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# Determination of Integral Antioxidant Activity of Phytoextracts on Rowan Fruits (*Sorbus aucuparia*) by Coulometric Titration

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This article presents the integral antioxidant activity (IAA) data of extracts based on the fruits of mountain ash (*Sorbus aucuparia*) obtained by coulometric titration with electrogenerated bromine and DPPH-test. A good agreement of the results confirmed the high antioxidant and antiradical activity of the studied phytoextracts. The influence of heat treatment method on the studied indicator was also estimated. The prospects and expediency of using the method of galvanostatic coulometry for determining the integral antioxidant activity of phytoextracts of Rowan fruits (*Sorbus aucuparia*) have been established.

**Keywords: *Sorbus aucuparia*, Coulometry, Antioxidant activity, Plant extracts.**

## INTRODUCTION

Development of society is characterized by increasing psychological and ecological tension, promptly changing the climatic conditions and the recently observed inability of the human immune system to adapt quickly to them, as a result of which the growth of various pathologies [1-5], including coronavirus infection [6-8], increases sharply. This prescriptions is the need to use adaptive foods and preparations in the human diet, among which tinctures, syrups and extracts from the fruits of mountain ash (*Sorbus aucuparia*) can be isolated, contributing to the normalization of the internal environment and increasing the body's resistance to the effects of dangerous eco-factors. They are characterized by a high content of vitamin C [9,10], organic acids [9,11], phenolic compounds [12-14], carotenoids [15,16], tannins [17], *etc.* This allows us to attribute mountain ash to valuable sources of biologically active substances that indicate its high antioxidant status, contributing to the alignment of the development of oxidative stress [18].

Nevertheless, there is a variety of mechanisms of action of antioxidants of different nature [19-22]. For example, (i) inhibition of radical forms of active oxygen metabolites capable of tearing off a hydrogen atom with the formation of organic radicals; (ii) a change in the structural organization that makes it difficult to oxidize; (iii) local reduction of $O_2$ concentration and avoidance of its inclusion in oxidation; (iv) interaction with organic radicals and avoidance of the development of chain oxidative processes; (v) binding or oxidation of metal ions of variable valence, which initiates the decomposition of peroxides and the formation of radicals; (vi) conversion of peroxides into stable oxidation products *viz.* alcohols, aldehydes, ketones, *etc.* has led to the development of methods for studying antioxidant activity, which differ in the type of oxidation source, the oxidized compound and the method of its measurement, as well as in the level of integrality (individual antioxidant/ integral antioxidant activity). The latter is preferable, given the multicomponent and polyfunctional pattern of adaptive preparations, including phytoextracts. In addition, the individual determination of individual antioxidants limits the lack of development of the instrumental and analytical base for the present. Therefore, research in the field of finding the most perspective method of measuring the integral antioxidant activity (IAA), that fully and reliably determines the value of this indicator, is relevant and in demand.

The known methods of analytical control for determining of antioxidants are divided into direct and indirect (Fig. 1). The latter are based on measuring the level of the cumulative action of antioxidants on the inhibition of the degree or rate of consumption of model reactants (indicator systems) or the formation of oxidation products. In this case, the antioxidant activity of the objects of study is expressed by the amount of a standard substance that produces an equivalent antioxidant effect. In present work, to determine the antioxidant activity of extracts based on natural and heat-treated rowan fruits, the coulometric titration method with electrogenerated bromine was used, followed by a comparison of the results with the data obtained by the DPPH method. This approach makes it possible to obtain more reliable information when interpreting experimental data, given the widespread use of the DPPH spectrophotometric method for the determination of IAA extracts rich in phenolic compounds. The choice of the first method is due to its advantages: there is lack of necessity for preliminary construction of calibration graphs, rapid, accessibility and ease of analysis, combined with high accuracy and reproducibility of the received results. And what is more important is the possibility of determining a larger number of antioxidants located in phytoextracts due to the formation of $Br^{\bullet}$, $Br_2$, $Br^{3-}$. As a result of electrochemical oxidation of bromides on a platinum electrode in an acidic medium, followed by their entry into radical and redox reactions, as well as reactions of electrophilic substitution and accession by multiple bonds [23].

