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Short communication

THE INFLUENCE OF TOBACCO BLEND COMPOSITION ON CARBON MONOXIDE FORMATION IN MAINSTREAM CIGARETTE SMOKE

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The aim of this study was to examine the impact of three main tobacco types (flue-cured FC, air-cured AC and sun-cured SC) and two tobacco-based materials (reconstituted tobacco - recon RT and expanded stem) on the formation of carbon monoxide (CO) in the gas phase of mainstream cigarette smoke. The results showed that the type of tobacco examined had a significant impact on the amount of carbon monoxide production in the gas phase of cigarette smoke. AC and SC tobaccos had the most evident impact. The amount of tobacco in mixtures M_1, M_2 and M_3 as well as the addition of expanded stems had an impact on the amount of CO formed in the cigarette smoke. There is weak correlation between CO content in the smoke and the chemical composition of the tobacco. Draw resistance had an impact on CO production. The research results are of great importance, since tobacco selection is the first step in the production of cigarettes with reduced emission of harmful elements contained in the smoke.

KEY WORDS: *AC tobacco, CO, expanded stem, FC tobacco, gas phase of tobacco smoke, recon (RT), SC tobacco*

Cigarette smoke is a complex aerosol that results from the incomplete combustion process or pyrolysis of a tobacco blend used in cigarettes. During the process of pyrolysis, a significant amount of carbon monoxide (CO) is formed. Earlier studies found that the chemical composition of smoke is a direct consequence of the chemical composition of the combusted material (1-3). Carbon monoxide occurs as a result of the thermal decomposition and combustion of various tobacco components such as starch, cellulose, sugars, organic acids, esters, etc. The mainstream tobacco smoke inhaled by a smoker contains 5 mg to 22 mg of carbon monoxide per one cigarette, whereas the amount of carbon monoxide in side-stream smoke is 9 mg to 35 mg per cigarette (4). Since side-stream smoke and the exhaled mainstream smoke form environmental tobacco smoke (ETS), it was found that passive smokers are left exposed to carbon monoxide from the tobacco smoke (5).

Carbon monoxide is an integral part of the gas phase of tobacco smoke and indicated as a very dangerous blood poison (6). Carbon monoxide enters into the blood through the lungs and creates a stable complex with haemoglobin (carboxyhaemoglobin), which leads to a disruption in the exchange of oxygen in the blood (7, 8). According to the Tobacco Act of both the Republic of Serbia and the Republic of Macedonia, starting with 2011, the content of carbon monoxide in cigarette smoke must not exceed 10 mg per cigarette (9, 10).

Different technological processes have been found to improve the rate of the combustion of cigarette tobacco and thereby reduce the amount of CO (11). So far, it has been established that the total amount of smoke and the consequently formed CO depend on various factors such as the technical specifications of a cigarette, filter material, filter ventilation, paper composition and permeability, and the composition of tobacco blends (12-15). Calafat et al. (16) found that the amount of carbon monoxide depends not only on the ventilation level, but also on the geographical origin of tobacco blends. They concluded that the content of carbon monoxide is significantly correlated with both variables - cigarette ventilation ($p = 0.0008$) and geographical origin ($p = 0.0136$). It was also found that adding 25 % of expanded stems reduces the amount of CO by 2 % in comparison with the control cigarette (17).

Only a few studies involving the influence of tobacco type and quantity on the production of CO have been conducted (18). Most of the studies refer to the identification of methods for determining the amount of CO in tobacco smoke (19-21).

Therefore, the primary aim of this study was to examine the impact of three tobacco types, as well as the impact of the reconstituted tobacco and expanded stems used in the blend for production cigarettes on the formation of CO in tobacco smoke. The obtained results will enable the determination of the optimal quantity of certain types of tobacco in a cigarette blend, which will then serve as the primary and simplest method for reducing CO production.

