Bulgarian Chemical Communications, Volume 51, Special Issue D (pp. 209 – 213) 2019

Coefficient of diffusion of tannins in extracts from physalis leaves (*Physalis peruviana* L.)

S. T. Tasheva[1], T. A. Ivanova[1*], V. T. Popova[1], I. Z. Iliev[1], S. S. Stankov[1], H. N. Fidan[1], N. N. Mazova[1], A. S. Stoyanova[1]

[1] *University of Food Technologies, 26 Maritsa blvd, 4002 Plovdiv, Bulgaria*

Received October 4, 2018; Revised January 4, 2019

Physalis peruviana L. (Cape gooseberry, Inca berry, goldenberry, physalis) is the most commercially important among the species of the genus *Physalis* (family Solanaceae). *P. peruviana* fresh or processed fruits are highly valued for their palatability and the high concentration of bioactive compounds. The other vegetative parts of the plants (calyx, leaves, stems) are relatively less studied and are currently unutilized. The aim of this study was to determine the coefficient of diffusion of tannins in extracts obtained from the leaves of two physalis genotypes grown in Bulgaria (provided by the Agricultural University, Plovdiv and by Versol bio-farm, Lik village). The dynamics of tannin accumulation in the extracts obtained by a scheme including four solvent concentrations (30, 50, 70 and 95 % ethanol) and three temperature regimes (20, 40 and 60°C) has been presented. Our data reveal that, regardless of the origin of the leaves, the quantity of extracted tannins decreases with process duration, as well as that the maximum amount of tannins is achieved at 60°C. Subsequently, the respective values of the coefficient of diffusion of tannins, known as a characteristic index of the extraction of plant materials, have been determined. The highest values of the coefficient were 0.28×10^{-9} m^2/s and 0.31×10^{-9} m^2/s (at 60°C), respectively for the two genotypes. To the best of our knowledge, these are the first reported results about the extraction of tannins from physalis leaves, and in particular – about the coefficient of diffusion. The outcomes of the study provide grounds for further investigation on the physalis varieties grown in Bulgaria, with the purpose of revealing more aspects of their composition, bioactivity, benefits and potential for use.

Keywords*: Physalis peruviana L., coefficient of diffusion, tannins, extracts.*

INTRODUCTION

Physalis peruviana L. (also called Cape gooseberry, Inca berry, goldenberry, Peruvian groundcherry, physalis, along with a number of domestic names) belongs to the genus *Physalis* of the Solanaceae family. Originally cultivated as a minor crop in the Andean zone, the production of physalis has expanded to the tropical and sub-tropical countries, the United States, Australia, New Zealand, and Europe [1, 2].

Although physalis has been evaluated as a promising crop under the environmental conditions of Bulgaria more than 15 years ago [3], currently its production is rather an exception, and the species remains comparatively unknown to the farmers. Still, there are a few Bulgarian farms (mainly organic), which produce physalis fruit based on introduced Colombian (or Peruvian) varieties, and supply selected markets and restaurants. Nevertheless, a local Bulgarian variety of physalis named "Plovdiv" has been selected (in the Department of Horticulture of the Agricultural University in Plovdiv), officially recognized in 2006 by the Executive Agency for Variety Testing, Field Inspection and Seed Control, and registered in the Official Variety List of Bulgaria [4]. In a series of publications, the research team of Prof. N. Panayotov determined the optimal agro-ecological conditions and technologies for the production of high-quality physalis fruit in Bulgaria, as well as the options of extending the post-harvest storage and market supply with locally-produced fruits [5-11].

The oval, golden-to-orange colored, calyx-protected fruits of *P. peruviana* are highly valued for their palatability (combining an excellent taste, flavor and texture) and attractiveness, but mostly for their nutritional and health benefits. The fruit is rich in fructose, pectin, vitamins A, B, C and K, phytosterols, tocopherols, polyunsaturated fatty acids, minerals, and many other macro- and micronutrients [1, 12-15]. The broad spectrum of medicinal properties of physalis fruit include antioxidant, antimycobacterial, immunomodulatory, anti-apoptotic, hypoglycemic, hepatoprotective, and many other activities [1, 15-19].

