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Chemometric Evaluation of the Colour and Smoke Aroma in Oriental Tobaccos Based on the Polyphenol and Valeric Acid Cultivar Characteristics as Influenced by the Genotype

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Abstract

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The influence of polyphenols and valeric acids of Oriental tobacco cultivars on the colour of dry tobacco and the aroma of tobacco smoke was studied by applying a chemometric approach. A group of Djebel basma genotypes and a local cultivar Djebel 576 used as a standard were involved in the study. To obtain primary data on the polyphenol pattern related to colour estimation high performance liquid chromatography (HPLC) was used, whereas capillary gas chromatography (CGC) was applied to get information on valeric acid content, related to aroma characteristics. Regression models involving the polyphenol data and the isovaleric acid content were used for colour and aroma estimation, respectively. The HPLC data were processed by means of pattern recognition method (PRM) and the similarity indexes (Is, %) between the selection lines, as well as between them and the standard, were calculated. It was established that all cultivars are distinct from the standard in the polyphenol pattern. The data obtained for the Oriental tobaccos studied point that the colour of dry tobacco and the aroma of tobacco smoke are determined by the genotype-specific composition of the polyphenol complex and of the valeric acids, varying in the different cultivars. The chemometric evaluation of tobacco colour and smoke aroma allows predicting the improved quality characteristics of the selection line L 3 with respect to the standard Djebel 576 and the other genotypes.

Key words: Oriental tobaccos, colour, smoke aroma, polyphenols, valeric acids, chemometrics
Abbreviations: CGC – capillary gas chromatography*,* HPLC - high performance liquid chromatography, MRA – multiple regression analysis, PRM – pattern recognition methods, Is – index of similarity

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Introduction

Chemometrics is widely applied for assessing food properties, such as aroma, colour, taste, etc., and for quality estimation and classification of essential oils, fruits, wines, plant oils, etc. (Adam et al., 2005; Beltran et al., 2006; Cozzolino et al., 2003; Csomos et al., 2002; Dimov et al., 1987; Goodacre et al., 2003 and Ranalli et al., 2002). Chemometrics involves mathematical and statistical methods of description and processing of multifactorial objects (Massart et al., 1988). The two general applications of this statistical approach are to predict a property of interest and to classify the sample into one of several categories (Massart et al., 1988).

The colour of dry tobacco and the aroma of tobacco smoke are quality characteristics which determine the usability of the Oriental tobaccos (Leffingwell, 2001). They are in strong relationship with the chemical characteristics, the polyphenols and the valeric acids (isovaleric and 3-methylvaleric), respectively (Leffingwell, 2001). On this basis regression models describing the colour (multiple regression analysis involving polyphenol data) and the aroma of smoke (monofactorial regression analysis involving isovaleric acid data) were developed (Dagnon et al., 2002 and Dagnon et al., 2003). The correlation coefficients of the models exhibit high values. The characteristic odor of the valeric acids (cheesy-sweaty-buttery-fruity) is well-defined aromatic note in the smoke of Oriental tobaccos (Leffingwell, 2001). Aroma in tobacco is commonly estimated by sensory evaluation, but given the subjectivity of the estimates, as well as the high cost and health hazards for expert panels, new methods allowing an objective determination of this important tobacco characteristic were developed (Zou and Wu, 2002). The pattern recognition methods (PRM) are shown to be a useful tool for classification of tobaccos with regard to colour performance, corresponding to the organoleptic evaluation (Dagnon and Edreva, 2003). The chemometric evaluation of colour and aroma diversity in the Oriental tobacco genotypes Djebel basma can contribute to the optimization of the breeding programs aimed at developing highly aromatic Oriental tobaccos.

The purpose of this paper is to evaluate the variations of the colour of dry tobacco and aroma of tobacco smoke in different Oriental tobacco genotypes Djebel basma as influenced by the polyphenol and valeric acids cultivar characteristics, and classify these tobaccos by using a chemometric approach. Important biological, morphological and chemical characters were also determined.

Materials and Methods

Dry fermented Ist class leaves of the following Oriental tobacco genotypes Djebel basma were analyzed: the cultivar Djebel 576 (as a standard) and the selection lines L 3, L 4, L 22 and L 29. All Oriental tobacco genotypes were from the collection of the Institute of Tobacco and Tobacco Products, Plovdiv, Bulgaria. Plants were grown in the year 2005 under the same agro-technical conditions suitable for the genotype. The plant density was 30000 plants da^{-1}. The tobacco gathering, drying, processing and fermentation were accomplished under equal conditions appropriate for Oriental tobaccos.

During the vegetation the following

morphological parameters were registered: height of plants (cm); number of leaves; size of the 7 th, 14 th and 21 th leaf (length, width, cm) at the stage of technical maturity.

The dry tobacco samples were examined for the following *chemical parameters*: content of polyphenol components (g/100g DM) by high performance liquid chromatography (HPLC) according to Snook and Chortyk (Snook and Chortyk, 1982) as described in details by Dagnon and Edreva (Dagnon and Edreva, 2003); content of valeric acids (µg/g DM) by capillary gas chromatography (CGC), by modifying the method of Arrendale et al. (Arrendale et al., 1984); nicotine and total sugar contents (g/100g DM) by continuous flow analysis according to ISO methods. The main components of the polyphenol complex and the valeric acids were identified by using reference substances.

