

Improvement in the nutritional value of legumes through inactivation of alkaloids

OTARI SESIKASHVILI – SERGEY ZVEREV – ELENE GAMKRELIDZE –
NODARI MARDALEISHVILI – SHALVA TSAGAREISHVILI

Summary

The study dealt with the current problem of increasing the nutritional value of some legumes through inactivation of undesirable substances, namely alkaloids, in food after the high-temperature micronization with infrared rays. Empirical relationships between the degree of alkaloids inactivation and the heat treatment time and temperature were obtained. It was established that the degree of alkaloids inactivation depends on the initial moisture content of the grain and the final heat treatment temperature. A mathematical model of inactivation of alkaloids was proposed, which was based on first-order equations of chemical kinetics with a reaction rate constant. The model was modified for conditions of the heat treatment with variable temperature. The model and the results of the experiments were processed by a mathematical package MathCad (Mathsoft, Cambridge, Massachusetts, USA). The average humidity of legumes on the market was 9–11 %. Under such humidity conditions, optimal inactivation of alkaloids began at temperatures of 75–100 °C and the average heat treatment time was 100 s. The optimal distance of the infrared panel was 75 mm and the maximum temperature was 110 °C. The further increase in temperature led to carbonization of the surface layer of the grain.

Keywords

bean; cereal; undesirable substance; mathematical model; alkaloid inactivation

Legumes form an important part of the population's diet due to the content of a large amount of plant proteins, fats, carbohydrates, essential minerals and vitamins. Green beans are considered a dietary and low-energy product. The energy value of raw beans is 2510 kJ·kg⁻¹, the energy value of boiled beans is 2770 kJ·kg⁻¹ and the energy value of stewed beans is 2380 kJ·kg⁻¹. Beans contain up to 40 % protein and can easily replace meat products in several aspects [1].

Beans are also a good source of cholesterol-lowering fibre. Beans are an excellent source of minerals, namely molybdenum, which is an essential component of the enzyme responsible for neutralizing harmful preservatives commonly added to prepared foods, as well as stabilizing blood sugar levels. The high content of fibre and pectins con-

tributes to the removal of heavy metal salts from the intestines, including radioactive isotopes [1].

Rich in potassium and folic acid, beans can be considered a health-promoting food. Beans are high in B vitamins, which reduce the risk of heart disease. Legumes have a beneficial effect on digestion, as they contain a lot of fibre and dietary fibre. Another beneficial property of beans is that they supply the human body with proteins without being accompanied by fats, which is always present in meat, even in lean. To provide the health-promoting effect, legumes should make up at least 8–10 % of the diet [1].

The chemical composition of beans is as follows (per 100 g): proteins 18.81 g, fats 2.02 g, carbohydrates 64.11 g, water 10.77 g, ash 4.3 g, vitamin B3 2.08 mg; vitamin B1 0.54 mg; vitamin B6 0.40 mg,

Otari Sesikashvili, Nodari Mardaleishvili, Department of Mechanical Engineering, Faculty of Engineering – Technical, Akaki Tsereteli State University, Tamar – Mepe Street 59, 4600 Kutaisi, Georgia.

Sergey Zverev, Federal Scientific Center for Food Systems V. M. Gorbатов, The All-Russian Research Institute of Grain and its Processing Products, Dmitrovskoe shosse 11, 127434 Moscow, Russia.

Elene Gamkrelidze, Department of Chemical Technology and Ecology, Faculty of Engineering – Technological, Akaki Tsereteli State University, Tamar – Mepe Street 59, 4600 Kutaisi, Georgia.

Shalva Tsagareishvili, LTD Kutaisi 21", Nikea Street 4, 4600 Kutaisi, Georgia.

Correspondence author:

Otari Sesikashvili, e-mail: otar.sesikashvili@atsu.edu.ge

vitamin B2 0.22 mg, vitamin C 4.60 mg, potassium 1540 mg, phosphorus 549 mg, magnesium 158 mg, calcium 103 mg, iron 6.95 mg, sodium 18 mg, zinc 3.50 mg, copper 0.79 mg, manganese 1.52 mg, sulfur 211 mg, nickel 76 μg , molybdenum 384 μg , cobalt 26 μg and boron 1160 μg [1, 2].