The other vegetative parts of the plant (calyx, leaves, stems, roots), however, are relatively less studied. Currently, they remain unutilized, although there is evidence of the presence of biologically active and other substances of potential interest [12], and they can be considered as a promising raw material for obtaining various extraction products. Ertürk et al. [20] identified a variety of phenolic and

* To whom all correspondence should be sent.
E-mail: tantonieva@mail.bg

volatile compounds in ethanol extracts prepared from different parts (fruit, seeds, leaves, roots, body) of *P. peruviana* collected from Trabzon province, Turkey. The total phenolic and flavonoid contents in the leaf extracts were 1.368 mg GA/g and 0.635 mg QE/g, respectively, and they demonstrated antimicrobial and antioxidant activity. Wu et. al. [21, 22] determined the total flavonoid and phenol contents in ethanolic, aqueous and supercritical carbon dioxide extracts from physalis leaves, as well as various aspects of their biological activity (antioxidant, anticancer, anti-inflammatory, etc.). In a study on non-structural carbohydrate partitioning between plant organs, Fischer et al. [23] showed that physalis leaves accumulated considerable quantities of glucose, fructose, sucrose, and starch.

The dynamics of the process of extraction of biologically active substances from physalis leaves, however, has not been studied. From a theoretical point of view, the driving force of extraction, until the moment of the transfer of the soluble substances onto the solid-phase surface (i.e. within the plant particles), is the molecular diffusion, which is carried out in a static medium and abides by Fick's first law of diffusion [24]. The transfer of substances in the extracting liquid (effectiveness of the extraction) is a resultant from this driving force, which encompasses the concentration gradient within the solid particle and on its surface, and the diffusion resistance. The diffusion resistance depends mainly on the structure and composition of cell walls and protoplasm, the nature of the solvent and the temperature. A generalized expression of the diffusive properties of the plant material in the solid-liquid extraction is the coefficient of internal molecular diffusion (D_{int}) [25, 26], which is individual for each solid body. It reflects the cumulative effect of various factors on the mass transfer of soluble substances from the solid matrix to the liquid medium, such as the structure and the physical properties of the plant material and the solvent, as well as the abundance of extractible molecules, the temperature and duration of the process, etc. The coefficient increases with temperature and varies with time, since plant tissues undergo physicochemical transformations during the extraction, which alter their permeability. The coefficient of molecular diffusion is currently determined only experimentally, due to the heterogeneity and complexity of solid bodies [27]. Data about the coefficients of diffusion of various aromatic and edible plants are summarized by Georgiev and Stoyanova [28].

To the best of our knowledge, there is no data available about the content of tannins, or about the diffusion coefficients of tannins in extracts from physalis leaves. Therefore, the aim of this study was to determine the coefficient of diffusion of tannins in ethanolic extracts obtained from the leaves of two physalis genotypes grown in Bulgaria.

EXPERIMENTAL

Plant material

Leaves of two genotypes of cultivated phyisalis (*Physalis peruviana* L.) were investigated. The first genotype represented the only Bulgarian variety named "Plovdiv" (PA), and was kindly provided by Prof. N. Panayotov from the Department of Horticulture at the Agricultural University, Plovdiv, Central South Bulgaria. The second genotype was organically-grown introduced variety, kindly provided by Mr. M. Peshovsky from Versol bio-farm, located in Lik village, municipality of Mezdra, North-West Bulgaria (PB).

Physalis leaves were hand-picked, air-dried at room temperature, and then stored in tightly closed plastic bags at a temperature of 5-8 °C until processing.

Methods

The raw material was characterized in terms of: moisture content – by drying it up to constant weight, at 105°C, and content of tannins – by titration of hot water extract with potassium permanganate solution using indigo carmine as indicator [29].

Determination of the diffusion coefficients

Extraction of dried physalis leaves was carried out in a batch static mode by maceration in the solvent under the following conditions: solvent – 30, 50, 70 and 95 % ethanol; size of leaf particles – 0.002 × 0.00125 m; ratio of raw material to solvent (hydromodule) = 1:10; temperature – 20, 40 and 60 °C; duration of extraction – 1 h. The solvent was fully replaced and analyzed for extracted tannins after each 10 min interval. The criterion for effectiveness of the process was the quantity of extracted tannins.