MATERIALS AND METHODS

The following tobacco and non-tobacco materials were used for the production of cigarettes analysed in this research:

1. Sun-cured tobacco (SC), origin from Macedonia (grade unik I-III), crop 2009;
2. Air-cured tobacco (AC), origin from Bosnia and Herzegovina (grade unik I-III), crop 2009;
3. Flue-cured tobacco (FC), origin from Bosnia and Herzegovina (grade unik I-III), crop 2009;
4. Reconstituted tobacco (RT), RECON GC-2, manufactured by LEMAN, France;
5. Expanded stem (50% FC, 50% AC), manufactured by the Sarajevo Tobacco Factory, Bosnia and Herzegovina;
6. Cigarette paper, permeability 42 CU (cm min^{-1} at 1 kPa), and tipping paper, non-porous, manufactured by PAPIERFABRIK WATTENS – Watenns, Austria;
7. Cellulose acetate filter 2,7 Y/35 000 and non-perforated cork paper TP 719 J, manufactured by Osterreichische Zigarettenfilter Gesellschaft, Hainburg, Austria;
8. Cigarette side-seam glue TURMERLEIM DNA 12/5 G and tipping glue TURMERLEIM MAX I/S, manufactured by TUMERLEIM Gm, Germany.

The experimental cigarettes were made by industrial machines Hauni Protos 90E (Hauni Maschinenbau AG, Hamburg, Germany) according to the specifications presented in Table 1.

Table 1 *Experimental cigarettes*

Cigarette	Blend composition
SC	100 % Oriental tobacco
AC	100 % Burley
FC	100 % Virginia
RT	100 % RECON
M_1	American blend: 10 % Oriental tobacco 30 % Burley 60 % Virginia
M_2	American blend: 5 % Oriental tobacco 25 % Burley 45 % Virginia 10 % RECON 15 % Expanded stem
M_3	American blend: 5 % Oriental tobacco 25 % Burley 30 % Virginia 10 % RECON 30 % Expanded stem

For the purpose of improving the hygroscopic properties, as well as preserving the moisture content in cigarettes (12±0.5) %, propylene glycol (Austria Tabak GmbH, Austria) was used in the amount of 2.5 % of the total amount of tobacco (dry weight). The total cigarette length was 85 mm. The length of the filter was 20 mm. Cigarettes for testing the composition of the tobacco blend were sampled on a random basis by cutting along the cigarette rod and taking out the tobacco blend. All seven tobacco cigarette samples (SC, AC, FC, RT, M_1, M_2 and M_3) were dried in an oven at a temperature of 40 °C. After drying, the samples were milled, sieved through a 0.5 mm sieve and placed in amber glass jars. Reducing sugar, total nitrogen and nicotine were determined using established methods on prepared samples (22). Reducing sugar was determined by the picric acid colorimetric method; total nitrogen was determined by the Kjeldalh method, while the nicotine level was determined using a UV spectrophotometer. Ash content was determined by using the standard AOAC method (23). All analyses were performed in triplicate. Prior to the analysis of physical parameters and chemical composition, the cigarettes were conditioned during 48 h in the Borgwaldt chamber for conditioning (Heinr. Borgwaldt GmbH, Germany) at a temperature of (22±2) °C and a relative humidity of (60±5) %, in accordance with the ISO 3402 standard (24). After that, they were smoked on the BORGWALDTH RM 20/CSR.

The environmental conditions for smoking were set by the ISO 3308 Standard (25), where puffing was (2±0.05) s, smouldering (58±0.05) s, butt length 23 mm, and the puffing volume (35±0.15) mL. In the gas phase of the smoke, the content of carbon monoxide (CO) was determined according to ISO 8454 (26). For the purposes of the analysis, 20 cigarettes were taken from each sample, whereas all the analyses were conducted in triplicate.