Among legumes, beans occupy leading positions regarding the starch content, which is approximately 470 $\text{g}\cdot\text{kg}^{-1}$ [2]. The seeds of this culture are also considered a good source of prebiotics, as they contain prebiotic carbohydrates at 120–140 $\text{g}\cdot\text{kg}^{-1}$ (dry weight basis). In addition, beans are relatively low in fat and sodium, but high in potassium, with sodium to potassium ratio of approximately 1:73. This also makes them an attractive dietary product [3].

However, along with many useful nutrients and micronutrients, legumes are characterized by a high content of substances undesirable in food, e.g. alkaloids [4, 5]. These may cause formation of toxins in the human body, abdominal distension, digestion problems and other adverse effects. Their partial inactivation takes place during the preparation process after thermal treatment.

Alkaloids are nitrogen-containing organic compounds with specific properties. Typically, they have a complex composition. The great majority of alkaloids are heterocyclic compounds. Plants contain alkaloids in the form of salts of various organic or mineral acids, and mostly they are contained as mixtures of several alkaloids [6].

Alkaloids in legumes play an important physiological role. They provide plants with a capability to resist to drought, low temperatures, pests, some fungal and viral diseases. They have a positive effect on the phytosanitary state of soil. Accordingly, the unit costs of labour are reduced and the yield of beans is increased [6].

Various types of legumes contain the following major alkaloids: lupanine, 13-hydroxylupanine, angustifolin, lupinine, sparteine and multiflorine. Along with the basic alkaloids, legumes contain up to 30 various alkaloids that can be considered as co-alkaloids. Their content is negligible as compared to the basic alkaloids. According to an increase in toxicity, alkaloids are classified as follows: lupanine, lupinine and 13-hydroxylupanine. Legumes containing less than 0.02 % of alkaloids are used in the food industry [6]. Such legumes include white lupin, various varieties of beans, lentils of different colours, pea and chickpea.

Heat treatment of various cereals using infrared rays is a quite common process to impart different properties to cereals. This method is mostly used by small enterprises that produce instant cereals and is known as high-temperature

micronization [7, 8]. Undesirable components for food belong to thermodegradable substances, so an affordable and relatively inexpensive way of their inactivation is heat treatment by PEREZ-MALDONADO et al. [9]. The high-temperature micronization can also be successfully used to inactivate undesirable nutrients in cereals, allowing to increase the nutritional value of legumes.

Many scientific papers have discussed the treatment of cereal crops with infrared rays, but little has been said about the inactivation of undesirable substances in cereals. In our study, we examined the process of inactivation of alkaloids by infrared rays using the high-temperature micronization method and determined the minimum number and type of parameters that satisfactorily describe the process of inactivation of alkaloids in legumes. We made a specific mathematical model of this process and determined the coefficients of the model. The reason was that the process is described by a non-linear relationship and determining the coefficients of the process experimentally is very difficult.

The purpose of the study was to determine the conditions for increasing the nutritional value of legumes (various varieties of beans and white lupin) through inactivation of alkaloids in the interest of the possibility of their use in health-promoting diet of the population.

Scientific hypothesis

Inactivation of undesirable nutrients during infrared treatment of legumes is a thermally activated process, therefore, the final content of undesirable nutrients will be primarily determined by the heat treatment time and the temperature of the product. Since the latter two variables are interdependent under infrared heat treatment and can be represented by each other, the inactivation process and its mathematical model can be represented by both the heat treatment time and the grain temperature. The initial moisture content under the heat treatment of the grain with infrared rays, the distance of the infrared radiation panel from the product and the temperature in the working environment also have an impact on the final content of undesirable substances in the product.

