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MODELLING OF THE PROCESS OF MICROMYCETUS SURVIVAL IN FRUIT AND BERRY SYRUPS

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Abstract. In order to develop methods for preserving fruit and berry syrup, which exclude the use of high-temperature sterilization and preservatives, the survival of spores of micromycetes (*B. nivea* molds) in model media with different concentration of food osmotically active substances (sucrose, ethyl alcohol, citric acid) at a certain concentration of lethal effects on microorganisms. It has been established that model media (juice based syrups from blueberries) with a mass content of 4 % and 6 % alcohol, 50 % sucrose, 1 % and 2 % titrated acids, have a lethal effect on spores of *B. nivea* molds. The regression equation is obtained expressing the dependence of the amount of spores of *B. nivea* molds on the concentration of sucrose, acid, alcohol and the storage time of syrups. The form of the dependence and direction of the connection between the variables is established – a negative linear regression, which is expressed in the uniform decrease of the function. The estimation of quality of the received regression model is defined. The deviations of the calculated data from the data of the initial set are calculated. The proposed model has sufficient reliability, since the regression function is defined, interpreted and justified, and the estimation of the accuracy of the regression analysis meets the requirements.

Key words: micromycetes, osmotically active substances, mold fungi, canning, syrups, regression model.

МОДЕЛЮВАННЯ ПРОЦЕСУ ВИЖИВАННЯ МІКРОМІЦЕТІВ У ФРУКТОВО-ЯГІДНИХ СИРОПАХ

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Анотація. Із метою розробки способів консервування фруктово-ягідних сиропів, які виключають застосування високотемпературної стерилізації та консервантів, вивчено виживання спор мікроміцетів (плісеневих грибів виду *B. nivea*) у модельних середовищах із різною концентрацією харчових осмотично-дієві речовин (сахарози, етилового спирту, лимонної кислоти), які виявляють при певних концентраціях летальну дію на мікроорганізми. Встановлено, що модельні середовища (сиропи на основі соку з ягід чорниці) з масовою часткою етилового спирту 4 % і 6 %, сахарози – 50 %, титрованих кислот 1 % і 2 % виявляють летальну дію на спори плісеневих грибів виду *B. nivea.* Отримано рівняння регресії, що виражає залежність кількості спор плісеневих грибів виду *B. nivea* від концентрації сахарози, кислоти, етилового спирту і тривалості зберігання сиропів. Встановлено форму залежності та напрямок зв'язку між перемінними – негативна лінійна регресія, яка виражається в рівномірному спаданні функції. Визначено оцінку якості отриманої регресійної моделі. Розраховано відхилення розрахункових даних від даних вихідного набору. Запропонована модель має достатню надійність, оскільки функція регресії визначена, інтерпретована і обґрунтована, а оцінка точності регресійного аналізу відповідає вимогам.

Ключові слова: мікроміцети, осмотично-дієві речовини, плісневі гриби, консервування, сиропи, регресійна модель.

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Introduction. Formulation of the problem

The main stimuli of microbe spoiling of fruit and berry syrups are micromycetus (mouldy fungi and yeast). The traditional technology of canned fruit and berry syrups provides for their sterilization or adding preservatives with the aim of providing the microbe stability. The lasting high temperature treatment of syrups (sterilization) results in formation the products of reaction of melanoidins formation, in particular oxymethylphurphurol, polymerization of phenol compounds, degradation of thermolabile biologically active compounds, deteriorating organoleptic indices and reducing food value of finished products. Adding of chemical preservatives is harmful for one's health and results in reducing of food and consumer value.

With the aim of developing the ways of canning of fruit and berry syrups, excluding application of high temperature sterilization and preservatives, survival of micromycetus in model mediums with different concentration of food substances with osmosis activity (saccharose, ethyl alcohol, citric acid), has been determined. Spores of mouldy fungi of *Byssochlamys nivea* type were used as a test-culture of micromycetus. Reactivation of the test-culture and obtaining of the spores were carried out by standard and well-known ways [1-6].

Main part

The model mediums were prepared on the basis of bilberry juice, saccharose, citric acid and ethyl alcohol. The ethyl alcohol was introduced into sterilized mixture of the juice with sugar and acid.

