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MODELING OF UREASE THERMAL INACTIVATION PROCESSES IN SOYBEAN AT HIGH-TEMPERATURE MICRONIZATION

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ABSTRACT

The use of soybean, in particular in forage production without preliminary heat treatment is not appropriate, and sometimes dangerous, because of the presence of antinutrients. As a marker in assessing safety of cakes and meals, there is often used urease in forage production. This paper describes the results of thermal inactivation of urease in soybean during the process of high-temperature micronization (heating of grain in the flux of infrared radiation). There have been obtained the empirical dependencies of the degree of its inactivation on time of heat treatment and energy exposure (the product of irradiation by the time of treatment). The similar dependences of urease activity on grain temperature are invariant to infrared heating (irradiation and time) regimes, but their nature is affected by the initial moisture content. The paper proposes the models of inactivation of antinutrients based on of the first-order equations of chemical kinetics with the reaction rate constant in various forms (Arrhenius and Hinshelwood, the transition state theory). The models have been tested on literature data on the inactivation of a trypsin inhibitor at a constant temperature. The models are further refined taking into account the variable (increasing) temperature and are reduced to the simplest form: $Y = k\,[\mathrm{Exp}\,(-\varepsilon_R/T) - T_0\,\mathrm{exp}\,(-\varepsilon_R/T_0)]$, where T, T_0 – are the current and initial temperatures of grain, k, ε_R – the empirical coefficients. The identification of the model coefficients was carried out based on the results of inactivation of urease during heating in the flux of infrared radiation. It has been established that the results of thermal inactivation of soybean do not depend on the IR processing regimes and are determined only by the initial moisture content of grain, and by the end heating temperature. The efficiency of inactivation is higher the higher is the used irradiation. There is a compensating effect - with the growth in one coefficient, another is also increased. The considered models can be used for the thermal degradation processes and other thermolabile substances.

Keywords: Soybean; urease; trypsin inhibitor; thermal inactivation; infrared heat treatment

INTRODUCTION

The use of native soybean, for example, in forage production, is not rational, and in some cases is dangerous because of the presence of antinutrients in it **(Zverev, Sesikashvili and Bulakh, 2013)**. Raw soybeans contain a number of antinutrients complicating the digestive process, which, however, can be inactivated by heat treatment. In particular, soyabean is characterized by high activity of inhibitors of digestive proteinases, and primarily of trypsin, which is responsible for protein digestion. In addition, among undesirable enzymes there are singled out:

- Lipoxygenase – an enzyme oxidizing the lipids. In addition, under its action, during continuous seed storage, there are created in them aldehydes and ketones, which impart soyabean a specific unpleasant odor and taste. As a result, the food benefits of soybean are thereby reduced.
- Urease — an enzyme, which performs hydrolytic cleavage of urea with the formation of ammonia and carbon dioxide. The level of its activity is important only for milk cattle breeding, when using soybean in feeds containing urea, since the interaction of urease with urea of feeds results in ammonia formation, which poisons the animal's body. In the initial soybean seeds, the share of urease may reach 6% from the amount of all proteins.

Since these undesirable components are thermolabile substances, heat treatment is the available and relatively inexpensive way to inactivate them **(Perez-Maldonado, Mannion and Farell, 2002)**. The listed substances have different degrees of heat resistance. The available data show that during the treatment of soybeans for 15 minutes, complete inactivation of lipoxygenase was observed at a temperature of 80 °C, of urease – at 90 °C, and of a trypsin inhibitor – at 100 °C. Similar results for

Table 1 Residual activity of antinutrients of soybean after the isothermical heat treatment.

Antinutrients	Heat treatment temperature, °C			
	60	65	70	75
Lipoxygenase	1	0.25	0	
Urease	1	0.8	0.53	0
Trypsin inhibitor	1	0.94	0.66	0

long (1 hour) heat treatment are given in Table 1 **(Reshetnik, 2007)**.

