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Chemical composition of Oriental tobacco of Krumovgrad ecotype in relation to quality and combustibility

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Abstract

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Oriental tobaccos of Krumovgrad ecotype are the most widely produced and one of the highest-quality tobaccos grown in Bulgaria. They have the characteristics of a true Basma type and are highly regarded for their rich, pungent flavor and good burning properties. The main objectives of the current study were: i) to investigate the chemical composition of Oriental tobaccos of Krumovgrad ecotype produced in different micro regions in Bulgaria, with the view of assessing their quality and applicability as a raw material in smoking products; and ii) to arrange the investigated tobaccos in groups according to their chemical indices and combustibility. The investigation was carried out in 2022, with samples of cured tobacco leaves from thirteen micro regions in three production regions in Southwestern Bulgaria. The concentrations of macroelements (K, Ca and Mg) and trace elements (Cd, Ni, Mn, Zn, and Cu) in Bulgarian Oriental tobacco of Krumovgrad ecotype differed significantly between the studied regions and micro regions. The main chemical indicators of the investigated tobaccos also showed variation, depending on the micro region of production: nicotine, 0.65–1.83%; reducing sugars, 13.30–21.20%; total nitrogen, 1.33–1.86%; ash, 7.95–11.70%; chlorine, 0.22–0.75%; as well as smoke tar (15.69–23.59 mg/cig) and nicotine (0.60–1.64 mg/cig). The applied hierarchical cluster analysis classified the tobaccos into two main clusters based on the measured chemical indicators. Tobacco combustibility, one of the key quality indicators, was evaluated by three combustibility indices, taking into account the concentration of inorganic elements in the cured leaves. The results spoke in favor of the good burning potential of Bulgarian Oriental tobacco of Krumovgrad ecotype. The ranking of tobaccos, based on the assessment of their combustibility, distinguished with the best burning properties those from Ablanitsa (region Nevrokop) and Dolno Osenovo (Gorna Gjumaya). The results obtained in the study can be used in evaluating the quality of tobacco as a raw material for smoking products, with a view to their optimal usability in tobacco blending.

Keywords: Oriental tobacco; mineral elements; nicotine; reducing sugars; leaf quality; tobacco combustibility

Introduction

Oriental tobaccos of Krumovgrad ecotype are the most widely produced and one of the highest-quality tobaccos grown in Bulgaria. Those tobaccos, together with other classical Bulgarian Oriental tobacco varieties (e.g. Djebel, Nevrokop, and East Balkan) have the characteristics of a true Basma type (Gilchrist, 1999). They are highly regarded for their rich, pungent flavor and good burning properties. In general, they tend to be fairly low in nicotine, around 1-1.5%, and have a relatively high sugar content of around 20%.

The complex of genetic and environmental factors, and production practices, determines the chemical and physical properties of tobacco leaves. The chemical composition of tobacco leaves can vary significantly among locations where the ecological conditions, such as light, humidity, precipitation, altitude, and temperature have different manifestations (Kurt, 2021). For Oriental tobacco, which cultivation is maintained with limited supplies of nitrogen, nutrients and water, there is an accumulation of carbohydrates, aromatic acids and resins at the expense of nitrogenous constituents (Leffingwell, 1999).

The combustibility of tobacco leaves (the leaf burning potential) is a function of the combined impact of the concentration of some inorganic and organic components, and the porosity of the leaf structure (Ivanov et al., 1973). Significant amounts of inorganic elements (calcium, potassium, magnesium, chlorine, phosphorus, sulfur, and others) are found in tobacco ash, and the presence of the major metallic anions strongly impacts the burning characteristics of tobacco leaf (accelerating or deteriorating leaf combustibility) (Mendell et al., 1984; Leffingwell, 1999). The importance of particular chemical constituents as factors controlling tobacco quality and leaf burn has been demonstrated in a series of studies dating back to the beginning of the 20th century, as well as in a number of investigations discussing those characteristics in Bulgarian tobaccos (Ivanov et al., 1973; Popova et al., 2006; Stoilova, 2008; Kirkova and Dyulgerski, 2015; Bozhinova et al., 2023). It is well-known that mineral (inorganic) components have different influence on tobacco combustion; positive for K_2O, negative for Cl^-, Na_2O, Fe_2O_3 and P_2O_5, and practically insignificant for CaO and MgO (Ivanov et al., 1973). According to Peterson and Tibbitts (1963), tobacco combustibility is significantly related to inorganic leaf constituents, positively (+) or negatively (-), in the following order of importance: K(+) > Cl(-) > SO_4-S(-) > Mg(-) > NO_3-N(-) > total N(-). The K/Cl ratio has a direct and strong relation with tobacco combustibility (Zahedi and Moghaddam, 2000).

As a product absorbed by the human body, the “safety” of cigarettes and other smoking items (i.e., the generated smoke composition) highly depends on the quality of tobacco raw materials. Along with others, the content of heavy metals is an important indicator of tobacco quality, and it is a cause for serious health concern (Huang et al., 2021). Heavy metals inhaled during tobacco smoking are easily absorbed in the body, from the upper respiratory tract and lungs through the bloodstream and from there – to various organs and parts of the body (Ali et al., 2019). Tobacco efficiently accumulates metals – primarily cadmium and zinc, in its leaves and could be a source of Cd intake by smokers (Kozak and Antosiewicz, 2023). Regarding the effect of heavy metals on the static burning rate (SBR), Radojičić et al. (2015) reported that the SBR values decreased as the amount of Cd in the tobacco samples increased. Regional differences and environmental changes are the main factors influencing the levels of heavy metal content in tobacco leaves. Therefore, determining the content of heavy metals in cured tobacco leaves of different origins, is a key link in the quality control of tobacco raw material (Huang et al., 2021).