Determination of cigarette weight and draw resistance was performed on the SODIMAT device (Sodim SAS, HAUNI, France). The module for draw resistance measuring was done according to the ISO 6565 method (27). A total of 120 cigarettes from each sample was analysed. The values of different physical parameters were expressed as mean value ± standard deviation. Cigarette hardness was set as a given parameter (67.95±0.45) %. All test cigarettes had the same diameter (7.95± 0.012) mm.

The results were statistically analysed using the Excel XP 2004 statistical program. Analysis of variance was used to determine the influence of the tobacco type and amount used in the blend on the CO content in the gas phase of a cigarette. Whilst determining statistical significance, we analyzed all possible pairs of comparison by applying the Tukey test (28). The relationship between the components in the smoke and the components in the blend were estimated by using the Pearson's correlation coefficient (29). To determine the strength of correlation, the classification by Chebyshev (30) was used.

RESULTS

Test cigarettes are manufactured by using different types of tobacco (SC, AC, FC and RT) containing different percentages of tobacco type in blend M_1 and expanded stems in blends M_2 and M_3. The results of the analysis of carbon monoxide content in the gas phase of mainstream cigarette smoke are presented in Table 2.

Based on the data presented in Table 2, it can be concluded that the type of tobacco had a significant impact on the amount of CO formed in the gas phase of cigarette smoke. The smoke from the cigarettes made with AC tobacco contained the highest average content of carbon monoxide (15.01 mg per cigarette). The average content of carbon monoxide in the smoke of cigarettes made with FC tobacco was 2.86 % lower. The amount of CO in the smoke from cigarettes made with SC tobacco was 12.33 % lower than in the AC cigarette smoke, and 9.75 % lower than in the FC smoke. The average content of CO in RT cigarette smoke was 11.78 % higher than in SC cigarette smoke, whereas the difference in relation to FC cigarettes was only 2.23 %. The difference in the average content of CO between AC and RT cigarettes was negligible, only 0.67 %.

The data presented in Table 2 leads to the conclusion that the different amount of tobacco in mixtures M_1, M_2 and M_3, as well as the addition of expanded stems, had an impact on the amount of CO that formed in the cigarette smoke. The largest amount of CO was found in the smoke of blend M_1, which consisted of 60 % FC, 30 % AC and 10 % SC tobacco.

Adding 10 % of RT tobacco and 15 % of expanded stems with simultaneous reduction of FC tobacco by 15 % and AC tobacco by 5 % caused a slight decline of 0.7 % of CO content. However, increasing the share of expanded stems from 15 % to 30 % in blend M_3 contributed to the formation of higher average CO concentrations, amounting to 6.52 % when compared

Table 2 *The content of CO in smoke of experimental cigarettes*

Cigarette	Content of CO in cigarette smoke / mg		
	$\bar{x}$	±	SD
SC	13.16	±	0.173
AC	15.01	±	0.169
FC	14.58	±	0.135
RT	14.91	±	0.338
M_1	15.17	±	0.167
M_2	14.47	±	0.066
M_3	15.12	±	0.207

to blend M_2, which can be explained by the influence of the chemical composition of experimental cigarettes and expanded stems (Table 3, Table 4). Expanded stems contain the highest amount of ash and the lowest amount of total nitrogen and proteins.

The performed analysis of variance showed that the type and amount of tobacco in a cigarette blend significantly affect the average content of CO and cigarette smoke (F_{est}=118.4; p<0.001).

The results of testing the significance of difference between mean values of CO content (Table 5) also show that experimental cigarettes differed amongst each other significantly.

The results suggest that SC cigarettes are significantly different from all other types of cigarettes for their production of CO in the gas phase of the smoke. AC cigarettes significantly differ from FC and SC cigarettes. There was no statistically significant difference between AC and RT cigarettes.

