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DEGRADATION KINETICS OF L-ASCORBIC ACID IN FOOD MATRIX OF JELLY DURING STORAGE

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The article presents the results of the study of the kinetics of degradation of ascorbic acid (AA in odel jelly during time storage at temperature 4 °C. Jelly samples based on concentrated apple juice and gelatin with a content of 2% and 3% were enriched with AA in the amount of 25, 50 and 75 mg/100 g of the sample.

Determination of AA content carried out by galvanostatic coulometric titration. It was found that the content of AA in samples of fruit jelly decreases with increasing storage time. The loss of AA is observed more pronounced in the initial period of storage and is more characteristic of the sample containing 2% gelatin compared with the sample containing 3% gelatin.

The mechanism of ascorbic acid degradation is considered as a reaction of its multi-stage oxidation with the dominant stage of oxidation to dehydroascorbic acid. The simulation of the degradation of ascorbic acid in the jelly during storage using the equations of the kinetics of the reaction of zero and first order. The goodness of model fitting to the experimental data was evaluated by adjusted coefficients of determination and the root mean square error. Compared with the zero order, the kinetics of degradation corresponded better to the first-order reaction model for all the studied samples. The reaction rate constant and half-life calculated for a first-order kinetic model are 0.00027 (1 / hour) and 107 days for samples with gelatin content of 2% and, respectively, 0.00015 and 193 days for jelly with a gelatin content of 3%. The obtained values of the first-order reaction rate constants for samples of model jelly correspond to similar values in other food products (juices, drinks, some solid products). The magnitude of the half reaction times allows the residual content of ascorbic acid to be estimated over time.

The results are important for the development of technology for ascorbic acid-rich foods.

Keywords: *ascorbic acid, fruit jelly, degradation kinetics, storage time, gelatin, apple juice.*

КІНЕТИКА РОЗКЛАДАННЯ АСКОРБІНОВОЇ КИСЛОТИ В ХАРЧОВІЙ МАТРИЦІ ЖЕЛЕ ПІД ЧАС ЗБЕРІГАННЯ

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Наведено результати дослідження кінетики розкладання аскорбінової кислоти (АК) у модельних желе під час зберігання за температури близько 4 °С. Желе з додаванням соку яблучного концентрованого та желатину з *вмістом 2% і 3% були збагачені АК у кількості 25, 50 і 75 мг/100 г зразка. Визначення вмісту АК проведено методом гальваностатичного кулонометричного титрування.*

Виявлено, що вміст АК у зразках желе зменшується зі збільшенням часу зберігання. Втрата АК більш виражена в початковий період зберігання і більшою мірою характерна для зразка з вмістом 2% желатину порівняно зі зразком із 3% желатину.

Проведено моделювання деградації АК під час зберігання з використанням рівнянь кінетики реакції нульового та першого порядку. Порівняно з нульовим порядком кінетика деградації краще відповідала моделі реакції першого порядку для всіх дослідних зразків. Розрахована для кінетичної моделі першого порядку константа швидкості реакції й період напіврозпаду дорівнюють 0.00027 (1/год) і 107 днів для зразків із вмістом желатину 2% та відповідно 0.00015 (1/год) і 193 дні для желе з вмістом желатину 3%.

Ключові слова: *аскорбінова кислота, желе, кінетика деградації, час зберігання, желатин, сік яблучний.*

КИНЕТИКА РАЗЛОЖЕНИЯ АСКОРБИНОВОЙ КИСЛОТЫ В ПИЩЕВОЙ МАТРИЦЕ ЖЕЛЕ ПРИ ХРАНЕНИИ

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Приведены результаты исследования кинетики разложения аскорбиновой кислоты (АК) в модельных желе при хранении при температуре около 4 °С. Желе с добавлением сока яблочного концентрированного и желатина с содержанием 2% и 3% были обогащены АК в количестве 25, 50 и 75 мг/100 г образца. Определение содержания АК проведено методом гальваностатического кулонометрического титрования.

