Asian Journal of Chemistry Vol. 20, No. 4 (2008), 3135-3142

Elemental Analysis of Tobacco Leaves by FAAS and WDXRF Spectroscopy

NECDET ÇAMAS*, BURHAN ARSLAN† and AHMET UYANIK‡
Department of Tobacco Growing and Processing, Vocational School of Bafra
Ondokuz Mayis University, Samsun 55400, Turkey
Fax: (90)(362)5426761; Tel: (90)(362)5426763
E-mail: necdetc@omu.edu.tr; cnecdetc@gmail.com

The intensity of the different elements in tobacco leaves was comparatively determined by using flame atomic absorption (FAAS) and wavelength dispersive X-ray florescence (WDXRF) methods. The accuracy of both methods was evaluated by the data obtained from standard reference materials (CRMs), tobacco leaves of CTA-OTL-1 (oriental tobacco leaves) and CTA-VTL-2 (virginia tobacco leaves). The results were in good agreement with the literature values. The WDXRF method is also found most suitable and easily applicable to the analysis of tobacco leaves. Besides the elemental content, the highest quality of tobacco type cultivated in Turkey was determined in accordance with the nicotine and the reducing sugar content.

Key Words: WDXRF, FAAS, Trace elements, Heavy metals, Tobacco varieties.

INTRODUCTION

Tobacco is an important industrial crop in all over the world. The leaves of this invaluable crop have been usually used in cigarette and related products and some of its trace elements are extremely hazardous for humans even at a very low level of intake[1]. As this product provides routes of entry into body system, it is particularly important to characterize its elemental composition that may have harmful and toxic properties[2].

The toxic elements present in tobacco leaves is an issue of increasing concern as many trace elements, in particular the heavy metals were generally resulted from the intensively fertilized soils[3]. Nitrate in the form of manure and sewage sludge and rock-derived phosphate fertilizers are

†Department of Field Crops, Faculty of Agriculture, Namik Kemal University, Tekirdag, Turkey.
‡Department of Chemistry, Faculty of Science and Arts, Ondokuz Mayis University, Samsun, Turkey.

implicated in the accumulation of heavy metals[4] and, unlike many organic contaminants, most inorganic contaminants are not removed by chemical or microbial degradation. Therefore, there is a need to determine the levels of trace elements, especially the heavy metals that can become readily bioavailable from soil and accumulate in the plant materials that are consumed as cigarettes[2].

Several elements are needed to be analyzed in tobacco samples in a wide range of concentration. The most tobacco analyses have been performed by using FAAS[5], ICP-MS[6] and INAA[7-10] in the literature. However, these methods requires chemical extraction or dissolution steps prior to analysis and more difficult and time consuming compared to the other analysis methods[11,12].

A rapid and accurate method is still required to analyze tobacco leaves with adequate sensitivity, involving minimal sample preparation and relatively short analysis time[2]. The advent of commercially available wavelength dispersive spectrometers for X-ray fluorescence (WDXRF) measurements has provided an economical and powerful tool for biological, environmental, clinical, chemical, geological and industrial analysis[13]. The WDXRF method is a non-destructive, fast, multi-element technique for all sizes and forms. Although XRF spectrometry measurement is simple for a quantitative study, accurate quantitative measurements often depend on matrix correction procedures[14-17].

In this study, the trace elements acquired by tobacco leaves from the atmosphere, soil or grow as bio-minerals within cell vacuoles or by fungal decay on leaf surfaces were determined by FAAS (except Na and K) and WDXRF method comparatively. The accuracy of the methods were evaluated by using standard reference tobacco leaves [CTA-OTL-1 (Oriental tobacco leaves) and CTA-OTL-2 (Virginia tobacco leaves)] to obtain a reliable source of information that could be used in the related studies in future. Besides the elemental content, the other aim was to determine the highest quality of tobacco type cultivated in Turkey in accordance with the nicotine and the reducing sugar content.

EXPERIMENTAL

Tobacco leaves from 14 different cities in Turkey was collected in year 2005 growing season. In each city, the number of high quality tobacco samples (described as leaves and smoking leaves for each of tobacco varieties) changing from 12 to 27 different parts of the city were collected. All the collected samples for each city were mixed to produce a composite sample. Each of the samples was consisted of about 5-12 kg. From this composite mixture of each city, an 250 g analysis sample was taken by random number table, prepared and analyzed.

A wavelength-dispersive X-ray fluorescence spectrophotometer (WDXRF, Rigaku ZSX-100e with Rhodium target) was used in the experiments with its given characteristics. Instrument was controlled by a computer running ZSX software[18]. Each of tobacco samples was dried in a Heraeus furnace and then ground in a Spex mill. To reduce particle size effects, the powder obtained were carefully sieved using a 400 mesh sieve and then homogenized by mixing for 20 min. A 2 g of this powder was spread homogeneously on a mylar film stretched across a fibre frame[19] and spectra were recorded on 35 mm pellets made from 2 g of this powder pressed under 15 tons[20].

