Bulgarian Chemical Communications, Volume 56, Special Issue D2 (pp. 05-09) 2024 DOI:10.34049/bcc.56.D.S2P17

Factors affecting the 5-hydroxymethyl-2-furfural content in the gas-phase of tobacco smoke of commercial cigarette brands

M. H. Docheva*, D. M. Kirkova, L. S. Stoyanova

Tobacco and Tobacco Products Institute, Agricultural Academy, Markovo 4108, Bulgaria

Received: November 3, 2023; Revised: April 11, 2024

Carbonyl compounds are some of the most harmful substances in tobacco smoke. They have mutagenic and carcinogenic properties and because of that, carbonyls are part of the controlled compounds in tobacco smoke in some countries. They cause burning and irritation of the upper respiratory tract of people during the smoking process. Acetaldehyde, acetone, acrolein, formaldehyde and 5-hydroxymethyl-2-furfural (5-HMF) are present in the largest quantities in tobacco smoke. 5-HMF is a product of the Maillard reaction and is found in many foods. In tobacco smoke, 5-HMF, together with the other carbonyl compounds, is formed by thermal combustion of the soluble sugars and the polysaccharides contained in the tobacco blends. 5-HMF has proven toxic and mutagenic properties. In this study, the factors affecting the 5-HMF content in the gas phase of tobacco smoke of different types of commercial cigarette brands are established. Three factors influencing the content of 5-HMF are investigated - type of blend, filter ventilation and type of filter. Higher content of 5-HMF is produced by Virginia blend cigarettes (63.0±5.1 µg/cig) compared to American blend cigarettes (28.0±2.2 µg/cig and 48.0±3.8 µg/cig).The cigarette brands with 30 - 40 % filter ventilation reduced the content of 5-HMF in tobacco smoke between 43 % and 58 % compared to the non ventilation filters. The content of 5-HMF is not significantly affected by the type of cigarette filter.

Key words: 5-hydroxymethyl-2-furfural (5-HMF), cigarette brands, cigarette blends, filter ventilation, cigarette filter

INTRODUCTION

There is a wide variety of smoking tobacco products on the world market to choose from, including cigarettes, cigars, cigarillos, bidis, chuttas and kreteks. Cigars, cigarillos, bidis, chuttas and kreteks consist only of tobacco leaves. Cigarillos are short, narrow cigars and are wrapped in tobacco leaves or brown, tobacco-based paper. They are smaller than regular cigars but usually larger than cigarettes. Bidis consist of a small amount of sun-dried, flaked tobacco hand wrapped in dried temburni or tendu leaf and tied with string. Despite their small size, bidis deliver more tar and carbon monoxide than manufactured cigarettes. Chuttas are homemade cigars and have a higher content of nicotine and total particulate matter as compared to cigarettes and Bidi. Kreteks are clove-flavored cigarettes. They may contain a wide range of exotic flavorings and eugenol, which have an anesthetic effect, allowing for deeper and more harmful smoke inhalation [1].

In the production of cigarettes, blended tobaccos are used - a mixture of two or more types of tobaccos in different proportions. The type of tobacco used in tobacco products has a decisive influence on the physicochemical nature and taste of the tobacco smoke they produce [1].

Tobacco smoke is a multicomponent system consisting of a solid-liquid phase, a gas phase, and environmental tobacco smoke. The qualitative composition of tobacco smoke is the same. The differences are only quantitative [2].

Tobacco smoke of the commercially sold cigarettes contains more than 6000 chemicals. They are formed during smoking processes, through pyrolysis, intra- or intermolecular interactions of the substances contained in tobacco. Most of these substances are potential toxicants, such as components of the groups of volatile organic compounds, polycyclic aromatic hydrocarbons, aromatic amines, tobacco-specific nitrosamines, phenolic compounds, carbonyl compounds [2-6].

More than 500 carbonyl compounds are identified in tobacco smoke, but ten of them are classified as probably and possibly carcinogenic to humans by the International Agency for Research of Cancer [3]. They cause burning and irritation of the upper respiratory tract of people when smoking. Carbonyls, including acetaldehyde, acetone, acrolein, formaldehyde, 5-hydroxymethyl-2-furfural (5-HMF), are present at high levels in the tobacco smoke [5].