The concentration of the ingredients with osmosis activity in model mediums was varied within the following limits:

– mass share of saccharose – 35 – 50 %;
– mass share of ethyl alcohol – 2 – 6 %;
– mass share of titrate acids – 1 – 2 %;
– actual acidity – 3,0 units pH.

Graphic representation of influence of osmosis active food ingredients concentration (ethyl alcohol, saccharose, and organic acids) on survival of mouldy fungi spores in model mediums has been shown in Fig. 1-3.

From the data, given in Fig. 1(a), we can draw a conclusion, that in case of concentration of 35 % of saccharose, 2 % of ethyl alcohol and 1 % of the acid in the model medium, we can note absence of lag-phase and presence of logarithmic phase, lasting for one month, at the stages of mouldy fungi growing. Permanency of titra-spores in the following months can be interpreted in two ways, but, most probably, spores experience the condition of stable anabiosis within six months of inoculation storage.

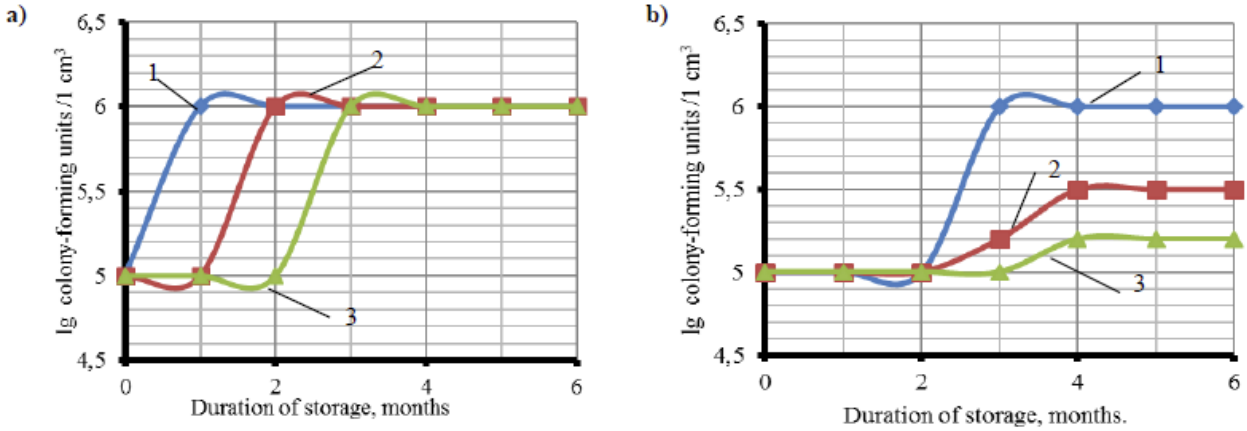

Fig. 1. Influence of ethyl alcohol concentration on survival of mould fungi spores in model mediums with mass share of saccharose – 35 %, titrate acids 1 % (a) and 2 % (b): 1, 2, 3 – mass share of ethyl alcohol is 2 %, 4 %, 6 % correspondingly.

For mouldy fungi in model medium of the above – mentioned composition, but with concentration of ethyl alcohol, making 4 %, presence of lag-phase lasting one month is typical, and with concentration of ethyl alcohol making 6 %, it lasts for 2 months. That is, with ethyl alcohol concentration increase up to 4 % and higher, adaptation of microorganisms becomes slower concerning time.

As the acidity increases up to 2 % (Fig. 1b), presence of a lag-phase of the test-culture has been noted under all concentrations of the ethyl alcohol. When concentration of the ethyl alcohol reaches 2 % and 4 %, it lasts for 2 months, under 6 % it lasts 3 months. Then the phase of logarithmic growth occurs (reproduction of the cells with their titer increase occurs, the value of the titer is in the inverse dependence from alcohol concentration), which occurs one month, and then comes the stage of anabiosis during the next six months of storage. At this stage the investigated model mediums of the above – mentioned composition render static effect on spores of mouldy fungi.

