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Characteristics of some components in tobacco smoke of organically and conventionally grown tobacco

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Abstract

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The study aimed to investigate the content of tar and nicotine in the particle phase, and the carbon monoxide (CO) and carbonyl compounds in the gas phase of tobacco smoke from organically and conventionally grown tobacco. The nicotine content in organically grown tobacco was lower than that in conventionally grown tobacco, while the tar and CO were higher. The total carbonyl content in gas-phase in organically grown tobacco is almost equal to that of conventionally grown tobacco. A good correlation between total sugars and acetaldehyde, total sugars and propionaldehyde, and total sugars and 5-hydroxymethylfurfural (5-HMF), was observed, whereas a weaker correlation was observed between glycerol and acetaldehyde, glycerol and propionaldehyde.

Keywords: tobacco smoke; carbonyl compounds; carbohydrates; glycerol; correlation

Introduction

Tobacco smoke is a complex mixture consisting of a particle phase (tar) and vapor phase (gas). There are over 6000 chemical components in tobacco smoke, of which about 1000 toxicants, which play a significant role in cigarette smoke toxicology (Smith and Fisher, 2001; Li and Hecht, 2022; Cai et al., 2019).

The particle phase contains well-known constituents such as nicotine, tobacco-specific N-nitrosamines (TSNA), polycyclic aromatic hydrocarbons (PAH), etc. The gas phase of tobacco smoke includes carbonyl compounds, volatile organic components, phenols, nitrosamines, nitrogen (N_2), oxygen (O_2), carbon dioxide (CO_2), carbon monoxide (CO) (Leffingwell, 2001; Wang et al., 2012; Rodgman and Perfetti, 2013). Some of these toxicants were selected and based on several criteria by the World Health Organization (WHO) Study Group on Tobacco Product Regulation (TobReg) (FCTC/COP1, 2007).

Carbonyl compounds in tobacco smoke affect organoleptic qualities of tobacco products and are considered to have a harmful effect on human health. They have mutagenic and carcinogenic properties (Smith and Fisher, 2001).

Some carbonyls, such as formaldehyde and acrolein are presented at low levels in tobacco smoke (between 7±2 μg/cig and 79±16 μg/cig), while acetaldehyde and acetone – at high concentration – from 184±28 μg/cig and 655±98 μg/cig (Docheva et al., 2019).

Organic farming in Bulgaria started in 2007 and until now, it has increased 10 times in the last 11 years. The main crops in organic farming are field crops, cereals, green fodders, and industrial crops (Mitova, 2021, Raei and Aghaei-Gharachorlou, 2015). In the region of southern Bulgaria, in the city of Goce Delchev, there is a designated organic-certified field for the production of organic tobacco from the Basmi variety group (Bozukov et al., 2019).

The study aims to investigate the content of tar and nicotine in the particle phase and carbonyl compounds and CO in

the gas phase of tobacco smoke of organically and conventionally grown tobacco variety from the Bulgarian region.

Materials and Methods

Reagents and materials

Perchloric acid, 2,4-dinitrophenylhydrazine, polyoxyethylene lauryl ether, acetic acid, glacial, hydrochloric acid, sodium hydroxide, calcium chloride hexahydrate, *p*-hydroxybenzoic acid hydrazide (PAHBAH), citric acid monohydrate were provided from Sigma-Aldrich (Germany, > 98% p.a.). HPLC gradient grade methanol and acetonitrile were purchased from Honeywell (USA). Standards for analysis were purchased from Sigma-Aldrich (Germany; nicotine, D-glucose, formaldehyde, acetaldehyde, acetone, propionaldehyde, acrolein, 5-hydroxymethyl-2-furaldehyde, glycerol, 1,3-butanediol).

Equipment

Analytical equipment for determination of carbonyl compounds: 8-port linear smoking machine Filtrona, England; High performance liquid chromatography (HPLC) Perkin Elmer equipped with LC 290 binary pump, UV/VIS detector, Kromasil LC18 column (150 mm, 5 μm, 4.6 mm i.d) (Supelco Park, USA). For determination of TNCO were used 20 – channel rotary smoking machine Borgwaldt 200A, Germany, and gas chromatograph Agilent 7890A with flame ionization detection and thermal conductivity detector (GC-FID/TCD). For the determination of carbohydrates, an Auto-Analyzer was used Seal Analytical – AA3 – continuous-flow analysis, while for determination of glycerol was used gas chromatograph Agilent 7890A with flame ionization detection. Analytical balance Sartorius (with 0.0001 g accuracy) was used for analysis.

