Секція 4. ХІМІЧНІ, ФІЗИЧНІ, МАТЕМАТИЧНІ МЕТОДИ ДОСЛІДЖЕНЬ ЯКОСТІ ПРОДУКТІВ ХАРЧУВАННЯ

UDC 664.38:577.164.2

DETERMINATION OF THE CONTENT OF L-ASCORBIC ACID IN FRUIT JELLY

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The article presents the results of the development of a method for the determination of L-ascorbic acid (AA) in model fruit jellies containing gelatin. In the determination of AA, a coulometric method with electrically generated iodine as a titrant in a galvanostatic mode with a current of 2–5 mA was used. As a research object, model fruit jelly with 2% and 3% gelatin content was used. In addition to gelatin, the systems contained apple juice concentrated clarified and potassium sorbate (used as a concerto). The peculiarity of the preliminary preparation of the samples was the stage, which envisaged the melting of the samples before the quantitative determination of AA.

In order to assess the suitability of the proposed methodology for the quantitative determination of AA in fruit jelly, a validation evaluation was performed on the following parameters: specificity, linearity and the range of an analytical method, limits of detection and determination, accuracy and reproducibility. Specificity has been studied on standard aqueous solutions of AA. It has been shown that under two conditions: 100% of current output and stoichiometricity of the oxidation reaction with iodine AA, the theoretical slope of the amount of the electrice curve from the analyte concentration with accuracy up to 0.2% coincides with the experimental one. The following parameters were determined: the working area of the technique 4.92–127.6 mg/100 g; limits of the determination 1.62 mg/100 g and quantitation 4.92 mg/100 g. The absence of a systematic error for standard and fruit jelly solutions was evaluated using the "introduced-found" method. Error of recovery did not exceed RSD<2.8%. Correctness and reproducibility were evaluated by the method of varying the weights at three levels of concentration using three sets at each level. Fischer and Student's criteria, which were used to compare average values, testified to the validity of the indicators of "correctness" and "reproducibility". Comparing the studied parameters, the best results were obtained for fruit jelly with a 2% gelatin content compared to a jelly with a content of 3%.

The obtained results testify to the possibility of application of the developed method of determination of ascorbic acid in real food systems.

Keywords: *ascorbic acid, fruit jelly, galvanostatic coulometry, validation.*

ВИЗНАЧЕННЯ ВМІСТУ АСКОРБІНОВОЇ КИСЛОТИ У ФРУКТОВОМУ ЖЕЛЕ

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Наведено результати розробки методики визначення аскорбінової кислоти в модельних фруктових желе, що містять желатин. Запропонована методика базується на кулонометричному титруванні електрогенерованим йодом у гальваностатичному режимі розплавлених зразків фруктового желе. Досліджено параметри валідаційної оцінки: специфічність, лінійність, робоча область методики, межі виявлення та визначення, правильність та відтворюваність. Отримані результати свідчать про можливість застосування розробленої методики визначення аскорбінової кислоти в реальних харчових системах.

***Ключові слова*:** *аскорбінова кислота, фруктове желе, гальваностатична кулонометрія, валідація.*

ОПРЕДЕЛЕНИЯ СОДЕРЖАНИЕ АСКОРБИНОВОЙ КИСЛОТЫ В ФРУКТОВОМ ЖЕЛЕ

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Приведены результаты разработки методики определения аскорбиновой кислоты в модельных фруктовых желе, содержащих желатин. Предложенная методика базируется на кулонометрическом титровании электрогенерированным йодом в гальваностатическом режиме расплавленных образцов фруктового желе. Исследованы параметры валидационной оценки: специфичность, линейность, рабочая область методики, пределы обнаружения и определения, правильность и воспроизводимость. Полученные результаты свидетельствуют о возможности применения разработанной методики определения аскорбиновой кислоты в реальных пищевых ситемах.

***Ключевые слова*:** *аскорбиновая кислота, фруктовое желе, гальваностатическая кулонометрия, валидация.*

Statement of the problem. L-ascorbic acid is also associated with its oxidation products (dehydroascorbic acid) to water-soluble vitamin C. This vitamin is an antioxidant, playing an important role in the regulation of oxidation-reducing processes. The role of this vitamin in the synthesis of collagen and procolagen, folic acid and iron metabolism, as well as in the synthesis of steroid hormones and catecholamines, etc., is known [1; 2].