The above considerations justify the main objectives of the current study: i) to investigate the chemical composition of Oriental tobaccos of Krumovgrad ecotype produced in different micro regions in Bulgaria, with the view of assessing their quality and applicability as a raw material in smoking products; and ii) to arrange the investigated tobaccos in groups according to their chemical indices and combustibility, the latter being one of the key indicators of tobacco quality, as it determines both the smoke sensory properties and the generation of harmful substances (tar, CO, and others) in the smoke.

Materials and Methods

Plant material

The investigation was carried out in 2022 with Oriental tobacco of Krumovgrad ecotype from three production regions in Southwestern Bulgaria (Nevrokop, Gorna Djumaya and Dupnitsa tobacco regions). Tobacco samples were taken on site from each of the micro regions and comprised of cured leaves from the upper stalk positions. All tobaccos were grown, picked and cured by the farmers according to the cultivation and processing practices common to the respective region and micro region.

Description of the tobacco production regions

a) *Regions Dupnitsa and Gorna Djumaya:* Both tobacco regions are located in the Sredna Struma sub-area in Southwestern Bulgaria. The topography of the tobacco-producing areas is defined as valley, semi-mountainous and mountainous. Climatic conditions are very suitable for growing tobacco, with average daily temperatures during the vegetation period (June-September) from 20.3°C (Dupnitsa) to 22.1°C (Gorna Djumaya), and average amount of precipitation from 156.1 mm (Gorna Djumaya) to 178.2 mm (Dupnitsa). The soils are mainly represented by eroded leached cinnamon, shallow leached cinnamon forest, alluvial and alluvial-meadow, deluvial and deluvial-meadow types (Timov et al., 1974; Tanov et al., 1978).

b) *Region Nevrokop:* The tobacco-producing areas in the region occupy the valleys along the upper and middle reaches of Mesta River – from the town of Yakoruda to the Greek border, as well as the slopes of the surrounding mountain heights of the Rhodopes and Pirin. The terrain is valley, foothill to mountainous and high-mountainous for some sections. Climatic conditions are characterized from particularly favorable to good for growing tobacco, varying by sub-region. The average daily temperature during the leaf maturing period (July-September) is from 17.7°C to 20.2°C, and the amount of precipitation is up to 176.7 mm. The soils are mainly represented by: alluvial and deluvial for the lower areas, eroded leached cinnamon – for the surrounding mountain slopes, and shallow brown forest soils for the high mountain areas (Timov et al., 1974; Tanov et al., 1978).

Data on the geographic location of the thirteen micro regions from which the tobacco samples were taken are presented in Table 1.

Chemical analysis

The tobacco samples were analyzed to determine the concentrations of elements influencing combustibility (N, K, Ca, Mg, and Cl), as well as the content of trace metals (Cd, Ni, Mn, Zn, and Cu) as an indicator of tobacco material quality. The preparation of tobacco samples for the analysis of K, Ca, Mg, Cd, Ni, Mn, Zn, and Cu was made by means of dry ashing and dissolution in 3 M HCl. The determination of the above macro- and microelement concentrations was made using an atomic absorption spectrometer „SpectrAA 220" (Varian, Australia). Chlorine (Cl) content was determined using continuous segmented flow analyzer SEAL AutoAnalyzer AA3 (SEAL Analytical, Mequon, USA), according to the procedure described by the equipment producer (Analytical Method MT24 G-267-01, SEAL Analytical).

The rest of the chemical traits influencing tobacco combustibility and quality (smoking characteristics) were determined by continuous-flow analysis with an AutoAnalyzer AA II C system (Technicon, USA), applying standardized methods: total alkaloids (as nicotine, %) – according to ISO 15152:2003; reducing sugars – ISO 15154:2003; total nitrogen – BDS 15836:1988; ash – ISO 2817:1999. The contents of tar and nicotine in tobacco smoke (mg/cig) were calculated using the models provided by Gueorgiev and Popova (1999). The obtained results are relevant to a non-ventilated filter-tipped cigarette, with length 84 mm, diameter 7.9 mm, and 21 mm 3/35000Y acetate filter.

Tobacco combustibility was analyzed by the values of three indices, expressing the quantitative relationships between constituents influencing tobacco burning capacity, as follows:

– Index I (*Index of Nessler*): $\frac{K_2O}{Cl}$ (Ivanov et al., 1973);

– Index II (*Index of Sastry and Kurup A*): $\frac{K_2O}{N + Cl}$ (Sastry and Kurup, 1958; Kurup and Sastry, 1962; Kurup et al., 1962);

– Index III (*Index of Sastry and Kurup B*): $\frac{K_2O + CaO}{MgO + N + Cl}$ (Sastry and Kurup, 1958; Kurup and Sastry, 1962; Kurup et al., 1962).

Statistical analysis

Experimental data were analyzed using the SPSS statistical package and the differences were assessed with the Duncan's multiple range test at the 0.05 probability level.

The method of Ward (1963) was used to group tobaccos from different micro regions in the hierarchical cluster analysis according to the following characteristics: concentra-

Table 1. Geographic coordinates and elevation of the tobacco-producing micro regions

Region	Sub-region	Micro region	Elevation, m	Geographic coordinates
Nevrokop	Foothill	Kornitsa	660	41.6406, 23.6764
Nevrokop	Foothill	Fargovo	1036	41.57715, 23.99968
Nevrokop	Foothill	Valkosel	783	41.528958, 23.989351
Nevrokop	Foothill	Godeshevo	714	41.474928, 24.049654
Nevrokop	Foothill	Slashten	679	41.497639, 24.018204
Nevrokop	Foothill	Zhizhevo	846	41.550757, 24.030489
Nevrokop	Foothill	Bogolin	947	41.540216, 23.957045
Nevrokop	Plain	Gotse Delchev	540	41.573684, 23.729077
Nevrokop	Foothill	Ablanitsa	650	41.536352, 23.935781
Nevrokop	Foothill	Tuhovishta	836	41.498524, 24.04463
Nevrokop	Field	Debren	883	41.584279, 23.82281
Gorna Djumaya	Yaka	Dolno Osenovo	840	41.955211, 23.240642
Dupnitsa	Foothill	Mursalevo	434	42.119847, 23.03867

Source: Authors' own elaboration

tion of potassium, calcium, magnesium, nitrogen, chlorine; nicotine, reducing sugars, and ash content. The distances between samples were calculated using Euclidean distances.

