Bulgarian Chemical Communications, Volume 51, Special issue D (pp. 113 – 118) 2019

Determination of carbonyl compounds in tobacco smoke and factors influencing their composition

M. H. Docheva*, D. M. Kirkova, Y. G. Kochev, A. B. Stoilova

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

Received October 31, 2018; Revised December 18, 2018

Over 500 carbonyl compounds were identified in tobacco smoke, but ten of them were classified as probably and possibly carcinogenic to humans. The aim of this study was to determine the content of carbonyl compounds in tobacco smoke of different varieties of Bulgarian tobaccos and industrial cigarettes brands and to establish the influence of the type of tobacco and the cigarette filter on their composition. Basic carbonyl compounds such as acetaldehyde, formaldehyde, acetone, propionaldehyde and acrolein were quantified by HPLC. The highest carbonyl content in tobacco smoke was found in Virginia tobacco varieties (average 1003±129 μg/cig), followed by Oriental tobacco varieties (average 868±112 μg/cig) and Burley tobacco varieties (850±47 μg/cig). The greatest variation in the total amount of carbonyls and individual components - formaldehyde, acetaldehyde and acetone was reported in tobacco smoke from Oriental tobacco varieties. The activation of menthol capsule in the filter, the charcoal filter and recessed and charcoal filter system increased the carbonyl content in cigarette brands compared to the acetate filter. Cigarettes with filter ventilation had lower carbonyl content in tobacco smoke compared to those with unventilated filters. Positive correlations between total carbonyl content and tar, nicotine, CO and individual carbonyls in cigarette brands were found.

Keywords: carbonyl compounds, tobacco, cigarette brands, cigarette filter, filter ventilation

INTRODUCTION

Tobacco smoke is a dynamic aerosol system including particulate phase and gas phase and consists of more than 5000 chemical components [1]. The particulate phase contains of well-known constituents such as nicotine, tar, tobacco-specific N-nitrosamines (TSNA), polycyclic aromatic hydrocarbons (PAH), etc. The gas phase contains highly reactive oxygen species (ROS) and various carbonyl compounds that play a significant role in cigarette smoke toxicology (IARC) [2-4].

Due to the complex composition of cigarette smoke, WHO study group on tobacco product regulation (TobReg) proposed beginning regulation with a limited set of high-priority toxic emissions, except tar, nicotine and CO (TNCO) [5, 6]. One of the chemical classes identified by TobReg as a priority, due to their impact on human health, are the volatile aldehydes [6, 7].

More than 500 carbonyl compounds have been identified and some of them have been classified as probably and possibly carcinogenic to humans by the International Agency for Research of Cancer, including formaldehyde, acetaldehyde, acrolein [3, 4].

Several analytical methods (HPLC/UV-VIS; HPLC/DAD, UHPLC/MS, GC/MS) and derivatization agents (2, 4-dinitrophenilhydrazine, 1-methyl-2-pyrazoline, cysteamine-thiazolidine, pentafluorophenyl hydrazine), used to investigate the carbonyl content in tobacco smoke, were reported in the literature [4, 6, 8]. Coresta special analytes sub group recommended derivatization with 2,4-dinitro-phenylhydrazine (DNPH) because it was the most suitable agent and thus was chosen as the basis of the Coresta recommended method [9].

The aim of this study was to investigate the content of carbonyl compounds in tobacco smoke and to establish the influence of tobacco type and cigarette filter on their composition.

EXPERIMENTAL

Reagents and equipment

Formaldehyde (CAS number 50-00-0), acetaldehyde (CAS number 75-07-0), acetone (CAS number 67-64-1), propionaldehyde (CAS number 123-38-6), acrolein (CAS number 102-07-8), 2,4-dinitrophenylhydrazine (CAS number 119-26-6), nicotine (CAS number 54-11-5); methanol (CH_3OH), acetonitrile (CH_3CN) and perchloric acid ($HClO_4$) were purchased from Merck, Germany.

- Single-channel smoking machine Borgwaldt, Germany;

- 8-channel linear cigarette smoking machine Filtrona 302, England;

- Gas chromatograph equipped with flame ionization detector and thermal conductivity detector GC-FID/TCD, Agilent 7890A; - HPLC

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

with UV/VIS detector, Perkin Elmer, USA.

