Bulgarian Chemical Communications, Volume 49, Special Issue G (pp. 212 –216) 2017

Basic chemical components and radical scavenging activity of tobacco extracts obtained by macroporous resin

M.H. Docheva*, M.B. Staykova, A.B. Stoilova, D. Dimanov

Tobacco and Tobacco Products Institute, Markovo 4108, Plovdiv, Bulgaria,

Received November 10, 2016; Revised December 21, 2016

The basic chemical components of tobacco extracts, containing flavonoids (E-Fl) and the radical scavenging activity of E-Fl was study. The extracts were prepared by using polymeric adsorbent Amberlite XAD 7. The tobaccos used in this study were selected from low to high content of nicotine, carbohydrates and polyphenols. The amount of flavonoids in extracts varied vastly depending on the content of flavonoids in the tobacco crop. The extracts contained flavonoids from 3.50 ± 0.26 mg.g^{-1} to 16.3 ± 1.3 mg.g^{-1} and phenolic acids are less than 0.6 mg.g^{-1}. The E-Fl extracts showed a high radical scavenging activity (IC_{50} data varied from 9.6 ± 0.8 μg.ml^{-1} to 33.4 ± 3.0 μg.ml^{-1}). In the extracts the nicotine content was less than 0.38 ± 0.02 mg.g^{-1} and depended strongly on its amount in tobacco, showing recovery mean 1.45 ± 0.31 %. The content of carbohydrates in extracts E-Fl was between 39 ± 1 mg.g^{-1} and 94 ± 4 mg.g^{-1} with mean recovery from tobacco 48%.

***Key words**: tobacco, tobacco extracts, polyphenols, nicotine, carbohydrates, radical scavenging activity*

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is a plant, containing a large number of chemical components. To date, approximately 4200 components have been identified in tobacco, which can be combined in the following large groups: alkaloids, carbohydrates, proteins, polyphenols, organic acids, essential oil, resins and etc. The type of tobacco (Virginia, Burley, Oriental) and how the tobacco is produced and cured affect the type and level of chemical compounds in tobacco leaf [1].

The chemical composition of tobaccos is a subject of extensive research in Bulgaria, especially the basic components as nicotine, carbohydrates, proteins, which are associated with processing technology, quality and the smoking properties of tobacco [2, 3, 4, 5]. In recent years, there is increasing interest on biologically active substances in tobacco - polyphenols, terpenes, alkaloids and etc [6, 7]. Tobacco leaves are rich in polyphenols, presented as phenolic acids (caffeoylquinic acids) and flavonoid glycosides. In the tobacco types Virginia and Oriental, their content can exceed 3% [8, 9, 10].

Phenolic acids possess a wide range of biological properties such as antibacterial, antioxidant, antimicrobial, anticancer and antimutagenic. Phenolic acids are active against human herpes simplex virus and adenoviruses [11]. The interest in bioflavonoids as antioxidants has been increased remarkably over the last decade because of their protective effect against different diseases, including cardiovascular, inflammatory and neurological diseases, as well as cancers. It is known that flavonoid-rich natural products exert a wide range of pharmacological properties. Flavonoids have been associated with a reduction in the incidence of diseases such as cancer and heart diseases [12].

Recently the interest in obtain and use of natural products as supplements has been growing continuously. In view of the high content of phenolic acids and flavonoids in tobacco and their biological properties, several attempts were made to obtain tobacco extracts, enriched in phenolic acids and flavonoids [9, 13, 14, 15]. The chemical composition and the properties of the obtained products are especially important.

The aim of this study was to determine the basic chemical components of tobacco extracts, containing flavonoids (E-Fl) and the radical scavenging activity of E-Fl. The recovery of nicotine and carbohydrates in the extracts was evaluated.

MATERIALS AND METHODS

Plant material

Dry leaves of Oriental tobaccos (Djebel basma 1 – Db 1, Basma 79 – B 79, Muymuynovo seme - Ms, Plovdiv 380 – Pl 380) and Virginia tobaccos (Virginia 385 – V 385 and Koker 254 – K 254) were used as a material. The cultivars were provide to us by Prof. D. Dimanov from the collection of the Tobacco and Tobacco Products Institute, Plovdiv,

* To whom all correspondence should be sent.
E-mail: margarita_1980@abv.bg

Bulgaria. The tobaccos used were selected from low to high content of nicotine, carbohydrates and polyphenols.