5-HMF is formed in many food items - dried fruit, fruit juice, caramel products. It is also detected in cigarette smoke [7]. Tobacco smoke is produced

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

by the thermal combustion of sugars in tobacco products, including complex polysaccharides such as cellulose, along with other carbonyl compounds [8]. 5-HMF has proven carcinogenic, hepatotoxic, nephrotoxic, neurotoxic, genotoxic, mutagenic, cytotoxic effect, reproductive and developmental toxicity [7].

The aim of this study is to investigate the factors affecting the content of 5-HMF in the gas phase of tobacco smoke in commercial cigarette brands.

EXPERIMENTAL

Material

The content of 5-HMF in tobacco smoke of 3 commercial cigarette brands (Virginia blends and two American blends) with different types of cigarette filters and filter ventilation were investigated. The cigarettes were purchased from the markets. The description of the investigated cigarettes is presented on Table 1.

Table 1. Description of the cigarette brands

Cigarettes
Brand A
Virginia blend A-nV-A –non-ventilation, acetate filter A-V35-A - 35 % ventilation, acetate filter A-nV-M –non-ventilation, menthol capsule
Brand B
American blend B-nV-A –non-ventilation, acetate filter B-nV-Ach –non-ventilation, acetate and charcoal filter (two sectors filter) B-V30-Ach – 30 % ventilation, acetate and charcoal filter (two sectors filter)
Brand C
American blend C_1-nV-A –non-ventilation, acetate filter C_1-V40-A – 40 % ventilation, acetate filter C_2-nV-ARCh – non-ventilation, acetate, recessed and charcoal filter system (three sectors filter) C_2-V40-ARCh – 40 % ventilation, a cetate, recessed and charcoal filter system (three sectors filter)

Three factors influencing the content of 5-HMF were investigated:

- Tobacco blends - Virginia blend and American blend (cigarette brands A-nV-A, B-nV-A and C_1-nV-A).
- Filter ventilation – non-ventilation, 30 % ventilation, 35 % ventilation, 40 % ventilation (cigarette brands A-nV-A and A-V35-A; B-nV-ACh and B-V30-Ach; C_1-nV-A and C_1-V40-A; C_2-nV-ARCh and C_2-V40-ARCh).
- Filter types – acetate; acetate and charcoal (two sectors); acetate, recessed and charcoal (three sectors) -A-nV-A and A-nV-M; B-nV-A and B-nV30-Ach; C_1-nV-A and C_2-nV-ARCh; C_1-V40-A and C_2-V40-ARCh.

Reagents

5-Hydroxymethyl furfural, 2,4-dinitrophenyl-hydrazine, acetonitrile, perchloric acid, glacial acetic acid, hydrochloric acid 37 %, sodium hydroxide, calcium chloride hexahydrate, *p*-hydroxybenzoic acid hydrazide (PAHBAH), citric acid monohydrate, D-glucose were purchased from Sigma Aldrich.

Methods

- *Determination of carbonyl compounds in tobacco smoke by HPLC-UV/VIS –Coresta recommended method №74, 2018 with some modifications* [9]. Two cigarettes were smoked according to ISO 3308-2012 [10] on an 8-channel linear smoking machine Filtrona 302 fitted with an impinger containing 40 ml of an acidified solution of 2,4-dinitrophenylhydrazine with a concentration of 3.396 mg/ml. The carbonyls in tobacco smoke were trapped by passing each puff through an impinger. The solution was left in the dark for at least 5 hours until the reaction is completed and the carbonyl hydrazone formed. The high-performance liquid chromatograph Perkin Elmer equipped with binary pump and UV/VIS detector was used. The chromatographic analysis was performed on an analytical column "Kromasil" C18, 5 µm, 150 mm. The mobile phase composition was: A = CH_3OH:H_2O (60:40); B = CH_3OH:H_2O (80:20). Gradient elution profile was 100% A, 0 min; 30 min to 0 % A, λ=360 nm.
- *Determination of the content of total sugars by continuous-flow analysis method using hydrochloric acid/p-hydroxybenzoic acid hydrazide (PAHBAH) – Coresta recommended method №89, 2019* [11]. An aqueous extract of the tobacco was prepared and the total sugar content of the extract was analyzed by continuous-flow analysis on Autoanalyzer AA3. The extract was heated in the presence of HCl at 90 °C, which hydrolyses any sucrose present to glucose and fructose. The reduced sample extract was passed through a dialyzer to eliminate interference from colored compounds in the sample and then reacted with PAHBAH in alkaline medium at 85 °C to produce a yellow

osazone complex. All measurements were performed at 420 nm.

