NEW DATA ON THE EVALUATION OF THE INFRARED (IR) SPECTRA OF SUBSTANCES OF COMPLICATED STRUCTURE AND THEIR APPLICATION FOR IDENTIFICATION WITH PRIMA PATTERN RECOGNITION METHOD. PART I

Investigation of the change in chemical composition of the tobacco plant during ripening

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Abstract

In our paper the change in chemical composition was studied on samples (leaf, tendril-flower, stem, complete plant) taken in a 2-week period (July 2—July 16) of the development of tobacco plant, grown by two different modes of cultivation (bed and ensilage) with classical analytical and infrared (IR) spectroscopic methods. IR spectra were evaluated by traditional spectroscopic method and by pattern recognition method PRIMA (Pattern Recognition by Independent Multicategory Analysis). The intensity of the IR bands was used for our investigations, which was given in relative % value, introduced by us. Data needed for mathematical processing were obtained by a new procedure, by feature selection resting on spectroscopical basis.

The dependence of cellulose and total nitrogen content, further the intensity of the IR spectral bands characteristic of these components, on the ripeness of the plant, on the quality of the plant part, on the mode of cultivation and on the place of the leaf on the plant were established. Various tobacco plant samples, having an identical IR spectrum on visual inspection, were separated and identified with the PRIMA pattern recognition method with a recognition power between 97 and 100% according to their origin (ripeness, mode of cultivation quality, place on the plant).

Introduction

In our paper chemical conversions during the development of the tobacco plant are studied by classical analytical and IR spectroscopic methods, and measuring results are processed by the pattern recognition process PRIMA (Pattern Recognition by Independent Multicategory Analysis).

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The basic principle of our process was described in our earlier papers [1, 2]. Its essence is summarized in the following.

The IR spectra of organic substances, containing many components of predominantly complicated structure, are for samples of identical type very similar to one another, show no visible differences, or if so, then only to a small measure. The reason of this is that the major part of these samples contain the same components, only in different quantitative ratios, depending on the various parameters.

The situation is the same in the case of the IR spectra of tobacco plant parts investigated by us. The quantitative composition of the different plant parts (leaf, tendril-flower, stem and average sample of the whole plant) is influenced besides by the quality of the plant also by its grade of ripeness, mode of cultivation, in the case of leaves by their position on the plant, etc., but in spite of this the IR spectra of parts of plants of different type and different degree of ripeness are practically the same, if only the number and place of absorption bands is investigated, because identical components (carbohydrates, proteins, etc.) absorbe at identical places. However, if the intensity behaviour of the IR spectral bands is also investigated, then considerably more data are obtained, on the basis of which samples of various types can be already separated.

It causes a problem that intensity data are subject to many errors (weight of sample, inhomogeneity, particle size, etc.), moreover, that the handling of many data is a complicated and lengthy operation.

Therefore, a new and simple process was developed (see later), by which substances of the said type can be separated, and using the PRIMA pattern recognition method, can be identified on the basis of their IR spectra.

In our process, the samples to be investigated are arrayed on the basis of their different properties—in the present case, the quality, ripeness, mode of cultivation and position on the plant of the parts—into classes (teaching program). The properties defining the class are difficult to measure (e.g. protein, cellulose, etc. content), therefore, classing is carried out on the basis of easily measurable properties, e.g. the intensity of their selected IR spectral bands. This is justified, because the properties defining the class (e.g. cellulose content depending on ripeness, etc.), are correlated with the IR spectrum, so that classification (identification) on the basis of properties difficult to measure, can be solved indirectly, with the aid of properties, easy to measure. This method is already known, was used since a long time by several authors [3—7]. Thus, the novelty of our work is not the application, but on one part, the selection of the substances studied (tobacco plant parts, not investigated so far by this method), and on the other part, our method developed for giving the intensity of the IR spectral bands. Moreover, it can be considered as novelty that the feature selection of data obtainable

from the IR spectrum is not done mathematically, but on the basis of their spectral knowledge. Places to be taken into consideration are selected so that the absorbability of the various substances in the set of samples shall differ at these places. It can be proved by data in the literature that the results of pattern recognition are improved, if the places of investigation are not selected equidistantly (e.g. each 5 or 10 cm^{-1}) by a computer, but by an expert with spectroscopic practice. Thereby, the number of places is considerably decreased, and with it computer time, efficiency increases and the solving of tasks becomes possible, which are already insoluble for practical spectroscopists working with traditional methods.