A flame absorption spectrometer (VARIAN Spectra AA-880 with air-acetylene flame) was used in the experiments with its given characteristics. The hollow cathode lamps, their wavelengths and currents were: Ca (422.7 nm, 8 mA), Mg (285.2, 7 mA), Fe (248.3, 9 mA), Al (309.3 nm, 10 mA), Cu (324.8 nm, 8 mA), Zn (213.9 nm, 6 mA) and Mn (279.5 nm, 5 mA). All the tobacco samples were dried 24 h at 85 °C in the oven and 0.4 g dried sample was weighed and added in to the digestion vessel (1.4 Mpa) with 5 mL concentrate nitric acid and 2 mL hydrogen peroxide[21]. The samples were burned down according to the wet digestion program of the microwave oven (CHEM MDS 2000). The resulted solutions were diluted with deionized water and the concentrations of the metals were determined by FAAS method. K and Na were then determined by flame photometer (PFP7 & PFP7C).

Analyses of nicotine and reducing sugar were carried out according to the modified UV spectroscopic method[22-24]. A UV-Vis spectrophotometer (Varian 50 UV-Vis spectrophotometer) was used in the experiments. The UV-Vis spectrophotometric nicotine measurements were taken at 236, 259 and 282 nm wavelengths following the steam distillation. The samples were also prepared for reducing sugar analysis[23,24] and UV-Vis spectrophotometric measurements were performed at 570 nm wavelength.

RESULTS AND DISCUSSION

The main purpose of this work was to investigate the content of the elements and trace elements such as K, Ca, Mg, Na, Fe, Al, Cu, Zn and Mn in the different tobacco leaves collected from the different cities of Turkey by using FAAS and WDXRF methods. The other aim was to determine the highest quality of tobacco type cultivated in Turkey up to some extend in accordance with the nicotine and the total reduced matter content.

The coordinates for the cities where the samples collected were Düzce (39° 55′ N 27° 19′ E), Sakarya (40° 47′ N 30° 24′ E), Akhisar (38° 58′ N 27° 58′ E), Gümüshaciköy (40° 55′ N 35° 11′ E), Bafra (41° 30′ N 35° 54′ E), Çarsamba (41° 15′ N 36° 42′ E), Samsun (41° 14′ N 36° 14′ E), Yenice

(39° 55′ N 27° 19′ E), Akçaabat (40° 57′ N 39° 32′ E), Yayladag (35° 58′ N 36° 6′ E), Bitlis (38° 19′ N 42° 8′ E), Adiyaman (37° 44′ N 38° 18′ E) and Mus (38° 51′ N 41° 33′ E).

However, various elements are essentially needed for the plant growth, over the critical concentrations toxicity problems for plants and human being usually emerge[25]. The average concentrations of the elements and the quality analysis results of the tobacco samples are given in Table-1.

TABLE-1
RESULTS OF ELEMENTAL ANALYSIS OF TOBACCO LEAVES FOR DIFFERENT VARIETIES BY USING FAAS AND WDXRF SPECTROSCOPY AND NICOTINE AND REDUCING MATTER CONTENTS