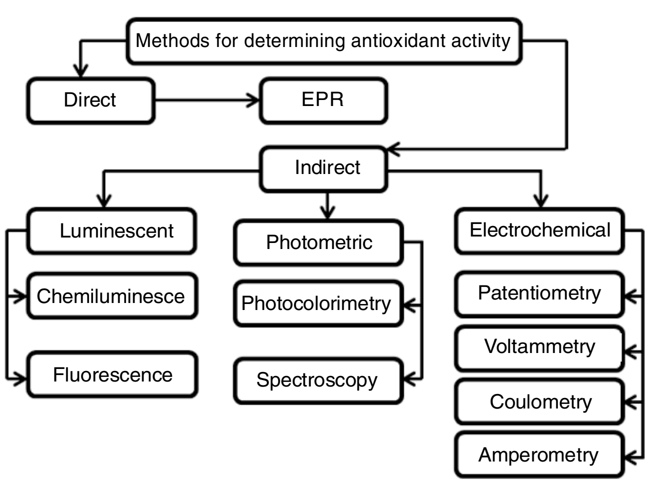


Fig. 1. Classification of methods for determining antioxidant activity

## EXPERIMENTAL

The convective drying of fruits of mountain ash *Sorbus aucuparia* was carried out at 45-50 ºC and freezing at -18 ºC. Extraction was carried out by maceration with stirring (500 rpm) for 1.5 h. The extractants were distilled water and ethanol. The ratio of raw materials:extractant was 1:6 [24,25]. The temperature mode of extraction was chosen taking into account the maximum possible preservation of thermolabile antioxidants (65 ºC) for obtaining aqueous extracts and 45 ºC for alcohol extracts. The assessment of the level of extraction of extractive substances was carried out by the quantitative determination of soluble solids by the gravimetric method [26].

The antioxidant activity of phytoextracts was evaluated by coulometric titration with electrogenerated bromine [18] and spectrophotometric titration using a stable DPPH radical [27]. For the calibration of instruments *viz*. Ekspert-006 coulometer and PE-5400 UV spectrophotometer, rutin [28] and Trolox, respectively were used as standards . In order to obtain the reliable results on the integral antioxidant activity of the studied extracts, firstly, the determination was carried out after reaching 3-4 subsequent device indications, the values of which did not exceed the specified error. The static processing of tje experimental data was carried out according to the “Stat” program.

The spectrophotometric determination of the antiradical activity of phytoextracts with a concentration of extractive substances of 0.5% was conducted at a wavelength of 517 nm in a 0.5 cm thick cuvette. As a comparison solution was used 80% ethanol. The antiradical activity of phytoextracts samples pronounced as a percentage of DPPH inhibition was calculated by the following formula:

$$\text{Inhibition DPPH}\,(\%) = 100 \times \frac{D_k - D_o}{D_k}$$

where $D_k$ is the optical density of control sample (in the absence of antioxidants); and $D_o$ is the optical density of the sample with phytoextracts.

## RESULTS AND DISCUSSION

The literature has sufficiently covered the issues related to the trace elements and organic composition of extracts, based on the fruits of mountain ash. As for the data on antioxidant properties [29-31], it is not possible to conduct a comparative analysis of determining their antioxidant properties under different conditions and by different methods. Therefore, it is of interest to conduct the comprehensive studies to assess the influence of processing of raw materials, extractants and methods for determining the integral antioxidant activity (IAA) of the studied phytoextracts on this indicator for extracts of rowan fruits obtained by galvanostatic coulometry.

The experimental results on the IAA extracts based on natural and heat-treated fruits of mountain ash obtained by coulometric titration electrogenerated bromine are given in Table-1. The value of IAA is presented in units of mg of standard/100 mL of extract with subsequent recovery to standard mg/g of absolutely dry extractable substances (ADS), which is explained by the possibility of fluctuations in the number of extractive substances in the individual samples of phytoextracts. As observed, for the aqueous extracts based on natural and dried fruits of mountain ash, there is a good reproducibility of the IAA value. On average, the values of this indicator were 16.6 and 16.1, respectively.

For frozen phytomaterials, a variation in the values of IAA was observed, which are higher compared to similar data