Samples

In the study, two varieties of tobacco from the Kabakulak variety group – Han Tervel 39 (HT 39) and Hanski 227 (H 227), which have an increased market demand, were used. The tobaccos were grown in the Agricultural Experimental Facility „Han Krum", Bulgaria. In addition, the only variety of oriental tobacco from the Basmi variety group – Krumovgrad 58 (Kr 58), grown under organic production conditions was analyzed. Organic oriental tobacco variety Kr 58 was grown in the Experimental tobacco field in Gotse Delchev, at the Tobacco and Tobacco Products Institute, Bulgaria. Tobaccos were grown under the standard agrotechnical requirements corresponding to the variety. The technology for harvest, drying, and fermentation of tobacco was preserved.

Dry tobacco leaves were divided into classes (I and II class). Laboratory cigarettes from all tobacco varieties were made and smoked on a smoking machine for the determination of TNCO and carbonyl compounds.

Determination of tar, nicotine and CO (TNCO) in tobacco smoke according to:

- ISO 4387:2021 Cigarettes – Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine;
- ISO 10315:2022 Cigarettes – Determination of nicotine in total particulate matter from the mainstream smoke – Gas-chromatographic method;
- ISO 8454:2012 Cigarettes – Determination of carbon monoxide in the vapor phase of cigarette smoke – NDIR method.

Determination of carbonyl compounds in tobacco smoke by HPLC-UV/VIS, according to the CORESTA recommended method No. 74 (2018), with some modifications described by Docheva et al. (2019).

Two cigarettes were smoked on an 8-channel linear smoking machine according to ISO 3308-2012, which was 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 was complete, and the carbonyl hydrazone was formed. The high-performance liquid chromatography (HPLC) was used. 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 glycerol content in tobacco by GC-FID, according to the SOP 06 – Standard operating procedure for determination of humectants in tobacco filler with some modifications.

For the analysis, methanol with internal standard 1,3-butanediol was used. Tobacco powder (2 g per sample) was measured on an analytical balance with 0.0001 g. The sample was transferred into a 50 ml flask for glycerol extraction and diluted with a 25 ml extraction solution. The sample was shaken for 60 minutes. An aliquot of the supernatant was taken to perform the assay.

Determination of the content of total sugars in tobacco by continuous-flow analysis method using hydrochloric acid / p-hydroxy benzoic acid hydrazide (PAHBAH) according to the Coresta recommended method № 89.

An aqueous extract of the tobacco was prepared. 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 an alkaline medium at 85 °C to produce a yellow osazone complex. Quantitation was by external standard using a series of glucose calibration standards (0.05 mg/ml – 2.5 mg/ml), prepared with the same extraction solution. All measurements were performed at 420 nm.

Results and Discussion

Content of tar, nicotine, and carbon monoxide (CO) of tobacco smoke

The mainstream tobacco smoke from the laboratory cigarettes made from the Basmi and Kabakulak variety group was analyzed for the content of tar, nicotine, and carbon monoxide. Cigarettes were smoked on a rotary smoking machine under the same conditions: puff volume – 35 ml, number of puffs per minute – 1, puff duration – 2 s, according to ISO 4387:2021. The results are shown in Table 1.

The tar of conventional tobaccos of the Kabakulak variety group (average 16.58 mg/cig) was lower than the tar of organically produced tobaccos (average 18.66 mg/cig). The tar in Kr 58 Bio II class was lower (17.82±1.19 mg/cig) compared to the Kr 58 Bio I class (19.51±1.3 mg/cig). The obtained tar data of Kr 58 Bio II class coincide with previous studies of conventionally produced tobaccos of Basmi variety group, which reported tar of 17.65 mg/cig (Docheva et al., 2019).

The levels of nicotine in tobacco smoke in organically grown tobacco were significantly lower than in conventional ones. The content of nicotine in Kr 58 Bio I class and II class varied between 0.79±0.048 mg/cig and 0.90±0.055 mg/cig, while in conventionally grown tobacco from Kabakulak variety group was between 1.20±0.07 mg/cig (H 227 I cl) and 1.64 ±0.10 mg/cig (HT 39, I cl) (Table 1). Docheva et al. (2019) established that conventionally grown tobacco from the Basmi variety group is characterized by a high content of nicotine in the tobacco smoke – over 1.5±0.091 mg/cig and reaching up to 2.00 mg/cig.