As can be seen, lipoxygenase is the least stable, the values of thermal stability of a trypsin inhibitor and urease are comparable and closely correlated, but the activity of urease is evaluated by much simpler method, therefore it is often used as a marker **(Erickson, 2002)**. Moreover, the activity of urease is rationed in soya cakes and meals intended for feed production **(Ruis, 2013)**.

The most commonly used model of thermal degradation is the differential equation well known in chemical kinetics in the form as follows **(Romanovsky, 2006)**:

$$Y = - K[T(t)] Y^n dt \quad (1)$$

where Y – quantitative measure of a reagent content; T(t) – the absolute temperature; t – time, K[T(t)] – reaction rate constant; n – reaction order.

We note that such an equation describes not only the processes in chemical reactions, but also, for example, the thermal inactivation of enzymes, bacteria, and so on.

The reaction rate constant in the generalized form can be represented as follows:

$$K[T(t)] = kT^m \exp(\frac{-\varepsilon}{RT}) \quad (2)$$

where k – coefficient of proportionality, c^{-1}; ε – energy of activation, J/mole; R=8.314 – universal gas constant, J/(mole K); T –temperature, K; m – coefficient.

Depending on the physical concepts of the mechanism of intermolecular interaction in the process of thermal degradation in various theories, m accepts value m = 0 (Arrhenius), m = 0.5 (kinetic theory of gases), m = 1 (transient state theory), m = -1 (Hinshelwood). The reaction rate constant in the Arrhenius form is most often used. The use of other forms somewhat changes the coefficients k and ε for their identification but does not significantly affect the accuracy of the approximation of the experimental data.

After the substitution of (2) for (1), separation of variables and integration, we obtain:

$$F(Y) = \int_{Y_0}^{Y} \frac{dY}{Y^n} = k \int_0^t T^m \exp(\frac{-\varepsilon}{RT}) dt \quad (3)$$

where Y, Y_0 – quantitative measures of the reagent content at the current and starting points in time.

In general,

$$F(Y) = \int_{Y_0}^{Y} \frac{dY}{Y^n} = Y^{n-1} - Y_0^{n-1}, \quad n \neq 1,$$

$$F(Y) = \ln(\frac{Y}{Y_0}), \quad n=1.$$

In the case of a constant temperature T = const, solving the equations (3) is not difficult:

$$F(Y) = -kT^m \exp\left(\frac{-\varepsilon}{RT}\right) t \quad (4)$$

Table 2 The values of the coefficients of a model (4) for some substances, when n = 1 and the reaction rate constant in the Arrhenius form (m = 0).

Substance	ε, kJ.mol^{-1}	k, sec^{-1}
Vitamins		
Folic acid (B_9)	70.3	2.10^{10}
Cyanocobalamine (B_{12})	17.2	10^{11}
Enzymes		
Malt amylase	177.1	10^{24}
Lipase		
– dry	104.7	10^{10}
– humid	192.6	10^{28}
Trypsin inhibitor	108.8	10^{12}

Taking the logarithm and subsequent transformations of the expression (4) lead to a nonlinear model:

$$y = k_0 + \varepsilon_R /T + \ln(t) + m \ln(T) \quad (5)$$

where $y = \ln[- F(Y)]$, $k_0 = \ln[k]$, $\varepsilon_R = - \varepsilon /R$,

When m = 0 (the kinetic coefficient is in the form of the Arrhenius), the model becomes linear and for the identification of coefficients it is possible to use the method of linear regression analysis. In this form, it describes the results of the experiments on inactivation of antinutrients of soybean **(Chen et al., 2014; Kargov et al., 2015)**.

In the contrary case, the nonlinear regression analysis methods are used, including also directly in respect to (4). By this means, the expression (4) allows us for evaluating thermal degradation of the object under isothermal conditions with the known coefficients k, m and ε. The values of the coefficients can be obtained as a result of their identification by the results of the experiments. For fixed n and m and involving the empirical data for the dependence Y (T, t = const), in the models (4) and (5), it is possible to identify the coefficients ε and k.

The models of thermal degradation of biorefinery of grain are of theoretical and practical interest. A series of evaluation of the model coefficients reported in the literature or obtained by processing the results of the described experiments, are given in Table. 2.