The chromatographic data were processed by the following *chemometric approach*:

- The colour of dry tobacco was assayed according to the multiple regression model involving polyphenol data (Dagnon et al., 2003);
- PRM using polyphenols components was performed according to the Package software program "Patreco" (Dimov et al., 1987). Processing of HPLC profiles of polyphenols was done using all peaks present in each sample as detailed by Dagnon and Edreva (Dagnon and Edreva, 2003). PRM data are presented as indexes of similarity (Is, %) between the items compared (the standard variety Djebel 576 and the cultivars studied). Values of Is above 98% are considered as not significant.
- The aroma of tobacco smoke was modelt by using regression equation based on isovaleric acid content in tobacco (Dagnon et al., 2002).

Statistics

Analyses were done in 5 replicates. The significance of differences was given by the *t*-test of Student at $P<5\%$.

Results and Discussion

Biological characters

Data for the morphological parameters are shown in Table 1. It is evidenced, that the obtained results (height of plants; number of leaves; size of the 7 th, 14 th and 21 th leaf) of all represented genotypes are characteristic for the Oriental tobaccos Dlebel basma. The selection lines L 3 and L 4 possess the smallest leaves (middle leaf 14 th) and their number is greater relatively to the standard Djebel 576. The plant height of these lines is also sharply different from the height of the standard. The biological characters of the plants point that the parameters of the leaves of lines L 3 and L 4 suit very close to the requirements for tobaccos of the genotype Djebel basma (Dimanov, 2005 and Nikolova et al., 2005).

Colour of dry tobacco

The contents of the total polyphenols and of the components of the polyphenol complex, as well as the color estimates calculated by the regression model are given in Table 2. The data reveal the small differences between the genotypes in regard to the total polyphenol content which vary from 2.51 g/100g to 2.88 g/100g. The standard Djebel 576 is characterized with a middle position (2.74 g/100g total polyphenols). The polyphenol content is highest in L 4 (2.88 g/100g) and lowest in

Table 1

Morphological parameters: height of plants (cm); number of leaves; size of the 7 th, 14 th and 21 th leaf (length, width, cm) in Oriental tobaccos Djebel basma

Genotypes	Height	Number of leaves	7th leaf		14th leaf		21th leaf	
			Length	Width	Length	Width	Length	Width
Djebel 576	89.0	24.0	20.0	12.0	17.0	8.9	12.0	7.9
L 3	72.0	27.6	17.3	10.8	13.8	9.8	10.8	7.3
L 4	73.0	28.8	17.3	11.3	13.3	9.5	10.0	6.3
L 22	80.0	26.0	18.0	10.0	17.0	9.0	12.0	7.0
L 29	85.0	31.0	18.0	10.0	17.0	9.0	11.0	7.0

Table 3

Classification of Oriental tobaccos Djebel basma according to colour performance, based on PRM (Is, %) of HPLC profiles of polyphenols

Genotypes	Djebel 576	L 3	L 4	L 22	L 29
	1	2	3	4	5
1	x	95.2	94.2	93.9	94.6
2		x	**96.4**	95.4	**91.7**
3			x	95.7	93.1
4				x	92.4
5					x

L 22 (2.51 g/100g), the difference of 0.31 g/100g between the genotypes being significant with respect to the uncertainty of the results (0.10 g/100g). The total polyphenols of lines L 3, L 4 and L 29 are practically equal (2.87 g/100g, 2.88 g/100g and 2.84 g/100g), respectively the lines exhibit also nearly the same colour (orange to orange-red) (Table 2). The colour of the Djebel basma genotypes studied as estimated by the regression model varies from yellow-orange (L 22) to orange-red (L 3). This variation in the calculated colours of the investigated Oriental genotypes to a great extent is in line with the organoleptical ranks describing the standard Djebel 576 and L 3, respectively, as yellow-orange with brown shades and yellow-orange to orange-red, pointing to the great correspondence between both estimates.

The classification of the tobacco

Table 2

Content of polyphenol components (g/100g DM) and colour of dry tobacco estimated by the regression model in Oriental tobaccos Djebel basma

Genotypes	Neo-chloro-genic aci	Chlorogenic acid	4-0-caffeoyl-quinic acid	Scopo-letin	Rutin	Kaempferol-3-rutino-side	Total	Estimated colour
Djebel 576	0.25	1.13	0.4	0.005	0.78	0.17	2.74	Orange
L 3	0.25	1.18	0.41	0.003	0.88	0.15	2.87	Orange to orange-red
L 4	0.28	1.15	0.46	0.003	0.84	0.15	2.88	Orange to orange-red
L 22	0.24	1.08	0.38	0.003	0.68	0.13	2.51	Yellow-orange to orange
L 29	0.34	1.07	0.53	0.005	0.71	0.18	2.84	Orange

genotypes Djebel basma with regard to the Is (%) based on the PRM processing of HPLC polyphenol profiles is presented in Table 3. All tobacco genotypes distinguish between them because all Is are less than 98% (considered as a limit value, above it differences are not significant). The chemometric evaluation of colour performance (regression model of colour and PRM of polyphenol patterns) points to the genotypic determined differences of lines L 3 and L 4 with regard to the standard Djebel 576. At the same time both lines are most similar to each other (Is 96,4%) and to the standard (Tables 2 and 3). The results obtained allow predicting that the colour of the selection line L 3 is of better quality than the standard Djebel 576.