No significant difference in CO content between organically produced tobacco from the Basmi variety group and conventionally produced tobacco from the Kabakulak variety group was observed. CO content varied from 10.04±0.60 mg/cig HT 39, II cl to 12.45±0.74 mg/cig H 227 I cl. Docheva found higher CO content in tobacco smoke of conventionally produced tobacco varieties from the Basmi variety group – 13.00±0.78 mg/cig and 17.90±1.07 mg/cig.

Content of carbonyl compounds in the gas phase of tobacco smoke

Carbonyl compounds (carbonyls) are important constituents of cigarette smoke. More than 500 carbonyl compounds have been identified in tobacco smoke. Some of them are toxic and may be carcinogenic or mutagenic to humans (Rodgman and Perfetti, 2013). Formaldehyde was included in Group I (Carcinogenic to humans), while acetaldehyde and acrolein in Group 2A (probably carcinogenic to humans), according to the International Agency for Research on Cancer (Cai et al., 2019).

In this study, the content of formaldehyde, acetaldehyde, acetone, propionaldehyde, acrolein, and 5-hydroxy-2-methyl furfural (HMF) in tobacco smoke in conventional and organically grown tobacco were determined. These substances are present in the largest amounts among the carbonyl compounds (Rodgman and Perfetti, 2013).

The highest content of carbonyl compounds in the gas phase of tobacco smoke of organic and conventionally produced tobacco varieties are those of acetaldehyde and ac-

Table 1. Content of tar, nicotine, and carbon monoxide (CO) in tobacco smoke from organic and conventionally grown tobaccos – Basmi and Kabakulak variety group, mg/cig ± SD

Type of production	Tobaccos		Tar	Nicotine	CO
	Variety group	Tobacco variety			
Organic	Basmi	Kr 58, I cl	19.51±1.30	0.79±0.05	12.19±0.73
		Kr 58, II cl	17.82±1.19	0.90±0.06	12.08±0.72
Conventionally	Basmi	Kr 988 Ref. (Docheva *et al.*, 2019)	17.8±1.19	2.00±0.12	17.90±1.07
		Kr 90 Ref. (Docheva *et al.*, 2019)	17.5±1.17	1.5±0.091	13.00±0.78
Conventionally	Kabakulak	HT 39, I cl	15.93±1.06	1.64±0.10	10.83±0.64
		HT 39, II cl	14.35±0.96	1.45±0.08	10.04±0.60
		H 227, I cl	17.94±1.20	1.20±0.07	12.45±0.74
		H 227, II cl	18.12±1.21	1.44±0.08	11.13±0.66

Source: Authors' own elaboration

etone. Acetaldehyde content varied between 553.4±82.4 μg/cig (H 227, I cl) and 639.6±95.2 μg/cig (Kr 58, II cl), while acetone ranged from 260.1±38.4 μg/cig (Kr 58, II cl) to 309.2±45.1 μg/cig (HT 39, I cl). No significant differences in the content of acetaldehyde and acetone in conventional and bio tobacco were observed.

The lower content of formaldehyde (between 30.7±8 μg/cig H 227, class II and 119.3±30 μg/cig Kr 58 Bio, class I), acrolein (from 37.3±7 μg/ cig Hanski 227, class II to 73.4±15 μg/cig Kr 58 Bio, class II) and 5-HMF (varies between 54.8±10 μg/cig H 227, class II and 108.6±25 μg/ cig HT 39 I class) was established. A greater variation in the content of formaldehyde and 5-HMF between organic and conventionally grown tobaccos was observed – Table 2. The higher values of formaldehyde, acetaldehyde, propionaldehyde, and acrolein were observed in the Kr 58 organic grown compared to conventional tobacco H 227 and HT 39 – Table 2. It was noteworthy that the levels of formaldehyde and 5-HMF were seriously increased in Kr 58 I class organically grown – 119.3±30 μg/cig and 101.3±22 μg/cig, respectively, compared to Kr 58 II class – 49.1±12 μg/cig and 60.5±12 μg/cig.