Table 3 The values of the parameters of a model (4), when n = 1.

Model coefficients				
m	**k**	**ε, kJ.mol^{-1}**	**ε_R, K**	**Squared multiple correlation, R^2**
1	7.327×10^{8} K^{-1} s^{-1}	103.8	12483	
0	7.754×10^{11} s^{-1}	107.0	12872	0.99
-1	8.205×10^{14} K s^{-1}	110.2	13261	

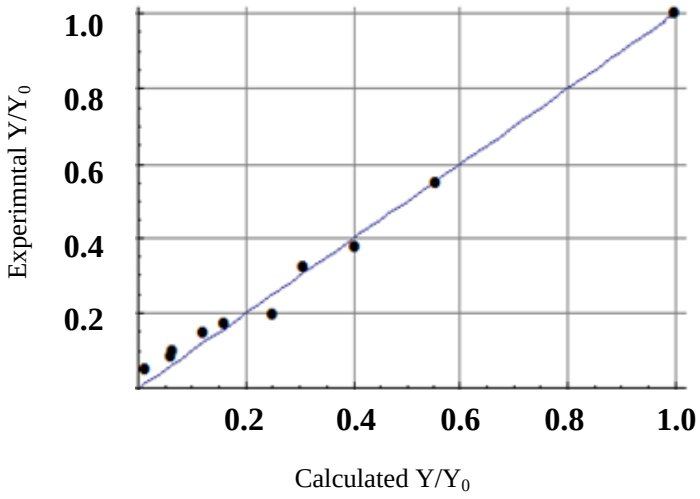

Figure 1 The results of the experiment and calculation on a model (4), when m = 1.

The values of the coefficients depend substantially on external factors, for example, with increased humidity, the parameters are also increased, as is the case with the example of lipase. Environment acidity plays an important role.

Let's consider the process of thermal degradation of a trypsin inhibitor in soybean **(Egorov et at., 1986)**. One of such methods of inactivation is autoclaving, for which, in a given paper, there are presented the experimental data on residual activity a trypsin inhibitor with variation of time, temperature and pressure within the ranges of 0 <t <900, c, 383 <T <398, K, 0.14 <P <0.23, MPa. Unfortunately, the experiment is not set up correctly, since the pressure also changed with the temperature, however, it is difficult to avoid this with this method of heat treatment. Therefore, the effect of pressure in explicit form was not taken into account, howe ver, it indirectly affects the energy of activation of the process, lowering it to some extent.

As a result of the identification, there have been obtained the values of the coefficients presented in Table 3, which,

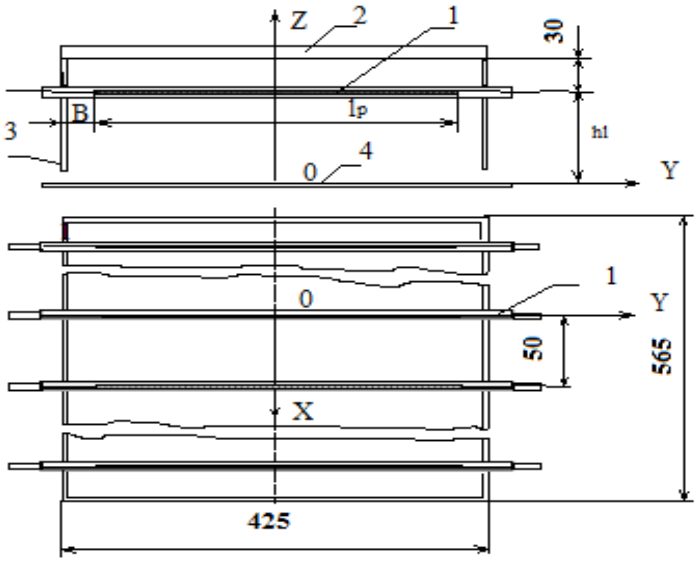

Figure 2 Diagram of the experimental-industrial block of the emitters: 1. The KGT-1000-220-type infrared source; 2 – Top reflector; 3 – Side reflector; 4 – A working surface of the processing zone.