Preparation of tobacco extracts, containing flavonoids (E-Fl)

The extracts were prepared by using polymeric adsorbent Amberlite XAD 7, according to the method described by M. Docheva with some modifications [15].

Dry tobacco powder (0.5 g) was extracted with 10 ml 60% (v/v) MeOH for 30 min on a mechanical shaker. The extract was filtered. The solution was added to 3 g macroporous resin Amberlite XAD7. The adsorption was carried out under static conditions for two hours. The flavonoids were desorbed from the resin with 65 ml 100% MeOH on a mechanical shaker for 2 hours.

Determination of polyphenols in tobacco and tobacco extracts E-Fl by HPLC analysis

0.1 g tobacco powder was sonicated for 30 min with 5 ml 60 % MeOH. The extract was filtrated under vacuum. The polyphenols were purified by passing the solution through cartridge C18 according to the method described by S. Dagnon and subjected to HPLC analysis [8].

Aliquot of the obtained extracts E-Fl was passed through a membrane filter 0.45 μm prior to HPLC analysis.

The limit of detection of the polyphenols pointed to LOQ * = 0.6 $\mu g.ml^{-1}$

Determination of nicotine in tobacco by continuous-flow analysis method

The nicotine content in tobaccos was determined according to the ISO 15152:2003 [16].

Determination of nicotine in tobacco extracts E-Fl by spectrometric analysis

The content of nicotine in the extracts was determined according to the ISO 3400:2009 with some modification [17]. Distillation of an aliquot portion of the extracts in two steps was done. Acidification of the solution with a sulfuric acid and remove the neutral and acid steam-volatile substances by distillation. By the next step were done alkalizing with sodium hydroxide solution and distillation of the alkaloids was done. The absorbance of the samples was measured at 236 nm, 259 nm and 282 nm with a spectrophotometer. The amount of tobacco was taken into account. The alkaloid content, expressed as nicotine in $mg.g^{-1}$ is given by formula:

$$Nic = \frac{AV_0V_2}{alV_1}$$

where:

a - absorptivity of nicotine in 0.025 mol/l sulfuric acid solution, i.e. 34.3 at the absorption maximum of 259 nm

A – corrected absorbance calculated from the absorbance measured at wavelengths of 236 nm, 259 nm and 282 nm

$$A = 1.059\left(A_{259} - \frac{A_{236} + A_{282}}{2} \right)$$

l – optical path length of the cell, in centimetres

V_0 - the volume of extracts, in millilitres

V_1 – the aliquot of portion of V_0 used for the distillation, in millilitres

V_2 – the volume of distillate from the alkaline distillation, in millilitres

The relative standard deviation of the method (RSD) was 1.1 %. The limit of detection (LOD) of the nicotine was 0.2 $\mu g.ml^{-1}$ and the limit of quantification (LOQ) was 0.7 $\mu g.ml^{-1}$, calculated by the formulas:

LOD = 3s/b and LOQ = 10s/b

where:

s - standard deviation of the lowest point of the calibration curve

b - slope of the calibration curve described by the equation y = a + bx

Determination of carbohydrates in tobacco and in tobacco extracts E-Fl by continuous-flow analysis method

The content of carbohydrates in tobacco was determined according to the ISO 15154:2003 [18].

Determination of radical scavenging activity of E-Fl by DPPH assay

The E-Fl extract was evaporated to dryness and its weight was measured. The dry extract was solved in 5 ml MeOH. The $DPPH^{\bullet}$ assay was carried out as described by M. Docheva [15].

Statistics

All experimental procedures were done in triplicate. The quantitative data were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Preparation of tobacco extracts, by various techniques, has already been the subject of many investigations. In the most cases, the extracts contain both phenolic acids and flavonoids [9, 13]. There have not been found enough data on the chemical composition of the tobacco extracts. Our aim was to study the content of nicotine, carbohydrates and polyphenols in tobacco extracts, obtained by macroporous resin.