Выявлено, что содержание АК в образцах желе уменьшается с увеличением времени хранения. Потеря АК более выражена в начальный период хранения и в большей мере характерна для образца с содержанием 2% желатина по сравнению с образцом с 3% желатина.

Проведено моделирование деградации АК во время хранения с использованием уравнений кинетики реакции нулевого и первого порядка. По сравнению с нулевым порядком кинетика деградации лучше соответствовала модели реакции первого порядка для всех исследованных образцов. Рассчитанная для кинетической модели первого порядка константа скорости реакции и период полураспада равны 0.00027 (1/час) и 107 дней для образцов с содержанием желатина 2% и соответственно 0.00015 и 193 дня для желе с содержанием желатина 3%.

Ключевые слова: *аскорбиновая кислота, желе, кинетика деградации, время хранения, желатин, сок яблочный.*

Statement of the problem. The process of creating food products enriched with functional components requires an integrated assessment of various aspects: the selection of a suitable raw material source, the detection of biologically active compounds in it, the use of methods for the separation and extraction of these compounds, the implementation of toxicological assessments and, finally, measurements of stability, activity and bioavailability [1]. In the case of enrichment with individual components with known properties, the issue of assessing the stability, primarily of the component being added, becomes a priority. Here we should talk about two types of stability. The first is related to the stability of the component itself in the food matrix of the finished product, both in the process of its creation and during storage during the shelf life. The second important issue is related to the stability of the component in the process of digestion in the gastrointestinal tract and the evaluation of its activity and bioavailability. At the initial stage of product creation, the first factor is fundamental and in many respects determines the efficiency of technological development. From this point of view, research conducted in this direction is relevant.

The above considerations fully apply to food products that include either L-ascorbic acid (AA) or vitamin C as an enriching component. It is known that L-ascorbic acid together with its oxidation product dehydroascorbic acid (DHAA), which has a biological activity equivalent to AA, is often considered in diets as water-soluble vitamin C [2]. This vitamin is an antioxidant, playing an important role in the regulation of oxidation-reducing processes. The role of vitamin C in folic acid and iron metabolism, the synthesis of collagen and procolagen, as well as in the synthesis of steroid hormones and catecholamines, etc., is known [3]. Vitamin C is often added to foods not only as a nutrient and an antioxidant, but it is added also in order to prevent the browning of fresh and canned fruits and vegetables, the acidification of meat and other [4]. Vitamin C is often added as a fortificant to fruit juices, drinks and jellies.

Jelly products, is a fairly traditional and popular food. Enriching it with various vitamins and vitaminized premixes allows you to consider this product as a source of essential nutrients in the human diet.

Review of the latest research and publications. Ascorbic acid decays by oxidation while DHAA is hydrolyzed. Various factors of influence on degradations of ascorbic acid, in particular in foods, are discussed in a large number of review publications.

So, according to reviews [2; 5; 6], a decrease in the concentration of ascorbic acid in food occurs by aerobic and/or anaerobic pathways. Their rates of decay are influenced by various factors such as their concentration in the sample, temperature, light, pH, dissolved oxygen, and the presence of oxidizing or reducing agents, water activity, and time of storage.

Numerous literature sources (for example, [7–9]) consider the process of degradation of ascorbic acid as a sequential chemical mechanism of reversible and irreversible reactions, the kinetic model of which can be represented by the equation (1):

$$AA \underset{k_2}{\overset{k_1}{\Leftrightarrow}} DHA \xrightarrow{k_3} products \qquad (1)$$

According to [9], the first stage of this model, associated with the transformation of AA into DHA, can be described in terms of a first-order kinetic equation. At the same time, in numerous studies, starting with the study of the degradation of AA in DHA in degassed juices [10], under the condition of an initial low oxygen content, a zero-order reaction model is applied to the experimental data. In this way, prediction of AA loss during storage and non-isothermal heat processes with non-linear kinetic models zero- and first-order reaction [11]:

$$\text{zero-order:} \qquad c_t = c_o - kt, \qquad (2)$$

$$\text{first-order:} \qquad c_t = c_o e^{-kt}, \qquad (3)$$

where c_o is AA concentration (mg/100g of fruit jelly) at the given time t=0, c_t is a concentration (mg/100 g of fruit jelly) at any given time (t), k is the reaction rate constant (mg/gh for zero-order or h^{-1} for first-order) and t is the storage time (h).