MATERIAL AND METHODS

Samples

Bean (*Phaseolus vulgaris*) variety Tsanava (Gori, Gori Region, Georgia), red kidney bean (*P. vulgaris*) variety Field Red (Tskhaltubo, Tskhaltubo Region, Georgia) and white lupin (*Lupinus albus*)

Tab. 1. Geometrical characteristics of grains.

Variety	Geometrical parameters			Weight of one bean [g]
	Length [mm]	Width [mm]	Thickness [mm]	
Tsanava bean	16.36 ± 0.25	8.62 ± 0.21	7.25 ± 0.18	0.636 ± 0.01
Field Red bean	12.29 ± 0.32	7.78 ± 0.37	5.58 ± 0.28	0.334 ± 0.01
White lupin	19.35 ± 0.45	13.45 ± 0.41	6.79 ± 0.33	0.868 ± 0.06

(Khoni, Khoni Region, Georgia) were used in the study. The initial moisture content of lupin grains was 9.7 % and of the two bean grains was 11.5 %, which corresponds to beans sold in markets. To obtain bean grains of different humidity, they were moistened to the specified humidity. Geometrical characteristics of grains are shown in Tab. 1.

Chemical reagents and sample preparation

For the experiment we used a 8% solution of trichloroacetic acid, which was prepared from a solution of 50% trichloroacetic acid (trichloroacetic acid, ≥99.5 %, Euro, Dnipro, Ukraine). 80 g of a 8% trichloroacetic acid solution was placed in a test vessel with a volume of 1 l, and then 920 g of distilled water was added and mixed.

To prepare the iodate-iodine solution, 13.0 g of potassium iodide and 2.0 g of potassium iodate were placed in a thermocup and dissolved in 200 ml of distilled hot water. After cooling, it was poured in a flask with a volume of 1 l, free potassium iodide was neutralized by adding 0.2 g of potassium hydroxide (≥99.7 %; LenReaktiv, St. Petersburg, Russia). The vessel was filled to the level of 1 l.

For the experiment, the zero solution was prepared in the following way. The experimental grains with initial moisture content were crushed in an electric mill and passed through a 0.8 mm sieve. An amount of 0.2 g of the sieved sample was placed in a 10 ml flask and 4 ml of 8% trichloroacetic acid was added. The suspension obtained was placed in a thermostat for 18 h at 22 °C, after which it was filtered through a paper filter and the resulting solution was used for experiments. Further experimental solutions were prepared in the same way [6].

Measurements

In a 50 ml beaker, to 3 ml of distilled water and 0.1 ml of an experimental zero solution, 0.15 ml iodate-iodine solution was added, mixed, incubated for 4 min and optical density of the coloured solution was measured using the device KFK-2 (PP ZOMZ, Zagorsk, Russia). We also prepared all the experimental samples and measured the optical density of the coloured solution [6], de-

termining the relative values of the optical densities in reference to the zero solution.

Equipment

For experiments on heat treatment of beans, a type of instrument QP1 model (Elcer, Odesa, Ukraine) of a panel of halogen quartz emitters (the panel composed of 7 emitters) was used as a source of infrared radiation. The dimensions of a panel for the quartz emitter were 247 mm × 62 mm. The length of the tube was 245 mm, tube step was 8.55 mm, rated power was 1 kW and emitter temperature was 750–800 °C.

The initial moisture content of beans was determined using an electronic digital moisture meter VSP-100 (PAtools, Kharkiv, Ukraine). Additionally, moisture loss during the infrared heating was estimated as the difference between the initial sample weight and its weight after the heat treatment. The sample weight of grain before and after the heat treatment was determined using an electronic digital analytical balance SF-400C model (Toms, Qilin, China) with a weighing accuracy of 0.01 g. The moisture content (W) after the heat treatment was calculated based on the initial moisture content (W_0) and mass loss (Δm) according to the formula based on the standards GOST 13586.5-2015 [10] and ISO 1446:2001 [11].