Tobacco varieties / Cities	Nicotine (%)/	Reducing matter (%)	Method	$K^{a,*}$	Ca^{a}	Mg^{a}	$Na^{b,*}$	Fe^{b}	Al^{b}	Cu^{b}	Zn^{b}	Mn^{b}
Virginia/	2.03	26.80	WDXRF	12.1	30.1	4.4	662	360	716	28.0	41.0	58.0
Duzce			FAAS	13.9	41.0	2.5	560	270	822	20.1	26.3	28.7
Burley/	3.83	1.20	WDXRF	25.9	45.6	6.5	662	652	542	25.0	25.0	87.0
Sakarya			FAAS	22.4	51.7	5.8	530	411	651	19.4	22.7	58.7
Izmir/	0.69	16.50	WDXRF	15.1	22.5	6.1	1511	2539	3637	16.0	26.0	66.0
Akhisar			FAAS	13.5	31.2	3.6	377	3156	3756	17.3	37.1	60.0
Basma/	1.57	8.25	WDXRF	19.4	49.2	6.9	559	834	2136	12.0	31.0	92.0
Ghacikoy			FAAS	17.4	48.1	3.5	511	723	1487	9.2	29.8	67.4
Bafra/	1.23	5.20	WDXRF	21.3	27.1	7.9	937	1278	2036	16.0	53.0	106.0
Bafra			FAAS	22.2	38.4	4.6	520	820	1460	21.2	64.9	70.0
Canik/	2.15	5.83	WDXRF	17.8	36.5	9.8	596	848	1011	29.0	52.0	75.0
Çarsamba			FAAS	17.2	41.9	4.8	576	782	811	19.4	46.1	49.6
Maden/	1.71	9.16	WDXRF	26.8	34.1	9.9	471	1344	1226	55.0	104.0	106.0
Samsun			FAAS	23.3	39.2	6.2	536	1131	887	54.4	107.1	108.0
Evkaf/	1.94	4.08	WDXRF	25.4	28.6	5.3	543	734	1399	22.0	44.0	73.0
Samsun			FAAS	25.6	39.0	5.2	584	708	760	15.0	31.3	78.0
Agonya/	2.41	15.71	WDXRF	20.3	21.9	7.9	543	611	1196	30.0	87.0	152.0
Yenice			FAAS	22.4	28.2	4.8	520	226	955	25.9	95.7	104.0
Trabzon/	3.56	0.90	WDXRF	15.8	38.8	27.2	915	626	560	24.0	42.0	224.0
Akçaabat			FAAS	16.0	44.4	17.2	531	550	543	28.0	36.5	232.5
Yayladag/	1.27	9.40	WDXRF	15.9	33.2	10.3	1741	876	1379	20.0	59.0	73.0
Yayladag			FAAS	18.1	35.9	6.9	410	774	1058	12.1	51.6	43.8
Bitlis/	0.81	24.30	WDXRF	10.7	22.5	5.6	733	774	1649	7.0	28.0	51.0
Bitlis			FAAS	10.7	27.9	3.9	496	550	1478	5.6	25.3	51.4
Adiyaman/	1.99	15.48	WDXRF	10.9	51.1	8.6	441	2412	2267	14.0	8.0	151.0
Adiyaman			FAAS	10.5	53.6	6.5	531	2581	2400	10.0	13.2	113.7
Mus/	0.77	16.30	WDXRF	10.9	39.4	11.5	568	1288	2470	12.0	15.0	98.0
Mus			FAAS	10.5	40.5	6.3	573	1020	1828	17.2	39.1	57.6
CERT/INFO/ CTA-VTL-2	3.84	-[+]		10.3	36.0	5.1	312	1083	1682	18.0	43.0	80.0
CERT/INFO/ CTA-VTL-1	1.72	-[+]		15.6	31.7	4.5	345	989	1740	14.0	50.0	412.0

[a]Unit g kg^{-1}; [b]Unit mg kg^{-1}; [*]Carried out by Flame Photometry; [+]No standard values for reducing sugar, results generally similar to the other literature values.

According to the FP results, the tobacco varieties with the highest potassium content were Evkaf, Bafra and Agonya and those with the lowest content were Mus, Adiyaman and Bitlis. As for the WDXRF method the highest potassium contents were observed in Maden, Burley and Evkaf varieties. Results for the other varieties were similar with those obtained using the FP method. Most of the tobaccos grown in Turkey has a potassium content approximately equal to the CTA-OTL-1 and CTA-VTL-2 standards. But, the Burley, Bafra, Evkaf and Agonya types have potassium content above these standards. One of the quality indicators of the tobacco leaves is the level of the potassium content which effecting burning quality and decreases the quantity of the chemical oxidant used in cigarette blends.

The results for sodium using both the FP and the WDXRF methods were generally similar with the all varieties studied. Except a few unexpectedly high and low results, almost all of the investigated samples showed a higher sodium content than that of CRMs used.

A comparison of the calcium content of the tobacco samples obtained by the FAAS and WDXRF methods showed that the content of the vast majority of the varieties were similar with the standard values and changes from 21.9 to 53.6 g kg^{-1}.

But, the magnesium content of the Akçaabat variety found by the FAAS and WDXRF methods (17.2 and 27.2 g kg^{-1}, respectively) was far above the standard values. Results for magnesium using the FAAS method for all of the varieties except for Akçaabat were similar with the standard values, but results for magnesium using the WDXRF method for Maden, Canik, Yayladag and Mus were found above the standard values.

The analytical results for iron and copper using both methods were agree with the standard values, but in the Izmir and Adiyaman varieties, the iron content was found above the CTA-OTL-1 standard and the same was observed for the copper content of the Maden and Agonya varieties. For the other varieties, the content of these elements were either similar to or below the CTA-OTL-1 standard. An important outcome must be emphasized here that, the copper content of Maden variety which cultivated near the copper smelting and other industrial plants was found higher than that of all varieties studied.

The aluminium contents of the Izmir, Mus and Adiyaman tobacco varieties were found above the values of Institute of Nuclear Chemistry and Technology (ICHTJ), but other samples were found in good agreement with CRMs and literature values.

Both methods used in this study were produced almost similar results for zinc and manganese elements in all studied varieties.

