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Original paper

Influence of waterpipe mixture composition on formation of components in particulate phase of mainstream smoke

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Abstract Due to the popularization of waterpipe smoking, the differences in the smoking protocols, differences in the mixture composition and the assumption that its smoke is less harmful than cigarette smoke, it is necessary to continuously carry out the chemical composition of smoke. Main goal of this research was to define how composition of tobacco mixture (tobacco, glycerin, glucose syrup, aroma), which is combusted in waterpipe, influences on basic components in particulate phase of smoke.The protocol of smoking session in laboratory conditions was based on researches performed on belgrade's waterpipe caffés and it represents the average smoking behavior of consumers. The mainstream smoke generation and sampling of particulate phase are performed on Smoke Collection System with Vacuum. The determination of smoke components is performed in accordance with ISO. The results showed that mixture's composition has a significant influence on formation of particulate phase in the waterpipe mainstream smoke. There was a statistically highly significant correlation between tobacco, glycerin and glucose syrup amounts in the mixture and tested characteristics of WTS. Aroma has no statistically significant influence on the components in particulate phase of the mainstream smoke. An important conclusion is that the mixture components such as glycerin and sugar components, which provide authenticity and specificity of the waterpipe mixture, actually additionally increase the harmful effects of smoke. However, it would be difficult to maintain taste characteristics with radically decreased amount of materials which are mentioned above.

Keywords Waterpipe, tobacco, waterpipe tobacco smoke (WTS), particulate phase

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Introduction

Powerful antismoking lobby and intensive campaigns against smoking has influenced on the reduction of cigarettes consumption. This reduction is not caused by reduction of the number of smokers, but it is caused by alternative tobacco consuming which is oriented on usage of waterpipe (Maziak & al., 2004 [1]; 2005 [2]; Jordan and Delnevo, 2010[3]). Waterpipe (Hookah, Narghile, Huqqa, Ghalyan, Aka shisha, Hubble Bubble) is a device for smoking in which the smoke is being chilled through water. The number of people who periodically smoke waterpipes has increased due to the sweet and fruity taste of smoke and in the meanwhile that they don't smoke cigarettes nor other tobacco products. Waterpipe use is also on the rise among adolescents and young adults on college campuses and beyond, even among people who explicitly refuse to smoke cigarettes (Eissenberg& al., 2008[4]; Barnett& al., 2009[5]; Braun & al., 2012[6]; Fielder & al., 2013[7]).

Investigations of waterpipe tobacco smoke (WTS) are relatively recently performed. In comparison with the cigarette smoke formation (Baker, 1980 [8]), WTS is being formed on different conditions (Voges, 1984 [9]).When a smoker inhales through the hose, a vacuum is created in the headspace of the water bowl sufficient to overcome the static head of the water above the inlet pipe, causing the tobacco smoke to bubble into the bowl. During each puff theair goes through heated charcoal and oxygen has impact in the charcoal combustion, the rest of the heated air goes through the tobacco and forms mainstream smoke (Shihadeh, 2003 [10]) so that the WTS is product from tobacco and charcoal combustion.

The WTS components is mostly formed during the distillation process and then by pyrolysis and combustion. There is a diference in the composition of material which combusted, as well as the combustion temperature, which has influence on the chemical composition of cigarette smoke and waterpipe smoke (Shihadeh, 2003 [10]).The conditions for forming of waterpipe smoke aerosol include lower combustion temperature (50-450oC) and high water content, formed waterpipe smoke contains less components. According to research published by El-Aasar and El-Marzabani (1991 [11]) there is 142 components in WTS,less than the 4.700 substances in mainstream cigarette smoke (Chaouachi, 2010 [12]). Next to that, the degree of retention of the mainstream smoke by the water column is between 71-81% in the first puffs and 11-59% in subsequent inhalation. The commonly used waterpipe tobacco is a moist paste like preparation made from tobacco that is mixed with honey, molasses, glycerin, and pulp of different fruits to add flavor. The chemical composition of WTS is given by the chemical composition of tobacco which combusted. So every variation in preparation compositon can make change in waterpipe tobacco smoke composition.

The nicotine amount in the smoke will directly depend from the tobacco amount in the mixture. Nicotine content in mainstream smoke waterpipe is inversely proportional to the water content in the waterpipe bowl (Shihadeh, 2003 [10]), which is due to high solubility of nicotine in water.