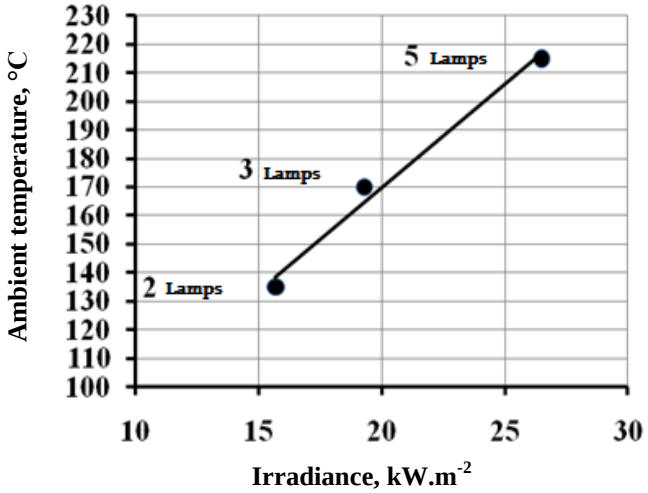

Figure 3 The dependency diagram of the ambient temperature in the treatment zone on the irradiance.

although they differ from each other, but, taking into account the activating influence of pressure, they fairly well correspond to the data of Table. 2.

Figure 1 graphically represents estimated and experimental data corresponding to the model (4).

We note that the obtained values of the coefficients are close to the values presented in Table 1 for a trypsin inhibitor, but they are applicable to the autoclaving operation, and how they correspond to other heat treatment methods, can be demonstrated only by the comparative experiments. The use of the reaction rate constants in the various forms (m = -1; 0; 1) leads to some change in the identified parameters (mostly of the coefficient of proportionality) but does not affect the accuracy of the approximation.

Scientific hypothesis

An essential part of antinutrients in soybean is a trypsin inhibitor – a substance of protein nature. At elevated temperatures, its activity decreases due to the destruction of its structure. In cakes, such inactivation is provided by toasting. Similar results can be expected for soybean grain when heating in a flux of infrared radiation. As a model of the inactivation process, it is expedient to test the modified Arrhenius equation taking into account the variable product temperature. The similar dependences on energy exposure are shown in Figure 5.

MATERIAL AND METHODOLOGY

High-temperature micronization (HTM) is the operation of heat treatment of product in the flux of infrared (IR) radiation **(Zverev, 2009)**. Heat transfer is carried out in two ways: convective from the air in the treatment zone, and radiative (IR radiation). Therefore, we can talk about the combined heat supply.

The industrial installations based on this principle of heating are used in small and medium-sized grain processing enterprises in the processes of the production of instant cereal, cereal flakes, feed ingredients, including for disinfection and inactivation of antinutrients. The heating process, as a rule, is carried out with a high thermal head and is limited by the time of the beginning of the browning of the grain surface. The temperature of product varies continuously throughout the entire treatment period, that is, the process is substantially non-isothermal.

The experiments on IR heating of soybean were carried out on a laboratory facility with the quartz halogen linear infrared emitters of the KGT-1000-220 type.

Diagram of a facility is given in Figure 2.

The experiments were carried out with a fixed height of the lamp h_l = 100 mm. Variation with irradiation was carried out by changing the number of lamps per unit area. Change in the number of lamps, that is, of the installed total power of the radiators in a fixed closed volume of the working area of a laboratory facility leads to increased ambient temperature. The graph of the interdependence of irradiance and ambient temperature in the treatment zone is given in Figure 3. The value of irradiation was estimated by a calculation according to a specially developed program, the activity of urease - according to State Standard GOST 13979.9-69 (Cakes and meals. Method – for performing measurements of urease activity), the initial moisture – according to State Standard GOST 13586.5-2015 (Grain. Method for determination of moisture content).

The soybean grain was placed in a monolayer on a pallet, which for a fixed time was placed in a heated treatment zone. Then, it was poured into a thermally insulated tank, where using a thermocouple and an electrnic thermometer, the average temperature of its mass was determined.