The daily needs of a person in AA depend on many factors: age, gender, work performed, climatic conditions, and so on. According to [3],

recommended dietary for AA allowances have been established for adult women of 75 mg/day and 90 mg/day for men. Lack of vitamin C in the human body causes hypovitaminosis, and with a long shortage of severe diseases – avitaminosis. Necessary measures for the prevention of vitamin C deficiency, along with the enrichment of the diet of vegetables and fruits, are the use of food products enriched with this vitamin. It should be considered a functional food ingredient, as it is an important bioactive compound with antioxidant properties [2]. However, the addition of vitamin C to the formulation often does not provide the necessary physiological effect on the body after consumption of this product. It is known that this substance is most sensitive to technological processing of production. The high sensitivity of AA to oxidation causes its degradation in the process of both production and during the period of preservation of the final product. Thus, the problem of quantitative determination of AA in enriched foods is important both for manufacturers and for consumers. Given this fact, the need is to develop analytical methods that take into account the preservation of samples, their preparation, as well as validation of the method of determination itself [4]. It is the validation technique that is a necessary step in the development of any method of quantitative analysis. This procedure provides for reliable results that reflect the quantitative determination of the correct substance, in the correct amount, and in the appropriate range for the intended samples [5]. Without validation in complex food matrices, the results obtained have significant uncertainty [6; 7]. That is why, not only the development and optimization of the analytical method is important for the development of the method, but also its correct verification.

Thus, the development and optimization of new methods for the quantitative determination of the content of ascorbic acid in specific food products with their subsequent validation is an urgent task.

Review of the latest research and publications. Various methods for determining ascorbic acid, in particular in foods, are discussed in a large number of review publications. This publication does not set the task of reviewing various physico-chemical methods for determining the AA, so the authors will not focus on it. Moreover, this issue was partly focused on the previous publication [8]. Wide application of titrimetric methods, in particular titrant 2,6-Dichloroindophenol sodium salt hydrate, was noted as the most commonly used AA definition. But in [9] it was shown that the standard method for determining AA by titration with sodium 2,6-dichloro-diphenolindophenolate gives low results relative to the reference method, which was explained by the ambiguous mechanism of oxidation of AA and the need for adequate sample preparation. The significantly lower results of the content of ascorbic acid in aqueous solutions of hydrocolloids, as

compared with the amounts of AA, are also indicated by chromatography [10–12]. The authors associate this with the sorption interaction of AA with molecules of hydrocolloids, in particular gelatin and agar. Similar results with respect to the uncertainty of the final result were obtained by the method of titrimetry [13].

The determination of ascorbic acid by the coulometric method with electrogenerated bromine was previously studied [8]. The results of this technique showed a reduced content of AA in a complex food matrix with gelatin. This fact is associated with the interaction of the components of the food matrix with the titrant. Bromine is not selective with respect to the analyte AA being detected and oxidizes non-stoichiometric gelatin. From this point of view, electro-generated iodine is a less powerful oxidizing agent than bromine, and according to coulometric data it reacts with AA. And with other antioxidants, such as polyphenolic compounds, does not react [14]. These facts serve as the basis for the development of a method for determining ascorbic acid in fruit jelly with electrogenerated iodine as a titrant.

The objective of the research was to develop and validate a method for determining of L-ascorbic acid amount in gelatin-based fruit jelly products by galvanostatic coulometry.

Presentation of the research material.

Materials. The following chemicals used in this study are as follows: potassium iodide, potassium sorbate (Reachim, Russia) of analytical grade and Standard titers for a buffer system with pH = 4.01 (Ukraine), distilled water, gelatin food (240 Bloom, 20 mesh) (Gelita Deutschland GmbH, Germany), apple juice concentrated clarified (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 no more 0.55 mS/m was used. The conductivity was measured by a conductometer CEL-1M2 (Analitpribor, Georgia).