Based on the results shown in Table 5, the influence of the amount of tobacco on CO production can also be confirmed. Blend M_2 significantly differs from blends M_1 and M_3. There was no statistically significant difference between M_1 and M_3. Considering the amount of tobacco in the blends, it was noted that the production of CO was significantly influenced by the type of tobacco. Bearing in mind that the chemical composition of smoke depends on the chemical composition of the blend, the basic quality parameters of the blends were subject to analysis: nicotine, protein, total nitrogen, sugars and ashes. The results are presented in Table 3.

Table 3 *Chemical composition of experimental cigarettes and expanded stem (%)*

Chemical composition	Non-blended tobaccos				Tobacco blends			Expanded stem
	SC	AC	FC	RT	M_1	M_2	M_3	/
Nicotine	1.67	2.42	1.88	0.84	2.06	1.73	1.49	0.53
Protein	7.17	9.11	6.01	5.96	7.45	6.72	6.20	4.11
Total nitrogen	2.63	3.66	2.15	2.03	2.87	1.91	2.56	1.90
Reducing sugars	10.75	1.20	17.47	5.66	11.26	11.38	11.20	13.84
Ashes	17.04	20.73	16.94	18.53	18.26	18.84	18.33	24.32

Table 4 *Persons's coefficient of correlation for content of CO in gas phase of smoke in relation to the chemical composition of tobacco*

Chemical composition	Coefficient of correlation
Nicotine	-0.197
Protein	-0.217
Total nitrogen	-0.022
Reductive sugars	0.127
Ashes	0.447

Table 5 *The test of differences in the carbon monoxide content*

Cigarette	Content of CO in cigarette smoke / mg	The values of the mean difference modality					
		M_3	M_2	M_1	RT	FC	AC
SC	13.16	-1.96**	-1.31**	-2.01**	-1.75**	-1.42**	-1.85**
AC	15.01	-0.11	0.54**	-0.16	0.10	0.43**	
FC	14.58	-0.54**	0.11	-0.59**	-0.33**		
RT	14.91	-0.21	0.44**	-0.26*			
M_1	15.17	0.05	0.70**		$W_{0.05}$	= 0.22	
M_2	14.47	-0.65**			$W_{0.01}$	= 0.27	
M_3	15.12						

* *statistical significance at the level 0.05*
** *statistical significance at the level 0.01*

In order to determine the content of CO in the gas phase of the smoke from the analysed cigarettes and its relation to the chemical composition of tobacco, correlation coefficients were calculated and are presented in Table 4.

Based on the data presented in Table 4, it can be concluded that there is weak negative correlation between the CO content in the smoke and the content of nicotine protein and total N in the cigarette blends. Weak positive correlation was noted between CO content and sugar content in the cigarette blends, whereas a slightly higher correlation coefficient was found to be present between the CO content and ash content. For these reasons, the M_3 tobacco blend, which contained a higher proportion of expanded stems, produced a larger amount of CO.

In our experiment, the hardness of cigarettes (67.95 ± 0.45) % was set as a fixed parameter for making cigarettes. As a result and due to a variety of physical structures of the used tobaccos, a change in the weight and draw resistance of the analyzed cigarettes was recorded (Table 6).

SC cigarettes had the highest average weight, while RT cigarettes had the lowest. The difference in weight was caused by differences in tobacco density. The results are consistent with previous studies (12).

If we compare the blends, M_1 had the highest average weight. Adding 10 % of recon and 15 % of expanded stems to blend M_2 caused an increase of tobacco filling power. As a result, the average weight of cigarettes was reduced by 9.3 %. A quantity of 30 % of expanded stems added to M_3 reduced the average weight by 6.5 % in comparison to blend M_2.

Based on the data presented in Table 6, it can be concluded that SC cigarettes had a minimum value of draw resistance, while the highest value was recorded in the RT cigarette. Different values of draw resistance are the result of different tobacco densities in instances when hardness of cigarettes is already assigned.