The coefficients of internal molecular diffusion (D_{int}) of tannins were determined for each 10-minute interval of extraction by the following equation [30]:

$$D_{int} = \frac{l^2\ 2.3\ \lg(E_1 - E_2)}{\pi^2\ (\tau_1 - \tau_2)} \quad (1)$$

where: l - size of the material, cm; $(\tau_1 - \tau_2)$ – duration of extraction, s; E_1, E_2 – initial and final concentration of tannins in the solid phase, %.

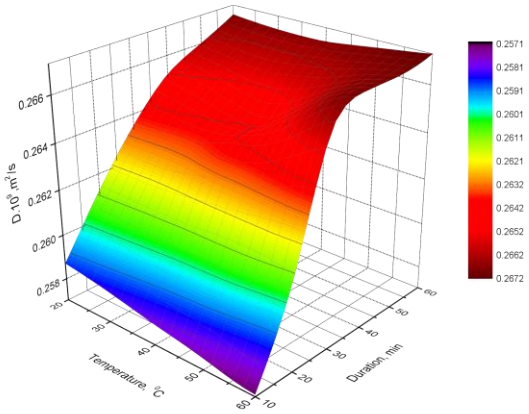
All experiments were carried out in threefold repetition and mean values are presented on the figures below (created with MicroCal™ Origin software).

RESULTS AND DISCUSSION

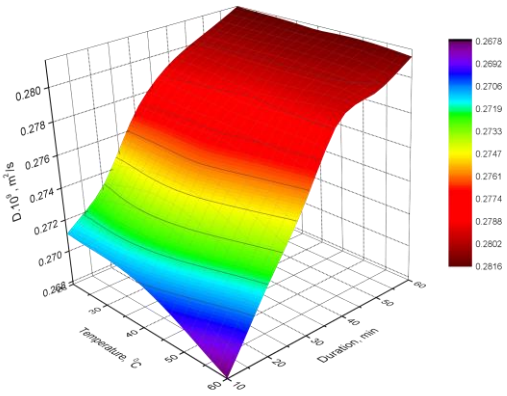
The moisture content of the analyzed physalis leaves from the two genotypes (PA and PB) was 8.32 % and 8.79 %, respectively. The total concentration of tannins in the leaves was estimated to 9.62 % and 10.58 %, respectively, for the leaves from PA and PB.

In order to compute the diffusion coefficients, the content of extracted tannins for each 10-minute interval was determined, and the values were subsequently used to calculate the initial and the final concentration of tannins in the solid phase for the respective time period. The analysis of these experimental data supported the strong dependency of tannin yield on temperature and duration of the extraction. The quantity of extracted tannins for 10 min decreased with time (i.e. there was a steady trend of decrease in each series of six consecutive 10-minute extractions), irrespective of temperature, solvent concentration or genotype. Regardless of the origin of the leaves, maximum amount of tannins was extracted at 60°C, which could be attributed to the positive influence of temperature on the extractive potential of the solvent. On the other hand, the solvent influenced the quantity of extracted tannins, too, and there were significant (at $p<0.05$) differences in the extraction efficacy between the respective concentrations of ethanol (30 and 50% vs. 70 and 95%). The profiles of tannin transfer dynamics (i.e. tannin concentration change for the entire 1-hour period of extraction) were similar for the two genotypes in the case of 30 and 50% (and partially – of 70 %) ethanol, but differed significantly for the 95% solvent. It reflects the impact of solvent nature (decelerated solubility of tannins in concentrated ethanol, the occurrence of oxidative transformations in water), as well as the influence of the altered structure of the solid matrix, the different diffusion resistance in and around the particle, and other complex mechanisms on tannin extraction, changing with time and temperature.

On the basis of these experimental data the diffusion coefficients of tannins (D_{int}) were calculated, and their variations are presented on figures 1 – 4.