Sampling. Standard solutions of ascorbic acid were prepared by weight and followed by a sequential weighing procedure 8 standard aqueous solutions of ascorbic acid (AA) in the range of 12.40 to 127.6 mg/100 g were prepared. This range corresponds to the variation in the content of AA in fortified foods at a level of 25–100 mg/100 g product. During the measurements, the solutions were in a dark cool place at a temperature of 5 °C (household refrigerator). A portion of the solution, which was pipetted into the cell, was weighed beforehand.

Two types of fruit jelly were prepared for research:

1) sample FJ-1 containing 2% gelatin, 8% apple juice concentrated clarified and potassium sorbate. The latter substance (potassium sorbate) is added to fruit jelly as a preservative;

2) sample FJ-2 containing 3% gelatin, 8% apple juice concentrated clarified and potassium sorbate.

For the preparation of fruit jelly, the required weight of gelatin was added to the water. The mixture was kept for 30 minutes to swell gelatin. The mixture was then heated to 45 °C. and stirred until complete dissolution of gelatin. Apple juice and potassium sorbate ware added. Subsequently, the sample was placed in a refrigerator to form a jelly and stored at a temperature of 5 °C. The samples without gelation were examined immediately after preparation and kept in liquid form.

Before entering the cell, the samples of fruit jelly under investigation were placed in a thermostat with a constant temperature of 37 °C. Within 5 minutes, the jelly was melted, and the resulting solutions were used for measurements. The molten solutions were kept in the thermostat during the measurements. The total time of measurement of one solution was not more than 20 minutes, which is the time spent by the samples in the thermostat.

The jelly melting operation is necessary for the correct quantitative determination of the AA content. Previous experiments have shown that inserting into the cell a jelly-like sample leads to non-reproducible results, even if this sample is completely dissolved in the background electrolyte. Occasionally the dissolution process lasted too long, for about 30 minutes, depending on the sample mass. On the other hand, the finding of the test sample in a thermostat at a temperature of 37 °C for 20 minutes does not make a significant contribution to the obtained quantitative value.

Methods. The amount of ascorbic acid in aqueous solutions and in fruit jelly samples was determined by the coulometry with electrogenerated iodine according to method [15]. The electrogeneration of iodine was performed using a PU-1 (ZIP, Belarus) potentiostat in a 0.1 M solution of KI in an phthalate buffer solution (pH=4,01), this was performed on a platinum electrode SM29-PT9 (Yokogawa Europa, Holland) under a constant current of 2.0-5.0 mA.

The endpoint of titration was established a potentiometric method with two platinum EPV-1 (ZIP, Belarus) and silver chloride EVL-1M3.1 (ZIP, Belarus) electrodes.

Monitoring and experimental data recording (electromotive force-time) was performed electronically [15].

The concentration of AA ω (mg/g sample) in candy caramels was established by the expression:

$$\omega = \frac{ItM}{nFm_p}, \quad (1)$$

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 96 485 C/mole, n is the number of electrons, participating in the reaction, m_p is mass of the solution.

When determining the time t, the time of preliminary electrolysis of the generating solution without the analyzed sample was taken into account. This is done in order to take into account the influence of impurities in the electrogenerating solution. As it was noted in [27], when interacting with halogens that are electrophoresed in a cell, AA is oxidized to dihydroascorbic acid with transfer of two electrons, corresponds to the value n = 2 in expression (1).

When calculating the ascorbic acid content in fruit jelly samples, the content of ascorbic acid in the concentrate of apple juice was taken into account. This parameter was determined coulometrically by this method. Thus, the published results of the content of AA in fruit jelly FJ1 and FJ2 are the sum of the amount of added pure ascorbic acid and ascorbic acid, which was included in the apple juice concentrate.

The method of determination and its validation assessment. The developed methodology should be based on the provisions and recommendations for the development and validation of methods of physico-chemical analysis [5; 16; 17]. Based on these settings, a validation assessment procedure was carried out on the following indicators: specificity, linearity and the range of an analytical method, limits of detection and determination, accuracy and reproducibility.

One approach to establish method selectivity is to prove the lack of response in blank matrix [6]. To study this issue, blank solutions were analyzed without the addition of gelatin and whith the addition of gelatin. The difference in titration time between these samples did not exceed the experimental errors. The results obtained were taken into account in the calculation of the time t for fruit jelly samples, by correction of the sample titration time.