A brief survey of literature dealing with the chemical composition and analysis of tobacco

For the qualitative evaluation of tobacco it is important to know the chemical composition, which is developed as a result of the metabolic processess of the plant, and depends on the variety of tobacco, on its circumstances of cultivation, its stage of ripeness and many other factors. Biochemical processes of nutrient uptake and metabolization continuously change in the single sections of the day and over longer periods, and the composition of the different plant parts changes accordingly [8—16]. The number of identified compounds, composing tobacco leaves, surpassed already in 1975 1500 [17]. In addition to the principal components (carbohydrates, proteins), there are many constituents occurring in small quantities, playing an important role in the forming of the organoleptic properties of tobacco.

Inorganic compounds are present in tobacco partly in the form of dissolved salts (nitrates, sulfates, phosphates, chlorides), and partly incorporated in organic substances (proteins, organic acids, chlorophyll, enzymes). The main elements are potassium, sodium, calcium, magnesium and iron; trace elements are copper, manganese, boron, molybdenum, cobalt, zinc, nickel [11].

The groups of compounds of *organic constituents* are the following: carbohydrates, compounds containing nitrogen, organic acids, polyphenols, volatile oils, resins, waxes dyestuffs, enzymes.

Carbohydrates are partly simple sugars, partly polysaccharides (starch, dextrins, cellulose). The total carbohydrate content of tobacco leaf is about 40%.

One part of *organic acids* participates in the regulation of the pH of cells, while their other part forms salts with cations or organic bases. Organic acids with carbon atom numbers C_1—C_{10} shape the organoleptic properties of smoke [18].

Compounds containing nitrogen are present in the form of proteins, alkaloids, amides, amines. The quantity of proteins depends on the variety, ripeness and cultivation conditions of tobacco [15]. There are skeletal proteins, further hormone, enzyme and plasma proteins. The major part of the alkaloids of tobacco is 3-pyridyl derivative (nicotine, nornicotine, anabasine), and amounts to 1—2% of the dry substance [16, 19, 20].

Polyphenols occur mainly as glycosides, linked to sugar, in a quantity of 2—5% [14].

Volatile oils, *waxes*, *resins* are secreted by the glandular hair of the plant, they do not participate in the metabolism of the plant, they protect the plant against the injurious effect of weather. Their quantity depends on cultivation, weather conditions and mechanical injuries, and may vary between 3 and 16% [11].

The more important *dyestuffs* are chlorophyll A and B, carotene, xanthophyll, rutin and anthocyan, occurring in a quantity between 0.5 and 4% [10, 11].

Enzymes contained in the tobacco plant can be divided into two main groups. Hydrolases catalyze conversions in conjunction with uptake or release of water, desmalases redox processes.

Without aiming at completeness, as termination of literature dealing with chemical composition, we briefly survey works *dealing with the changes of chemical composition occurring during the ripening of tobacco, and with their determination.*

The technological ripeness of tobacco leaf is still determined today mostly subjectively, though more and more research is done on the development of an objective measuring technique, based e.g. on the measurement of coloring matters or other chemical constituents (e.g. NIR spectroscopy).

— *Changes of carbohydrates:* the quantity of simple carbohydrates is highest at the time of blooming, while the content in composite carbohydrates decreases in this period, then afterwards it increases up to the yellowing of the leaves [12].

— *Changes of compounds containing nitrogen:* protein nitrogen content is low in the seedling period, but then it begins to increase up to the blooming period, then it decreases, and afterwards it increases again, up to turning yellow. Nicotine nitrogen content reveals gradual steady increase [12].

— *Changes of pectins and coloring matters:* the disintegration of pectins begins after the full development of the leaves, therefore it is important to choose well the time of gathering in, because too much pectin causes hygroscopicity and spoilage, while insufficient pectin bad water absorption capacity and breakability [11].