Based on earlier researches (Talhout& al., 2006[13]), it has been established that added invert sugars in tobacco mixture increases the concentration of acetaldehyde for about 0,6%, acrolein 0,2%, formaldehyde for 22% and nitrogen oxides (NOx) for 5.8%. Aldehyde can be find in smoke gas phase as well, formaldehyde can be find in particulate phase because its easily soluble in water solutions (Al Rashidi& al., 2008 [14]).

Glycerin C3H5(OH)3 is added in cigarette mixture in quantity of 1 – 5%, to improve the ability to retain moisture. Since is also a natural constituent of tobacco (Rodgman, 2014 [15]), it was shown that the smoke formed consisting of large amounts of glycerin and water (probably more than 80%) and these substances are not hazardous for health (Chaouachi, 2009 [16]).

Investigation of particulate phase constituents involve generating smoke aerosol from a waterpipe using a smoking machine that is programmed to produce a given puffing regimen, and then sampling the particulate phase of WTS for subsequent analysis. Numerous factors can influence particulate phase amount and constituents, including the composition of the tobacco mixture smoked, the quality of the charcoal used, the design and construction of the waterpipe (e.g. volume of the water bubbler head space, the fresh air infiltration rate of the hose during a puff), and puff topography (e.g. the number of puffs drawn, the puff volume and duration, and the interval between successive puffs).

The claim that the waterpipe smoke is less harmful than cigarette smoke should be interpreted with caution because recent research show that all harmful substances in the waterpipe smoke occupy the same amount as in cigarette smoke or in quantities that are several times higher. Unlike cigarettes, waterpipe inhalation process has a relatively high volume and low draw resistance, which is typical for a free inhalation (WHO TobReg, 2005[17]). Smokers during smoking a cigarette that lasts 5-7 minutes, carry out the puff of 8-12 with volume of 40-75 mL. During it they inhaled 0.5-0.6 L of smoke (Djordjevic& al., 2000[18]). In contrast, one session of waterpipe smoking may takes 20-80 minutes during which smoker carries 50-200 puff from 0.15 to 1 L volume (Shihadeh& al., 2004 [19]), so waterpipe consumer during the current session of smoking may inhale smoke volume equivalent to smoking 100 cigarettes. It could be said that typical session of smoking consists 100 puffs and takes about an hour, during which drawn is 100 L of smoke.

The procedure of tobacco smoke aerosol gathering from waterpipes still is not completely defined and standardized. That is the reason why waterpipes smoke results aren't fully reliable. Also, that is the reason why it is necessary to continuously carry out the smoking protocols and the chemical composition of smoke.

Materials and Methods

1. Waterpipe tobacco mixture samples

For preparation of tobacco mixture samples following materials were used:

- Tobacco type Virginia, Serbian origin (unique 1-3), 2011 crop, in strips;
- Glycerin, 86-88% (Zorka, Sabac, Serbia),
- Aroma Bahraini apples, 06324WP (Curt Georgi GmbH & Co. Germany),
- Glucose syrup, dry matter 84% (Starch Industry, Pancevo, Serbia),

In experiment performed during this study 1.5 liters of water were used for each tobacco mixture sample.

Tobacco type Virginia was conditioned for moisture content, from the initial 7.98% to 16%. Conditioning was performed with hot distilled water, after which the tobacco was left for 24 hours. Determination of moisture content of tobacco was performed according to the method described (Radojičić, 2011[20]). Then the tobacco was manually shredded. During the preparation of the sample remaining quantity of water was added.

Six different moist pastes like preparation for waterpipe have been made for the purposes of this study (Table 1).

Table 1. Composition of waterpipe tobacco mixture sample

Sample No.	Ingredients (%)			
	Tobacco	Glycerin	Glucose syrup	Aroma
1	95	5	-	-
2	65	35	-	-
3	60	5	35	-
4	95	5	-	2.5
5	30	35	35	-
6	30	35	35	2.5

Waterpipe tobacco mixture samples were prepared by intensive mixing shreded tobacco, glycerin, water, aroma and glucose syrup in a given ratio. Samples containing the glucose syrup were prepared by preheating glucose syrup up to 65°C.

Thus prepared samples were wrapped in aluminum foil and placed in plastic boxes and stored in the refrigerator (4°C) for a period of 72 hours for the purpose of equalization. To sample No. 4 and sample No. 6 flavoring was added after which they were left standing for another 8 hours in the refrigerator because of homogenization.

2.2. System for smoke generation and sampling of particulate phase

The mainstream smoke generation and sampling of particulate phase are performed on Smoke Collection System with Vacuum shown in Fig. 1. The smoke aerosol existing mouthpiece was split into three streams and each stream dawn trough a single 92 mm glass fiber filter pad (Bogwaldt, Germany) in polycarbonate holder to collect the particulate phase.