The data, given in Fig. 2(a) testify that in model medium with mass share of saccharose – 42 %, ethyl alcohol – 2 %, titrate acids – 1 %, both a lag-phase and a logarithmic phase of growing are absent in growing of the mouldy fungi spores. Most probably, that in the medium of the above-mentioned composition the spores of micromycetus are in the condition of stable anabiosis. As concentration of ethyl alcohol increases, after two months of stable anabiosis, the phase of cells dying off occurs, which lasts 1 month if the concentration of the alcohol is 4 %; and 2 months if the concentration is 6 %, then the condition of stable anabiosis begins again.

Increase of acids concentration up to 2 % intensifies lethal effect of ethyl alcohol; the data, given in Fig. 2b, testifies to it. So, when the concentration of the alcohol reaches 2 %, the condition of anabiosis lasts the first three months, then the phase of cells dying off occurs and it lasts one month and then comes stable anabiosis again. When the concentration of the alcohol reaches 4 %, the condition of anabiosis lasts one month, then the phase of dying off occurs and stable

anabiosis comes again. When the concentration of the alcohol reaches 6 %, we can watch destruction of spores (decrease of a titer by one order), then the condition of stable anabiosis comes.

The given results testify that model mediums of the above – mentioned composition make mouldy fungi growing difficult, make partial lethal effect, but don't lead to their complete destruction.

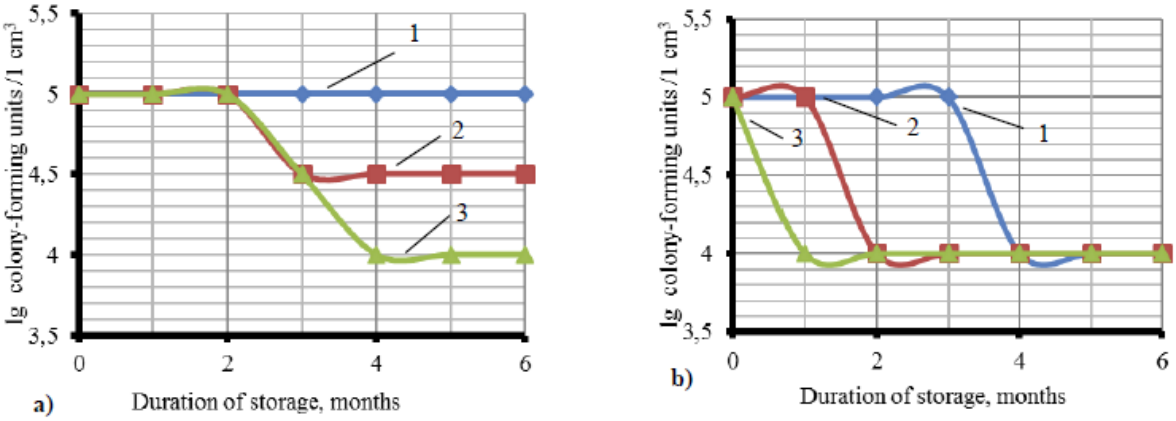

Fig. 2. Influence of ethyl alcohol concentration on survival of mouldy fungi spores in model syrups with mass share of saccharose – 42 %, titrate acids 1 % (a) and 2 % (b): 1, 2, 3 – mass share of ethyl alcohol is 2 %, 4 %, 6 % correspondingly

The data, given in Fig. 3, testify that model mediums with mass share of saccharose 50 %, titrate acids 1 % and 2 %, ethyl alcohol 4 % and 6 %, make a lethal effect on mouldy fungi spores, as during the whole period of storage, cells dying off occurs; reduce of titer testifies to it. The test-culture in the mediums with mass share of ethyl alcohol – 2 %, titrate acids – 1 %, Fig. 3(a), are in condition of anabiosis during one month, which transfers to the phase of cells dying off within a month, but further stable anabiosis occurs again. This very model medium, but with mass share of titrate acids 2 %, intensifies the effect of ethyl alcohol, as we can watch the phase of dying off the test-culture at once; this phase lasts for one month and transfers to the phase of anabiosis.