On the other hand, the absence of a signal in a blank matrix means 100% of the presence of a signal in a standard solution. For this purpose standard aqueous solutions of potassium iodide were investigated. Based on experimental data, in terms of coulometric titration of standard solutions, the values of the concentration of AA in standard solutions were calculated according to expression (1). On fig. 1 shows the dependence of the amount of electricity from the mass of ascorbic acid in a standard solution at the level of 8 concentrations and 4 measurements of each solution. The specified acid mass m is equal to the product ω and m_p.

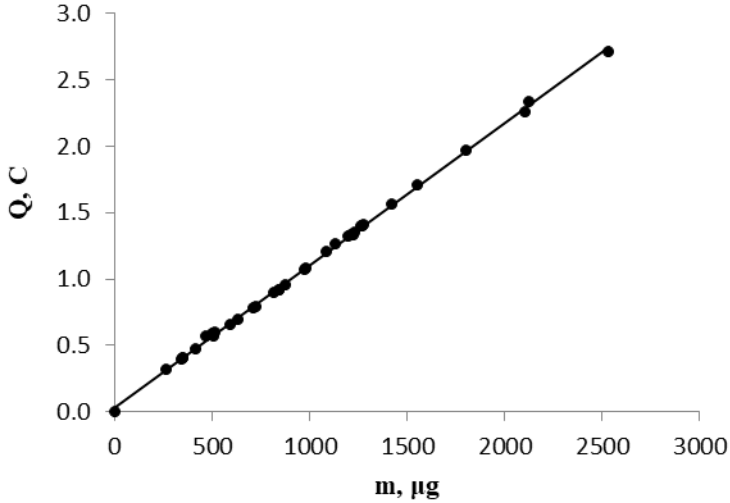

Fig. 1. Dependence quantity of electricity Q from amount m acid in aliquot of standard aqueous solutions of ascorbic acid

In this case, the graph gives all the experimental points without averaging over the concentration, based on the fact that in determining the variation of both the mass of the sample and the magnitude of the current in the range 1–5 mA. That is, each of the 5 measurements at a given concentration had an inherent set of mass of the sample and electric current strength. As expected, this dependence has a linear character with a very high correlation coefficient of 0.9995, which proves the linearity condition and the possibility of quantitative determination of AA in this analytical field in the proposed technique. The method of coulometric titration used by us is absolute, that is, it requires no calibration. The reason for this is the physically grounded Faraday equation. So according to this law, the analytical signal in the form of the amount of electrical energy needed to generate the titrant, associated with the concentration of analyte is given by the expression (2).

$$Q = It = n(\frac{F}{M}m) = kf(m). \quad (2)$$

Thus, observance of the linear relationship between the amount of electricity and the mass of AA (Fig. 1) is possible under two conditions:

1) 100% of the output of an electric current, which means the flow of only one electrochemical reaction associated with the generation of titrant, in this case, iodine;

2) the reaction between the titrant and the analyte in the stoichiometric ratio according to equation (3), which determines the number of electrons in the generation of titrant through the equivalent:

$$C_6H_8O_6 + I_2 = C_6H_6O_6 + 2I^- + 2H^+ \cdot \quad (3)$$

The above, means that the value of the electrochemical equivalent k in expression (2) for a given pair of analyte-titanium has a certain value, which allows us to judge the feasibility of the above conditions. Regarding the first condition, the verification of the efficiency of coulometric titration on the standard titre "Sodium sulfate-oxide 0.1 mol / l", which amounted to 99.9±0,2%.

Calculation of the angle of inclination of the curve, that is, the sensitivity coefficient gives a value of 1,0969 at the expression of the mass of the analyte in milligrams. The theoretical coefficient, which should be for potassium iodide from expression (2) is 1,0956 and differs from the experimental by 0,12%. From the value k we obtain the calculation n = 2, which corresponds to two electrons involved in the oxidation-reduction reaction (3).