Statisic analysis

Nonlinear modeling was carried out by using an application software package “STADIA-6", developed at the M.V. Lomonosov Moscow State University, **(Kulaychev, 1999)**.

Scoping the adequacy of the models is a complex procedure, requiring high computational costs, which are rapidly growing with dimensions of space of external parameters. By the volume, this task may greatly exceed the task of parametric optimization of a model itself (especially in the case of a nonlinear model), that’s why for the newly-designed objects, it may not be resolved. Some indication of the adequacy of the models is provided by the Squared multiple correlation, R2. In addition,

directly in the diagrams we can see that the residual dispersion and the dispersion medium differ considerably.

RESULTS AND DISCUSSION

The independent variables for the IR heating of the soybean monolayer were the irradiance on the surface of the grain monolayer, the moisture content of the grain, and the processing time. Dependent variables are the temperature of grain and urease activity.

Figure 4 illustrates the empirical dependences of urease activity on the heat treatment time in the range of variation with the initial moisture content W0 = 8-30% for different irradiations on the surface of the monolayer of grain.

Similar dependences on the energy exposure (the product of irradiance for the processing time) are given in Figure 5.

Figure 6 illustrates the experimental data of urease activity depending on the soybean heating temperature with different initial humidity in a wide range of irradiation variation.

The dependences of the activity of urease in soya on time of heat treatment and energy exposure are invariant to its initial moisture content, but depend on the irradiation on the surface of a monolayer of grain. With the growth in the irradiation, with equal processing time or energy exposure, the degree of inactivation increases. It should be noted that during the process of the experimnet the irradiation was changing by increasing the number of IR emitters, which also led to a temperature riuse in the treatment zone.

That is, With the growth in the irradiation, the intensity of heat transfer was increased, including due to the growth in ambient temperature.

If we consider the dependence of urease activity on the temperature of product, they will be be invariant to the heating regimes, but depend on the initial humidity of grain.

As a model of the process of urease inactivation, we use the dependence (3). In the case of infrared heat treatment, the process proceeds in an unsteady period, that is, the temperature is a function of time, which, in this case, leads to the nonlinear dependencies. The identification of coefficients of such models according to experimental data is a much more complex task, although a number of packaged applications have already been developed. The reliability of the estimates is higher, the less is the number of the estimated parameters, and the more information is available. Often, the values of the parameters obtained, depend on the initial values, since the residual functions (in particular, the sum of the least squares) may have several minimum values. So, there is always the need to check the coefficient values obtained on their compliance with a common sense and with data from independent sources. For example, knowingly we have to abandon negative values if, from physical considerations, the value of the coefficient must be positive, and so on.

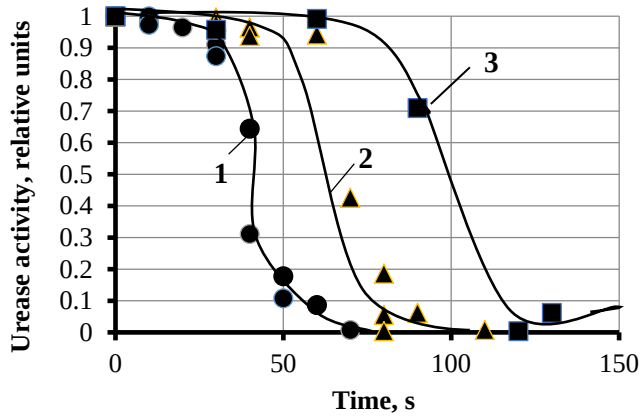

Figure 4 The dependence of urease activity in soybean (humidity 8 – 30%) on processing time with the irradiation as follows: 1 – 26.5 kW.m^{-2}; 2 – 19.3 kW.m^{-2}, 3 – 15 kW.m^{-2}.