In absence of detailed smoking topography we have used a pilot study conducted by Shihadeh (2003 [10]) to provide data for the number of smoke puff, their duration, frequency and volume. The system was programmed to produce smoking regimen consisting of 50 puffs of 100 ml and duration 3 s, with 57 s between puffs. The average rate of smoking was set up100 ml/s and total duration of session per sample was 50 minutes.

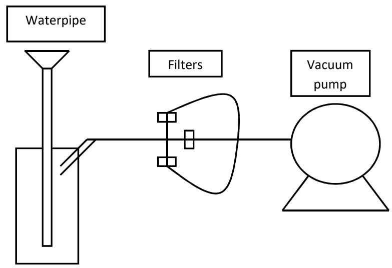

Figure1. Schematic of system set for collecting WTS

Protocol session of waterpipe smoking in the laboratory was established on the basis of research waterpipe caffés in Belgrade and represents the average tobacco user. Thus established protocol is different from those established in the territory of the Middle East. The differences are the result of habits of smokers in the Middle East where this form of smoking is traditional while on Europe this way of consuming is relatively unfamiliar.

Eight grams of each prepared tobacco mixture was loaded in the waterpipe head and covered with aluminum foil, perforated with 36 holes pattern. A single-light charcharcoal (Three Kings, Holland) was lit and placed on top of foil at the beginning of the smoking session. For each waterpipe samples the three sessions of smoking was done. No filter changes was required during each session because 92-mm glass fiber filter pad specifies that up to 600 mg of tobacco smoke condensates may be collected on it (ISO 4387, 2000 [21]).

2. Analysis of smoke condensate

The waterpipe head was weight before and after smoking session to determine the amount of ashes. Subtracting the values obtained from the weight of the sample (8 g), obtained the mass of burnt tobacco for each sample. To measure the mass of the sample and glass fibre filter pads mass before and after smoking it was used analytical balance (Kern &Sohn GmbH) accuracy 0.1 mg, capable of measuring to four decimal places.

Total particulate matter (TPM) and nicotine were determined for three replicate smoking sessions. In accordance with standard (ISO 4387, 2000[21]), TPM was determined gravimetrically as the difference in filter plus holder weight before and after the each smoking session. The determination of water in TPM is performed in accordance with ISO (ISO 10362-1, 1999[22]).

Each filter pad was analyzed for nicotine by gas chromatography (GC-FID) in accordance with standard (ISO 10315, 2013 [23]). Total amounts of nicotine free dry particulate matter (NFDPM) or 'tar', is as difference between DPM and amounts of nicotine. The amount of dry particulate matter (DPM), given by difference between amount of TPM and the amount of water.

3. Statistical analysis

Data obtained from the experiments were analyzed and the results were expressed as mean ± SD. Statistic were performed using SPSS 17.0 software ANOVA with post hoc test analyses based on Tukey was used to compare differences between samples.

Results and discussion

Data in Table 2 presents that different mixture of tobacco for the waterpipe have shown different ashes yield.

The highest amount of combusted tobacco has the tobacco mixture No. 4, and the lowest tobacco mixture No. 1. The combusted tobacco amount of 4.22 g in tobacco mixture No. 2 was slightly higher compared with the mixture No. 3.

Based on the data presented in Table 2 it can be concluded that fastest combustion has sample No. 4, which had lowest mass of ashes (3.18 g) and statistically significant mass of combusted tobacco (4.82 g). Sample No. 1 and sample No. 6, where it was found significant largest mass of ashes (4.32 g and 4.26 g, respectively) and the lowest mass of combusted tobacco (3.67 g and 3.74 g respectively), had statistically significant slowest combustion. As it can be seen from Table4, a statistically significant medium strong correlation was found between the amount of tobacco in the mixture and the amount of combusted tobacco (r = 0.48).

Sample No. 4 differs from the sample No. 1 only in aroma contains (2.5%). Sample No. 6 also distinguishes from sample No. 5 only in aroma content (2.5%). However, both of these samples contained a large amount of glycerin (35%) and glucose syrup (35%) when compared to the samples No. 1 and 4. Thus, it can be concluded that addition of glucose syrup to the mixture has effect on decreasing combustion (r = -0.43). For this reason glucose syrup in the sample No. 3 (35%), resulted in the decrease of the combustion tobacco rate of this sample (4.12 g) as compared to sample No. 2 (4.22 g).