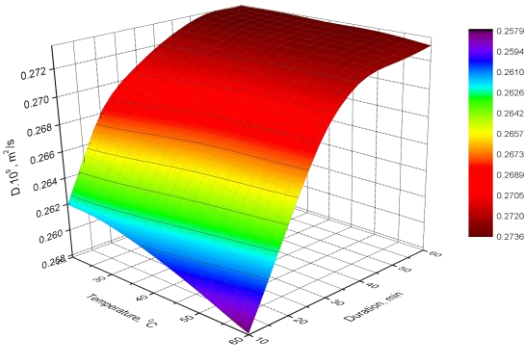


1a) Genotype PA

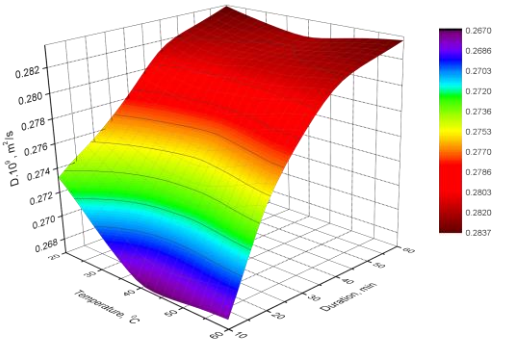


1b) Genotype PB

Fig. 1. Diffusion coefficients of tannins in extracts from physalis leaves with 30 % ethanol.

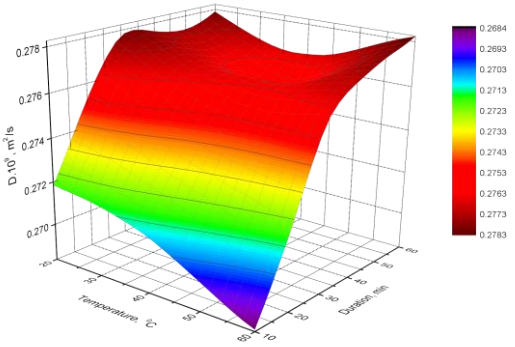


2a) Genotype PA

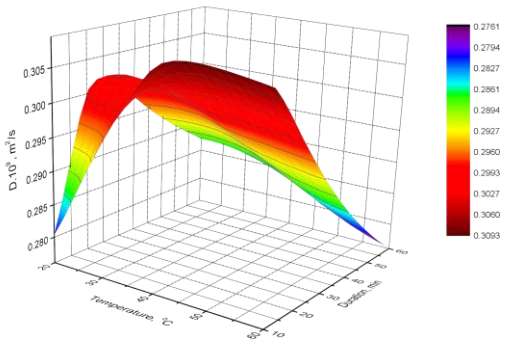


2b) Genotype PB

Fig. 2. Diffusion coefficients of tannins in extracts from physalis leaves with 50 % ethanol.

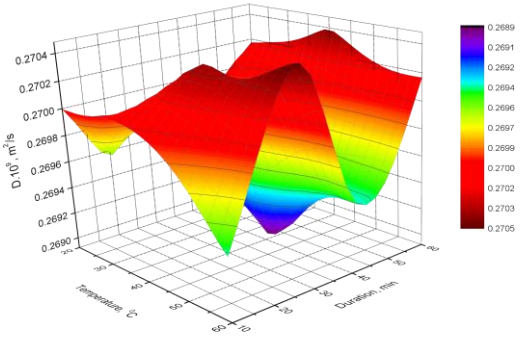


3a) Genotype PA

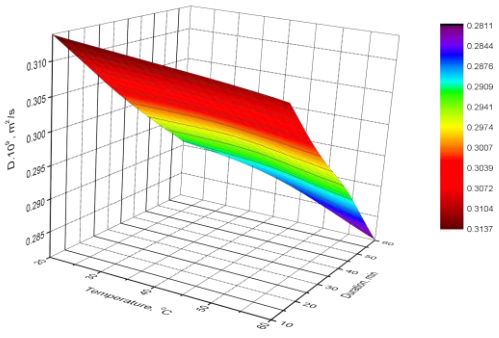


3b) Genotype PB

Fig. 3. Diffusion coefficients of tannins in extracts from physalis leaves with 70 % ethanol.