The linear dimensions of beans were determined using an electronic digital caliper Vinca DCLA-0605, 150 mm (Neiko Tools, Lu Chu Hsiang, Taiwan). The surface temperature of the beans was measured under the middle lamp of the emitter, being determined as follows: beans were placed on the pallet in a monolayer that, for a fixed amount of time (30, 60, 90 and 120 s), was placed in a heated infrared-treatment zone. Then, the pallet was quickly removed and the temperature of beans was determined using an AR360A+ infrared laser thermometer (Simzo, Long, China). The temperature measurement interval varied from –50 °C to +360 °C and the temperature measurement error was 0.5 °C due to heat loss, which could be taken as insignificant.

To determine the percentage of alkaloids in the samples, 5 samples were prepared of calibrating

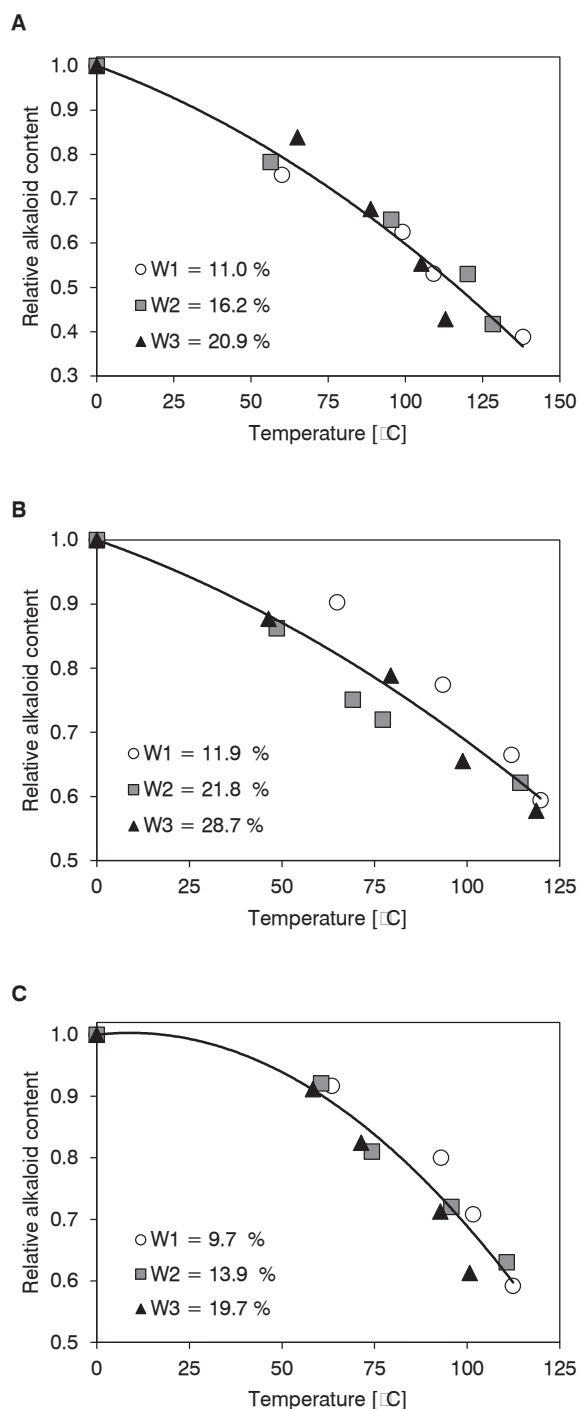


Fig. 1. The relationship between the relative alkaloid content and the heat treatment of beans and white lupin with various humidities.

A – Tsanava beans, B – Field Red beans, C – white lupin. Heat treatment at a fixed distance of a 75 mm infrared panel from the grain surface. Heat treatment time was between 0 s and 120 s with an interval of 30 s. W1, W2, W3 – grain humidity.

with different alkaloids contents and their optical density was determined using the optical density metering device KFK-2. The samples of beans and lupin were crushed by a laboratory grain crushing machine (Retsch, Kiev, Ukraine) and passed through a laboratory sieve No. 08 (Retsch).