Basic chemical components of tobaccos

The content of nicotine, carbohydrates and polyphenols in different varieties of tobacco is present in Table 1. The data in Table 1 show that Oriental tobaccos Db 1, B 79, Ms and P 380 contain more polyphenols (average 31.8 $mg.g^{-1}$) by comparing to Virginia tobaccos – average 17.9 $mg.g^{-1}$. Tobacco variety Ms is characterized with the highest content of polyphenols – 51 ± 4 $mg.g^{-1}$, followed by B 79 – 35.6 ± 2.8 $mg.g^{-1}$, while variety P 380 and V 385 contain lowest amount of polyphenols (approximately 15 $mg.g^{-1}$).

The amount of carbohydrates, as primary metabolites, varies widely in tobaccos - from 82.8 ± 3.3 $mg.g^{-1}$ (V 385) to 193 ± 8 $mg.g^{-1}$ (Db 1). The average carbohydrates amout in Oriental tobaccos is 147 $mg.g^{-1}$, while in Virginia tobaccos is lower - 118 $mg.g^{-1}$. These results confirm the established correlation between the content of carbohydrates and polyphenols [19] where tobaccos with high content of carbohydrates exert higher polyphenols content.

The content of nicotine in Oriental tobaccos Db 1, B79 and Ms is lower than 6.7 $mg.g^{-1}$ (0.67 %). An exception can be seen by Pl 380 - 16.3 ± 0.3 $mg.g^{-1}$. Virginia tobaccos V 385 and K 254 are characterized with higher nicotine content – 26.9 ± 0.5 $mg.g^{-1}$ and 15.4 ± 0.3 $mg.g^{-1}$ respectively. These results are in accordance with the varietal characteristics of tobaccos [20, 21].

Extracts, containing flavonoids (E-Fl)

Table 2 shows the content of flavonoids, phenolic acids, carbohydrates and nicotine in tobacco extracts.

The E-Fl extracts contain flavonoids from 3.50 ± 0.26 $mg.g^{-1}$ (V 385) to 16.3 ± 1.3 $mg.g^{-1}$ (Ms). The amount of flavonoids in the extracts is proportional to the content of flavonoids in tobaccos.

The carbohydrates amounts in E-Fl extracts vary between 39 ± 1 $mg.g^{-1}$ (K 254) and 94 ± 4 $mg.g^{-1}$ (B 79) with mean recovery from tobacco about 48%.

The E-Fl extracts contain minimum amount of phenolic acids and nicotine. The nicotine content varies from ≤LOQ to 0.38 ± 0.02 $mg.g^{-1}$ and shows recovery maximum 2 %. The recovery of phenolic acids in these extracts is from 1.8 % to 5 % and relate to the amount of the phenolic acids in tobaccos.

The data reveal that is no correlation between the amount of carbohydrates in extracts and in tobaccos. Oriental tobacco variety B 79 and Virginia tobacco K 254 contain an equal amount of carbohydrates – 155 $mg.g^{-1}$ (Table 1), while their extracts show a significant difference in carbohydrates content. The extract derived from B 79 contains 94 ± 4 $mg.g^{-1}$ carbohydrates, whereas the extract derived from K 254 – 39 ± 1 $mg.g^{-1}$ (Table 2).

Table 1. Content of nicotine, carbohydrates and polyphenols in different varieties of tobacco

Tobacco varieties	Basic chemical components in tobaccos, $mg.g^{-1}$			
	Flavonoids	Phenolic acids	Carbohydrates	Nicotine
Oriental tobaccos				
Djebel basma 1	12.2 ± 1.0	13.7 ± 1.1	193 ± 8	2.00 ± 0.04
Basma 79	15.5 ± 1.2	20.1 ± 1.6	156 ± 6	4.30 ± 0.09
Muymuynovo seme	23.3 ± 1.9	27.8 ± 2.2	129 ± 5	6.70 ± 0.13
Plovdiv 380	6.8 ± 0.5	8.1 ± 0.6	112 ± 5	16.3 ± 0.3
Virginia tobaccos				
Virginia 385	4.51 ± 0.36	10.4 ± 0.8	82.8 ± 3.3	26.9 ± 0.5
Koker 254	7.4 ± 0.5	13.8 ± 1.1	154 ± 6	15.4 ± 0.3