TABLE-1
INTEGRAL ANTIOXIDANT ACTIVITY (IAA) DATA OF ROWAN FRUIT EXTRACTS

| Extractant-water | | | | Extractant-ethanol | | | |
|---|---|---|---|---|---|---|---|
| ADS (%) | IAA (mg/100 mL) | IAA medium (mg/100 mL) | IAA (mg/g) | ADS (%) | IAA (mg/100 mL) | IAA medium (mg/100 mL) | IAA (mg/g) |
| Natural | | | | | | | |
| 10.39 | 175.45<br>175.45<br>172.98 | 174.53 ± 3.37 | 16.80 | 15.91 | 175.11<br>170.07<br>176.23 | 173.80 ± 3.28 | 10.92 |
| 10.25 | 167.26<br>176.23<br>175.22 | 172.90 ± 4.91 | 16.86 | 10.59 | 127.24<br>127.24<br>127.24 | 127.24 ± 0.00 | 12.01 |
| 8.31 | 143.27<br>136.15<br>138.45<br>137.19 | 137.26 ± 1.15 | 16.52 | 11.26 | 164.78<br>129.36<br>130.45<br>128.45 | 129.42 ± 2.49 | 11.49 |
| 8.81 | 141.97<br>144.36<br>144.97 | 143.76 ± 1.59 | 16.31 | 11.47 | 125.43<br>123.96<br>125.43 | 124.94 ± 0.85 | 10.89 |
| Frozen | | | | | | | |
| 4.91 | 101.23<br>101.23<br>101.35 | 101.27 ± 0.06 | 20.61 | 10.8 | 142.83<br>146.86<br>141.82 | 143.83 ± 2.67 | 13.32 |
| 4.59 | 84.64<br>83.63<br>83.52 | 83.93 ± 0.62 | 18.29 | 12.7 | 175.56<br>181.05<br>181.05 | 179.22 ± 3.17 | 14.11 |
| 5.12 | 96.14<br>96.34<br>99.89 | 97.46 ± 5.24 | 19.03 | 10.32 | 156.45<br>160.24<br>159.24 | 158.64 ± 1.96 | 15.36 |
| 3.96 | 80.46<br>82.49<br>80.46 | 81.14 ± 2.91 | 20.47 | 9.14 | 145.39<br>149.31<br>145.36 | 146.69 ± 2.27 | 16.05 |
| Dried | | | | | | | |
| 8.06 | 132.85<br>127.24<br>127.24<br>132.85 | 130.04 ± 3.26 | 16.13 | 8.76 | 131.84<br>127.24<br>132.85 | 130.64 ± 2.99 | 14.91 |
| 9.42 | 151.46<br>150.48<br>148.96 | 150.30 ± 1.26 | 15.96 | 13.79 | 213.57<br>207.96<br>218.16 | 213.23 ± 5.11 | 15.46 |
| 8.7 | 142.41<br>140.69<br>144.15 | 142.42 ± 1.73 | 16.37 | 11.2 | 169.34<br>171.39<br>198.46 | 169.73 ± 1.50 | 15.15 |
| – | – | – | – | 11.47 | 183.19<br>180.38<br>185.79 | 183.12 ± 2.71 | 15.96 |

for extracts based on natural and dried rowan fruits. The most probable explanation of the established fact is due to the damage of protoplasm during freezing of fruits and their storage at -18 ºC, which results in the subsequent transition of intracellular antioxidants into the extract [32], as well as the possible formation of additional antioxidants during its freezing. Similar observation was also reported [33], where the low temperatures lead to a sharp increase (2-3 times) in the amount of unsaturated fatty acids, which are antioxidants.

The observed variation of the presented values of IAA extracts based on frozen fruits is probably associated with some differences in the freezing conditions of certain portions of phytomaterials, however, according to previous report [33], depending on the cooling speed and various types of detected ice formation, since slow freezing causes minor cell death, while rapid cooling, results in the protoplasm surface layers damages.

According to the results, the antioxidant activity of the water extracts of mountain ash fruits (averaged values given) in rutin equivalents is extracts based on frozen raw materials > extracts based on natural fruits ~ extracts based on dried phytonutrients.

Comparison of IAA results of water extracts of mountain ash obtained by two analytical methods showed a similar tendency to change the values of this indicator for natural and heat treated fruits (Fig. 2). However, there is a more significant difference in the values of the antioxidant capacity of the studied samples as frozen fruits > natural fruits > dried fruits.

In case of alcohol extracts, there is a evident decrease in the values of their integral antioxidant activity, as well as a different series of values of the IAA of the studied samples (Fig. 3) obtained by galvanostatic coulometry.