Material

Dry leaves of Oriental tobaccos (Krumovgrad 988 – Kr988, Krumovgrad 90 – Kr90, Muymuynovo seme – Ms, Djebel basma 1 – Db1, Basma 13- B13, Basma 79 – B79, Linia 30 –L30, Srednogorska yaka - Sy, Plovdiv 380 – Pl380, Kozarsko 339 – Kz339), Virginia tobaccos (Virginia 385 – V385, Koker 254 – K254 and Linia 543- L254) and Burley tobaccos (Burley – Parvomay - BPar, Burley – Haskovo – BH, Burley – Plovdiv - BPl) were used as a material. The cultivars were from the collection of the Tobacco and Tobacco Products Institute, Plovdiv, Bulgaria. Laboratory cigarettes from all tobacco varieties were made.

Three industrial cigarette brands A, B and C with different composition and ventilation of filters were used for analysis. The description of the samples is presented in Table 1.

Table 1. Description of the analyzed cigarette brands

Cigarette	Cigarette filter	Filter ventilation
A1Ø	Acetate filter	No
A1V	Acetate filter	35 %
A2	Acetate filter *with activated menthol capsule*	No
A2V	Acetate filter *with activated menthol capsule*	35 %
B1Ø	Acetate filter	No
B2	Acetate *and charcoal filter (two sectors filter)*	No
B2V	Acetate *and charcoal filter (two sectors filter)*	30 %
C1Ø	Acetate filter	No
C1V	Acetate filter	40 %
C2	Acetate, *recessed and charcoal filter system (three sectors filters)*	No
C2V	Acetate, *recessed and charcoal filter system (three sectors filters)*	40 %

Ø -controls

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 on a single-channel smoking machine according to ISO 3308-2012, that was fitted with impinger, containing 40 ml of acidified solution of 2,4-dinitrophenylhydrazine with concentration of 3.396 mg/ml. The carbonyls in tobacco smoke were trapped by passing each puff through an impinge. The solution was left in the dark for at least 5 hours until the reaction was complete and the carbonyl hydrazone formed.

The high performance liquid chromatography (HPLC) equipment used was Perkin Elmer equipped with binary pump and UV/VIS detector. The chromatographic analysis was performed on an analytical column “Кromasil” C_{18}, 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. All the analyses were conducted in triplicate.

Determination of tar, nicotine and carbon monoxide (TNCO) in tobacco smoke according to ISO 4387-2000, ISO 10315-2013 and ISO 8454-2007. The automatic process of smoking cigarettes was performed on the smoking machine according to ISO 3308-2012 [10]. The cigarettes were smoked under ISO smoking conditions (puff volume – 35 ± 0.3 ml, puffs frequency – once per minute, puff duration - 2.0±0.2 s) and the total particulate matter (TPM) as specified in ISO 4387-2000 was collected [11]. Gas chromatograph with flame ionization detector for determination of nicotine and NDIR analyzer for determination of CO in tobacco smoke was used [12, 13].

RESULTS AND DISCUSSION

Carbonyl content of different tobaccos and cigarette brands

Influence of type of tobacco. The content of carbonyl compound in the gas phase of tobacco smoke of Bulgarian tobaccos of three variety groups – Oriental, Virginia and Burley was investigated. Table 2 shows both the individual components of carbonyl compounds in tobacco smoke - formaldehyde, acetaldehyde, acetone, propionaldehyde and acrolein and the total carbonyl content (TCC).

Acetaldehyde and acetone with a maximum content of 655±98 μg/cig (K254) and 344±52 μg/cig (BPar) respectively were found to be predominant in the carbonyl content for all tobaccos, followed by acrolein - 86±17 μg/cig (Sy), formaldehyde - 80±20 μg/cig (Sy) and propionaldehyde - 48±10 μg/cig (Sy). The highest content of acetaldehyde, propionaldehyde and acrolein was reported in Virginia tobacco smoke, acetone - in Burley tobacco smoke, and formaldehyde in Oriental tobacco smoke. The largest variation of formaldehyde from 4±1 μg/cig (BPar) to 80±20 μg/cig (Sy) was found.