4a) Genotype PA



4b) Genotype PB

Fig. 4. Diffusion coefficients of tannins in extracts from physalis leaves with 95 % ethanol.

Reasonably, with the increase in the temperature, the values of the diffusion coefficient also increased (with maximums established at 60°C), for the two leaf genotypes.

The highest values of the coefficient of internal molecular diffusion at 60°C for the leaves from PA genotype were as follows: with 30% ethanol – 0.267×10^{-9} m^2/s, with 50% ethanol – 0.273×10^{-9} m^2/s, with 70% ethanol – 0.278×10^{-9} m^2/s, and with 95% ethanol – 0.271×10^{-9} m^2/s.

The highest values of D_{int} for the leaves from PB genotype (at 60°C) were as follows: with 30% ethanol – 0.281×10^{-9} m^2/s, with 50 % ethanol – 0.283×10^{-9} m^2/s, with 70 % ethanol – 0.308×10^{-9} m^2/s, and with 95 % ethanol – 0.310×10^{-9} m^2/s.

The differences in the values of the coefficients on a solvent-concentration base were minimal, for the two genotypes studied. Still, as the graphics reveal, slightly higher values of the coefficient were obtained in the case of extraction with 50 and 70% ethanol, which supports the suggestion that these concentrations favor the diffusion of tannins.

Although the respective values of D_{int} were slightly higher for the leaves from PB genotype, the numerical differences between the genotypes were insignificant. The outline of the graphical dependencies of the diffusion coefficient was practically equal for genotypes PA and PB in the case of extraction with 30 and 50 % ethanol. The coefficient of diffusion took higher values in the final time intervals, which could be explained by the higher concentration of the extracted molecules, due to the overcome diffusion resistance in the end of the process. An exception was the decreasing trend of D_{int} variation during the extraction of genotype PB with 95% ethanol (and to some extent - with 70%). It could be attributed to the maximal amounts of substances extracted in the initial periods of the process, and their reduced availability by its end.

Our study on physalis leaves showed lower values (0.28×10^{-9} – 0.31×10^{-9} m^2/s) of the diffusion coefficients of tannins, compared to data about ethanol extracts from other plants, for example – from laurel leaves (2.05×10^{-9} m^2/s) [31], from sage leaves (16.61×10^{-9} m^2/s) [32], from hawthorn leaves (9.82×10^{-9} – 548×10^{-9} cm^2/s) [33], from paulownia leaves (68.9×10^{-12} cm^2/s) [34]. These results are

connected to the nature of the raw material (cell structure, porosity, size, etc.), the available bioactive substances and the specific conditions of the extraction process – solvent and temperature.

CONCLUSIONS

To the best of our knowledge, this study presents for the first time results about the extraction of tannins from physalis (*P. peruviana* L.) leaves, and in particular – about the coefficient of diffusion of tannins, known as a characteristic index of the extraction of plant materials. The highest values of the diffusion coefficients for the two studied physalis genotypes were obtained at 60°C – 0.28×10^{-9} m^2/s and 0.31×10^{-9} m^2/s, respectively for PA and PB genotypes. The outcomes of the study provide grounds for further investigation on the physalis varieties grown in Bulgaria, with the purpose of revealing more aspects of their composition, bioactivity, benefits and potential for use.

Acknowledgements: *We acknowledge the financial support from the Science Fund of the University of Food Technologies, project 04/18-N. We are most thankful to Prof. N. Panayotov from the Agricultural University, Plovdiv and to Mr. M. Peshovsky from Versol Bio-farm, Lik village for providing the physalis leaves.*