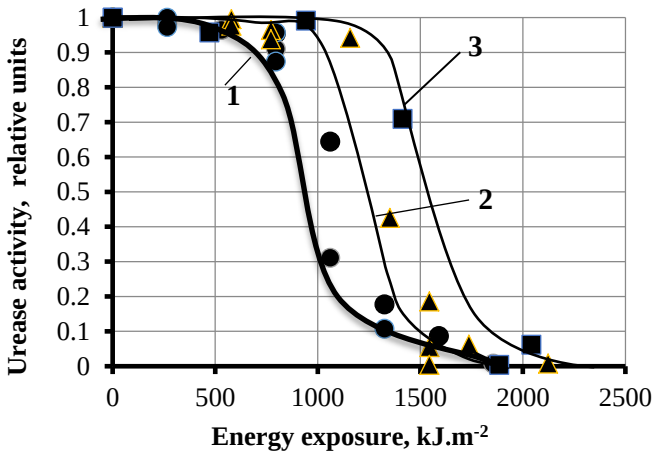

Figure 5 The dependence of urease activity in soybean (humidity 8-30%) on energy exposure with the irradiation as follows: 1 – 26.5 kW.m^{-2}; 2 – 19.3 kW.m^{-2}, 3 – 15 kW.m^{-2}.

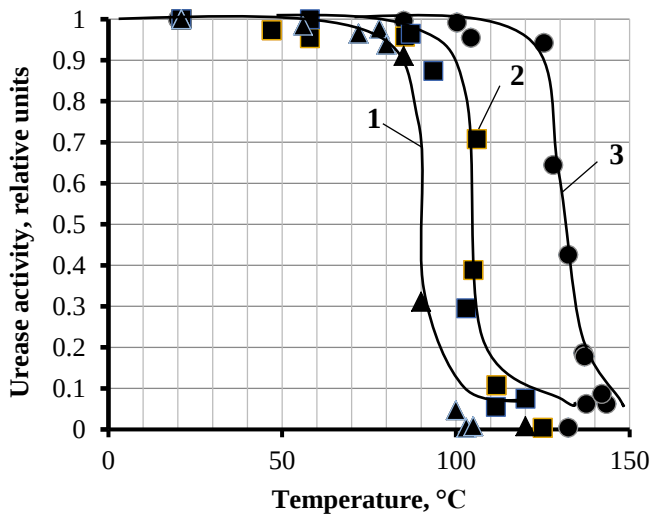

Figure 6 The dependence of urease activity in soybean on itys temperature (E = 15,7–26,5 kW.m^{-2}) with humidity: 1 – 30%; 2 – 17%, 3 – 8%.

Table 3 The values of the coefficients of the models (10) and (11), when C = ∞, n = 1.

Model	W, %	k	ε, kJ.mol^{-1}	ε_R, K	Squared multiple correlation, R^2
(10)	8	1.368×10^{16}	113	13541	0.93
	17	4.172×10^{22}	138	16603	0.99
	30	3.530×10^{86}	582	70378	0.99
(11)	8	2.460×10^{12} K^{-1}	117	14081	0.89
	17	2.384×10^{16} K^{-1}	138	16595	0.99
	30	4.966×10^{81} K^{-1}	585	70376	0.99

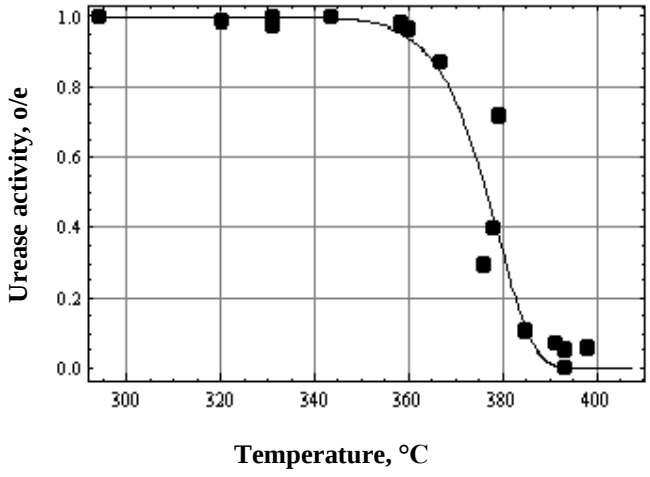

Figure 7 Experimental and calculating dependences of urease in soybean with the irradiation *E* = 15,7 – 26,5 kW.m^{-2} and humidity 17%.