All experiments were performed at least three times. 5-HMF yield in tobacco smoke was calculated as mg per cigarette. Total sugars in tobacco blends were calculated as %. All data are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Type of blend

The most commonly used cigarette blends are American blend and Virginia blend [1, 12].

The content of 5-HMF in tobacco smoke of two brands of American blend cigarettes and one brand of Virginia blend cigarettes were investigated. In order to eliminate the influence of the filter type and filter ventilation, cigarettes were selected with the same type of filter – non-ventilation, acetate filter (Table 1). The results are presented in Table 2. The higher 5-HMF emissions were produced by Virginia blend cigarettes (63.0±5.1 µg/cig - A-nV-A) compared to American blend cigarettes (48.0±3.8 µg/cig – C_1-nV-A and 28.0±2.2 µg/cig – B-nV-A). It was found that the difference between the highest and the lowest content of 5-HMF in tobacco smoke is 35 µg/cig.

Table 2. Content of 5-HMF in tobacco smoke of different cigarette blends, µg/cig±SD

Tobacco blend	Cigarette brands	5-HMF
Virginia blend	A-nV-A	63.0±5.1
American blend	B-nV-A	28.0±2.2
American blend	C_1-nV-A	48.0±3.8

The obtained results can be explained by the composition of the different tobaccos included in the tobacco blends. In Virginia blends there is an approximate ratio on a weight-weight basis (w/w) of Virginia tobacco and Burley 68:27 (w/w). The ratio can be skewed in favor of either tobacco type or manipulation of the deliveries. Oriental tobacco makes up less than 5% w/w of the tobacco blend [13]. The American blend consists of Virginia, Burley and Oriental tobacco in a ratio of approximately 50:37:13 (w/w/w) [13]. In the Virginia blend, the total amount of Virginia and Oriental tobacco is higher than in the American blend. Kirkova *et al.* found that the highest content of 5-HMF was produced in tobacco smoke by Virginia tobaccos (between 56 µg/cig and 76 µg/cig) and Oriental tobaccos (between 34.2 µg/cig and 108 µg/cig), compared to the Burley tobacco (between 15.6 µg/cig and 35.0 µg/cig) [14].

Filter ventilation

Filter ventilation dilutes tobacco smoke with air, which is achieved through filter holes, which are usually one or more rings of small holes or perforations and serve to dilute the smoke with air [12]. Ventilated filters are now common on cigarettes sold in EU countries. Filter ventilation reduces standard toxin yields, such as tar, nicotine and CO [12, 15], but the influence of filter ventilation on the content of 5-HMF in tobacco smoke is not investigated. In this regard, ventilated cigarettes A-V35-A, B-V30-Ach, C_1-V40-A and C_2-V40-ARCh were selected. In the research both ventilated cigarettes and closed ventilated cigarettes were used.

The content of 5-HMF in cigarette brands with non-ventilated filters varied between 63.0±5.1 µg/cig (A-nV-A) and 28.0±2.2 µg/cig (B-nV-ACh), while the content of 5-HMF with filter ventilation varied from 12.2±0.9 µg/cig (B-V30-ACh) to 32.6±2.6 µg/cig (A-V35-A) – Table 3. The data revealed that the cigarette brands with 30% - 40% filter ventilation reduced the content of 5-HMF in tobacco smoke between 43% (Brand B) and 58% (Brand C) compared to non-ventilated filters. The results are presented in Table. 3.

Table 3. Content of 5-HMF in tobacco smoke of cigarette brands with different filters ventilation, µg/cig±SD

Filter ventilation	Cigarette brands	5-HMF
Non ventilation	A-nV-A	63.0±5.1
35 % ventilation	A-V35-A	32.6±2.6
Non ventilation	B-nV-ACh	28.0±2.2
30 % ventilation	B-V30-ACh	12.2±0.9
Non ventilation	C_1-nV-A	48.0±3.8
40 % ventilation	C_1-V40-A	20.0±2.8
Non ventilation	C_2-nV-ARCh	40.0±3.2
40 % ventilation	C_2-V40-ARCh	22.8±3.5

The content of 5-HMF in the gas phase of tobacco smoke decreases in proportion to the decrease in the content of tar, nicotine and CO in the solid-liquid phase of tobacco smoke. Docheva *et al.* 2022 established that the content of tar and CO in cigarettes with 30 % ventilation decreased by about 92 %, while the nicotine content - by 36 % compared to non-ventilation cigarettes [16].