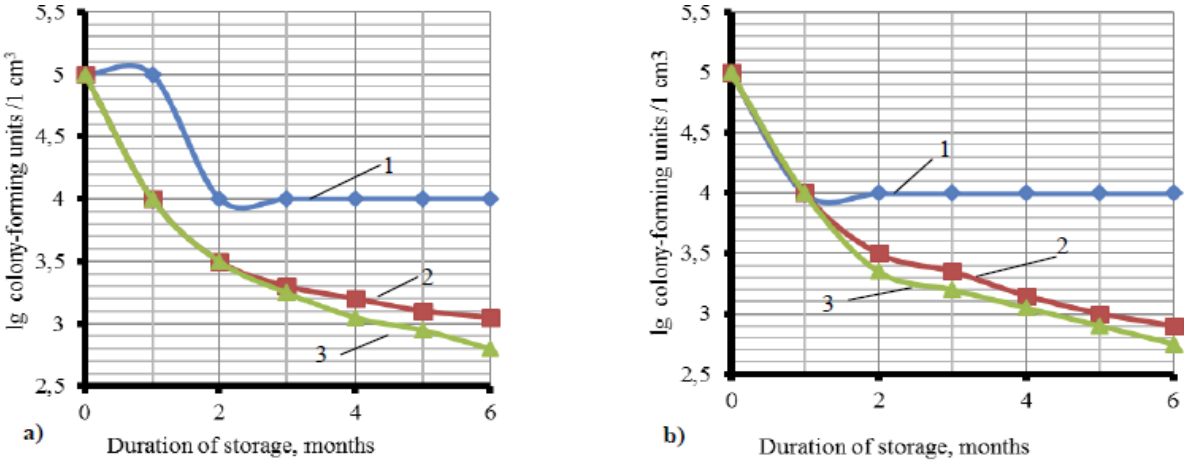

Fig. 3. Influence of concentration of ethyl alcohol on survival of mouldy fungi spores in model syrups with mass share of saccharose – 50 %, titrate acids 1 % (a) and 2 % (b): 1, 2, 3 – mass share of ethyl alcohol is 2 %, 4 %, 6 % correspondingly.

Mathematical modeling. As the results of the experiments always contain some error, it's necessary to carry out correlation and regressive analysis, which allows to approximate the experimental data.

The main task of the correlation analysis is determining of tightness and direction of the connection between the values being studied. The components of the correlation matrix are given in table 1.

The analysis of the data, given in table 1, allows to make the following conclusions:

– correlation dependence exists only between survival of mouldy fungi spores of *B. nivea* type, concentration of every ingredient from the fruit and berry syrups composition (saccharose, organic acids, alcohol) and storage duration;
– dependence between the components wasn't revealed;

– the coefficient of correlation makes 0.

Therefore, a regressive model for determining the analytical expression of relation of the dependent random value Y – the amount of mouldy fungi spores of *B. nivea* type (effective sign) with independent random values; X_1 – storage duration; X_2 – saccharose; X_3 – acid; X_4 – alcohol (factors), can be composed.

For fulfilling the regressive analysis, the following stages of modeling of spores survival of *B. nivea* mouldy fungi in fruit and berry syrups, have been chosen.

1. Determining of the analytical form of a regression equation and determining of the regression parametres.

2. Revealing the degree of stochastic interrelation of the resulting criterion and factors in the regression, checking of the common quality of the regression equation.

3. Checking of the statistic importance of every coefficient of the regression equation and determining of their confidential intervals.

The results of the regressive statistics have been given in table 2.

Table 1 – Components of the correlation matrix

Signs and factors of the system	Amount of spores *B. nivea*, Y	Duration, X_1	Saccharose, X_2	Acid, X_3	Alcohol, X_4
Amount of spores *B. nivea*, Y	1				
Duration, X_1	-0,18	1			
Saccharose, X_2	-0,79	0	1		
Acid, X_3	-0,16	0	0	1	
Alcohol, X_4	-0,23	0	1,13E-17	0	1

Table 2 – The results of the regressive statistics

Regressive statistics	
Plural R	0,86
R^2	0,73
Fixed R^2	0,72
Standard mistake	0,48
Observations	126

The results of the regressive statistics, in particular, $R^2 > 0,7$ point out that the variation of the resulting criterion Y is stipulated, mainly, by influence of X-factors included in the regressive model.