In leaves near to ripeness chlorophyll A begins to be enzymatically decomposed, the leaf becomes lighter. The yellow colour of the ripe leaf is

caused by carotenes. The changes of green and yellow coloring matters during ripening was investigated by several authors [21—23].

The brown coloring matters on dried and fermented tobacco amount to 6.5%, and are composite compounds, consisting of water-soluble polyphenols of high molecular mass and of proteins [21].

Finally, we mention some works, dealing with *spectroscopic methods, suitable for the analysis of the composition of natural organic substances*, among others, of parts of tobacco plant.

The authors of Ref. 24 assembled a survey containing 260 references on the application of IR and Raman spectroscopy for the investigation of biological systems.

Near infrared reflection (NIR) spectroscopy is the most modern, most simple method of investigation for the quantitative analysis of multicomponent systems, thus of natural organic substances, without previous separation. Wetzel [25] gives a survey on the principle of the method and its possible applications.

For the determination of the moisture, sugar, nicotine and cellulose content of tobacco a NIR spectroscopic process was developed by Long [26, 27].

The reducing sugar content of dried tobacco [28], the alkaloide content of sauced tobacco [29], the quantity of polyphenols [30], further the total nitrogen content [31] were measured by the authors cited with spectrophotometric, Fourier transform and NIR spectrocopic methods, and results obtained were compared [32].

Samples investigated and experimental methods

— *Samples*

Two varieties of tobacco (of Heves and coarse tobacco) were investigated. Coarse tobacco was grown by two ways of cultivation (ensilaged or twin-row and bed cultivation [12]). In both cases of cultivation samples were obtained from different parts of the plant (leaf, tendril-flower, stalk, total plant). In the case of coarse tobacco, the whole 2-week period (July 2—July 16) was investigated. In the case of tobacco from Heves, leaves were taken from different heights of the tobacco plant.

Characteristic data of our samples investigated are contained in Table 1.

— *Classical analytical tests*

— *Preparation of samples*

Analyses were carried out on samples pulverized by grinding and dried to constant weight.

— *Determination of cellulose content by the method of Kirschner and Hoffer*

Table 1

Characteristic data of the tobacco plant samples investigate

Type of tobacco	Ser. No.	Mode of cultivation	Time of sampling 1987	Part of the plant	Number of leaf on the plant
1	2	3	4	5	6
coarse	1	ensilaged I	July 2	total	—
coarse	2	ensilaged I	July 2	leaf	—
coarse	3	ensilaged I	July 2	tendril-flower	—
coarse	4	ensilaged I	July 2	stem	—
coarse*	5	ensilaged I	July 2	total	—
coarse	6	ensilaged II	July 9	total	—
coarse	7	ensilaged II	July 9	leaf	—
coarse	8	ensilaged II	July 9	tendril-flower	—
coarse	9	ensilaged II	July 9	stem	—
coarse	10	ensilaged III	July 16	total	—
coarse	11	ensilaged III	July 16	leaf	—
coarse	12	ensilaged III	July 16	tendril-flower	—
coarse	13	ensilaged III	July 16	stem	—
coarse	14	bed I	July 2	total	—
coarse	15	bed I	July 2	leaf	—
coarse	16	bed I	July 2	tendril-flower	—
coarse	17	bed I	July 2	stem	—
coarse*	18	bed I	July 2	total	—
coarse	19	bed II	July 9	total	—
coarse	20	bed II	July 9	leaf	—
coarse	21	bed II	July 9	tendril-flower	—
coarse	22	bed II	July 9	stem	—
coarse	23	bed III	July 16	total	—
coarse	24	bed III	July 16	leaf	—
coarse	25	bed III	July 16	tendril-flower	—
coarse	26	bed III	July 16	stem	—
Heves	27	—	—	leaf	1
Heves	28	—	—	leaf	2
Heves	29	—	—	leaf	3
Heves	30	—	—	leaf	5
Heves	31	—	—	leaf	7

Note: Samples 5 and 18 marked with x are identical with samples 1 and 14, respectively. In the evaluation they were taken into consideration as parallel samples.