REFERENCES

1. L. Puente, C. Pinto-Munoz, E. Castro, M. Cortes, *Food Res Int.*, **44,** 1733 (2011).
2. J.G. Alvarez-Herrera, H.E. Balaguera-López, G. Fischer, *Acta horticulturae*, **928**, 163 (2012).
3. N. Panayotov, S. Tsorlianis, *Acta Hortic*., **579,** 373 (2002).
4. N. Panayotov, *Agrarian Sciences*, **1,** 9 (2009) (in Bulgarian).
5. N. Panayotov, *Agriculture and Food*, **4,** 115 (2016).
6. N. Panayotov, A. Popova, *Turkish J. Agric. Nat. Sci.*, **SI 1**, 1134 (2014).
7. N. Panayotov, A. Popova, *Turkish J. Agric. Nat. Sci.*, **SI 1**, 1141 (2014).
8. N. Panayotov, A. Popova, *Agro-knowledge J*., **17**, 267 (2016).
9. N. Panayotov, A. Popova, *Acta Horticulturae et Regiotecturae*, **SI 1,** 18 (2016).
10. N. Panayotov, M. Dimitrova, L. Krasteva, D. Dimova, D. Svetleva, *Agro-knowledge J.*, **13**, 547 (2012).
11. N. Panayotov, D. Dimova, A. Popova, V. Ivanova, D. Svetleva, *Optimization of ornamental and garden plant, technologies and environment*, **7,** 157 (2016).
12. Y.-J. Zhang, G.-F. Deng, X.-R. Xu, S. Wu, S. Li, H.-B. Li, *Int. J. Food Nutr. Saf.*, **3,** 15 (2013).
13. M. Yilmaztekin, *Sci. World J.*, **2014,** 8 pages (2014).
14. M. Sathyadevi, S. Subramanian, *Asian J. Pharm. Clin. Res.*, **8,** 152 (2015).
15. M. Ramadan, A. El-Ghorab, K. Ghanem, *J. Arab Soc. Med. Res.*, **26,** 56 (2017).
16. M. Dkhil, S. Al-Quraishy, M. Diab, M. Othman, A. Aref, A. Abdel Moneim, *Food Chem. Toxicol.,* **74,** 98 (2014).
17. A. Puspaningtyas, *Int. Curr. Pharm. J.*, **3,** 265 (2014).
18. A. Eken, B. Únlű-Endirlik, A. Baldemir, S. Ilgűn, B. Soykut, O. Erdem, G. Akay, *J. Clin. Anal. Med.*, **7,** 291 (2016).
19. D. Dag, M. Kilercioglu, M. Oztop, *LWT – Food Sci. Technol.*, **83,** 86 (2017).
20. O. Ertürk, M. Colayvaz, Z. Can, Ü. Karaman, K. Korkmaz, *Indian J. Pharm. Educ. Res.*, **51**, 213 (2017).
21. S. Wu, J. Tsai, S. Chang, D. Lin, S. Wang, S. Huang, L. Ng, *J. Ethnopharmacol*., **108,** 407 (2006).
22. S. Wu, S. Chang, D. Lin, S. Wang, F. Hou, L. Ng, *Food Chem. Toxicol.*, **47,** 1132 (2009).
23. G. Fischer, C. Ulrichs, G. Ebert, *Agronomía Colombiana*, **33**, 155 (2015).
24. A. Kasatkin. Basic processes and apparatuses in chemical technology, Nauka I izkustvo, Sofia, 1960 (in Bulgarian).
25. V. Kafarov. Basics of mass transfer, Visshaya shkola, Moskva, 1962 (in Russian).
26. G. Akselrud, V. Lisyanskii. Extraction in the system "solid – liquid", Khimia, Leningrad, 1974 (in Russian).
27. A. Stoyanova, E. Georgiev. Technology of essential oils, Acad. publ. house UFT, Plovdiv, 2007 (in Bulgarian).
28. E. Georgiev, A. Stoyanova. A guide for the specialist in aromatic industry, BNAEOPC, Plovdiv, 2006 (in Bulgarian)
29. Russian Pharmacopoeia, 11th Edition, Moscow, 1990.
30. V. Beloborodov, V. Dementii, B. Voronenko, *Sci. Works VNIIJ*, **28,** 102 (1971) (in Russian)
31. G. Stefanova, S. Tasheva, S. Damianova, A. Stoyanova, *Sci Works UFT*, **64,** 70 (2017).
32. S. Mollova, PhD Thesis, UFT, Plovdiv (2017) (in Bulgarian).
33. M. Ergezen, PhD Thesis, UFT, Plovdiv (2014) (in Bulgarian).
34. S. Tasheva, DSc Thesis, UFT, Plovdiv (2014) (in Bulgarian).