The dependence of the temperature of product on time during IR heating is well described by the expression **(Egorov et al., 1986)**.

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

whence it follows that $dt = dT/[K_t(C - T)]$, (7)

where t – time;

$C = T_0 + \Delta T_{\infty} > T$;

T_0 – initial temperature of grain;

ΔT_{∞}, K_t – coefficients.

Upon integrating (3), we obtain

$$Y = k\{- T \exp(-\varepsilon_R/T) - C \exp(-\varepsilon_R/C) Ei(z) + +(C - \varepsilon_R) Ei(-\varepsilon_R/T), \quad m = 1 \quad (8)$$

$$Y = k \{Ei(-\varepsilon_R/T) - Ei(-\varepsilon_R/T_0) + \exp(-\varepsilon_R/C) [Ei(z_0)] - - Ei(z)], \quad m = 0 \quad (9)$$

and $Y = k [Ei(z_0) - Ei(z)]$, $m = -1$ (10)

$z = \varepsilon_R(1/C - 1/T)$, $z_0 = \varepsilon_R(1/C - 1/T_0)$, $C = T_0 + \Delta T_{\infty} > T$

where Ei – an exponential integral function (Eulerian function));

k, C and ε_R – empirical coefficients.

Comparative analysis of the integrals (8, 9, 10) in a wide range of variation by the parameters ($50000 < \varepsilon_R < 1000$, K; $T_0 = 273$, K; $75 < \Delta T_{\infty} < 200$, K; $0.005 < K_t < 0.5$, c^{-1}; $0 < t < 120$, c) shows that their graphs lie well on each other with an appropriate selection of the constant factor k. Therefore, in the indicated range of variation by variables, it is advisable to use the simplest form (10) as a model, identifying the coefficients k, ε R and C from the results of the Y (T) experiments.

However, the identification of three parameters might cause difficulty. Additional information on the value of C can be obtained by assessing the parameters of the temperature dependence (4), according to the results of the experiments T (t).

If we make one more assumption, namely, C is much larger than T, i.e. $z \approx -\varepsilon_R/T$, then the expressions (10) can be simplified further, reducing them to a two-parameter model.

The Eulerian function is not always convenient for work. Let us therefore use its property for large values of the argument. In this case the following expansion is fair

$Ei(z) = [\exp(z)/z] (1 + 1/z + 1/z^2 + \ldots)$,

which, taking into account the first term, will lead (10) to the expression:

$$Y = k[T \exp(-\varepsilon_R/T) - T_0 \exp(-\varepsilon_R/T_0)] \quad (11)$$

The values of the identified coefficients are given in table 3.

Experimental data and the approximating curve for humidity W = 17% are shown in Figure 7.

CONCLUSION

Heat treatment, including in a flux of IR radiation, provides effective inactivation of urease and a trypsin inhibitor in soya when product is heated above 100°C.

The dependences of the level of urease activity on time and energy exposure are invariant to the initial moisture content of product, and the similar temperature dependences - to the initial moisture content.

The results of thermal inactivation under conditions of increasing temperature do not depend on the IR processing regimes, in particular on time, and they are determined only by the initial moisture content of grain, and by the end heating temperature. The lower is the humidity, the higher the temperature is needed for heating.

Since the energy exposure reflects the energy inputs for heating of product located on the unit of the working surface of the treatment area, the inactivation efficiency is the higher, the higher is the used irradiation. However, quick browning of the grain surface imposes restrictions on the upper limit of the intensity of heat supply.

The proposed model of inactivation of a trypsin and urease inhibitor, under conditions of unsteady temperature, describes quite well the experimental results. We can try to generalize the model by including humidity as an independent variable.

The considered models can be used for the thermal degradation processes and other thermolabile substances under the similar heating conditions.

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