Statistical analysis

For statistical processing of the obtained data, we determined the mean root square deviation and the arithmetic-mean dispersion, at which point we determined the mean root square deviation of the arithmetic mean. We conducted each experiment at least three times and determined the arithmetic mean of the computed value. We obtained the value of the reliability coefficient which was $p < 0.05$, that is, the confidence probability was at least 0.95. Statistical data were processed using the mathematical package MathCad 15 (Mathsoft, Cambridge, Massachusetts, USA) [12].

RESULTS AND DISCUSSION

During the high-temperature heat treatment of beans and lupin with infrared rays, the initial moisture content of grain and the heat treatment time were taken as independent variables. As dependent variables, temperature of grain and the alkaloid content of grain were taken. The change in the alkaloid content in grain was determined by the relative values in comparison to the initial value. The average content of alkaloids in Tsanava and Field Red beans reached $1.5 \text{ g}\cdot\text{kg}^{-1}$ on dry matter basis, while the value permitted by the norm is $0.2 \text{ g}\cdot\text{kg}^{-1}$. The value for lupin was, on average, $1.2 \text{ g}\cdot\text{kg}^{-1}$ on dry matter basis.

In our experiments, we tried to determine the temperature of the grain surface and its heat treatment time between 0 s and 120 s, with an interval 30 s, during which the maximum possible inactivation of the alkaloids in grain occurred. The empirical relationship between the alkaloid content before and after treatment of the seeds at a fixed distance of a 75 mm infrared panel from the grain surface is shown in Fig. 1. Relative alkaloid content represents the ratio of the initial value and the value after treatment. The experimental data for Tsanava beans with grain humidity from 11.0 % to 20.9 % is shown in Fig. 1A. Fig. 1B presents the experimental data for Field Red beans under the heat treatment with changes in grain humidity from 11.9 % to 28.7 %. Fig. 1C presents the experimental data for white lupin under the heat treatment with changes in grain humidity from 9.7 % to 19.7 %. Fig. 2 illustrates the empirical relation-

ship between the alkaloid content in the Tsanava beans, Field Red beans and white lupin depending on the temperature of the grain surface with different humidities at a fixed distance of a 75 mm infrared panel from the grain surface. As can be seen from Fig. 2, the alkaloid content changes in all three cases by almost the same law and depends little on the initial moisture content.

As seen in the figures, the dependence of the alkaloid content on the time of heat treatment (from 0 s to 120 s, with a 30 s interval) in the grains of beans and lupine is invariant to the initial moisture content of the grain, but depended on the temperature of the grain surface. The rate of inactivation of alkaloids increased intensively in the temperature range of 75–100 °C. Such a wide range of temperatures in inactivation of alkaloids can be explained by the different size of the two varieties of beans and lupin.

The empirical data on the effect of treatment temperature on the alkaloid content in beans and lupin in a wide range of humidity can be approximated in a simple case by means of a polynomial equation of the second degree

$$y = 1 - ax - bx^2 \quad (1)$$

The identified coefficients are shown in Tab. 2.

Our recent studies [13] revealed that for thermal inactivation in chemistry and biology, as a model of thermal degradation of reagents or microflora contained in a product, a first-order differential equation can be effectively used. This is known as Arrhenius equation and in its simplest form has the form of Eq. 2 [14]:

$$dY = -K[T(t)]Y^n dt \quad (2)$$

where Y is a quantitative measure of the reagent content (in grams), T is absolute temperature (in degrees Kelvin), t is time (in seconds), $K[T(t)]$ is the reaction rate constant and n is the reaction order.

The reaction rate constant in a generalized form can be represented as Eq. 3 [14]:

$$K[T(t)] = kT^m \exp\left(-\frac{\varepsilon}{RT}\right) \quad (3)$$

where k is the proportionality coefficient (in reciprocal Kelvin), ε is the activation energy (in joules per mole), R is the universal gas constant ($R = 8.314 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$) and m is the coefficient (dimensionless).