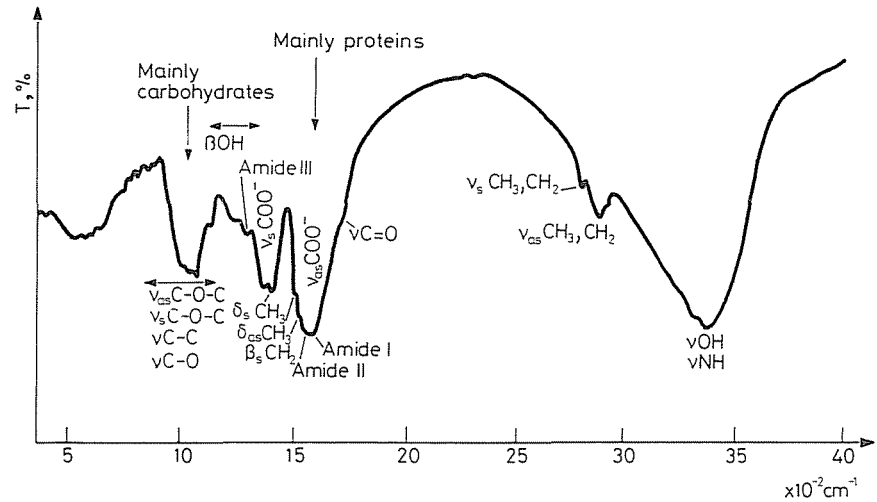

Fig. 1. IR spectrum of a tobacco leaf sample and its interpretation

The principle of the method is that constituents other than cellulose can be solubilized by cooking the tobacco plant part in a mixture of alcohol and nitric acid. The mass of residual cellulose is weighed [13].

— *Determination of total nitrogen with Kjeldahl's method*

The total nitrogen content of tobacco plant was measured after decomposition with concentrated sulfuric acid. Ammonia, set free with sodium hydroxide from ammonium sulfate formed, was distilled into 0.1 n hydrochloric acid, and excess acid was titrated back [13].

— *IR spectroscopic analysis*

From the pulverized tobacco plant samples, dried to constant weigh, 1—1.5 mg was homogenized with 300 mg of potassium bromide by triturating in an agate mortar for 5 minutes, then compressed to pills at a pressure of 2.10^4 KPa. Three parallel measurements were made for each sample. Spectra were taken with a Zeiss Specord 75 spectroscope.

Measuring results and their evaluation

— *Investigation of the IR spectra*

The IR spectrum of a tobacco leaf sample is shown in Fig. 1. The spectrum of the substance, containing many and predominantly high-molecular organic components, seems simple, the absorption bands of the principal components—carbohydrates and proteins—dominate in them. These bands were formed by the summation of the absorption of all the components, but at certain positions the absorption of certain types of components dominates.

The assignment of the bands is shown in Fig. 1 and Table 2, using the symbol system of Sohár—Holly—Varsányi [33], where *ν*: stretching vibration,

Table 2

Characteristic absorption positions of the IR spectra of the tobacco plant samples investigated, their assignement and bands selected for computerized evaluation

Interpretation (Mode of vibration)	Bands selected for evaluation	Position of the band (cm^{-1})	Remark
νNH, νOH	1	3400	broad, intensive
ν_{as} CH_3, ν_{as} CH_2	2	2980	weak bands, difficult to separate
ν_s CH_3, ν_s CH_2		2870	
νC=O	3	1710	shoulder indicative of the presence of aldehydes, ketones, characteristic of simple sugars, formed in the decomposition of polysaccharides
νC=O+νC—N	4	1600	amide I band, characteristic of the protein part
βNH+νC—N	5	1550	amide II band, characteristic of proteins
β_s CH_2	6	1470	diffuse band system of medium intensity, the single modes of vibration cannot be separated, it arises mainly from hydrocarbon groups in the different components and the OH deformation vibrations of carbohydrates
ϑ_{as} CH_3	7	1430	
βOH	8	1400	
ϑ_s CH_3	9	1375	
νC—N+βNH	10	1300	amide III band, weak, characteristic of proteins
ν_{as}C—O—C	11	1240	intensive merged band system, the band of different vibrations cannot be separated, this region is characteristic of the the carbo hydrate part
νC—C	12	1130	
νC—O	13	1080	
ν_sC—O—C	14	1020	
βO=C—N, γNH, γC=O		720—600	amide IV, amide V, amide VI merged bands of low intensity