Results and Discussion

The data presented in Table 2 revealed that the content of macroelements (K, Ca and Mg) in tobacco depended on the agro-ecological conditions of the cultivation area.

A significant influence of regions and micro regions was found regarding the leaf potassium content ($P < 0.05$). The results were fully compliant with the observations by Tang et al. (2020), who reported that potassium content in tobacco leaves was significantly influenced by climatic factors. The same authors point out that slightly acidic soil conditions, improved soil physical and chemical properties, and appropriate soil nitrogen content can promote the absorption and accumulation of potassium in tobacco. Therefore, the different environmental conditions (climatic factors and soil properties) of the regions and micro regions in the study affected the potassium uptake by plants and the K_2O concentration in tobacco leaves, which ranged from 1.97% to 4.19%. Potassium has been linked to optimizing leaf quality and combustibility of tobacco, and according to Volodarskiy (1971) 3–5% K_2O concentration in the leaves has beneficial influence on the burning properties of tobacco. The content of potassium in tobacco grown in two of the micro regions (Ablanitsa and Dolno Osenovo) was within that range. The concentration of K_2O in the tobaccos from the other micro regions studied remained below 3% and these levels are not sufficient to improve the burning properties of Oriental tobacco.

Calcium concentration in the leaves was in the range from 1.90% to 3.43%. These values were lower or similar to those reported by Yancheva (2002) for Krumovgrad 58 variety. Literature data on the influence of Ca on the combustibility of tobacco are not consistent. Peterson and Tibbitts (1963) concluded that there was no significant correlation between leaf burn and Ca content in tobacco. On the other hand, Radojičić et al. (2015) established strong positive correlation between Ca concentration and the static burning rate in Virginia tobacco, while Stoilova (2008) found a negative relationship between the combustibility of the three types of tobacco (Oriental, Virginia and Burly) and the Ca content in leaves. In the current study, Ca content of tobacco samples differed significantly between the micro regions. The concentration was the highest in the samples from the Bogolin, Slashten, Tuhovishta and Kornitsa micro regions, and, respectively, the lowest in the raw material from Zhizhevo, Debren and Valkosel.

Magnesium content has been correlated positively with the combustibility of Oriental (Stoilova, 2008) and Virginia (Radojičić et al., 2015) tobacco. The actual availability of Mg during the growing season depends on various environmental factors (such as precipitation) and site-specific conditions (soil type, availability of other nutrients, etc.) (Gransee and Führs, 2013). Climatic and soil factors of the different

Table 2. Macroelement concentration in Oriental tobaccos of Krumovgrad ecotype depending on the region and micro region (% of dry weight)

Region	Micro region	K_2O	CaO	MgO
Nevrokop	Kornitsa	2.48 ± 0.07^{e}	3.09 ± 0.16^{b}	0.61 ± 0.05^{b}
Nevrokop	Fargovo	2.18 ± 0.10^{fg}	2.32 ± 0.17^{e}	0.56 ± 0.07^{bc}
Nevrokop	Valkosel	2.33 ± 0.07^{ef}	2.06 ± 0.08^{f}	0.56 ± 0.07^{bc}
Nevrokop	Godeshevo	2.70 ± 0.13^{d}	2.59 ± 0.19^{cd}	0.61 ± 0.04^{b}
Nevrokop	Slashten	2.42 ± 0.09^{e}	3.15 ± 0.13^{b}	0.63 ± 0.03^{b}
Nevrokop	Gotse Delchev	2.48 ± 0.12^{e}	2.41 ± 0.19^{de}	0.58 ± 0.07^{b}
Nevrokop	Ablanitsa	4.19 ± 0.23^{a}	2.30 ± 0.12^{e}	1.05 ± 0.11^{a}
Nevrokop	Zhizhevo	2.93 ± 0.11^{c}	1.90 ± 0.13^{f}	0.46 ± 0.04^{cd}
Nevrokop	Tuhovishta	2.05 ± 0.12^{gh}	3.12 ± 0.11^{b}	0.95 ± 0.06^{a}
Nevrokop	Bogolin	2.42 ± 0.15^{e}	3.43 ± 0.08^{a}	0.43 ± 0.03^{d}
Nevrokop	Debren	1.97 ± 0.12^{h}	2.04 ± 0.08^{f}	0.60 ± 0.06^{b}
Gorna Djumaya	Dolno Osenovo	3.66 ± 0.09^{b}	2.48 ± 0.15^{cd}	0.63 ± 0.04^{b}
Dupnitsa	Mursalevo	2.86 ± 0.09^{cd}	2.67 ± 0.15^{c}	0.58 ± 0.07^{b}
CV, %		23.22	18.99	26.56

Mean ± standard deviation

Different letters within each column indicate that the means are significantly different ($P < 0.05$)

CV – Coefficient of variation

Source: Authors' own elaboration

regions and micro regions in the study had significant influence on the magnesium content of oriental tobacco leaves. The obtained MgO values (0.43–1.05%) were similar to those reported by Yancheva (2002) and Stoilova (2008). The concentration of the element was the highest in the tobaccos from Ablanitsa and Tuhovishta micro regions, and the lowest – in those from Bogolin and Zhizhevo.

The coefficients of variation (*CV*) described the variability for each element in the study (Table 2). The lowest *CV* of 18.99% was observed for CaO, while the calculated *CV*s for K_2O and MgO were 23.22% and 26.56%, respectively.

Data on the trace elements' concentrations in the analyzed tobacco leaves are presented in Table 3.