In previous studies, Docheva et al. (2019) have studied the carbonyl content in the tobacco mainstream of two varieties from the Basmi variety group – Krumovgrad 988 and Krumovgrad 90, which were conventionally grown, and reported a lower content of formaldehyde, acetaldehyde, propionaldehyde, acrolein and 5-HMF, and the content of acetone is approximately the same – Table 2.

Correlation between carbohydrates and glycerol in tobacco and gas-phases in tobacco smoke

Sugars are naturally present in tobacco and the most abundant sugars in dried tobacco leaves are glucose, fructose, and sucrose (Banožić et al., 2020). Sugars generate acetaldehyde, which has addictive properties and acts synergistically with nicotine in rodents. Also, sugars increase the level of formaldehyde, acetone, acrolein, and 2-furfural in tobacco smoke (Baker, 2007). Reported toxic compounds in high-sugar tobacco were up to 150%. The lower-sugar tobaccos had a lower percentage of compounds such as acetone, acrolein, 2-furfural, and formaldehyde in smoke.

Glycerol is also naturally present in tobacco. It is known that glycerol produces carbonyls during the smoking process.

In this regard, the content of sugars and glycerol in organic and conventionally grown tobaccos Basmi and Kabakulak variety groups was studied. The results are presented in Figure 1. The total sugar content is between 14.3±0.14% (H 227, I class) and 23.1±0.23% (HT 39, I class). The average content of total sugars in Krumovgrad 58, organically grown was 19.71%, and was lower than variety HT 39 and higher than variety H 227.

The average glycerol content in organically grown tobacco Krumovgrad 58 I class and II class was 0.135%. Tobacco variety H 227 was characterized by a higher level of glycerol content – 0.18%, compared to the HT 39 variety. A greater variation in the glycerol values was observed in organically produced tobacco I and II classes.

The correlation between total sugars and glycerol in tobacco and carbonyl content in the gas-phase of tobacco smoke from organic and conventionally grown tobacco Basmi and Kabakulak variety groups was examined. Correlation is a statistical measure that expresses the extent to which two variables are linearly related (Bewick et al., 2003).

A good correlation between total sugars and acetaldehyde ($R^2 = 0.7623$), followed by total sugars and propionaldehyde ($R^2 = 0.6575$) and total sugars and 5-HMF ($R^2 = 0.6647$) was

Table 2. Content of carbonyls in the gas phase of tobacco smoke from organic and conventionally grown tobaccos – Basmi and Kabakulak variety group μg/cig ± SD

Type of production	Tobaccos		Carbonyl compounds, μg/cig ± SD					
	Variety group	Tobacco variety	Formaldehyde	Acetaldehyde	Acetone	Propionaldehyde	Acrolein	5-HMF
Organic	Basmi	Kr 58, I cl	119.3±30	635.7±94.1	278.4±41.6	73.6±13.8	68.7±14.5	101.3±22.3
		Kr 58, II cl	49.1±12.3	639.6±95.2	260.1±38.4	70.9±13.2	73.4±15.6	60.5±12.6
Conventionally		Kr 988, Ref. (Docheva *et al.*, 2019)	16±4	410±61	222±33	32±6	43±9	38.4±6
		Kr 90, Ref. (Docheva *et al.*, 2019)	16±4	558±84	316±47	42±8	55±11.3	50±9
Conventionally	Kabakulak	HT 39, I cl	66.2±16.4	639.0±94.4	309.2±45.1	73.9±14.3	57.1±11.4	108.6±25.1
		HT 39, II cl	61.1±15.1	592.8±88.6	285.1±42.9	67.6±12.5	49.7±10.2	104.3±24.3
		H 227, I cl	63.5±16.5	553.4±82.4	299.5±44.1	62±11.7	37.3±7.9	66.6±12.8
		H 227, II cl	30.7±8.1	555.2±82.3	262.6±39.2	67.7±12.5	61.6±12.2	54.8±10.4