ϑ: bending vibration, β: in plane, γ: out of plane deformation vibration, *s*, *as*: symmetric, asymmetric vibration, amide I, II, III: marking band series arising from the amide group. Vertical arrows mark those two absorption regions, in which mainly proteins or mainly carbohydrates absorb. It can be seen from the figure, where the more important groups, bonds, of the plant components appear with bands in the spectrum.

For the comparison of classical qualitative analysis data and data calculable from the IR spectra, further for evaluation with the PRIMA pattern recognition method, the intensity of the bands at the absorption positions of

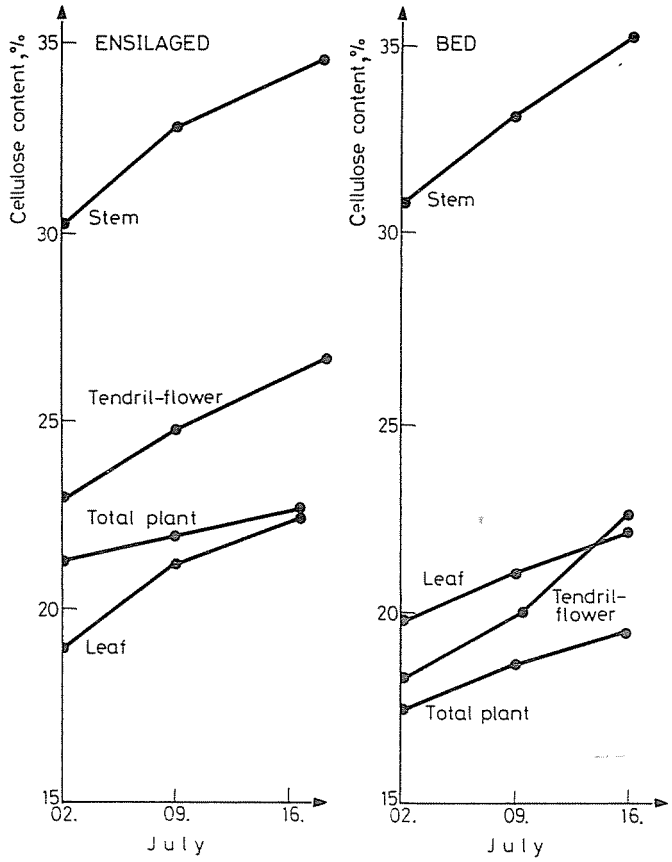

Fig. 2. Change in cellulose content of tobacco plant samples cultivated in different modes

the different groups was also determined. Absorbances were calculated with the base line correction method. Intensity data were given in two ways:

1. absorbance/weight of sample [mg]
2. absorbance [relative %]

This latter mode of giving the results is also new [34], in so far as it does not calculate with absorbance falling to unit of sample, but gives the results in relative %, by determining the absorbance values (A_i) at an arbitrarily selected number (n) of characteristic absorption positions, summarizes these $\left(\sum_{i=1}^{n} A_i\right)$, and gives as percent of the sum the measure of absorption at the single positions: $A_i[\text{relative } \%] = \dfrac{A_i}{\sum_{i=1}^{n} A_i} \cdot 100$. Data obtained in this way