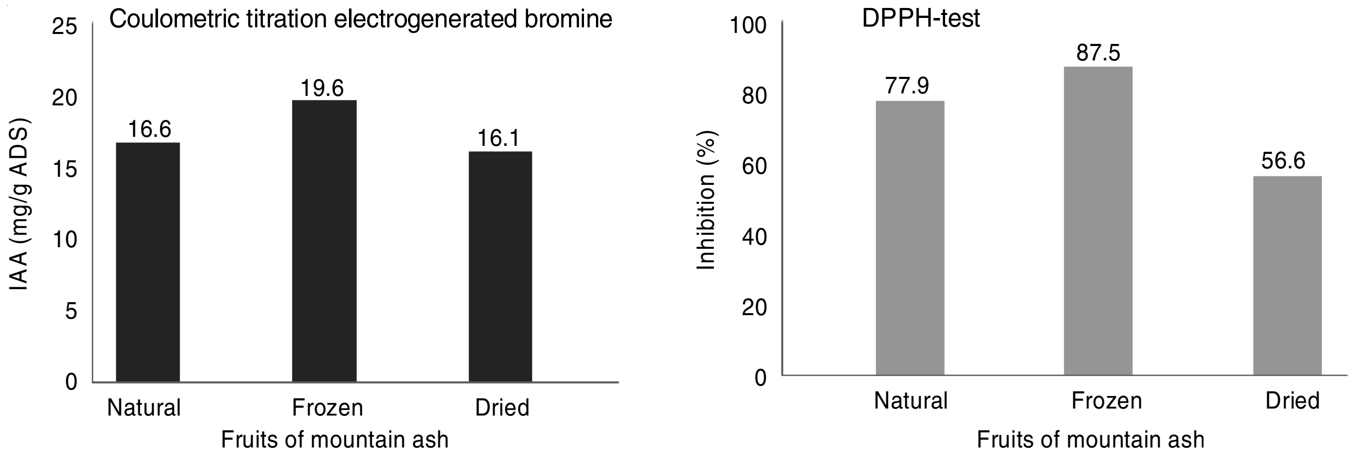

Fig. 2. Averaged values of IAA of rowan fruit extracts

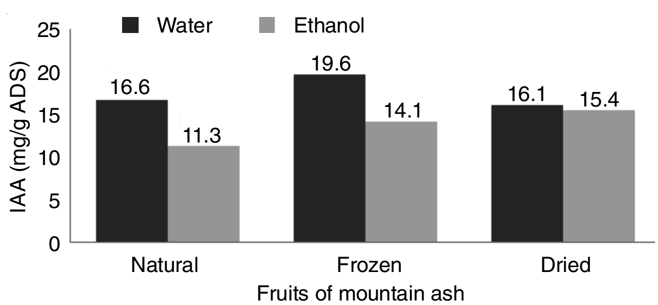

Fig. 3. Averaged antioxidant activity of phytoextracts in rutin equivalents

The obtained results indicated a higher extracting ability of water in relation to the antioxidants of the studied fruits, which are consistent with the literature data. According to Bae *et al*. [34], an increase in the portion of alcohol in the water-alcohol extractant reduces the concentration of water-soluble antioxidants and the reverse tendency is typical for lipophilic biological active substances. A similar pattern was established when determining the antiradical activity of the studied phyto-extracts samples (Fig. 4).

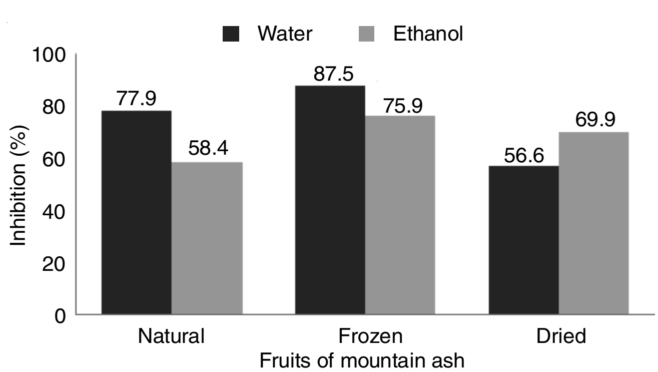

Fig. 4. Averaged antioxidant activity of phytoextracts in trolox equivalents

However, the exception is the extracts based on dried fruits of mountain ash. The obtained data are probably conditioned by the difference in the extractive ability of two studied extra-ctants related to both the content of lipophilic components in phytomaterials and their inability to enter into the antiradical reactions with DPPH. According to Magalhães *et al*. [35], many antioxidants interacting with radical oxygen forms do not react with DPPH. In addition, it is necessary to take into account the possible passing of destructive processes during the heat treatment of rowan fruits, which lead both to a decrease in the reactive hydroxyl groups interacting with the test-radical or their blocking and a decrease in the lipophilic components of antioxidants, soluble mainly in ethanol.