Table 2. Content of flavonoids, carbohydrates and nicotine in tobacco extracts E-Fl

Tobaccos varieties	Basic chemical components in E-Fl, $mg.g^{-1}$			
	Flavonoids	Phenolic acids	Carbohydrates	Nicotine
Oriental tobaccos				
Djebel basma 1	8.1 ± 0.6	>LOQ*	55 ± 2	>LOQ**
Basma 79	13.3 ± 1.0	1.02 ± 0.08	94 ± 4	0.0500 ± 0.0033
Muymuynovo seme	16.3 ± 1.3	0.51 ± 0.04	82 ± 4	0.090 ± 0.006
Plovdiv 380	5.03 ± 0.37	0.34 ± 0.03	67 ± 3	0.32 ± 0.02
Virginia tobaccos				
Virginia 385	3.50 ± 0.26	>LOQ*	48 ± 2	0.38 ± 0.02
Koker 254	4.77 ± 0.35	>LOQ*	39 ± 1	0.15 ± 0.01

LOQ * = 0.6 $\mu g.ml^{-1}$, LOQ ** = 0.7 $\mu g.ml^{-1}$

Table 3. Radical scavenging activity of E-Fl extracts

Tobaccos	Flavonoids in extracts, %	IC_{50} μg.ml^{-1}
Oriental tobaccos		
Djebel basma	22.5 ± 3.0	19.8 ± 1.7
Basma 79	25.6 ± 3.1	11.3 ± 1.0
Muymuynovo seme	37 ± 4	9.6 ± 0.8
Plovdiv 380	10.9 ± 1.2	16.9 ± 1.4
Virginia tobaccos		
Virginia 385	10.2 ± 1.2	33.4 ± 3.0
Koker 254	20.3 ± 2.3	16.4 ± 1.4
Rutin		3.20 ± 0.20

The free radical scavenging activity of E-Fl is presented as IC_{50} μg.ml^{-1} in Table 3. Lower IC_{50} values correspond to higher radical scavenging activity of the extracts. Rutin, well-known antioxidant compounds with a structure similar to those of the tobacco flavonoids, is employed as reference compound with IC_{50} = 3.20 ± 0.20 μg.ml^{-1}. The data in Table 3 reveal the highest scavenging activity of Ms extract (IC_{50} =9.6 ± 0.8 μg.ml^{-1}) which can be associated with the highest content in flavonoids – 37 ± 4 %.

The content of flavonoids in extracts, obtained from Oriental tobacco P 380 and Virginia tobacco V 385 are equal (average 10.5 ± 0.4 %), while the radical scavenging activity of V 385 extract (IC_{50} = 33.4 ± 3.0 μg.ml^{-1}) is twice lower than P 380 extract (IC_{50} = 16.9 ± 1.4 μg.ml^{-1})

The obtained data coincide with the results in previous studies where, despite the lower content of flavonoids in the extract from Virginia tobacco, its radical scavenging activity is higher than that of extracts from Oriental tobaccos. This fact confirms that there are some other chemical components in the extracts, other than flavonoids, which are variety depending and influence the DPPH radical scavenging activity [15].

CONCLUSION

In this study the content of phenolic acids, flavonoids, nicotine and carbohydrates in tobacco extracts, containing flavonoids (E-Fl), obtaining by macroporous resin, was investigated. All extracts were purified from phenolic acids and nicotine. The amounts of flavonoids, phenolic acids and nicotine in extracts strongly depend on their amount in tobaccos. The content of carbohydrates in extracts (E-Fl) did not depend on its amount in tobaccos. E-Fl showed a high radical scavenging activity.