Type of filters

To determine the effect of the type of cigarette filters on the content of 5-HMF, cigarette brand A and cigarette brand B were smoked with block vents (non-ventilation filter). Cigarette brand C was smoked with filter ventilation and filter non-ventilation. The results are presented in Table 4.

Table 4. Content of 5-HMF in tobacco smoke of cigarette brands with different filter types, µg/cig±SD

Filter types	Cigarette brands	5-HMF
Acetate	A-nV-A	63.0±5.1
Acetate menthol capsule	A-nV-M	70.0±6.2
Acetate	B-nV-A	28.0±2.3
Acetate and charcoal filter (two sectors filter)	B-nV-ACh	39.0±3.2
Acetate	C_1-nV-A	48.0±3.8
Acetate, recessed and charcoal filter system (three sectors filter)	C_2-nV-ARCh	40.0±3.2
Acetate filter	C_1-V40-A	20.0±2.8
Acetate, recessed and charcoal filter system(three sectors filter)	C_2-V40-ARCh	22.8±3.5

Cigarette filters with an activated menthol capsule (A-nV-M) and acetate and charcoal filter (two sectors) (B-nV-ACh) increased the content of 5-HMF in tobacco smoke. The amount of 5-HMF in cigarette brands with acetate filter with activated menthol capsule (A-nV-M) is 70.0±6.2 µg/cig and it is 7.0 µg/cig more than the acetate filter (A-nV-A). Cigarettes with acetate and charcoal filter (B-nV-ACh) produced 39.0±3.2 µg/cig 5-HMF, while those with acetate filter (B-nV-A) - 28.0±2.3 µg/cig. For cigarette brand C with non-vented three-sector filter (C_2-nV-ARCh) a reduction in content of 5-HMF of 8.0 µg/cig is reported compared to non-vented acetate filter C_1-nV-A. Cigarette brand with three-sector ventilated filter (C_2-V40-ARCh) showed approximately the same 5-HMF content (22.8±3.2 µg/cig) compared to acetate ventilated filter (C_1-V40-ARCh) - 20.0±0.8 µg/cig.

The content of 5-HMF in tobacco smoke in one cigarette is significantly lower than that of the toxic effect of 5-HMF - 2–30 mg per person/day [17, 18].

Content of soluble sugars in cigarette tobacco blends

The main degradation product in the caramelization reaction of sugars is 5-HMF [8]. In this regard the content of soluble (total) sugars in tobacco blends A, B and C was analyzed. The results are presented in Table 5. The content of soluble sugars in the Virginia blend (13.7±0.14 %) is higher than in the American blend – 8.5±0.08 % and 10.8±0.11 %. The results obtained are consistent with the ratio of the different types of tobacco in the blends and the content of total sugars in tobaccos. Virginia blend has more than 68% Virginia tobacco content, while American blend has less Virginia tobacco content - about 50% [13].

Table 5. Content of soluble sugars in tobacco of cigarette brands, %±SD

Tobacco blend	Total sugars
Virginia blend (A)	13.7±0.14
American blend (B)	8.5±0.08
American blend (C)	10.8±0.11

Correlation between 5-HMF in tobacco smoke and soluble sugars in cigarette brands

Additionally, the ratio between the content of 5-HMF in tobacco smoke and total sugars in cigarette brands with different type of blends and different filter ventilation was investigated. The correlation (R^2) between the content of 5-HMF in tobacco smoke and total sugars in cigarette brands with different type of blends and different filter ventilation is presented in Table 6.

Table 6. Correlation (R^2) between the content of 5-HMF in tobacco smoke and soluble sugars in cigarette brands with different type of blends, different filter ventilation and different filter type.

Correlation	Soluble sugars, %		
	Type of blends	Filter ventilation	Filter type
5-HMF, µg/cig	0.978	0.9864	0.2283

A high correlation between the content of 5-HMF in tobacco smoke and total sugars in cigarette brands with different type of blends and different filter ventilation was found. The content of 5-HMF is not affected by the type of filter.