Table 2. The yield of ashes depending on the waterpipe tobacco mixture (g)

Sample No.	Mixture mass (MM)	Ashes mass (AM)	Combusted tobacco (MM-AM)
1	8.00003 ± 0.00006	4.32 ± 0.24^{a}	3.67 ± 0.235^{e}
2	8.00030 ± 0.00010	3.78 ± 0.17^{d}	4.22 ± 0.165^{b}
3	8.00003 ± 0.00006	3.88 ± 0.02^{c}	4.12 ± 0.016^{c}
4	8.00030 ± 0.00010	3.18 ± 0.07^{e}	4.82 ± 0.007^{a}
5	8.00020 ± 0.00010	4.16 ± 0.31^{b}	3.84 ± 0.072^{d}
6	8.00090 ± 0.00020	4.26 ± 0.71^{a}	3.74 ± 0.072^{e}

Mean values with different letters in a column for each group are significantly from one another (p <0.05

Table 3. Mean values ±SD (mg/session) of TPM, water, DPM, NFDPM and TAR content in particulate phase samples

Sample No.	TPM	Water	DPM	NFDPM	Nicotine
1.	235.68 ± 0.73^{c}	34.66 ± 0.28^{f}	201.02 ± 0.45^{d}	189.04±0.68^{d}	11.98 ± 0.23^{a}
2.	467.90 ± 0.30^{c}	78.67 ± 1.42^{d}	389.23 ± 1.72^{c}	379.38±1.41^{c}	9.85 ± 0.31^{b}
3.	282.07 ± 2.18^{d}	84.33 ± 0.65^{c}	198.40 ± 1.63^{d}	188.83±1.54^{d}	9.57 ± 0.35^{b}
4.	216.86 ± 1.24^{f}	49.28 ± 0.70^{e}	167.58 ± 1.32^{e}	156.04±1,12^{e}	11.53 ± 0.31^{a}
5.	687.35 ± 1.91^{a}	147.92 ± 0.51^{a}	539.43 ± 1.42^{a}	530.06±1.20^{a}	9.37 ± 0.30^{b}
6.	568.93 ± 3.09^{b}	132.26 ± 1.15^{b}	436.66 ± 2.02^{b}	427.53±1.84^{b}	9.13 ± 0.18^{b}

Mean values with different letters in a column for each group are significantly from one another (p<0.05)

Table 4. Pearson's correlation between composition of mixture, amount of combusted tobacco and WTS

	Tobacco	Glycerin	Glucose syrup	Aroma	Weight of ashes	Combusted tobacco	TPM	NFDPM	Water	Nicotine	DPM
Tobacco	1										
Glycerin	-.784**	1									
Glucose syrup	-.846**	.332	1								
Aroma	.001	.000	.000	1							
Weight of ashes	-.482*	.350	.432	-.386	1						
Combusted tobacco	.482*	-.349	-.432	.387	-1.00**	1					
TPM	-.905**	.928**	.578*	-.068	.458	-.457	1				
NFDPM	-.861**	.946**	.496*	-.101	.463	-.463	.995**	1			
Water	-.982**	.778**	.823**	.050	.397	-.397	.927**	.883**	1		
Nicotine	.911**	-.701**	-.783**	.059	-.290	.290	.755**	-.708**	-.864**	1	
DPM	-.859**	.946**	.492*	-.101	.463	-.463	.994**	1.000**	.881**	-.704**	1

* Correlation is significant at the 0.01 level
** Correlation is significant at the 0.05 level

Table 3 presents the amounts of total particulate matter, dry particulate matter, nicotine free dry particulate matter, water, and nicotine in the particulate phase of tobacco mixtures.

In Table 4 are shown Pearson's coefficient of correlation to determine the dependencies between composition of tobacco mixture, amount of combusted tobacco and WTS.

Analysis of variance showed that the mixture composition has a significant impact on the composition of TPM. According to results shown in Table 3, it can be concluded that there were differences in yield of TPM. The significantly highest amount of TPM was observed in sample No. 5 (687.35 mg/session). On the other hand, sample No. 4 had a significantly lowest amount of TPM (216.86 mg/session). The characteristic of this sample is highest amount of tobacco (95%) and low amount of glycerin (5%).