The results show that the metal concentrations in tobacco were in the following order: Mn > Zn > Cu > Ni > Cd. The concentrations were significantly influenced by the growing regions and micro regions ($P < 0.05$). According to Golia et al. (2009) trace metals content in Oriental tobacco grown in Central Greece varied in the following ranges: Cd (0.68–9.9 mg kg^{-1}), Ni (20–80 mg kg^{-1}), Mn (118–510 mg kg^{-1}), Zn (3.2–50 mg kg^{-1}), and Cu (3.3–109 mg kg^{-1}). Zaprjanova and Hristozova (2018) found that the concentrations of trace elements in Oriental tobacco varieties were as follows: Cd (2.3–2.8 mg kg^{-1}), Mn (43.9–55.6 mg kg^{-1}), Zn (68–79.7 mg kg^{-1}), and Cu (9.4–11.9 mg kg^{-1}). Pelivanoska et al. (2014) analyzed 117 samples of tobacco leaves from Skopje production region and reported that Cd concentration ranged from 0.55 to 7.6 mg kg^{-1}, Mn concentration was from 30.4 to 670.7 mg kg^{-1}, Zn (6.73–76.97 mg kg^{-1}) and Cu (2.9–12.65 mg kg^{-1}). Nickel content in tobacco from three growing regions in Macedonia was in the range from 0.6 to 16 mg kg^{-1} (Jordanoska et al., 2018). In our study, the content of Cd in the tobacco samples was from 0.1 to 1.0 mg kg^{-1}, Ni concentration varied between 0.3 and 4.4 mg kg^{-1}, and the concentrations for the other elements were: Mn (31.9–102.4 mg kg^{-1}), Zn (16.1–48.0 mg kg^{-1}) and Cu (3.7–31.7 mg kg^{-1}). Therefore, it could be summarized that the concentrations of the analyzed trace elements are entirely within the ranges for Oriental tobacco grown in Bulgaria, Greece and Macedonia (Golia et al., 2009; Pelivanoska et al., 2014; Jordanoska et al., 2018; Zaprjanova and Hristozova, 2018).

The coefficients of variation indicated that the concentration of trace elements (Cd, Ni, Mn, Zn, and Cu) varied highly ($CV > 30\%$) (Table 3). Large coefficients of variation (> 30%) are often associated with increased experimental variability (Taylor et al., 1999).

Data about the chemical composition of cured tobacco leaves as a raw material for smoking products are important for the development of new blends, or for maintaining the quality of existing ones, with a view to ensuring consistency of their smoking properties (Kurt, 2021). The results from the determination of the main chemical traits, typically regarded as decisive for tobacco quality – nicotine, reducing sugars, total nitrogen, and ash, are presented in Table 4.

As it is known, some of the most important factors determining alkaloid levels in tobacco leaves are related to the genetic potential of the variety, the environmental conditions of the region and crop year, the applied agricultural practices

Table 3. Trace element concentration in Oriental tobaccos of Krumovgrad ecotype depending on the region and micro region (mg kg^{-1} dry matter)

Region	Micro region	Cd	Ni	Mn	Zn	Cu
Nevrokop	Kornitsa	0.1 ± 0.06^{e}	0.73 ± 0.15^{de}	31.9 ± 3.15^{f}	20.0 ± 2.00^{fg}	5.3 ± 0.46^{efg}
Nevrokop	Fargovo	0.2 ± 0.10^{cd}	0.70 ± 0.10^{de}	102.4 ± 7.21^{a}	30.5 ± 1.51^{c}	3.7 ± 0.26^{h}
Nevrokop	Valkosel	0.3 ± 0.12^{cd}	1.80 ± 0.17^{c}	38.2 ± 1.93^{ef}	16.5 ± 1.35^{h}	4.4 ± 0.66^{h}
Nevrokop	Godeshevo	0.2 ± 0.10^{cd}	3.90 ± 0.66^{ab}	43.9 ± 4.11^{e}	19.3 ± 1.76^{fg}	7.0 ± 0.62^{ef}
Nevrokop	Slashten	0.8 ± 0.06^{ab}	3.70 ± 1.04^{ab}	56.3 ± 6.75^{cd}	21.5 ± 1.14^{ef}	10.0 ± 1.00^{d}
Nevrokop	Gotse Delchev	1.0 ± 0.50^{a}	2.20 ± 0.56^{c}	57.4 ± 5.90^{cd}	37.8 ± 2.76^{ab}	31.7 ± 1.82^{a}
Nevrokop	Ablanitsa	0.4 ± 0.10^{cd}	0.80 ± 0.05^{de}	63.3 ± 3.70^{bc}	26.9 ± 1.91^{d}	14.1 ± 0.85^{bc}
Nevrokop	Zhizhevo	0.4 ± 0.12^{cd}	0.70 ± 0.17^{de}	39.2 ± 3.68^{ef}	16.1 ± 1.45^{h}	5.2 ± 0.44^{efg}
Nevrokop	Tuhovishta	0.3 ± 0.06^{cd}	3.40 ± 0.78^{b}	53.6 ± 2.84^{d}	16.3 ± 2.46^{h}	6.4 ± 0.44^{ef}
Nevrokop	Bogolin	0.3 ± 0.10^{cd}	0.30 ± 0.10^{e}	66.6 ± 4.41^{b}	19.3 ± 2.79^{fg}	5.0 ± 1.00^{gh}
Nevrokop	Debren	0.5 ± 0.12^{cd}	1.60 ± 0.46^{cd}	67.3 ± 4.35^{b}	23.8 ± 1.91^{de}	12.6 ± 2.19^{c}
Gorna Djumaya	Dolno Osenovo	0.1 ± 0.10^{e}	3.30 ± 0.44^{b}	41.3 ± 3.32^{e}	25.7 ± 2.89^{d}	7.4 ± 0.52^{e}
Dupnitsa	Mursalevo	0.6 ± 0.17^{bc}	4.40 ± 0.50^{a}	51.6 ± 2.35^{d}	48.0 ± 2.36^{a}	14.9 ± 1.49^{b}
CV, %		75.0	69.34	33.07	37.58	75.56