Source: Authors' own elaboration

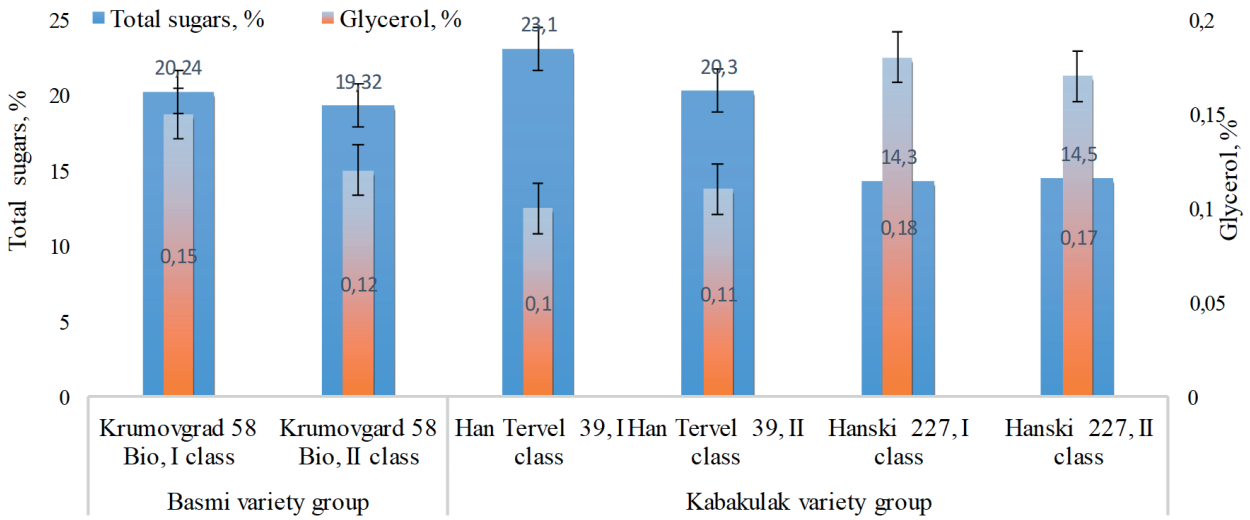


Fig. 1. Total sugars and glycerol content in organic and conventionally grown tobaccos – Basmi and Kabakulak variety group, %

Source: Authors' own elaboration

Table 3. Correlation between the content of total sugars and glycerol in tobacco and carbonyls in tobacco smoke from organic and conventionally grown tobaccos – Basmi and Kabakulak variety group µg/cig ± SD

Correlation	Formaldehyde	Acetaldehyde	Acetone	Propionaldehyde	Acrolein	5-HMF
Total sugars, %	0.1842	0.7623	0.1025	0.6575	0.135	0.6647
Glycerol, %	0.0110	0.5247	0.0408	0.3859	0.0856	0.3889

Source: Authors' own elaboration

established. No correlation between total sugars and formaldehyde ($R^2 = 0.1842$), total sugars and acetone ($R^2 = 0.1025$), and total sugars and acrolein ($R^2 = 0.135$) was observed.

The correlation between the glycerol content and carbonyl substances was smaller than that between total sugars and carbonyls. But a similar trend was observed – a higher correlation between glycerol and acetaldehyde ($R^2 = 0.5247$), glycerol and propionaldehyde ($R^2 = 0.3859$), and glycerol and 5-HMF ($R^2 = 0.3889$) was observed.

There was no correlation dependence between glycerol and formaldehyde ($R^2 = 0.0110$), glycerol and acetone ($R^2 = 0.0408$), and glycerol and acrolein ($R^2 = 0.0856$) was observed. The results are presented in Table 3.

The studied result contradicts the results reported by Baker et al. (2024), but we should note that the dependence was traced only for naturally occurring glycerol in tobacco and not for added glycerol.

Conclusion

A comparative study of tar and nicotine in the particle phase of tobacco smoke, as well as carbonyl content and carbon monoxide in the gas phase, was conducted for organically and conventionally grown tobacco varieties. The nicotine content in organically grown tobacco was lower, while the tar and CO levels were comparable to those of conventionally grown tobacco. The total carbonyl content in the gas phase (formaldehyde, acetaldehyde, acetone, propionaldehyde, acrolein, and 5-HMF) in organically grown tobacco was 1215.5 µg/cig, which was nearly equal to that of conventionally grown tobacco (1132 µg/cig). A strong correlation was observed between total sugars and acetaldehyde, total sugars and propionaldehyde, and total sugars and 5-HMF, whereas a weaker correlation was observed between glycerol and acetaldehyde, glycerol and propionaldehyde, and glycerol and 5-HMF.

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