The total carbonyl content ranges from 723±144 μg/cig (Kr988) to 1137±227 μg/cig (K254). The largest amount of carbonyl compounds, as average content, was reported in tobacco smoke of Virginia tobacco varieties, while the smallest - in Burley tobacco varieties. The largest variation in total carbonyl content of Oriental variety emissions - from 723±144 μg/cig (K988) to 1064±212 μg/cig (Sy), was due to the diversity of ecotypes and varieties.

Table 2. Content of carbonyl compounds in tobacco smoke of Bulgarian tobaccos, μg/cig±SD

Tobaccos		Form-aldehyde	Acet-aldehyde	Acetone	Propion-aldehyde	Acrolein	TCC
Oriental tobaccos	Kr988	16±4	410±61	222±33	32±6	43±9	723±144
	Kr90	16±4	558±84	316±47	42±8	55±11	987±197
	Ms	12±3	451±68	290±43	34±7	55±11	841±168
	Db1	44±11	410±61	260±39	31±6	59±12	804±160
	B13	20±5	397±59	283±42	31±6	53±11	784±156
	B79	28±7	479±72	280±42	36±7	64±13	887±177
	L30	16±4	530±±79	281±42	40±8	66±13	933±186
	Sy	80±20	560±84	290±43	48±10	86±17	1064±212
	Pl380	13±3	379±57	254±38	32±6	48±10	726±145
	Kz339	46±11	487±73	282±42	39±8	72±14	926±185
Virginia tobaccos	V385	14±3	600±90	275±41	39±8	63±13	991±198
	K254	28±7	655±98	331±50	44±9	79±16	1137±227
	L543	15±4	522±78	241±36	44±9	58±12	880±176
Burley tobaccos	BPar	4±1	373±56	344±52	29±6	46±9	796±159
	BH	7±2	524±79	258±39	37±7	55±11	881±176
	BPl	7±2	453±68	330±49	28±6	55±11	873±174

Table 3. Content of carbonyl compounds in tobacco smoke of different cigarette brands, μg/cig±SD

Cigarette	Form-aldehyde	Acetaldehyde	Acetone	Propion-aldehyde	Acrolein	TCC
A1Ø	40±10	481±72	237±35	33±7	61±12	852±170
A1V	17±4	323±48	184±28	24±5	43±9	591±118
A2	55±14	524±79	253±38	34±7	77±15	943±188
A2V	23±6	286±43	156±23	20±4	39±8	523±104
B1Ø	18±4	458±69	234±35	36±7	56±11	802±160
B2	26±6	484±73	245±37	33±7	62±12	851±170
B2V	11±3	335±50	177±26	24±5	41±8	588±117
C1Ø	18±4	510±76	274±41	34±7	60±12	896±179
C1V	8±2	186±28	125±19	14±3	20±4	353±70
C2	24±6	541±81	261±39	37±7	70±14	933±186
C2V	9±2	298±45	163±24	24±5	36±7	530±106

Carbonyl compounds in tobacco smoke are usually generated by pyrolysis of the saccharides (sugars and cellulose) or emitted directly from unburnt tobacco [14]. The result obtained for the carbonyl content was comparable to the sugar content of the tobaccos leaves [15, 16]. Virginia tobacco was characterized with the highest content of sugars while Burley tobacco – with the lowest [1].

The content of carbonyl compounds in tobacco smoke of different cigarette brands is shown in Table 3. The qualitative composition of the carbonyl compounds in cigarette brands was the same like tobaccos – with the highest content was acetaldehyde, following acetone, acrolein, propionaldehyde and formaldehyde. The total carbonyl content varied from 353±70 μg/cig (C1V) to 943±188 μg/cig (A2), which was lower than that of tobaccos and due to the influence of different cigarette filters.

Influence of type of cigarette filters

The most widely used are acetate filters. In recent years, however, there has been a wide variety of cigarette filters - acetate filters containing activated charcoal or menthol capsule, recess filters and other. Sometimes the filters are combined and consist of two or three sectors. Filters can reduce "tar" and nicotine smoke yields up to 50%, with a higher removal rate for other classes of compounds (e.g. phenols), but are ineffective in filtering toxins such as carbon monoxide [17]. An investigation on the effect of filters on carbonyl compounds is insufficient.