A very strong negative correlation (Table 4) was observed between TPM values and quantity of tobacco (r= -0.91) in the mixture. However, it has been found a strong positive statistical correlation between the amount of TPM-a and glycerin (r = 0.93), as well as glucose syrup content (r = 0.58) in mixture. Accordingly, in samples No. 5 and 6, which are composed of 30% tobacco, 35% glycerol and 35% of glucose syrup, established the largest amount of TPM. The highest amount of water in smoke particulate phases have also samples No. 5 and 6, as the result of adding 35% glycerin (r = 0.78) and glucose syrup (r = 0.82) in the mixture. A high share of glycerin in tobacco mixture, significantly increased water content in smoke particulate phase which is in accordance with literature data (Carmines and Gaworski, 2005 [24]).

These samples have the lowest amount of tobacco in the mixture, which is statistically very significant negative correlation with water content in the particulate phase (r = -0.98). The least amount of water was in a WTS of samples No. 1 and 4, where no added glycerin and glucose syrup in the mixture.The results showed that the glycerin and glucose syrup contents in tobacco mixture also had an influence on the DPM content in smoke particulate phase (Table 6), as indicated by a very strong positive correlation between DPM and glycerin (r = 0.95)and medium strong correlation with glucose syrup content (r = 0.49).

In other hand, by increasing of tobacco content in the mixture, the quantity of DPM was decreased in WTS (r = -0.86). Thus, the highest amount of DPM is established in samples No. 5 and 6 and the lowest in the sample No. 4.

Amounts of NFDPM in samples were in range from 156.04 mg to 530.06 mg/session. The highest NFDPM amount was contained in sample No. 6, then No. 5 and finally No. 2. Sample No. 4 contained the lowest amounts of NFDPM. Also, it is important to emphasize that there was a statistically very strong positive correlation between the NFDPM yield and glycerin content (r = 0.95) in the tobacco mixture (Table 4). According to the literature data, the addition of glycerin in the tobacco mixture affects the increase of the 'tar' (Carmines and Gaworski, 2005 [24]). The results of this experiment directly confirm this fact, because samples No. 2, 5 and 6 containing 35% glycerol in the mixture. Based on the results presented in Table 3, it can be concluded that glucose syrup also has a significant impact on increasing the content of the NFDPM (r = 0.50).

From the results presented in the Table 3 it can be seen that concentration of nicotine in WTS was highest in samples No. 1 and 4 (11.98 mg/session and 11.53 mg/session). There was no significant difference between amounts of nicotine content in these samples (95%). The results (Table 4) showed that there was a very strong positive correlation between the nicotine content in WTS and amount of tobacco in the mixture (r = 0.91). The concentration of nicotine in WTS was smallest in samples No. 6 and 5 (9.13 mg/session and 9.37 mg/session). These samples contained the lowest amount of tobacco (30%). On lowering the nicotine content in WTS also influenced the big content of glycerin and glucose syrup in the mixture. According to the results in Table 4 it is established a statistically highly significant negative correlation between the amount of nicotine in the WTS and amount of glycerin (r = -0.70) and glucose syrup (r = -0.78) in a mixture. For the same reason, lower glycerin yield (only 5%) in sample No. 3, compared to sample No. 2 (35%), reduces the amount of nicotine in WTS. These data are in agreement to data previously reported (Carmines and Gaworski, 2005 [24]).

Conclusion

The results obtained from this study indicate the following conclusions:

The amount of tobacco in the mixture has the greatest impact on the amount of TPM, DPM, NFDPM, water and nicotine in WTS particulate phase. There was a statistically highly significant correlation. With increasing amounts of tobacco, increases the nicotine content (r = 0.91), while decreases the TPM content (r= -0.91), DPM (r = -0.86), NFDPM (r = -0.86) and water content (r =- 0.98) in the WTS.There was a statistically highly significant correlation between the content of glycerin in the mixture and tested characteristics of WTS. With increasing content of glycerin decreases nicotine content (r = -0.70), while increases the TPM content (r = 0.93), DPM (r = 0.95), NFDPM (r = 0.95) and water (r = 0.78) in the smoke particulate phase.

There was a statistically significant correlation between the content of glucose syrup in the mixture and tested characteristics of WTS. With increasing content of glucose syrup decreases nicotine content (r = -0.78) and increases the TPM content (r = 0.58), DPM (r = 0.49) NFDPM (r = 0.50) and water (r = 0.82) in the particulate phase of smoke.

Aroma has no statistically significant influence on the components of particulate phase of the mainstream smoke. An important conclusion is that the mixture components such as glycerin and glucose syrup, which give authenticity and specificity of the composition of the waterpipe mixture, actually add more harmful effects on waterpipe tobacco smoke.

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