## Conclusion

The conducted studies have confirmed the high anti-oxidant and antiradical activities of the aqueous and ethanol extracts of the fruits of mountain ash (*Sorbus aucuparia*) with the prevalence of the former. The influence of the heat treatment of phytomaterials on the integral antioxidant activity was observed, which indicated an increase in the antioxidant capacity of extracts based on frozen fruits. The convenience and perspective of using coulometric titration electrogenerated bromine method is also established for assessing the integral antioxidant activity (IAA) of multicomponent phytoextracts. A good coordination of the results on the integral antioxidant activity of the studied extracts obtained by galvanostatic coulometry and DPPH analyzed is also proven.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

## REFERENCES

1. A. Ghavami, F. Khorvash, S. Khalesi, Z. Heidari and G. Askari, *J. Funct. Foods*, **86**, 104738 (2021); https://doi.org/10.1016/j.jff.2021.104738
2. A. Ionescu-Tucker and C.W. Cotman, *Neurobiol. Aging*, **107**, 86 (2021); https://doi.org/10.1016/j.neurobiolaging.2021.07.014
3. R. Thanan, S. Oikawa, Y. Hiraku, S. Ohnishi, S. Pinlaor, P. Yongvanit, N. Ma, S. Kawanishi and M. Murata, *Int. J. Mol. Sci.*, **16**, 193 (2014); https://doi.org/10.3390/ijms16010193
4. R.R. Goldblum, M. McClellan, S.J. Gonzalez, B.R. Thompson, K. White, H.X. Vang, H. Cohen, L.A. Higgins, T.W. Markowski, T.-Y. Yang, J.M. Metzger and M.K. Gardner, *Dev. Cell*, **56**, 2252 (2021); https://doi.org/10.1016/j.devcel.2021.07.004
5. S.M. Coulibaly, A. Mesfioui, I. Berkiks, A. Ennaciri, Y. Chahirou, Y. Diagana, A. Ouichou, A. El Midaoui and A. El Hessni, *Neuroscience*, **476**, 1 (2021); https://doi.org/10.1016/j.neuroscience.2021.09.008