CONCLUSION

In this study three factors affecting the 5-HMF content in the gas phase of tobacco smoke in different types of commercial cigarette brands were investigated - type of blend, filter ventilation and type of filter. 5-HMF is a degradation product on the caramelization reaction of sugars during the smoking process. The highest content of 5-HMF was found in tobacco smoke of Virginia blend cigarettes, followed by American blend cigarettes. Filter ventilation reduced the 5-HMF content between 43 % and 58 % compared to the non ventilation filters. Cigarette filters with an activated menthol capsule and acetate and charcoal filter increased the content of 5-HMF in tobacco smoke. Cigarette brand with acetate, recessed and charcoal filter system showed approximately the same 5-HMF content compared to acetate ventilated filter.

***Acknowledgement**: This research is supported by the Bulgarian Ministry of Education and Science under the National Program "Young Scientists and Postdoctoral Students – 2".*

REFERENCES

1. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 83. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, Lyon (FR), International Agency for Research on Cancerm 2004.
2. F. Soleimani, S. Dobaradarn, G.Torre, T. Schmidt, R. Saeedi, *Sci Total Environ.,* **813**, 152667 (2022).
3. A. Rodgman, A. Perfetti A. The Chemical Components of Tobacco and Tobacco Smoke, 2nd edn., CRC Press; Boca Raton, Florida, 2013.
4. J. Leffingwell, Chemical Constituents of Tobacco Leaf and Differences among Tobacco Types, Science Direct Working Paper No S1574-0331 (04) 70433-6, 1–60 (2001).
5. T. Horinouchi, T. Higashi, Y. Mazaki, S. Miwa, *Biol Pharm Bull*., **39 (6),** 909 (2016).
6. WHO Elaboration of guidelines for implementation of the Convention (decision FCTC/COP1(15)) Article 9: Product regulation Conference of the Parties to the WHO Framework Convention on Tobacco Control Second session, 2007
7. M. Farag, M. Alagawany, M. Bin-Jumah, S. Othman, A. Khafaga, H. Shaheen, D. Samak, A. Shehata, A. Allam, M. El-Hack, *Molecules,* **25: 1941** (2020).
8. M. Banožic, S. Jokic, D. Ackar, M. Blažic, D. Šubaric, *Molecules,* **25,** 1734 (2020).
9. CRM 74 Determination of Selected Carbonyls in Mainstream Cigarette Smoke by High Performance Liquid Chromatography (HPLC), 2019.
10. ISO 3308:2012. Routine analytical cigarette-smoking machine - Definitions and standard conditions, 2012.
11. CRM 89 Tobacco – Determination of the content of total sugars – continuous-flow analysis method using hydrochloric acid / *p*-hydroxybenzoic acid hydrazide (PAHBAH), 2019.
12. D. Hoffmann, I. Hoffmann, in: Risks Associated with Smoking Cigarettes with Low Machine-Measured Yields of Tar and Nicotine (Smoking and Tobacco Control Monograph No. 13; NIH Publ. No. 02-5074), Bethesda, MD, National Cancer Institute, p. 159 2001.
13. J. Wigand, Additives, Cigarette Design and Tobacco Product Regulation. A Report to World Health Organization, Tobacco Free Initiative, Tobacco Product Regulation Group, 28 June-2 July 2006, Kobe, Japan, 2006.
14. D. Kirkova, M. Docheva, Y. Kochev, *Proceed. of National Sci. Conf. with Intern. Participation "Ecology and Health"* 25-26 June 2020, Plovdiv, Bulgaria, 100 (2020).
15. L. Kozlowski, R. O'Connor, *Tobacco Control*, **11**:i40-i50 (2002).
16. M. Docheva, Y. Kochev, M. Kasheva, H. Bozukov*Kem. Ind*., **71 (3-4):** 157−161 (2022).
17. K. Appel, R. Gürtler, K. Berg, G. Heinemeyer, A. Lampen, K. Appel, *Mol. Nutr. Food Res.,* **55**: 667–678 (2011).
18. S. De La Cueva, J. Álvarez, A. Vegvari, J. Montilla-Gómez, O. Cruz-López, C. Delgado-Andrade, J. Henares, R. Ángel, *Mol. Nutr. Food Res.*, **61,** 1600773 (2016)