Mean ± standard deviation

Different letters within each column indicate that the means are significantly different ($P < 0.05$)

CV – Coefficient of variation

Source: Authors' own elaboration

Table 4. Chemical indexes of Oriental tobaccos of Krumovgrad ecotype depending on the region and micro region (%)

Region	Micro region	Indexes of tobacco (%)					
		Nicotine	Reducing sugars	RS/Nic[1)]	N (total)	Ash	Chlorine
Nevrokop	Kornitsa	0.87 ± 0.07	18.80 ± 1.92	21.61	1.33 ± 0.01	8.23 ± 0.05	0.28 ± 0.01
Nevrokop	Fargovo	0.86 ± 0.06	17.00 ± 1.63	19.77	1.75 ± 0.01	8.23 ± 0.05	0.66 ± 0.01
Nevrokop	Valkosel	0.93 ± 0.11	21.20 ± 1.87	22.80	1.35 ± 0.01	8.14 ± 0.05	0.22 ± 0.01
Nevrokop	Godeshevo	0.97 ± 0.09	17.80 ± 1.94	18.35	1.71 ± 0.02	9.10 ± 0.07	0.75 ± 0.01
Nevrokop	Slashten	0.94 ± 0.07	14.20 ± 1.23	15.11	1.58 ± 0.02	9.12 ± 0.07	0.48 ± 0.01
Nevrokop	Zhizhevo	1.03 ± 0.09	14.90 ± 1.64	14.47	1.53 ± 0.02	9.77 ± 0.07	0.34 ± 0.01
Nevrokop	Bogolin	1.25 ± 0.09	13.40 ± 0.99	10.72	1.75 ± 0.02	9.43 ± 0.07	0.60 ± 0.01
Nevrokop	Gotse Delchev	1.83 ± 0.10	15.70 ± 1.41	8.58	1.75 ± 0.02	11.19 ± 0.99	0.28 ± 0.01
Nevrokop	Ablanitsa	0.75 ± 0.09	15.50 ± 1.35	20.67	1.65 ± 0.02	11.23 ± 1.01	0.32 ± 0.01
Nevrokop	Tuhovishta	0.98 ± 0.09	13.50 ± 1.28	13.78	1.59 ± 0.02	8.60 ± 0.09	0.51 ± 0.01
Nevrokop	Debren	0.65 ± 0.03	18.10 ± 1.96	27.85	1.35 ± 0.01	7.95 ± 0.08	0.32 ± 0.01
Gorna Djumaya	Dolno Osenovo	1.25 ± 0.11	13.30 ± 1.22	10.64	1.86 ± 0.02	11.70 ± 0.99	0.29 ± 0.01
Dupnitsa	Mursalevo	1.30 ± 0.15	15.30 ± 1.39	11.77	1.69 ± 0.02	9.63 ± 0.98	0.24 ± 0.01

RS/Nic – Reducing sugars/nicotine
Mean ± standard deviation
Source: Authors' own elaboration

(e.g. fertilization, irrigation), the leaf stalk position, the degree of maturity at harvest, and other factors. As seen from the data in Table 4, the nicotine content in the studied tobaccos of Krumovgrad ecotype varied in a wide range – from 0.65% in the sample from Debren to 1.83% in that from the Gotse Delchev micro region. Nicotine levels in the present study were fully comparable to the results obtained by Kinay et al. (2020) and Kurt (2021), but higher than the data reported by Ekren (2018) and lower than those determined by Karim and Mohammad (2020) for Oriental tobaccos from different crops and regions of Turkey.

The content of sugars in the cured tobacco leaves depends on the variety, the crop year and, above all, on the curing conditions (temperature, relative air humidity and duration). If the curing process involves the maintenance of high temperatures (as is the case with flue-cured or sun-cured tobaccos), the final sugar content is high (Banožić et al., 2020). In general, high-sugar varieties are considered to be of higher quality in all bright tobacco types (Camlica and Yaldiz, 2021). Previous studies have reported that the total amount of sugars in tobacco leaves is up to 30%, with 22% being reducing sugars (Zilkey et al., 1982). Unlike other carbohydrates (starch, cellulose, pectin), reducing sugars have a positive impact on the smoking quality of tobacco, improving smoke taste and aroma (Pang et al., 2007). As seen from Table 4, the content of reducing sugars in the examined tobaccos also varied significantly – from 13.30% in the sample from Dolno Osenovo to 21.20% in the sample from Valkosel. The tobaccos from Bogolin and Tuhovishta micro regions were indistinguishable from the low limit of that range, while those from Kornitsa, Debren, Godeshevo, and Fargovo had similar sugar contents that were closer to the high data range limit. The obtained results were very close to those presented by Camlica and Yaldiz (2021) and Ekren (2018) for Turkish tobaccos (respectively, 9.70–21.30% and 12.9–22.8%), and by Trajkoski et al. (2015) for Macedonian tobaccos from Prilep ecotype (13.67–17.99%), but higher than those reported by Tsaliki et al. (2023) for Greek tobaccos (2.71–9.78%). The reducing sugars-to-nicotine ratio, which primarily determines the flavor balance of the smoke, took an optimal value in the tobacco from Gotse Delchev micro region, 8.58. The samples from Dolno Osenovo and Bogolin were positioned very close to the high limit of the accepted optimal range of the index (i.e., between 6.00 and 10.00), with ratio values, respectively, of 10.64 and 10.72. The rest of the compared tobaccos showed considerably higher than optimal values, which would predetermine a certain imbalance in the smoking properties.

Nitrogenous compounds, along with sugars, are the most important precursors of tobacco flavor (Leffingwell, 1999; Roemer et al., 2012). The total nitrogen content in the studied tobaccos varied from 1.33% in the sample from Kornitsa (1.35% in the samples from Valkosel and Debren, respectively) to 1.86% in the sample from Dolno Osenovo. There was no significant difference in that indicator between the rest of the tobaccos, as they were close to the highest reported content (1.53–1.75%). The data were compatible to those reported by Karim and Mohammad (2020) for Oriental tobaccos from Iraq (1.57–2.45%).