In our study, we investigated filters with acetate filter, acetate filter with menthol capsule and combined filters (Table 1). Cigarette with different filter composition (activated menthol capsule, two and three sectors) increased the carbonyl content in tobacco smoke 943±188 µg/cig (A2), 851±170 µg/cig (B2) and 933±186 µg/cig (C2), compared to the controls 852±170 µg/cig (A1Ø), 802±160 µg/cig (B1Ø) and 896±179 µg/cig (C1Ø) (Table 3).

The highest content of formaldehyde (55±14 µg/cig) and acrolein (77±15 µg/cig) was reported upon the activation of the menthol capsule in filter - cigarette brand A2 and of the acetaldehyde (541±81 µg/cig), acetone (261±39 µg/cig) and propionaldehyde (37±7 µg/cig) in the C 2 cigarettes (three-sector filter).

Various results were described for the content of carbonyl compounds in tobacco smoke. This is due to the different methods and derivatization agents that used authors. Pang and Lewis [4] found that acetaldehyde was predominant, varying from 72.6 µg/cig to 187 µg/cig; the content of formaldehyde, acetone and propionaldehyde was above 20 µg/cig, while acrolein varied from 10 to 20 µg/cig. The studies were performed by GC/MS and pentafluorophenyl hydrazin derivatization [4].

Fujioka [8] quantified carbonyl compounds in 14 cigarette brands by derivatization, solid phase extraction and gas chromatography methods with a nitrogen phosphorus detector. The content of formaldehyde varied from 87 ±3 µg/cig to 243 ± 11 µg/cig, acetaldehyde – from 1518 ± 63 µg/cig to 2101 ± 28 µg/cig; propion-aldehyde from 87±3 µg/cig to 176 ± µg/cig and acrolein from 285 ± 22 µg/cig to 468 ± 17 µg/cig [8].

Influence of filter ventilation

Cigarettes without ventilation and with ventilation of 35% - 40% were analyzed (Table 1). Cigarettes with unventilated acetate filters A1Ø, C1Ø and unventilated filters with different composition A2, B2, C2 had the higher carbonyl content (average 895±43 µg/cig) compared to the ventilated filters A1V, C1V and A2V, B2V, C2V (average 517±96 µg/cig). The results confirmed our previous studies founding that filter ventilation had the most significant impact on reduction of carbonyls in tobacco smoke [18]. The data obtained are consistent with the studies of Pauwels (2018). It was found that trapping the filters vents, thereby eliminating the tip filter ventilation to 0 % lead to an increase in aldehyde yields in cigarette smoke [6].

The influence of filter ventilation on the reduction of carbonyls was so strong that it compensates the greatest expansion for cigarettes with activated menthol capsule in the filter, which was observed in our research. The results are shown in Table 3: Activation of menthol capsules in cigarette filters increased the content of carbonyl compounds in non-ventilated filters A2 (943±188 µg/cig) compared to the control A1Ø (852±170 µg/cig), while cigarette with ventilation filters A2V (523±104 µg/cig) contained lower carbonyls than A1V (591±118 µg/cig).

Content of TNCO of different tobaccos and cigarette brands

The content of tar, nicotine and CO of different tobaccos and cigarette brands is presented on fig. 1.