In addition to these expressions (2) and (3), to describe the degradation of AA, more complex kinetic models are used that take into account the behavior of the first stage of the mechanism (1) [12].

In tab. 1, the literature data [12; 14–23] on the study of the degradation of ascorbic acid in some food systems are given. The focus is on food systems that contain fruit. At the same time, they proceeded from the fact that jelly contains fruit as the main ingredient [13], despite some national peculiarities and differences in the definition of the product jelly.
As can be seen from the table 1, foodstuffs in the form of fresh fruits, various juices and jams made from them are mainly objects in such studies. Data analysis allows us to trace the following patterns that are important when conducting the experiment on degradation of AA in jelly:

1. In all studies, the fact of the degradation of ascorbic acid, leading to a decrease in its content in the process of increasing shelf life.

Table 1

Research of ascorbic acid degradation kinetics in foodstuffs

Food matrix	Degradation kinetic models	Influence parameters on AA degradation	Result	Ref.
1	2	3	4	5
Fresh strawberry juices	Zero-order	Storage, temperatures and sugar addition	Ascorbic acid concentration in all strawberry juices were decreased with time upon storage. The degradation reaction rate constants were decreased when the juices were stored at the refrigerated temperature and also upon sugar addition	[14]
Orange; orange juice	Zero-order first-order	Storage, temperatures	Ascorbic acid concentration decrease with increase in storage time and temperature. The degradation kinetics was better fitted to the *first order* reaction model for both orange and orange juice	[15]
Blanched and unblanched peas	First order reversible consecutive	Frozen storage	The loss of vitamin C during frozen storage depends on the balance of oxidation and reduction capacities. Inactivation of oxidative enzymes and decrease of atmospheric oxygen in the tissues may be the reasons for the increased retention of vitamin C in blanched peas during frozen storage	[12]
Four citrus juices concentrates	First-order	Storage, temperatures	Ascorbic acid in citrus juice concentrates decreesed with increasing temperature. The loss of ascorbic acid in citrus juice concentrates at all storage temperatures was described as a *first-order* reaction	[16]

Continuation of Tabl. 1

1	2	3	4	5
Pasteurised blood orange juice	Zero-order half-order first-order second-order	Storage, temperatures, degassing	Ascorbic acid degradation loss during storage were best explained by *first-order* kinetic model. The impact of storage temperature and degassing are the most influent factors in the degradation of ascorbic acid	[17]
Pasteurised and high pressure processed reconstituted orange juice	Second stage degradation: first-order and zero-order	Storage, temperatures	Ascorbic acid degradation rates were lower for high pressurised juice, leading to an extension of its shelf life compared to conventionally pasteurised juice. The more rapid decrease of ascorbic acid concentration at the beginning of storage can be attributed to the immediate reaction of an amount of ascorbic acid with the dissolved oxygen	[18]
Cornelian cherries dried	First-order	Storage, temperatures	*First-order* reaction kinetics was found for the degradations of AA and its content of dried samples decreased depending on decreasing drying temperature	[19]
Fruit yogurt	–	Storage	L-ascorbic acid concentration in yogurts was affected by storage time and decreased significantly	[20]
Red raspberry jams	–	Storage	During the first three months of storage, AA concentration decreased on average by 83%	[21]
Mixed cerrado fruit jam	–	Storage, temperatures	Vitamin C value showed a decrease at the two temperatures, 25 and 35 °C, during storage	[22]
Strawberry jam	First-order	Storage, temperatures	Ascorbic acid degradation followed *first-order* kinetics where the rate constant increased with an increase in the temperature	[23]

2. The process of AA degradation proceeds more intensively at a higher storage temperature.

3. To describe the process of degradation of ascorbic acid, kinetic models of reactions of zero and first orders are used in most studies.