6. B. Karkhanei, E. Talebi Ghane and F. Mehri, *New Microbes New Infect.*, **42**, 100897 (2021); https://doi.org/10.1016/j.nmni.2021.100897
7. M. Samadi, H. Shirvani and S. Rahmati-Ahmadabad, *Apunts Sports Med.*, **55**, 115 (2020); https://doi.org/10.1016/j.apunsm.2020.06.003
8. P.R. Augusti, G.M. Conterato, C.C. Denardin, I.D. Prazeres, A.T. Serra, M.R. Bronze and T. Emanuelli, *J. Nutr. Biochem.*, **97**, 108787 (2021); https://doi.org/10.1016/j.jnutbio.2021.108787
9. I.A. Gostishchev, V.I. Deineka, I.P. Anisimovich, M.Yu. Tretyakov, P.A. Myasnikova, L.A. Deineka and V.N. Sorokopudov, *Regional Geosystems*, **10**, 374 (2010) (In Russian).
10. O.M. Blinnikova, *Bull. Michurinsk State Agrarian Univ.*, **1**, 89 (2013).
11. E.V. Sergunova and D.O. Bokov, *Pharmacogn. J.*, **11**, 996 (2019); https://doi.org/10.5530/pj.2019.11.157
12. M. Rutkowska, J. Kolodziejczyk-Czepas, A. Owczarek, A. Zakrzewska, A. Magiera and M.A. Olszewska, *Food Res. Int.*, **147**, 110526 (2021); https://doi.org/10.1016/j.foodres.2021.110526
13. M. Olszewska, *J. Pharm. Biomed. Anal.*, **48**, 629 (2008); https://doi.org/10.1016/j.jpba.2008.06.004
14. F. Aladedunye and B. Matthäus, *Food Chem.*, **159**, 273 (2014); https://doi.org/10.1016/j.foodchem.2014.02.139
15. R. Bobinaitë, C. Grootaert, J. Van Camp, A. Šarkinas, M. Liaudanskas, V. Zvikas, P. Viškelis and P. Rimantas Venskutonis, *Food Res. Int.*, **136**, 109310 (2020); https://doi.org/10.1016/j.foodres.2020.109310
16. K.P. Šavikin, G.M. Zdunic, D.B. Krstic-Miloševic, H.J. Šircelj, D.D. Steševic and D.S. Pljevljakusic, *Chem. Biodivers.*, **14**, e1700329 (2017); https://doi.org/10.1002/cbdv.201700329
17. J. Suvanto, L. Nohynek, T. Seppänen-Laakso, H. Rischer, J.P. Salminen and R. Puupponen-Pimiä, *Planta*, **246**, 227 (2017); https://doi.org/10.1007/s00425-017-2686-8
18. Yu.V. Shcherbakova. Eds.: Yu.V. Shcherbakova, F.Yu. Akhmadullina and E.A. Egorova: Prospects for Using the Method of Galvanostatic Coulometry to Assess the Integral Antioxidant Activity of Milk and Dairy Products: Monograph, Ministry of Education and Science of Russia, Kazan, National Research Technological University, Kazan: Publishing House of KNRTU, p. 128 (2019) (In Russian).
19. K. Lemañska, H. Szymusiak, B. Tyrakowska, R. Zieliñski, A.E. Soffers and I.M. Rietjens, *Free Radic. Biol. Med.*, **31**, 869 (2001); https://doi.org/10.1016/S0891-5849(01)00638-4
20. I. Pinchuk and D. Lichtenberg, *Prog. Lipid Res.*, **41**, 279 (2002); https://doi.org/10.1016/S0163-7827(01)00026-1
21. R. Castañeda-Arriaga, A. Vivier-Bunge and J. Raul Alvarez-Idaboy, *Comput. Theor. Chem.*, **1077**, 48 (2016); https://doi.org/10.1016/j.comptc.2015.10.012
22. M.A. Soobrattee, V.S. Neergheen, A. Luximon-Ramma, O.I. Aruoma and T. Bahorun, *Mutat. Res. Fundam. Mol. Mech. Mutagen.*, **579**, 200 (2005); https://doi.org/10.1016/j.mrfmmm.2005.03.023
23. G. Ziyatdinova and H. Budnikov, *Chemosensors*, **9**, 91 (2021); https://doi.org/10.3390/chemosensors9050091
24. S.N. Leonidovna, T.D. Alexandrovich, B.K. Nikolayevna, G.S. Kamilevna, L.A. Pavlovna, F.I. Mikhaylovich and B.T. Gennad'yevich, *Asian J. Chem.*, **31**, 2672 (2019); https://doi.org/10.14233/ajchem.2019.22366
25. N. Sharonova, E. Nikitin, D. Terenzhev, A. Lyubina, S. Amerhanova, K. Bushmeleva, A. Rakhmaeva, K. Fitsev and K. Sinyashin, *Plants*, **10**, 1279 (2021); https://doi.org/10.3390/plants10071279
26. M. Ashraf-Khorassani, M. Ude, T. Doane-Weideman, J. Tomczak and L.T. Taylor, *J. Agric. Food. Chem*., **50**, 1822 (2002); https://doi.org/10.1021/jf011389s
27. K. Hara, T. Someya, K. Sano, Y. Sagane, T. Watanabe and R.G.S. Wijesekara, *Data Brief*, **17**, 870 (2018); https://doi.org/10.1016/j.dib.2018.02.013
28. Rutin-Standard Sample, TU 9369-141-04868244-07 (2019).
29. L.A. Ostroumov, O.V. Krieger, K.V. Karchin and M.P. Shchetinin, *Food Process.: Techniq. Technol.*, **4**, 38 (2014).
30. I.A. Safonova, V.Ya. Yatsyuk and N.V. Kostebelov, *Scientific Statements of BelSU Series Medicine-Pharmacy*, **22**, C173 (2011) (In Russian).
31. S.E. Fomenko, N.F. Kushnerova, V.G. Sprygin, E.S. Drugova and T.V. Momot, Chemistry of Plant Raw Materials, vol. 2 (2015) (In Russian).
32. L.G. Kosulina, E.K. Lutsenko and V.A. Aksenova, Physiology of Plant Resistance to Adverse Environmental Factors, Rostov-on-Don: Rostov University Publishing House, p. 240 (1993) (In Russian).
33. G.A. Samygin, Causes of Plant Death, *Science* (1974) (In Russian).
34. H. Bae, G.K. Jayaprakasha, J. Jifon and B.S. Patil, *Food Chem.*, **134**, 1912 (2012); https://doi.org/10.1016/j.foodchem.2012.03.108
35. L.M. Magalhães, M.A. Segundo, S. Reis and J.L. Lima, *Anal. Chim. Acta*, **613**, 1 (2008); https://doi.org/10.1016/j.aca.2008.02.047