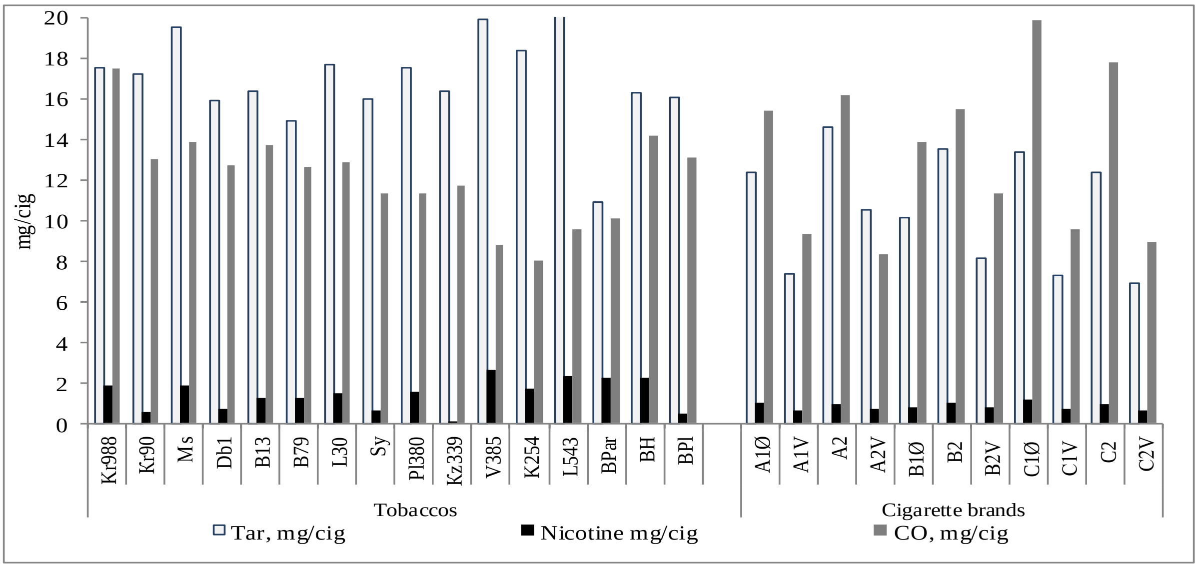

Fig. 1. Content of TNCO of different tobaccos and cigarette brands

Tobaccos were characterized with higher content of tar (average 17.01±2.38mg/cig) and nicotine (average 1.43±0.75 mg/cig) compared to cigarette brands – average 10.59±2.83 mg/cig, 0.83±0.17 mg/cig, respectively, while CO was approximately the same - average 12.15±2.34 mg/cig for tobaccos and 12.96±3.99 mg/cig for cigarette brands. Cigarette brands contain blends of different types of tobaccos and additives. Three main types of tobacco are commonly used in these blends; Virginia, Burley and Oriental in different ratios and over 500 additives, resulting in reduction in tar and nicotine content [19].

The results show the highest average tar for Virginia tobaccos (20.0±1.65 mg/cig), followed by Oriental tobaccos (16.9±1.27 mg/cig) and Burley tobaccos (14.4±3.06 mg/cig). The average nicotine content of smoke in tobaccos decreases as follows: Virginia (2.19±0.45 mg/cig), Burley (1.67±1.01 mg/cig), Oriental (1.12±0.59 mg/cig), while CO decreased in the order: Oriental tobaccos (13.06±1.80 mg/cig), Burley tobaccos (12.5±2.12 mg/cig) and Virginia tobaccos (8.77±0.8 mg/cig).

Trapping the cigarette filter leaded to increased tar yields between 39 % and 84 %, nicotine yield between 26 % and 72 % and CO yield between 37 % and 108 % (Fig. 1). The results are in accordance with Pauwels *et al*. [6].

Activation of the menthol capsule in the filter and the two-sector filter increase the TNCO content of tobacco smoke compared to controls, while the three-section filter decreases them (Fig. 1).

Correlation between carbonyl content and TNCO

Additionally, the ratio between total carbonyl content (TCC) and individual carbonyls and TCC and TNCO in tobaccos and cigarette brands was investigated.

A better correlation between TCC and individual carbonyl components of cigarette brands was found compared to tobaccos. There was a strong correlation between TCC and acetaldehyde (R^2=0.9957), acetone (R^2=0.9710), propionaldehyde (R^2=0.9232) and acrolein (R^2=0.9312) in tobacco smoke of cigarette brands (Table 4). No correlation was observed between TCC and formaldehyde both in tobaccos and in cigarette brands. The content of formaldehyde in tobaccos and cigarette brands varied widely – from 4±1 μg/cig to 80±20 μg/cig in tobaccos and from 8±2 μg/cig to 55±14 μg/cig in cigarette brands (Table 3) and therefore it was difficult to find correlations.