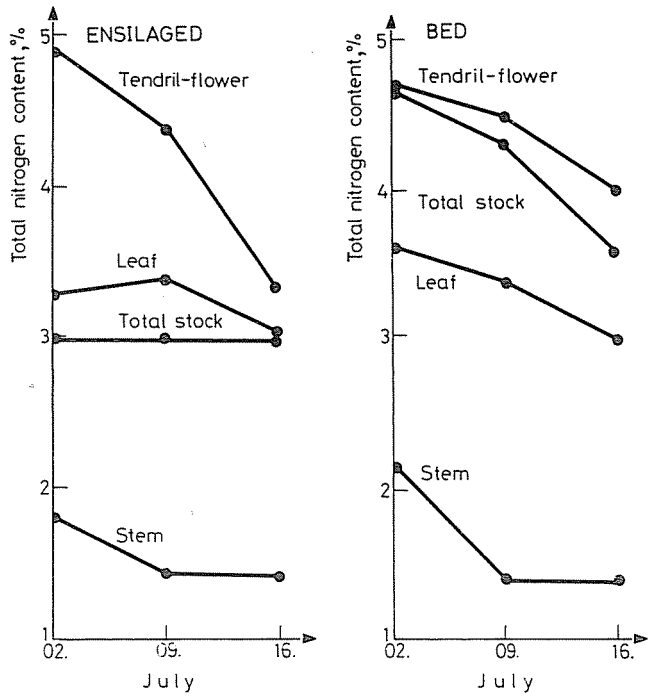

Fig. 3. Change in total nitrogen content of tobacco plant samples cultivated in different modes

characterize well in the case of a sample set the quantity of functional groups absorbing at the selected positions in relation to one another.

— *Evaluation of classical quantitative analysis data*

— *Dependence of cellulose content on the ripeness of the plant, on the quality of the plant part, and on the mode of cultivation*

Figure 2 shows the changes of the cellulose content of the whole plant, of leaf, tendril-flower and stem samples, obtained by ensilaged and bed cultivation, as a function of growing time for a selected 2-week period of ripening. The figure shows that the cellulose content of each sample increased with time, there is a difference only with respect to the magnitude of cellulose concentrations and to the rate of increase, depending on the quality of the plant part and on the mode of cultivation. The cellulose content of the parts of the plant (with the exception of the stem), grown by ensilaged cultivation, is higher than that of the samples of bed cultivation.

— *Total nitrogen content as a function of the ripeness of the plant, of the quality of the plant part and of the mode of cultivation*

Changes of total nitrogen content, as a function of the parameters above are shown in Fig. 3. Nitrogen content decreased during the two weeks investigated with one exception (ensilaged, complete plant), and for two samples

(ensilaged, stem, bed stem) data of samples taken at the end of the first and second week are identical, i.e. there was no change in the second week. In the case of samples obtained by bed cultivation, nitrogen contents are higher for each plant part, than in ensilaged cultivation. This trend is opposite to the behaviour of cellulose content. The only exception was found only for stem samples, where both the cellulose contents and the nitrogen contents are the same in the two modes of cultivation.

— *Comparison of classical analytical and IR spectral evaluation data*

Chemical changes in composition during the ripening of the tobacco plant were studied by analysing the samples, taken in the selected 2-week interval of ripening, with classical analytical method described above, then the IR spectra of the same samples were also taken. Our investigations were aimed to clear, whether the time-, labour- and chemical-intensive classical methods of analysis can be replaced by IR spectroscopic measurements. Therefore, the behaviour of the cellulose content of the samples was compared with the change of intensity of such IR spectral bands, which arise mainly from cellulose components, then we investigated, whether the tendency of the change in ratio of cellulose and total nitrogen concentrations is similar to the change of the intensity ratio of two such bands of the IR spectrum, which arise from the cellulose and protein components.

As may be seen from Figs 4—7, the change of intensity data, calculated from the IR spectra—of which the absorbance of the band at 1080 cm^{-1} was given in relative %, that of the band at 1025 cm^{-1} in values falling to unit of sample—follow in each case for all the tobacco plant parts the tendency of the change of cellulose concentration. Evidently, slopes are not identical, but neither are the data characteristic of cellulose content, however, it can be seen unequivocally from the figures that if we wish to study as a function of time chemical conversions occurring during the ripening of a plant, classical analysis can be replaced by simpler IR spectroscopic analysis, by measuring the change in intensity of the IR spectral band, characteristic of the component to be investigated. These data follow within a set of samples the change in quantity of the given component. If not only the tendency of changes, but the absolute value of concentration is important, then we can prepare a calibration from a few "teaching" samples of known concentration.