In general, the tobaccos in the study showed relatively low ash contents, with the lowest values recorded for the samples from Debren (7.95%), Valkosel (8.14%), Kornitsa and Fargovo (8.23%). The tobaccos from Dolno Osenovo (11.70%), Ablanitsa (11.23%) and Gotse Delchev (11.19%) had the highest ash content, without a significant difference between them. The obtained results were comparable to the values reported in a number of previous studies (Ekren, 2018; Şahin and Ekren, 2022; Tepecik and Ongun, 2020), and were significantly lower than those found for tobaccos in Macedonia (14.29–16.66%; Trajkoski et al., 2015) and Iraq (17.15–24.56%; Karim and Mohammad, 2020).

A previous study on chlorine accumulation in Oriental and semi-Oriental tobacco leaves reported a range between 0.38% and 2.68% (Darvishzadeh et al., 2011). It is considered that high quality Virginia tobaccos should contain less than 1% chlorine, as leaves with higher Cl concentration are of poor quality and have reduced burning rate (Karaivazoglou et al., 2005). The chlorine content in the examined Oriental tobaccos was below 1%, with the highest concentration found in the samples from Godeshevo (0.75%), Fargovo (0.66%) and Bogolin (0.60%), and the lowest – in the samples from Valkosel (0.22%), Mursalevo (0.24%), Kornitsa (0.28%), Gotse Delchev (0.28%), and Dolno Osenovo (0.29%). Previous studies on the chlorine content of domestically produced Krumovgrad (Bozhinova et al., 2023) and Burley (Popova et al., 2006) tobaccos showed variation in the ranges 0.26–0.92% and 0.06–0.84%, respectively, which is in full compliance with the current data. The low chlorine content is characteristic of Bulgarian tobaccos and is indicative of their good combustibility (Stoilova, 2008).

Fig. 1 presents the results from the determination of the main indices of tobacco smoke composition (nicotine and tar, mg/cig) for the studied tobaccos of Krumovgrad ecotype.

Smoke nicotine is an important indicator of tobacco quality, both for cigarette consumers and manufacturers, in view of the addictive nature of nicotine and the harmful consequences of smoking, on one hand, but also in view of its contribution to smoke sensory properties and perception (Djordjevic and Doran, 2009; Tassew and Chandravanshi, 2015). The data shown on Fig. 1 identified the highest smoke nicotine content in the tobacco from Gotse Delchev (1.64 mg/cig), followed by the samples from Mursalevo, Dolno Osenovo and Bogolin, with no significant differences between them (1.12–1.07 mg/cig). With the lowest smoke nicotine content were the tobaccos from Debren and Ablanitsa, 0.60 mg/cig and 0.67 mg/cig, respectively. Smoke condensate (tar) levels correlate strongly with the major health risks associated with smoking (e.g., lung cancer, cardiovascular and respiratory diseases), and they depend on tobacco nature, tobacco growing conditions, tobacco additives, the smoking conditions, and many other factors (Jebet et al., 2018; Lee, 2018). The highest tar content was registered in the smoke of the tobacco from Debren – 23.59 mg/cig, followed by those from Tuhovishta and Fargovo, respectively, 23.12 mg/cig and 22.42 mg/cig. The sample from Ablanitsa showed the lowest tar content, 15.69 mg/cig. The tar/nicotine ratio took a maximum value in the tobacco from Debren (39.32), and minimum – in that from Gotse Delchev (12.76).

The general chemical assessment of the investigated tobaccos of Krumovgrad ecotype presented above apparently revealed certain differences both within the sample pool and when compared to the data in the literature, which confirmed the significant influence of the variety, the cultivation prac-

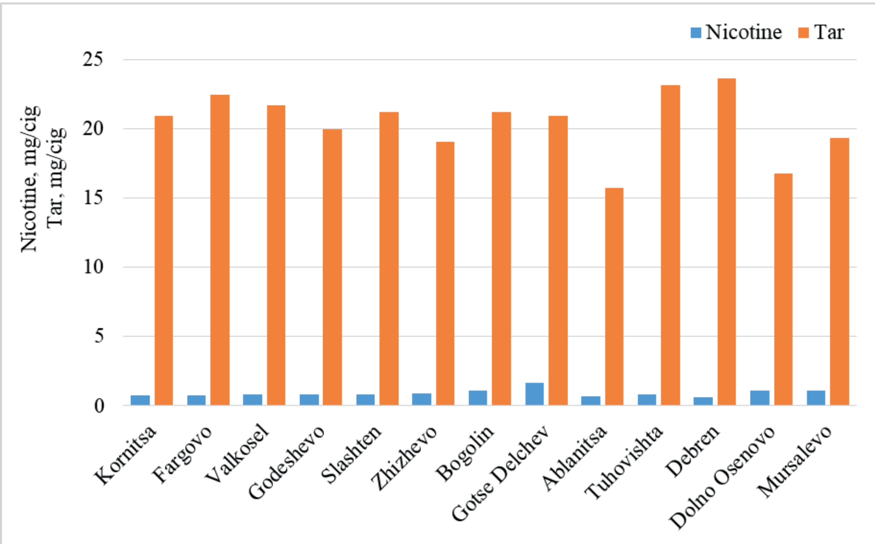

Fig. 1. Tar and nicotine (mg/cig) in tobacco smoke of Oriental tobaccos of Krumovgrad ecotype depending on the region and micro region

Source: Authors' own elaboration

tices and the environmental conditions (the production area and crop year conditions) on the formation of the chemical composition of Oriental tobacco.

As a result of the conducted hierarchical cluster analysis, it was found that the tobacco samples from different micro regions were grouped in two main clusters (Fig. 2), according to the concentration of K_2O, CaO, MgO, total N, chlorine, nicotine, reducing sugars, and ash in the cured leaves (Tables 2 and 4).