4. In the majority of studies, the statistical analysis of experimental data on the determination of the order of the AA degradation reaction in these systems testifies to the better descriptive possibility of the first order.

Apparently, these patterns will be true for the process of preserving AA in the food multicomponent jelly matrix.

Another important factor affecting the loss of AA is the presence of such a component as sugar in the jelly recipe. Based on the data [14], the addition of this component will contribute to the safety of AA.

Comparison of constants in various fortified foods shows that the rate constant of the AA decomposition reaction for both the solid matrix and for liquid matrices is described by a first-order equation [4]. In this case, the values themselves are at the level of the order of 0.0001 1/h and depend on many factors, including the nature of the product matrix.

It should also be noted review of the stability of biocomponents, including ascorbic acid, in fruit jams and jelly [24]. Many of the facts described above are reflected in this review.

The objective of the research was to research of kinetic degradation of L-ascorbic acid in gelatin-based jellies products.

Presentation of the research material.

Materials. The following chemicals used in this research are as follows: potassium iodide (Reachim, Russia), standard titers for a buffer system with pH = 4.01 (Ukraine), gelatin food (240 Bloom, 20 mesh) (Gelita Deutschland GmbH, Germany), apple juice concentrated (mass fraction of dry soluble substances 70%, mass fraction of titrated acids in terms of malic acid 3%, ascorbic acid content 79 mg/100 g of product) (Royal Fruit Garden, Ukraine).

For preparation of the solutions distilled water with electric conductivity 3 μS/sm was used. This parameter was measured by a conductometer CEL-1M2 (Analitpribor, Georgia).

Sampling. Model jellies were prepared according to formulations (table 2). During the addition of ascorbic acid, the recommended daily dose was taken into account in accordance with [25; 26].

Samples of jelly were prepared as follows. A portion of gelatin is soaked in room temperature water for 30–40 minutes to swell. The swollen gelatin is dissolved by heating, constantly stirring. After the gelatin is completely dissolved, the sugar is added. The solution of gelatin with sugar was cooled and the concentrate of juice with the required amount of ascorbic acid was rapidly added with stirring. The obtained samples were poured into 20 ml containers and placed in a refrigerator for solidification. The total soluble solids (TSS) content in each sample is 28%.

Table 2

Formulations of jelly samples

Code of sample	Amount of ascorbic acid	Compound of jelly, g/100 g simple			
		gelatin	apple juice concentrated	sugar	water
J1	0.025	2.0	14	16	up to 100 g
J2	0.050	2.0	14	16	up to 100 g
J3	0.075	2.0	14	16	up to 100 g
J4	0.050	3.0	14	16	up to 100 g

Subsequently, the sample was placed in a refrigerator to form a jelly and stored at a refrigerated storage (4 °C).

When determining the amount of AA in the samples, used the procedure described in [27].

Determination of TSS. TSS values of each jelly were measured with a hand-held refractometer model Digital Brix MA871 (Milwaukee Electronics Kft., Hungary).

Determination of ascorbic acid. The amount of ascorbic acid in jelly samples was determined by the coulometry with electrogenerated iodine according to method [27–29]. The electrogeneration of iodine was performed on a platinum electrode SM29-PT9 (Yokogawa Europa, Holland) under a constant current of 2.0-5.0 mA. В эксперименте использовали a PU-1 (ZIP, Belarus) potentiostat. Генерацию iodine производили in a 0.1 M solution of potassium iodide in an phthalate buffer solution (pH=4,01). The endpoint of titration was established a potentiometric method with two indicators electrodes: platinum EPV-2 (ZIP, Belarus) and silver chloride EVL-1M3.1 (ZIP, Belarus).