The calculated values of the regression coefficients and their statistic estimations are given in table 3.

The calculated and given in the table regression coefficients a_i allowed to bring out the equation, determining the dependence of the amount of mouldy fungi spores of *B. nivea* type on concentration of saccharose, acid, alcohol and storage duration.

Table 3 – Values of the regression coefficients and their statistic estimations

Signs and factors of the system	Regression coefficients, a_i	Standard mistake	t-statistics	P-value	Lower 95 %	Upper 95 %
Y-crossing	10,71	0,35	30,97	0,00	10,03	11,40
Duration, X_1	-0,08	0,02	-3,89	0,00	-0,13	-0,04
Saccharose, X_2	-0,12	0,01	-16,71	0,00	-0,13	-0,10
Acid, X_3	-0,30	0,09	-3,49	0,00	-0,47	-0,13
Alcohol, X_4	-0,13	0,03	-4,82	0,00	-0,18	-0,07

$$y = 10,71 - 0,08x_1 - 0,12x_2 - 0,3x_3 - 0,13x_4$$

The value of the plural determination coefficient R = 0,86 means that 86 % of the general variation of the resulting criterion is explained by the variation of factor characteristics X_1, X_2, X_3, X_4.

It means that the chosen factors essentially influence the amount of spores of mouldy fungi of *B. nivea* type, and it affirms correctness of including them into the suggested model.

Regression coefficients a_i are significant; P = 0 testifies to it, because it is less than the given level of significance a = 0,05 (table 3).

Conclusions

1. It has been determined that the model mediums (syrups on the basis of the juice from bilberries) with mass share of alcohol 4 % and 6 %, saccharose – 50 %, titrate acids 1 % and 2 % make a lethal effect on mouldy fungi spores of *B. nivea* type.

2. The regression equation, expressing dependence of the amount of spores of the mouldy fungi of *B. nivea* type on concentration of saccharose, acid, alcohol and duration of syrups storage, has been obtained. The form of dependence and direction of interrelation between the variables – negative linear regression, which is expressed in uniform reduction of the function, have been determined.

3. The estimation of the quality of the received regressive model has been determined.

4. The deviations of the rated data from the data of the primary kit, have been calculated.

5. The suggested model possesses sufficient reliability, because the regression function is determined, interpreted and substantiated, and the estimation of exactness of the regressive analysis meets the requirements.

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МОДЕЛИРОВАНИЕ ПРОЦЕССА ВЫЖИВАЕМОСТИ МИКРОМИЦЕТОВ ВО ФРУКТОВО-ЯГОДНЫХ СИРОПАХ

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Аннотация. С целью разработки способов консервирования фруктово-ягодных сиропов, исключающих применение высокотемпературной стерилизации и консервантов, определяли выживаемость спор микромицетов (плесневых грибов вида *B. nivea*) в модельных средах с различной концентрацией пищевых осмотически деятельных веществ (сахарозы, этилового спирта, лимонной кислоты), оказывающих при определенной концентрации летальное действие на микроорганизмы. Установлено, что модельные среды (сиропы на основе сока из ягод черники) с массовой долей спирта 4 % и 6 %, сахарозы 50 %, титруемых кислот 1 % и 2 % оказывают летальное действие на споры плесневых грибов вида *B. nivea*. Получено уравнение регрессии, выражающее зависимость количества спор плесневых грибов вида *B. nivea* от концентрации сахарозы, кислоты, спирта и продолжительности хранения сиропов. Установлена форма зависимости и направление связи между переменными – отрицательная линейная регрессия, которая выражается в равномерном убывании функции. Определена оценка качества полученной регрессионной модели. Рассчитаны отклонения расчетных данных от данных исходного набора. Предложенная модель обладает достаточной надежностью, поскольку функция регрессии определена, интерпретирована и обоснована, а оценка точности регрессионного анализа соответствует требованиям.

Ключевые слова: микромицеты, осмотически деятельные вещества, плесневые грибы, консервирование, сиропы, регрессионная модель.

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