Figure 2: Grouping of Oriental tobaccos of Krumovgrad ecotype from different micro regions according to their chemical characteristics

The first cluster at the bottom of the dendrogram was represented by tobaccos from five micro regions – Fargovo, Debren, Godeshevo, Kornitsa, and Valkosel. In this cluster, the respective indicators fell within the following ranges: K_2O (from 1.97% to 2.70%), CaO (2.04–3.09%), MgO (0.56–0.61%), total N (1.33% – 1.75%), chlorine (0.22–0.75%), nicotine (0.65–0.97%), reducing sugars (17.0–21.2%), and ash (7.95–9.10%).

The second cluster included tobacco from eight micro regions (Zhizhevo, Mursalevo, Gotse Delchev, Ablanitsa, Slashten, Tuhovishta, Bogolin, and Dolno Osenovo) and had higher nicotine content (0.75–1.83%) and lower reducing sugar content (13.3–15.7%). The variation of other indicators was as follows: K_2O (2.05–4.19%), CaO (1.90% – 3.43%), MgO (0.43–1.05%), total N (1.53–1.86%), chlorine (0.24–0.60%), and ash (8.60–11.70%).

Table 5 presents the obtained values of the selected combustibility indices, which express the relationship between the most important inorganic constituents and tobacco leaf burning potential.

As stated earlier, combustibility is one of the key quality indicators for tobacco consumed by smoking, since, on the one hand, it is related to the manifestation of smoking properties, and on the other hand – it largely determines the production of tar, carbon monoxide, carbonyls, and many other harmful components in tobacco smoke. The combined influence, either positive or negative, of the most important inorganic elements on tobacco combustibility finds reflection in the application of various indices (combustibility coefficients), the most characteristic of which is the potassium/chlorine ratio (*Index of Nessler*). In accordance with the ranges set by Zahedi and Moghaddam (2000) for assessing tobacco leaf burning rate by the K/Cl ratio (namely, below 1 – deteriorated combustibility, the tobacco leaf does not burn; 1÷4 – low burning rate; 5÷20 – high burning rate; above 20 – very high burning rate), the following grouping of the studied tobaccos of Krumovgrad ecotype in the different micro regions could be made:

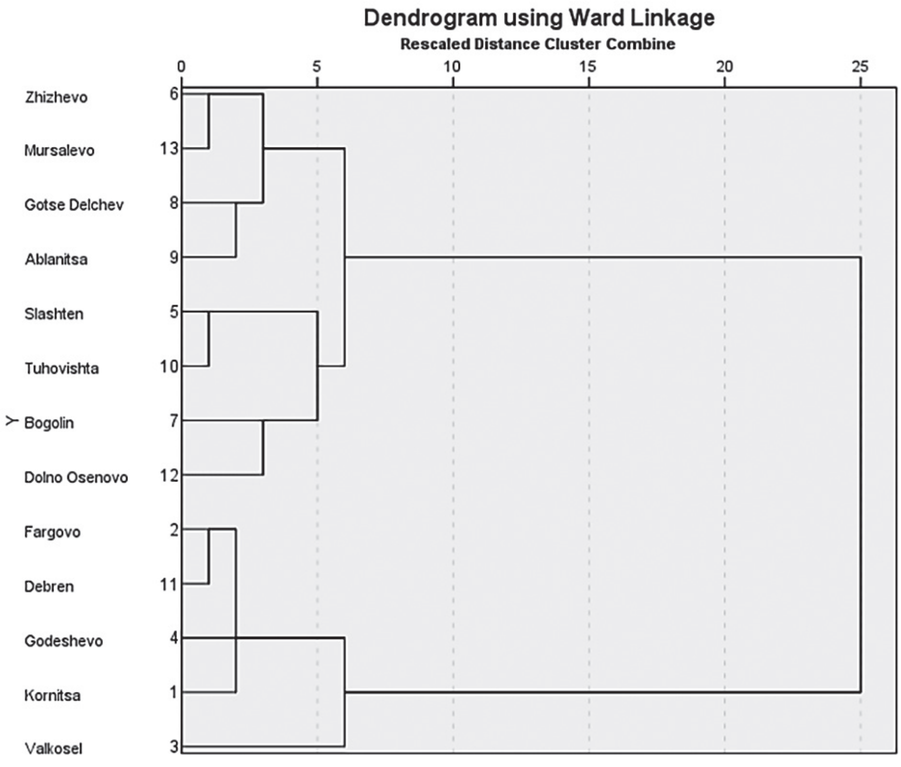

Fig. 2. Grouping of Oriental tobaccos of Krumovgrad ecotype from different micro regions according to their chemical characteristics
Source: Authors' own elaboration

- Group I – tobaccos with weak combustibility. The group included the samples from Fargovo, Godeshevo, Tuhovishta, and Bogolin micro regions (with K/Cl values from 3.30 to 4.03);
- Group II – tobaccos with good combustibility. The highest burning rate according to the index was associated with the samples from Ablanitsa (13.09), Dolno Osenovo (12.62), Mursalevo (11.92) and Valkosel (10.59), followed by the rest of the samples (with K/Cl ratios from 5.04 to 8.86).

As the data in Table 5 show, among the investigated tobaccos there were no ones with deteriorated combustibility.

According to the second combustibility index (*Index of Sastry and Kurup A*; Table 5), the best combustibility was observed again for the tobaccos from Ablanitsa and Dolno Osenovo micro regions (2.13 and 1.70, respectively), followed by those from Zhizhevo, Kornitsa, Valkosel, Mursalevo, etc. (with index values descending from 1.22 to 0.90).

The highest burning potential, according to the values of the third combustibility index regarded (*Index of Sastry and Kurup B*; Table 5), was revealed by the sample from Kornitsa (2.51), followed by those from Dolno Osenovo (2.21), Mursalevo (2.20) and Ablanitsa (2.15), and the rest of the studied tobaccos.