The correlation between TCC and CO (R^2=0.8225), TCC and tar (R^2=0.7671) and TCC and nicotine content (R^2=0.6584) only in different cigarette brands was established (Table 5). Positive correlations between total carbonyl content, tar yield and CO yield was found by Pang and Lewis too [4]. Correlations between TCC and TNCO in tobaccos were not detected.

.

Table 4. Correlation between TCC and individual carbonyls

Samples		Formaldehyde	Acetaldehyde	Acetone	Propionaldehyde	Acrolein
Tobaccos	TCC	0.1792	0.8690	0.2007	0.6488	0.7216
Cigarette brands	TCC	0.3917	0.9957	0.9710	0.9232	0.9312

Table 5. Correlation between TCC and TNCO

Samples		Tar	Nicotine	CO
Tobaccos	TCC	0.0494	0.0209	0.3201
Cigarette brands	TCC	0.7671	0.6584	0.8225

CONCLUSIONS

The content of five main carbonyl components in tobacco smoke of tobaccos and cigarette brands such as formaldehyde, acetaldehyde, acetone, propionaldehyde and acrolein and factors influencing their composition were investigated. The qualitative composition of the carbonyl compounds in tobaccos and cigarette brands was the same. The highest content of acetaldehyde, followed by acetone, acrolein, propionaldehyde and formaldehyde was found in tobacco and cigarette brands. The highest carbonyl content in tobacco smoke of Virginia tobacco varieties was established, followed by Oriental tobacco varieties and Burley tobacco varieties. The total carbonyl content in the investigated cigarette brands was lower than that of tobaccos. The activation of menthol capsule in the filter, the charcoal filter and recessed and charcoal filter system increased the carbonyl content in cigarette brands compared to the acetate filter. Cigarettes with filters ventilation had lower carbonyl content in tobacco smoke compared to those with unventilated filters. Positive correlations between total emission of carbonyls, tar, nicotine, CO and individual carbonyl content only in cigarette brands were found.

REFERENCES

1. J. Leffingwell, *Leffingwell Reports*, 1 (2), 1 (2001).
2. IARC working group on the evaluation of carcinogenic risks to humans, *IARC Monogr. Eval. Carcinog. Risks Hum.*, **83**, 1 (2004).
3. A. Rodgman, T. Perfetti, CRC Press, USA, 2013.
4. X. Pang, A. Lewis, *Science of Total Environmental* **409**, 5000 (2011).
5. Tobacco Products Directive (2014/40/EU), *Official Journal of the European Union*, EU, 2014.
6. C. Pauwels, W. Klerx, J. Pennings, A. Boots, F.J. van Schooten, A. Opperhuizen, R. Talhout, *Chem. Res. Toxicol*. **31,** 462 (2018).
7. World Health Organization. *Second report of a WHO study group*, 2008, p. 1.
8. K. Fujioka, T. Shibamoto, *Environ. Toxicol.* **21** (1), 47 (2006).
9. Coresta recommended method № 74. Determination of selected carbonyls in mainstream cigarette smoke by HPLC, 2018.
10. ISO 3308:2012. Routine analytical cigarette-smoking machine - Definitions and standard conditions.
11. ISO 4387:2000. Cigarettes - Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine.
12. ISO 10315:2013. Cigarettes - Determination of nicotine in smoke condensates - Gas-chromatographic method.
13. ISO 8454:2007. Cigarettes - Determination of carbon monoxide in the vapour phase of cigarette smoke - NDIR method.
14. R. Baker, *Food Chem. Toxicol.*, **42**, 53 (2004).
15. E. Roemer, K, Schorp, J Piade, I. Seeman, E. Leyden, J. Haussmann, *Crit. Rev. Toxicol.* **42**, 244 (2012).
16. P. Cheah, PhD Thesis, Maastricht, 2016.
17. H. Bradford, *Tobacco Control*, 20 (1): 10-16 (2011).
18. A. Stoilova, M. Docheva *Jubilee International Scientific Conference “70 Years of Tobacco and Tobacco Products Institute”,* 06 – 08 November, Plovdiv, Bulgaria (2014).
19. N. Djulancic, V. Radojicic, M. Sbinovska, *Arh. Hig. Rada Toksikol.*, **64**, 107 (2013).