The fulfillment of both conditions indicates the selectivity of the determination with respect to the analyte, in this case, in standard solutions of ascorbic acid. But this condition can also be used as a criterion of selectivity in the determination of AA in complex food matrices, which is not a contradiction with regard to the essence of this notion [18]. Apparently, one should speak about the fact that this circumstance is a sign of this absolute method, which distinguishes it from the physicochemical methods that require calibration of the analytical signal of the analyte.

Averaging the data for each concentration of the standard solution was used to calculation of limits of determination LOD and quantitation LOQ:

$$DL = \frac{3\sigma}{S}, QL = \frac{10\sigma}{S}, \quad (4)$$

The results of calculations and parameters of linear regression are given in table 1.

Table 1

Regression output (Y=*a* + *b*·X)

Parameter	Data
Range, mg/100 g	4.92-127.6
Regression equation:	*Y=a+bX*
Slope, b·	1.0966
Intercept, a	35.238
Regression coefficient, r	0.9993
Standard deviation of the analytical signal, σ	5.3519
LOD, mg/100 g	1.62
LOQ, mg/100 g	4.92

That is, based on the data of the table 1, the working area of quantitative determination of AA was 4.92-127.7 mg/100 g. The upper border of this interval was determined based on the maximum concentration of standard solution of potassium iodide used in the experiment. Of course, this does not indicate the maximum possible concentration for determining the indicated method, which, apparently, is much more significant. But this does not correspond to the expediency of adding such an amount of AA for the purpose of enrichment of food products. On fig. 2 shows the dependence of the amount of electricity Q required for generating an equivalent mass of titrant in the equation (3) of the AA content in an aliquot of the sample FJ-1 mass m_p. Dependence is linear with a gradient of 1.0955 for the studied samples of fruit jelly based on 2% gelatin with the addition of potassium sorbate. The resulting value coincides with the theoretical value of 1.0956. That is, the addition of components to the fruit jelly formulation (gelatin, apple concentrate, sodium sorbate) does not affect the specificity of the determination of the content of ascorbic acid by this method. This indicates the absence of the influence of the matrix effect of the medium in comparison with standard solutions.

As shown in fig. 3, the Q-f(ω) dependence is linear with a gradient of 1.0964 for the studied samples of 3% gelatin fruit jelly. The resulting value has a difference of 0.2% with a theoretical value of 1.0956. This is another evidence of the possibility of using this criterion to analyze the specificity of the method of quantitative determination of analyte content in complex matrices.

The absence of a systematic error in the method for determining the AA for standard solutions was evaluated using the "introduced-found" method. According to the data of the table 2 results obtained at the level of values RSD < 0,83%, which does not exceed the value of 1%. This indicates an absence of systematic error.

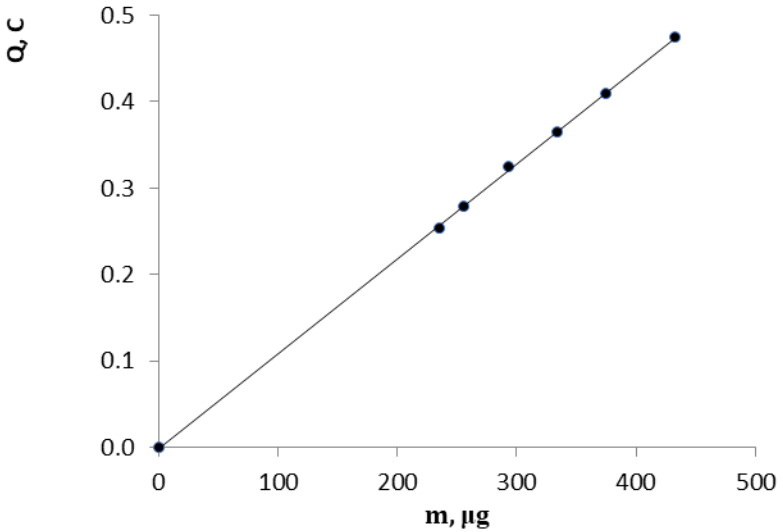

Fig. 2. Dependence quantity of electricity Q from amount m of ascorbic acid in weighing the sample for fruit jelly FJ-1 10 hours after the addition of AA (with gelling)