Potassium and calcium concentrations are always found higher in tobacco leaves. Toxic elements are of great importance in toxicological studies, as these elements are partly or completely volatilized in the smoke and are inhaled or absorbed through smoking[19]. When the content of heavy metals is higher or above the standards, the risk of damaging effect to human health can be significant especially to the respiratory and urinary systems.

The results of FAAS and WDXRF methods were compared in statistical terms in Fig. 1. In the statistical analysis of FAAS *vs.* WDXRF graphs, the correlation coefficients (r) for Fe, Mg, K, Al, Ca, Mn, Zn, Cu and Na were found very high as 97.29; 96.39; 94.73; 93.85; 93.28; 92.82; 92.28; 91.21 and 85.69 %, respectively. The regression analysis for each element shows that both analysis methods are valid and produce similar and accurate values for the investigated elements in the studied concentration range. It may be said that one method may be used instead of other without remarkable systematic errors in the elemental analysis of tobacco leaves.

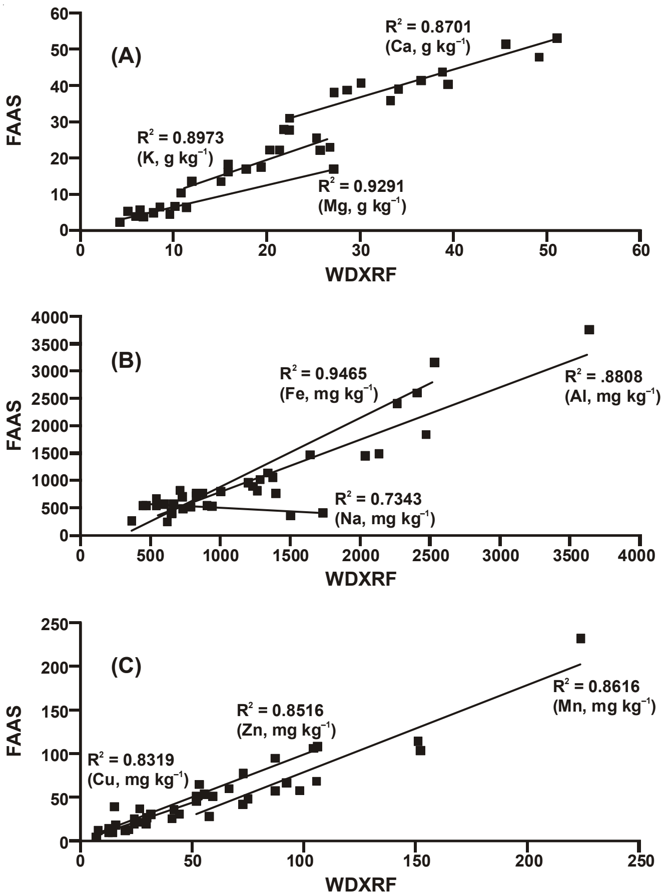

Fig. 1. Statistical evaluation of the results of FAAS and WDXRF method (a) K, Ca, Mg (b) Na, Fe, Al (c) Cu, Zn, Mn

In addition to elemental analysis, the average nicotine content of the investigated varieties were ranged from 0.69 to 3.83 %, while reducing sugar ranged from 0.90 to 26.80 % in the tobacco leaves (Table-1). While the nicotine content of the Burley, Akçaabat, Agonya, Canik and Virginia varieties were found the highest, Izmir, Bitlis and Mus varieties were found the lowest.

Similarly, the reducing sugar content of the Virginia and Bitlis varieties were found the highest, but Akçaabat and Burley varieties were found the lowest. The results for nicotine and reducing sugar of this study agree with the other similar studies in the literature for example Turkish Standards Institution-Turkish Tobacco Standards[26,27]. In addition to this, the obtained nicotine values in good agreement with the National Research Centre for Certified Reference Materials GBW 08514 (Oriental tobacco) and GBW 08515 (Virginia tobacco) values[28].

Conclusion

About 6 million tons of tobacco leaves and 5.5 million tons of cigarettes are produced in the world every year. Therefore, the elemental analysis of tobacco leaves is very important for the human health point of view and simplified techniques are still required for widespread application and for controlling the agricultural parameters for Good Agricultural Practices. The nicotine and reducing sugar analysis showed that the obtained values are in good agreement with the literature. According to the elemental content results, the FAAS and WDXRF methods produce almost similar values and no significant difference is observed between the concentration of elements. But, the WDXRF method is found most suitable and easily applicable to the analysis of tobacco leaves than that of more sensitive, but more difficult FAAS method. As a conclusion, it may be said that the WDXRF method used in this study may not be used instead of FAAS methods in any such analysis, but using this technique extremely simplifies the time consuming analysis and provides relatively fast information about the elemental content of tobacco leaves without sacrificing the accuracy.

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(*Received*: 11 September 2007; *Accepted*: 19 January 2008) AJC-6226