Monitoring and experimental data recording (electromotive force-time) was performed electronically as in [28]. The concentration of AA ω (mg/g sample) in jellies was calculated by the expression:

$$\omega = \frac{ItM}{nFm_p}, \quad (4)$$

where I is current strength, t is the time of the titration end-point, M is the molar weight of AA, F is Faraday's constant 96485 C/mole, n is the number of electrons, participating in the reaction, m_p is mass of the solution.

Ascorbic acid is oxidized to dihydroascorbic acid with transfer of two electrons, corresponds to the value n = 2 in expression (1).

The results of the amount of AA in jellies are the sum of the amount of added pure ascorbic acid and its in the juice apple concentrated [27].

Degradation kinetics model of ascorbic acid during storage. The kinetics of AA degradation was considered for both models and the calculation of the response rate constant was made from experimental data using the equations (2) and (3).

The goodness of model fitting to the experimental data was evaluated by adjusted coefficients of determination (R^2_{adj}) [17]. The regression line seeks to minimize the sum of the squared errors of prediction. The square root of the average squared error of prediction is used as a measure of the accuracy of prediction. This measure is called the root mean square error (RMSE) and the formula for RMSE is:

$$RSME = \sqrt{\frac{Y - Y'}{N - p}}\,, \quad (5)$$

where Y and Y' – experimental properties and it's the prediction, N is the number of the data, and p is the number of parameters.

The highest the (R^2_{adj}) values and the lowest the SEoE values, the better the fitting of the model to the experimental data.

Statistical analysis. A one-way ANOVA was applied to the AA contents in order to detect differences due to the storage period. In all cases, data normality was assessed (data not shown). The post hoc LSD test was applied to identify differences that were set at $p < 0.05$. In order to afford a predictive model of ascorbic acid degradation as a function of storage time, a regression test was applied. The SigmaPlot for Windows Version 10 (Systat Software Inc., USA) statistical software packages was used throughout.

Evolution of ascorbic acid content during storage of jelly. Ascorbic acid content change in jelly during storage at refrigerated temperature were represented in fig. 1 and 2.

The analysis of the obtained dependences allows us to trace the following patterns:

– for the samples studied, both with a gelatin content of 2% (fig. 1) and a gelatin content of 3% (fig. 2), an increase in AA loss with an increase in storage time is observed;

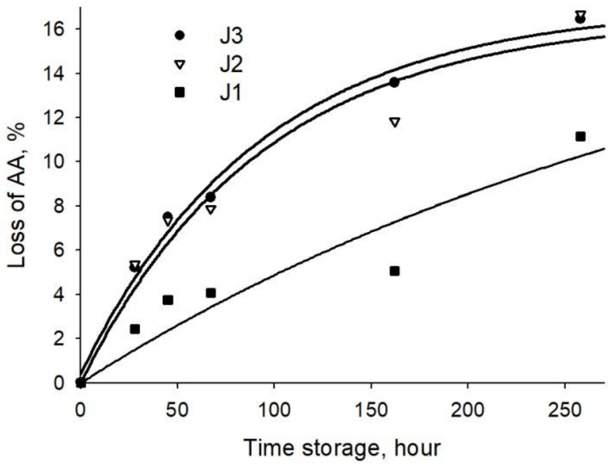

Fig. 1. Ascorbic acid loss during storage of jellies samples (with 2% gelatin) at the refrigerated temperature