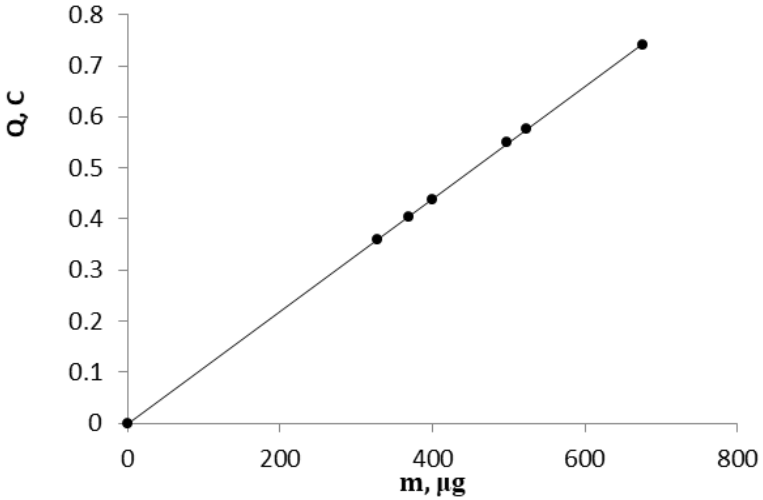

Fig. 3. Dependence quantity of electricity Q from amount m of ascorbic acid in weighing the sample for fruit jelly FJ-2 30 minutes after the addition of AA (without gelling)

Table 2

Results of analysis of standart solutions of ascorbic acid (n = 5, P = 0.95)

Amount taken, mg/100 g	Amount found, mg/100 g	Recovery±RSD, %	д, %
12.40	12.42±0.16	100.2±0.83	+0.16
25.08	25.22±0.20	100.5±0.49	+0.44
48.25	48.30±0.30	100.1±0.39	+0.23
64.61	64.77±0.76	100.3±0.74	+0.25
85.24	84.85±0.30	99.5±0.23	–0.46
119.0	118.6±1.1	99.7±0.72	–0.25
127.7	128.0±0.8	100.2±0.39	+0.23

Adding components to the jelly formulation also does not affect the result of determining the contents of the AA. This is evidenced by the results of research in the "added-found" method for the fruit sample. In tables 3, 4 show data for 6 samples of FJ-1 with the introduced content of AA in the narrowed range compared to standard solutions, namely 48–57 mg/100 g.

In table 3 shows the results of determining the content of AA at a time corresponding to the interval 30 minutes after the addition of ascorbic acid in a cooled jelly. That is, in fact, the AA content in the solution with the FJ-1 composition was measured without the formation of jelly as a result of the gelatin gel process. In table 4 shows the results of the study of AA content in the jelly formed after gel formation 10 hours after the addition of the acid. In this case, before measurements, the jelly was melted in accordance with the procedure described above. Similar operations were performed using both FJ-1 and FJ-2 samples.

According to table 3 and 4 the correctness of the technique was obtained lower for fruit jelly on the basis of gelatin 2% with the addition of potassium sorbate. The difference in the results between the added and found value of the AA content up to 2% should be considered as a valid value for the method of quantitative determination of AA as a method for controlling the quality of the enriched food product with a RSD < 2.6% (for FJ2 RSD < 2.8%). Comparison of data table. 3 and 4 indicates that the AA content in the sample obtained after the melting of the fruit jelly is within the determination error of an identical AA content in the jelly 30 minutes after the injection. This makes it possible to carry out quality control of the finished product on the contents of the AA immediately after its

introduction, guaranteeing the index in the finished product, that is, after the gelation process with the formation of the jelly structure.

Table 3

Results of analysis of ascorbic acid in FJ-1 (30 minutes after the addition of AA, without gelling) (*n* = 5, P = 0.95)

Amount taken, mg/100 g	Amount found, mg/100 g	Recovery±RSD, %	д, %
57.64	57.49±0.07	99.7±1.6	–0.27
53.69	54.46±0.58	100.9±2.3	+0.88
50.04	49.02±0.70	97.9±1.2	–2.06
48.16	47.91±1.53	97.4±2.6	–0.51
53.46	54.25±0.30	101.5±0.4	+1.47
56.76	57.49±0.07	99.2±0.3	–0.80

