obtained models were used to calculate the output parameters – the amylolytic, proteolytic, lipolytic enzymes and urease activity at the given input parameters. The closest data to the experimental ones were obtained in all cases using a second degree polynomial.

According to the dependences (1) - (4), the authors found the values of variables $x_i$, $i=1,2$, when the amylolytic ($Y_1$), proteolytic ($Y_2$) and lipoxygenase activity ($Y_3$) chickpea seeds germinated enzymatic activity values increased, but urease activity ($Y_4$) decreased, i.e. it is necessary to solve the two criteria optimization problem and determine the $x_1$ and $x_2$ values:

$$Y_1, Y_2, Y_3 \rightarrow \max, \quad Y_4 \rightarrow \min$$  
(5)
The input parameters varied within the following limits: \( x_1 = 12 - 14 \) °C, \( x_2 = 64 - 72 \) h. in order to find the optimal process conditions of bioactively seeds chickpeas. At these values, the \( \beta \)-amylase and lipoxygenase activity had the best values in the experiments, the proteases activity increased 2.5 times in comparison with initial, and urease activity reduced to 0.035 units/g.

In the modeling process, the parameters were determined to achieve chickpea seeds enzymatic activity optimal values: temperature - 13.6 °C, process duration - 71.5 h. and the output factors values: \( Y_1=5.76 \) units/g; \( Y_2=0.58 \) units/g; \( Y_3=24.73 \) units/g and \( Y_4=0.36 \) units / g.

4. Conclusion

The chickpea seeds germination, before their use as a prescription component of food products of increased biological value, can reduce the unwanted enzymes activity and increase the necessary seed enzymes activity. Processed seeds are a source of essential amino acids, unsaturated fatty acids and vitamins. It allows enriching foodstuff with the listed substances, thereby giving to these products functional properties.

With the help of experimental data mathematical processing, dependences of the enzyme activity on chickpea seeds germination conditions were obtained. The experimental and calculated data are consistent. It confirms the optimization efficiency, based on experiment mathematical planning in order to determine optimal parameters of the chickpea seeds germination that affect the bioactivated grain enzymatic activity.

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