Increased draw resistance caused an inhibited flow of gases through the cigarette, which significantly changed the conditions of burning and smoke production. If we compare the results from tables 2 and 6, the lowest draw resistance was present in SC cigarettes, which caused them to have the lowest CO production. In RT cigarettes, where the greatest draw resistance was noted, CO production is still lower when compared to AC cigarettes, which can be explained by the influence of the chemical composition of tobacco. A slightly higher correlation coefficient was found between CO content and ash content in the blends.

If we compare the blends, M_3 had the highest draw resistance. In addition to the previously stated explanations about the effects of chemical composition, the higher production of CO in comparison with blend M_2 can be explained by an increase of draw resistance.

CONCLUSION

The results showed that the type of tobacco (SC, AC, and FC) and reconstituted tobacco (RT) had a significant impact on the amount of CO formed in the cigarette smoke. AC and SC tobaccos had the most evident impact. No significant difference was noted between the impact of AC and RT tobacco.

The amounts of tobacco in blends M_1, M_2 and M_3, as well as the addition of expanded stems, had an

Table 6 *Physical characteristics of experimental cigarettes*

Cigarette	Weight / g $\bar{x}$ ± SD	Draw resistance / mm H_2O $\bar{x}$ ± SD
SC	1.1392 ± 0.013	47.26 ± 0.41
AC	0.9608 ± 0.011	78.42 ± 0.47
FC	1.0982 ± 0.015	57.84 ± 0.49
RT	0.8811 ± 0.018	83.67 ± 0.43
M_1	1.0570 ± 0.023	52.35 ± 0.34
M_2	0.9567 ± 0.011	55.27 ± 0.33
M_3	0.8949 ± 0.013	63.25 ± 0.46

impact on the content of CO formed in the gas phase of the smoke. Considering the amount of tobacco in the blends, it was noted that the production of CO was significantly influenced by the type of tobacco.

There is weak correlation between the CO content in the smoke and the chemical composition of the tobacco. Draw resistance had an impact on CO production.

The M_2 blend had the most optimal content in terms of the type and quantity of tobacco and formed a lower content of CO in the gas phase of the smoke.

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Sažetak

UTJECAJ SASTAVA MJEŠAVINE DUHANA NA FORMIRANJE UGLJIKOVA MONOKSIDA U GLAVNOJ STRUJI CIGARETNOG DIMA

Cilj rada bio je ispitivanje utjecaja triju osnovnih tipova duhana (virdžinijski, sušen u sušarama, eng. *flue cured* – FC; berlej, sušen na zraku, eng. *air cured* – AC i duhan sušen na suncu, eng. *sun cured* – SC) i dvaju materijala na bazi duhana (rekonstituirani duhan, eng. *reconstituted tobacco, RT* i ekspandirano duhansko rebro) na formiranje ugljikova monoksida (CO) u plinskoj fazi glavne struje cigaretnog dima. Rezultati istraživanja pokazali su da je tip duhana utjecao na količinu formiranog CO u plinskoj fazi cigaretnog dima. Količine duhana u mješavinama M_1, M_2 i M_3, kao i uvođenje ekspandiranog duhanskog rebra, utjecale su na formirani sadržaj CO. Ustanovljen je značajniji utjecaj tipa duhana na produkciju CO u odnosu na količine duhana u mješavinama.
Nije utvrđena statistički značajna povezanost između prosječnog sadržaja CO i kemijskog sastava mješavina. Ustanovljen je utjecaj otpora na uvlačenje na produkciju CO.
Optimalnu zastupljenost tipa i količine duhana u cilju formiranja manjeg sadržaja CO u plinskoj fazi dima imala je mješavina M_2. Rezultati istraživanja veoma su važni s obzirom na to da je izbor prikladne mješavine prvi korak u proizvodnji cigareta sa smanjenom produkcijom štetnih elemenata dima.

KLJUČNE RIJEČI: *AC duhan, ekspandirano duhansko rebro, FC duhan, plinska faza duhanskog dima, rekonstituirani duhan (RT), SC duhan, ugljikov monoksid (CO)*

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