Table 5. Combustibility indexes of Oriental tobaccos of Krumovgrad ecotype from different micro regions

Region	Micro region	Index I[1)]	Index II[2)]	Index III[3)]
Nevrokop	Kornitsa	8.86	1.54	2.51
Nevrokop	Fargovo	3.30	0.90	1.52
Nevrokop	Valkosel	10.59	1.48	2.06
Nevrokop	Godeshevo	3.60	1.10	1.72
Nevrokop	Slashten	5.04	1.17	2.07
Nevrokop	Zhizhevo	8.62	1.57	2.07
Nevrokop	Bogolin	4.03	1.03	2.10
Nevrokop	Gotse Delchev	8.86	1.22	1.87
Nevrokop	Ablanitsa	13.09	2.13	2.15
Nevrokop	Tuhovishta	4.02	0.98	1.70
Nevrokop	Debren	6.16	1.18	1.77
Gorna Djumaya	Dolno Osenovo	12.62	1.70	2.21
Dupnitsa	Mursalevo	11.92	1.48	2.20

[1)] Index of Nessler (K_2O/Cl);
[2)] Index of Sastry and Kurup *A* ($K_2O/(N + Cl)$);
[3)] Index of Sastry and Kurup *B* ($(K_2O + CaO)/(MgO + N + Cl)$)
Source: Authors' own elaboration

Table 6. Ranking of Oriental tobaccos of Krumovgrad ecotype by combustibility indices

Region	Micro region	Rank by combustibility index value			ΣXij	Ranking
		Index of Nessler	*Index of Sastry and Kurup A*	*Index of Sastry and Kurup B*		
Nevrokop	Kornitsa	5.5	4	1	*10.5*	3
Nevrokop	Fargovo	13	13	13	*39.0*	13
Nevrokop	Valkosel	4	5.5	7	*16.5*	5
Nevrokop	Godeshevo	12	10	11	*33.0*	11
Nevrokop	Slashten	9	8.5	7	*24.5*	8
Nevrokop	Zhizhevo	7	3	7	*17.0*	6
Nevrokop	Bogolin	10.5	11	5	*26.5*	9.5
Nevrokop	Gotse Delchev	5.5	7	9	*21.5*	7
Nevrokop	Ablanitsa	1	1	4	*6.0*	1
Nevrokop	Tuhovishta	10.5	12	12	*34.5*	12
Nevrokop	Debren	8	8.5	10	*26.5*	9.5
Gorna Djumaya	Dolno Osenovo	2	2	2.5	*6.5*	2
Dupnitsa	Mursalevo	3	5.5	2.5	*11.0*	4

Source: Authors' own elaboration

The resulting differences in the ranking orders of the examined tobaccos of Krumovgrad ecotype are reasonable in view of the involvement of different chemical components in each of the selected coefficients. In order to achieve a more complex assessment of the investigated tobaccos, based on the results of the above-discussed combustibility indices, the samples were ranked according to the value of the corresponding index, taking into account its positive correlation with tobacco leaf combustibility (Table 6). In the case of undistinguishable differences in indices' values the samples shared the respective rank positions (i.e., they were awarded equal average rank). Ranking reliability was validated by Kendall's concordance test, and the significance of results – by Fisher's test (at a confidence level $\alpha = 0.05$).

The ranking of the tobaccos shown in Table 6 was statistically reliable and significant (Kendall's concordance coefficient $W = 0.86$), which gave reason to make the following grouping (in decreasing order of combustibility) of Krumovgrad ecotype tobaccos produced in the thirteen micro regions:

– Group I – tobaccos from Ablanitsa (region Nevrokop) and Dolno Osenovo (Gorna Djumaya);
– Group II – tobaccos from Kornitsa (Nevrokop) and Mursalevo (Dupnitsa);
– Group III – tobaccos from Valkosel and Zhizhevo (Nevrokop);
– Group IV – tobaccos from Gotse Delchev, Slashten, Bogolin, and Debren (Nevrokop);
– Group V – tobaccos from Godeshevo, Tuhovishta and Fargovo (Nevrokop).

Conclusions

The concentrations of macroelements (K, Ca and Mg) in Bulgarian Oriental tobacco of Krumovgrad ecotype differed significantly between the studied regions and micro regions. The lowest *CV* (18.99%) was observed for CaO, while the calculated *CV*s for K_2O and MgO were 23.22% and 26.56%, respectively. The trace element concentrations (Cd, Ni, Mn, Zn, and Cu) found in the studied tobaccos from Nevrokop, Gorna Djumaya and Dupnitsa producing regions were within the ranges reported by other researchers. Therefore, the tobacco grown in the studied area can be used by the cigarette manufacturers without accelerated health risks associated with metal accumulation.

The assessment of the main chemical indicators of the investigated tobaccos of Krumovgrad ecotype showed variation, depending on the micro region of production: nicotine, 0.65–1.83%; reducing sugars, 13.30–21.20%; total nitrogen, 1.33–1.86%; ash, 7.95–11.70%. The tobaccos from micro regions Gotse Delchev (region Nevrokop), Dolno Osenovo (Gorna Djumaya) and Bogolin (Nevrokop) had a more balanced chemical composition. The applied hierarchical cluster analysis classified the tobacco samples from the thirteen micro regions into two main clusters based on the measured chemical indicators.

Tobacco combustibility, one of the most important tobacco quality indicators, was evaluated by different combustibility indices, taking into account the concentration of inorganic elements in the cured leaves. The results spoke in favor of the good burning potential of Bulgarian Oriental tobacco of Krumovgrad ecotype, as observed previously. The ranking of tobaccos, based on the assessment of their combustibility, distinguished with the best burning properties those from Ablanitsa (Nevrokop) and Dolno Osenovo (Gorna Gjumaya).

The results obtained in the study can be used in evaluating the quality of tobacco as a raw material for smoking products, with a view to their optimal usability in tobacco blending.

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