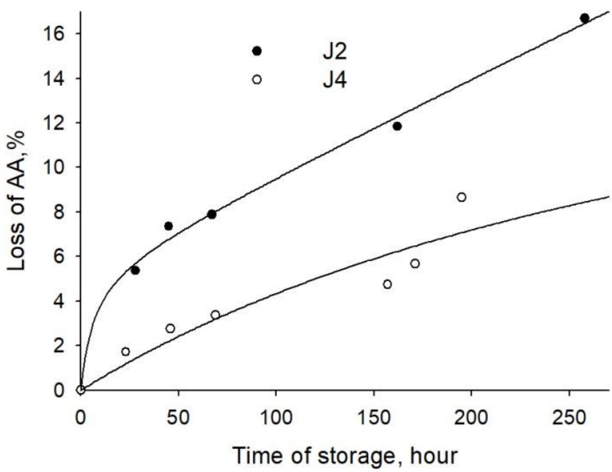

Fig. 2. Ascorbic acid loss during storage of jellies samples with amount AA 50 mg/100 g sample at the refrigerated temperature for jelly with 2% gelatin (J2) and with 3% gelatin (J4)

– for a sample with a content of 3% gelatin, the rate of degradation of AA at the level of 8% in about 200 hours less than at the same time for samples with a gelatin content of 2%;

– faster AA loss rate in the first 28 hours; this is followed by a reduction in the rate of loss with an increase in storage time.

The latest trend was noted in a number of papers [15; 18] These papers are devoted to the degradation of AA in other systems. The authors of these publications explain the higher rate of loss at the beginning of storage by the immediate reaction of a certain amount of ascorbic acid with dissolved oxygen.

Ascorbic acid degradation rate and reaction order during storage. The order of reaction was estimated graphically (fig. 3) by comparing the adjusted coefficients of determination (R^2_{adj}) and root mean square error obtained from plots of AA concentration change as a function of storage time.

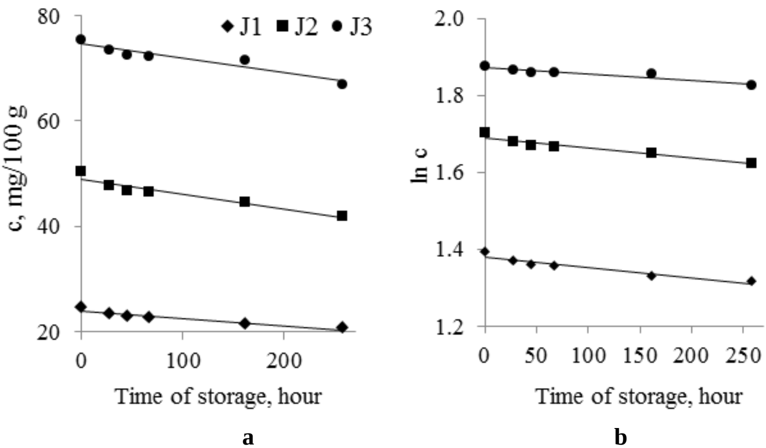

Fig. 3. Ascorbic acid degradation during storage of jellies samples at the refrigerated temperature: a) zero-order kinetic model; b) first-order kinetic model

The calculated data on the reaction rate constants of both orders are given in table 3.

The reaction rate constant (k) of AA increased from 0.014 to 0.042 mg/gh for jelly samples in range J1, J2 и J3 with increased the amount AA. For the first-order reaction, we obtain a constant, within the error of the calculation, the reaction rate constant at the level $2.7 \cdot 10^{-4}$ 1/h. This fact corresponds to the ideas of the formal kinetics of the independence of this quantity from the initial concentration of the reaction participants, in contrast to the results of the description by the reaction of zero order.

Table 3

Ascorbic acid degradation rates k of reaction order during storage of jellies samples

Sample	Zero-order			First-order		
	k, mg/gh	R^2_{adj}	RSME	$k\cdot10^{-4}$,1/h	R^2_{adj}	RSME
J1	0.014±0.03	0.8639	0.5370	2.7±0.4	0.8854	0.0095
J2	0.028±0.05	0.8845	0.9738	2.7±0.4	0.9043	0.0084
J3	0.042±0.01	0.9857	0.4881	2.6±0.1	0.9898	0.0026
Mean	0.028			2.7		
J4	0.020±0.03	0.8746	0.5776	1.5±0.2	0.9155	0.0037

As can be seen from table 3, a good fit was obtained by nonlinear regression with range values R^2_{adj} ($0.8854 < R^2_{adj} < 0.9898$) and RMSE (0.0026< RMSE < 0.0095) for the first-order. Thus, the degradation of AA in samples jellies J1, J2 and J3 are better fitted with the first-order while those determination coefficients were higher value in zero-order model (specially, 0.4881 < RMSE < 0.9738).