REFERENCES

1. Rodgman A., T. Perfetti: The chemical components of tobacco and tobacco smoke. CRC Press Taylor & Francis Group, New York, 2013.
2. A. Stoilova, M. Docheva D. Dimanov, *Bulgarian tobacco*, **1** ,18 (2015).
3. Y. Dyulgerski, *Genetics and Breeding*, **38, 3-4,** 145 (2009).
4. Y. Dulgerski, *Тутун /Tobacco* **64, 7-12,** 13 (2014).
5. M. Kasheva, J. Kochev, *International scientific-practical conference "Food technology and health"*, 12 (2012).
6. T. Ivanova: PhD Thesis, UHT, Plovdiv, 2014.
7. V. Popova, DSc Thesis, UHT, Plovdiv, 2015.
8. S. Dagnon, A. Edreva, *Contributions to Tobacco Research*, **20**, 5, 355 (2003).
9. H. Wang, M. Zhao, B. Yang, Y. Jiang, G. Rao, *Food Chemistry* **107,** 1399 (2008).
10. G. Xiang, H. Yang, L. Yang, X. Zhang, Q. Cao, M. Miao, *Microchemical Journal* **95,** 198 (2010).
11. http://cdn.intechopen.com/pdfs/29974.pdf
12. A. Scalbert, I. Johnson, M. Saltmarsh, *American Journal of Clinical Nutrition* **81, 1** 215 – 217 (2005).
13. J. Zhang, S. Zhang, J. Wu, N. Li, J. Wang, *Journal of Medicinal Plants Research* **7, 24,** 1784 (2013).
14. M. Docheva, S. Dagnon, S. Statkova-Abeghe, *Nat Prod Res.*, 1328 (2014).
15. M. Docheva, S. Dagnon, *Comptes rendus de l'Académie bulgare des Sciences*, **68, 2,** 183 (2015).
16. ISO 15152:2003 – "Tobacco – Determination of the content of the total alkaloids as nicotine – Continuous-flow analysis method".
17. ISO 3400:2009 – "Cigarettes – Determination of alkaloids in smoke condensates – Spectrometric method".
18. ISO 15154:2003 – "Tobacco – Determination of the content of reducing carbohydrates – Continuous-flow analysis method".
19. J. Wooten, N. Kalengamaliro, D. Axelson, *Phytochemistry*, **70,** 940 (2009).
20. D. Dimanov, R. Todorova, J. Kochev, *Scientific papers – Kardzhali*, **4, 2,** 168 (2010).
21. D. Dimanov, *Тутун/ Tobacco* **61,** 130, (2011).

ОСНОВНИ ХИМИЧНИ КОМПОНЕНТИ И РАДИКАЛ УЛАВЯЩА АКТИВНОСТ НА ТЮТЮНЕВИ ЕКСТРАКТИ ПОЛУЧЕНИ ЧРЕЗ АДСОРБЦИОННА СМОЛА

М. Х. Дочева*, М. Б. Стайкова, А. Б. Стоилова, Д. Диманов

Институт по тютюна и тютюневите изделия, Марково 4108, Пловдив, България,
e-mail: margarita_1980@abv.bg

Постъпила на 10 ноември 2016 г.; приета на 21 декември 2016 г.

(Резюме)

Изследвано е съдържанието на основни химични компоненти в тютюневи екстракти, съдържащи флавоноиди (Е-Фл), получени чрез използване на адсорбционна смола Amberlite XAD 7. Определена е радикал-улавящата активност на екстрактите. Тютюните са подбрани с различно съдържание на никотин, въглехидрати и полифеноли. Количеството на флавоноидите в екстрактите варира значително и е в пряка зависимост от съдържанието им в тютюневата суровина. Съдържанието на флавоноиди в Е-Фл е между 3.50 ± 0.26 $mg.g^{-1}$ и 16.3 ± 1.3 $mg.g^{-1}$. Установено е по-малко от 0.6 $mg.g^{-1}$ фенолни киселини. Екстрактите Е-Фл показват висока радикал улавяща активност (стойностите на IC_{50} варират от 9.6 ± 0.8 $\mu g.ml^{-1}$ до 33.4 ± 3.0 $\mu g.ml^{-1}$). Съдържанието на никотин в Е-Фл е по-ниско от 0.38 ± 0.02 $mg.g^{-1}$ и е пропроционално на това в тютюните. Максималният добив на никотин, изчислен спрямо съдържанието му в тютюна е до 2 %. Количеството на въглехидратите в екстрактите (Е-Фл) варира между 39 ± 1 $mg.g^{-1}$ и 94 ± 4 $mg.g^{-1}$. Отчетен е среден добив от 48 %.

Ключови думи: *тютюн, тютюневи екстракти, полифеноли, никотин, въглехидрати, радикал-улавяща активност*