Depending on the physical concepts of the mechanism of intermolecular interaction in the process of thermodegradation in various theories, m takes the values of 0, 0.5, 1 or -1 [14]. When $m = 0$ (the kinetic coefficient is in the form of the

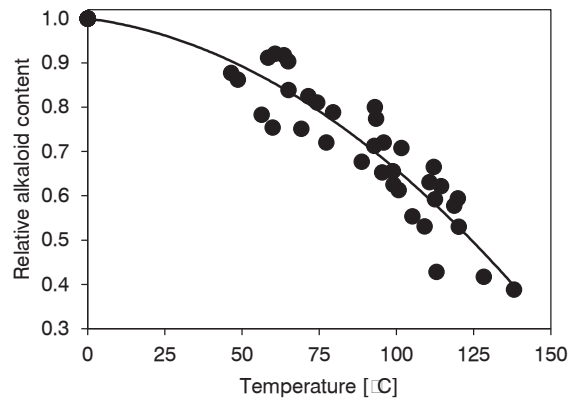


Fig. 2. The relationship between the relative alkaloid content in Tsanava bean, Field Red bean and white lupin with different humidities and the heat treatment.

Heat treatment at a fixed distance of a 75 mm infrared panel from the grain surface. Heat treatment time was between 0 s and 120 s with an interval of 30 s.

Humidity: Tsanava bean 11.0–20.9 %. Field Red bean 11.9–28.7 %, white lupin 9.7–19.7 %.

Tab. 2. Values of coefficients of Eq. 1.

Variety	a	b	R^2
Tsanava bean	0.0025	1×10^{-5}	0.97
Field Red bean	0.0020	1×10^{-5}	0.94
White lupin	–0.0007	4×10^{-5}	0.95
Tsanava bean and white lupin	0.0009	3×10^{-5}	0.91

a , b – empirical coefficients (dimensionless), R^2 – square of the coefficient of multiple correlation.

Arrhenius equation), the model becomes linear and, for identification of coefficients, it is possible to use the method of linear regression analysis. In this form, it was used to describe the results of the experiments on inactivation of antinutrients in soybean [15, 16].

For simplicity of further calculations, we put $m = -1$. After substituting Eq. 3 into Eq. 2, separating the variables and integrating, we get

$$K[T(t)] = \int_{Y_0}^Y \frac{dY}{Y^n} = k \int_0^t T^m \exp\left(-\frac{\varepsilon}{RT}\right) dt \quad (4)$$

where Y , Y_0 is a quantitative measure of the reagent content at the current and initial time points (in grams). If the temperature is constant ($T = \text{const}$), the solution of Eq. 3 gives

$$F(Y) = \ln\left(\frac{Y}{Y_0}\right) = -kT^m \exp\left(-\frac{\varepsilon}{RT}\right)t \quad (5)$$

The dependence of the product temperature on time during infrared heating is well described by the Eq. 6 [9]:

$$\Delta T(t) = \Delta T_\infty [1 - \exp(-K_t t)] \quad (6)$$

from where it follows

$$dt = \frac{dt}{[k_t(C - T)]} \quad (7)$$

where

$$C = T_0 + \Delta T_\infty > T \quad (8)$$

t is time (in seconds), T_0 is initial grain temperature (in degrees Kelvin), ΔT_∞ and k_t are coefficients.

Integrating Eq. 4 by $m = -1$, we get Eq. 9

$$F(Y) = k[Ei(z) - Ei(z_0)] \quad (9)$$

where

$$z = \varepsilon_R \left(\frac{1}{C} - \frac{1}{T} \right) \quad (10)$$

$$z_0 = \varepsilon_R \left(\frac{1}{C} - \frac{1}{T_0} \right) \quad (11)$$

If we make the assumption that C (Eq. 8) is significantly greater than T , then $z \approx -\varepsilon_R/T$, we use the property of the Euler function for large values of the argument, expand in a series and take into account the first term, then Eq. 9 can be represented in the form of Eq. 12 [13]:

$$F(Y) = k \left[T \exp\left(-\frac{\varepsilon_R}{T}\right) - T_0 \exp\left(-\frac{\varepsilon_R}{T_0}\right) \right] \quad (12)$$