Taking into account the results obtained for a jelly with a gelatin content of 2%, the determination of the rate of the AA degradation reaction constant in a jelly with a gelatin content of 3% was carried out on one sample J3 with the amount of ascorbic acid 50 mg/100 g simple. At the same time allowed the same kinetic model, regardless of the content of gelatin in the sample. For sample J4, a first-order reaction rate constant was obtained $1.5\cdot10^{-4}$ 1/h. Thus, an increase in the gelatin content by one and a half times from 2% to 3% reduces the reaction rate of AA degradation by almost two times. It is likely that the semi-solid state of aggregation of the jelly significantly hampers the access of atmospheric oxygen for the oxidation of AA, which is inside the jelly food matrix. At the same time, the gel network, the branching and strength of which increases with increasing gelatin concentration, contributes to the enhancement of this barrier.

Comparison of the obtained rate constants for the degradation of AA in jelly with the data of this process in various liquid and solid products shows a similar level of magnitudes of the order of 10^{-4} 1/h [4]. However, the authors of [4] present data mainly at temperatures above 15 °C. Only the data from two studies [30,31] for fruit juices make it possible in general to compare the order of magnitudes of the AA degradation constants during storage in liquid juices and semi-solid jelly at the same temperature level. This comparison is conditional due to the difference in the nature of the matrix, but allows us to state an order of magnitude greater constant in a liquid medium.

The half-life of AA degradation reactions in jelly. Based on data on the rate constants of the reaction of degradation of ascorbic acid the half-life time $t_{1/2}$ calculated for first-order reaction from equations:

$$t_{1/2} = \ln 2/k, \quad (6)$$

For a better perception of the values of $t_{1/2}$ in table 4 given in units of measure (day). The half-life time of AA in jelly with a gelatin content of 2% is about 107 days, increasing to 193 days for jelly with a gelatin content of 3% (table 4).

Table 4

The half-life time $t_{1/2}$ and residual amount of ascorbic acid degradation during storage in jellies samples

Sample	The half-life time $t_{1/2}$, (day)	The predicted residual AA content at the end of the shelf life of 60 days, (%)
J1, J2, J3	107	68
J4	193	81

Based on the obtained data on the constants of the rate of degradation of ascorbic acid, the predicted AA content at the end of the shelf life (expected 60 days) in jelly samples with a gelatin content of 2% will be 68% of the entered amount, and for jelly with a content of 3% gelatin 81%. It should be noted that these figures are valid at storage temperatures in the refrigerator not higher than 5 °C. It is natural to assume that these figures require correction and the residual AA content will be lower with an increase in temperature to the maximum allowable 25 °C.

Conclusion. The conducted researches allow us to formulate the following conclusions:

1. There is a decrease in the amount of ascorbic acid added to the jelly for all samples with different levels of AA concentration and gelatin content.

2. The kinetics of degradation of ascorbic acid in fruit jelly is described better by a first-order reaction compared to a zero-order reaction.

3. The calculated first-order reaction rate constants have the values $2.7 \cdot 10^{-4}$ and $1.5 \cdot \cdot 10^{-4}$ (1/hour) for jelly with a gelatin content of 2% and 3%, respectively. Thus, an increase in the gelatin content in jelly contributes to a greater preservation of AA.

4. The calculated half-lifes of AA were 107 and 193 days for jelly with a gelatin content of 2% and 3%, respectively.

5. The predicted amount of AA in jelly after storage for 60 days was 68% with a gelatin content of 2% and 81% with a gelatin content of 3%.

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