Aroma of tobacco smoke

Valeric acids in tobacco (isovaleric and 3-methylvaleric) occur as salts, as well as glucose and sucrose tetraesters (Gwynn et al., 1985 and Leffingwell, 2001). Evidence was obtained that the sugar tetraesters of valeric acids are the dominant thermally labile precursors of aroma components responsible for the specific Oriental flavor (Ashraf-Khorassani et al., 2005). Single dominant gene located on the chromosome A controls the synthesis of valeric acids, this being particularly important for Oriental tobacco breeding (Leffingwell, 2001). The contents of valeric acids (isovaleric, 3-methylvaleric and total), nicotine and total sugars are given in Table 4. The data reveal the differences between the genotypes with regard to the parameters studied. They show that generally isovaleric acid and 3-methylvaleric acid in the leaves occurred in similar amounts. When comparing the total valeric acids content

Table 4

Content of valeric acids (µg/g DM), nicotine (g/100g DM) and total sugars (g/100g DM) in Oriental tobaccos Djebel basma

Genotypes	Isovaleric acid	3-methyl-valeric acid	Total valeric acids	Nicotine	Total sugars
Djebel 576	46.4	53.6	100	0.56	14.3
L 3	49.9	59.9	109.8	0.55	15
L 4	39.4	40.7	80.1	0.4	17.4
L 22	42.1	42.9	85	0.65	14.9
L 29	29	30.4	59.4	1.24	9.42

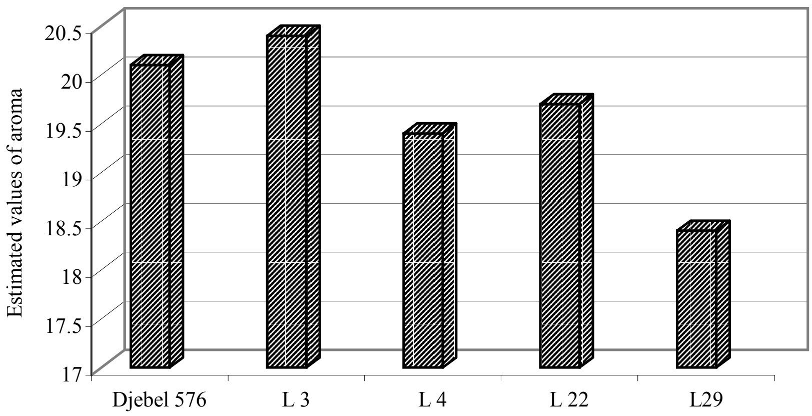

Fig. 1. Estimated values of smoke aroma in Oriental tobaccos Djebel basma calculated by using the regression model involving isovaleric acid content in the leaves

three situations can be observed:

a) total valeric acids content 100 and above 100 µg/g (the standard Djebel 576 and L 3);

b) total valeric acids content 80 - 85 µg/g (L 4 and L 22);

c) total valeric acids content about 60 µg/g (L 29).

The same grouping refers to the nicotine and total sugars content. Their data are similar in the standard Djebel 576 and L 3 (Table 4). Evidence was shown that the correlation between valeric acid content and smoke aroma exhibits high value in tobaccos containing similar amount of nicotine and sugar, like the genotypes Djebel 576, L 3, L 4 and L 22 (Dagnon et al., 2002). It must be noticed that the nicotine content of most lines is lower than the typical values for the Oriental tobaccos Djebel basma (1% and above) which can influence negatively the smoke characteristics (Leffingwell, 2001 and Nikolova et al., 2005).

Figure 1 shows the aroma scores of the smoke in the Djebel genotypes calculated from the regression model involving isovaleric acid content in the leaves. The highest score is obtained by the L 3 (20.4) followed by the standard Djebel 576 (20.1) and the other genotypes (below 20) (Figure 1). All obtained results in this study point to the ordinary aroma of the smoke in the Oriental tobaccos studied. The selection line L 3 is distinguished by its improved aroma characteristics in comparison with the standard Djebel 576.

Conclusion

The genotypes Djebel basma and the cultivar Djebel 576 used as a standard, although grown at equal agroecological conditions, exhibited variation in the biological, morphological and chemical parameters scored, as well as in colour and aroma performance. The data point to the strong influence of the genotype on the characters of Oriental tobaccos studied. The colour of dry tobacco and the aroma of tobacco smoke are determined by the genotype-specific composition of the polyphenol complex and valeric acids, respectively, varying in the different cultivars. The chemometric evaluation of tobacco colour and smoke aroma allows predicting the improved quality characteristics of Line 3 with respect to the standard Djebel 576 and the other genotypes.

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