Table 4

Results of analysis of ascorbic acid in FJ-1 (10 hours after the addition of AA, with gelling) (*n* = 5, P = 0.95)

Amount taken, mg/100 g	Amount found, mg/100 g	Recovery±RSD, %	д, %
57.64	57.62±0.23	100.0±0.3	+0.04
53.69	54.33±0.38	101.2±0.6	+1.20
50.04	49.33±0.46	98.6±0.6	–1.43
48.16	47.82±0.44	98.5±0.8	–0.70
53.46	54.08±0.16	101.2±0.3	+1.15
56.76	56.54±0.50	99.6±0.7	–0.39

Data of table 4 indicate that the specificity of the method is achieved for fruit jelly with different values of the content of added AA. So at the level of the content of AA 25-75 mg/100 g in jelly, results were obtained at the level of values δ, which does not exceed the value of 1.43%. Similar results have also taken place for FJ-2 fruit jelly with a high level of gelatin.

According to the guidelines [7], the correctness and reproducibility were assessed by the method of varying the weight at three concentration levels of approximately 25, 50 and 75 mg/100 g of the sample using three

weights at each level (the data presented in Table 3, 4 and 5 is part of these studies). Metrological parameters of one of the samples of fruit jelly FJ-2 for the level of concentration AA 57.6 mg/100 g, characterizing the reproducibility of results at the level of one concentration, are given in table 5.

Table 5

Results of analysis of ascorbic acid in FJ-2 (30 minutes after the addition of AA, without gelling) (n = 5, P = 0.95)

Amount taken, mg/ 100 g	n	Current strengt, мА	Massa of aliquot, g	Time of the titration, s	Amount found, mg/100g	Statistic data
57.64	1	2.97	0.910	194	57.8	$\bar{\omega}$ = 57.62 $\Delta\bar{\omega}$ = 0.23 S_x = 0.094 S_r = 0.004 RSD = 0.28%
	2	2,97	0,865	185	58,0	
	3	0,99	0,570	362	57,4	
	4	2,97	0,640	136	57,6	
	5	2,97	1,175	249	57,5	
	6	2,98	0,695	147	57,5	

As a result of using the experimental data for the Fisher test, statistically insignificant results were obtained that indicate the homogeneity of the data. A comparison of the averages within the Student's criterion indicates their statistical insignificance. Thus, the method of coulometric titration is valid for indicators of accuracy and reproducibility.

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

1. The method of determination of ascorbic acid in model systems by the method of galvanostatic coulometry with the optimal conditions for determination: a background electrolyte – 0.1 M solution of potassium iodide in a buffer solution with pH = 4.01, current strength of 2 to 5 mA is developed. As model systems, fruit jelly with a mass fraction of gelatin 2 and 3% were used. In addition, in the jelly, apple juice concentrate and sodium sorbate as a preservative was added.

2. The validation procedure of the developed method is approved for the following parameters: operating range with boundaries of determination, correctness and reproducibility, detection limit and limit of quantitative determination. The following results were obtained: the working area of the method 4.92–127.6 mg/100 g; limit of determination 1.62 mg/100 g and quantitation 4.92 mg/100 g; Correct and reproducible results of content determination with RSD ≤ 2.6%.

3. Comparison of the studied parameters showed better results for fruit jelly with 2% gelatin content compared to 3% gel content.

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DOI: 10.5281/zenodo.2364885

УДК 621.3.011.5:612.79

ВИЗНАЧЕННЯ ДІЕЛЕКТРИЧНОЇ ПРОНИКНОСТІ ВОЛОГОЇ ШКІРИ

В.О. Захаренко, С.В. Сорокіна, В.О. Акмен, О.В. Смоленський, С.О. Колієнко

Сучасний український ринок формується під впливом багатьох взаємопов'язаних чинників, кожен із яких у певній ситуації може як стимулювати, так і стримувати його розвиток. До основних чинників належать: обсяг і структура вітчизняного виробництва та імпорту, стан ринку сировини та матеріалів, кількість населення та ін.

Електричні властивості капілярно-пористих систем однозначно визначаються кількістю та видом зв'язку вологи в капілярах і порах з основним матеріалом речовини. Знаючи залежність електричних