As a model of the alkaloids inactivation process, we used Eq. 12, which is relatively simple and has only two empirical coefficients that require identification. The values of the identified coefficients are given in Tab. 3. From Tab. 2 and Tab. 3 it follows that, with a change in the type of grains, the coefficients of the equations are changing. When the coefficient a in Eq. 1 decreases, the coefficient b remains unchanged in the case of beans. In the case of lupin, the coefficient a decreases relative to the coefficient a of beans, but the coefficient b increases almost 4-fold.

The proportionality factor k in Eq. 12 decreases with decreasing the bean size, and ε_R also decreases. In the case of lupin, the size of which exceeds the size of beans, the proportional-

ity factor k increases and ε_R also increases. Here, a compensatory effect takes place, i. e. with an increase or a decrease in one coefficient, the other one increases or decreases [13]. As can be seen, the values of the coefficients in Eq. 12 for beans and lupin differ significantly. The larger the grain size, the greater the coefficients. The small size of Field Red beans compared to lupin allows infrared radiation to penetrate and heat up its entire volume faster, which results in a lower activation temperature ε_R .

In the case of the infrared heat treatment, the process proceeds in a non-stationary manner, that is, the temperature is a function of time, which in this case leads to non-linear dependencies. The identification of the coefficients of such models from experimental data is a much more difficult task. The smaller the number of estimated parameters, the higher the reliability of estimates. Often the values of the parameters obtained depend on the initial values, since the residual functions (in particular, the sum of least squares) can have several minimum values. Therefore, there is always a need to check the obtained values of the coefficients for their compliance with common sense and data from independent sources. For example, we must consciously give up the negative values if, for physical reasons, the value of the coefficient must be positive, and so on.

CONCLUSION

Based on the results obtained, it can be concluded that alkaloids are intensively inactivated under conditions of high-temperature micronization in legumes at a temperature of 75–100 °C. At the end of the heat treatment, the alkaloid content decreases by almost 60 %. The results show that change in humidity does not have much effect on the results of alkaloid inactivation. The authors of the article believe that inactivation of alkaloids at different distances of the infrared panel from the product will be the more intense, the smaller the distance of the infrared panel from the processed product, since at the same heating times, the temperature of grain and in the heat treatment zone increases. Based on the results of our research, we consider 75 mm to be the rational distance of the infrared panel from the processed product, with the heat treatment time of 90–120 s at a maximum temperature of 110 °C. Any further increase in temperature leads to carbonization of the surface layer of grain. At high humidity, the heat treatment time is slightly shorter than at low humidity. During the high-temperature heat treat-

Tab. 3. Values of coefficients of Eq. 12 for $C = \infty$, $n = -1$.

Variety	k [K ⁻¹]	ε_R [K]	R^2
Tsanava bean	0.291	2 051	0.98
Field Red bean	0.084	1 667	0.98
White lupin	11.215	3 653	0.98
Tsanava bean and white lupin	1.754	2 865	0.96

k – proportionality coefficient, ε_R – empirical coefficient, R^2 – square of the coefficient of multiple correlation.

ment of legumes with infrared rays, due to a decrease in the content of anti-nutritional substances (alkaloids) in them, it can be considered that their consumer qualities are improving. Legumes processed in this way will have a higher nutritional value in the traditional diet of the population. This is especially true for the low-alkaloid white lupin (an alkaloid content less than 0.1 %), which contains less alkaloids compared to beans and both of them are not safe for frequent consumption but, after proper heat treatment, they can be used both in feed production and the food industry. The proposed mathematical models of alkaloid inactivation under non-stationary temperature conditions describe the results of experiments quite well and can be used to predict the processes of thermal degradation of other anti-nutrients.

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