In Figs 8—11, the changes of cellulose and total nitrogen concentration ratios, characteristic of cellulose/protein ratio, and the change in intensity ratio ($A_{1080\ cm^{-1}}/A_{1600\ cm^{-1}}$) of the band at 1080 cm^{-1}, characteristic of cellulose, and of the band at 1600 cm^{-1}, characteristic of proteins, can be studied as a function of time. Our experiences are the same as above, and show that IR transmission spectra can be used both in the investigation of the development of the plant and in tobacco technological research, as they yield rapidly and simply data, suitable to follow also numerically in the whole

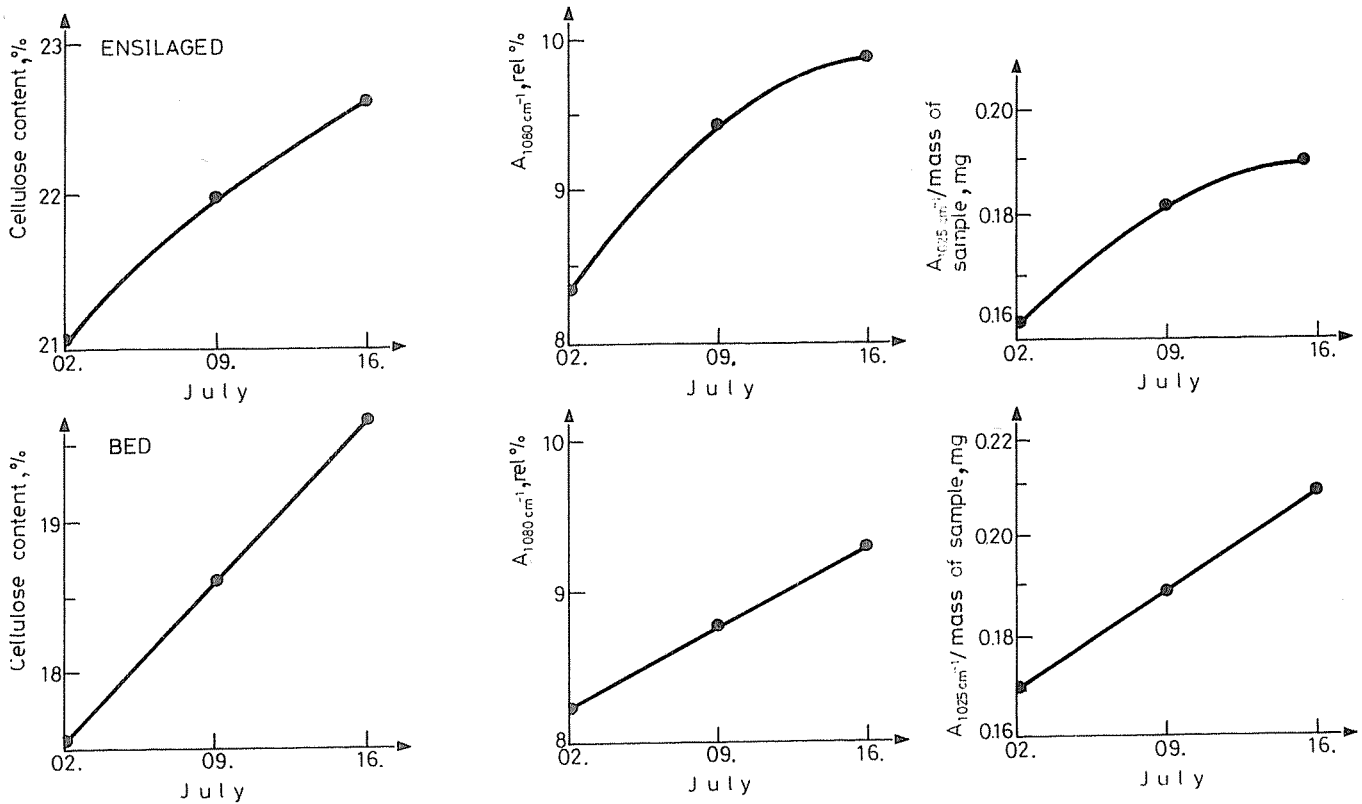

Fig. 4. Change in cellulose content of the total tobacco stock

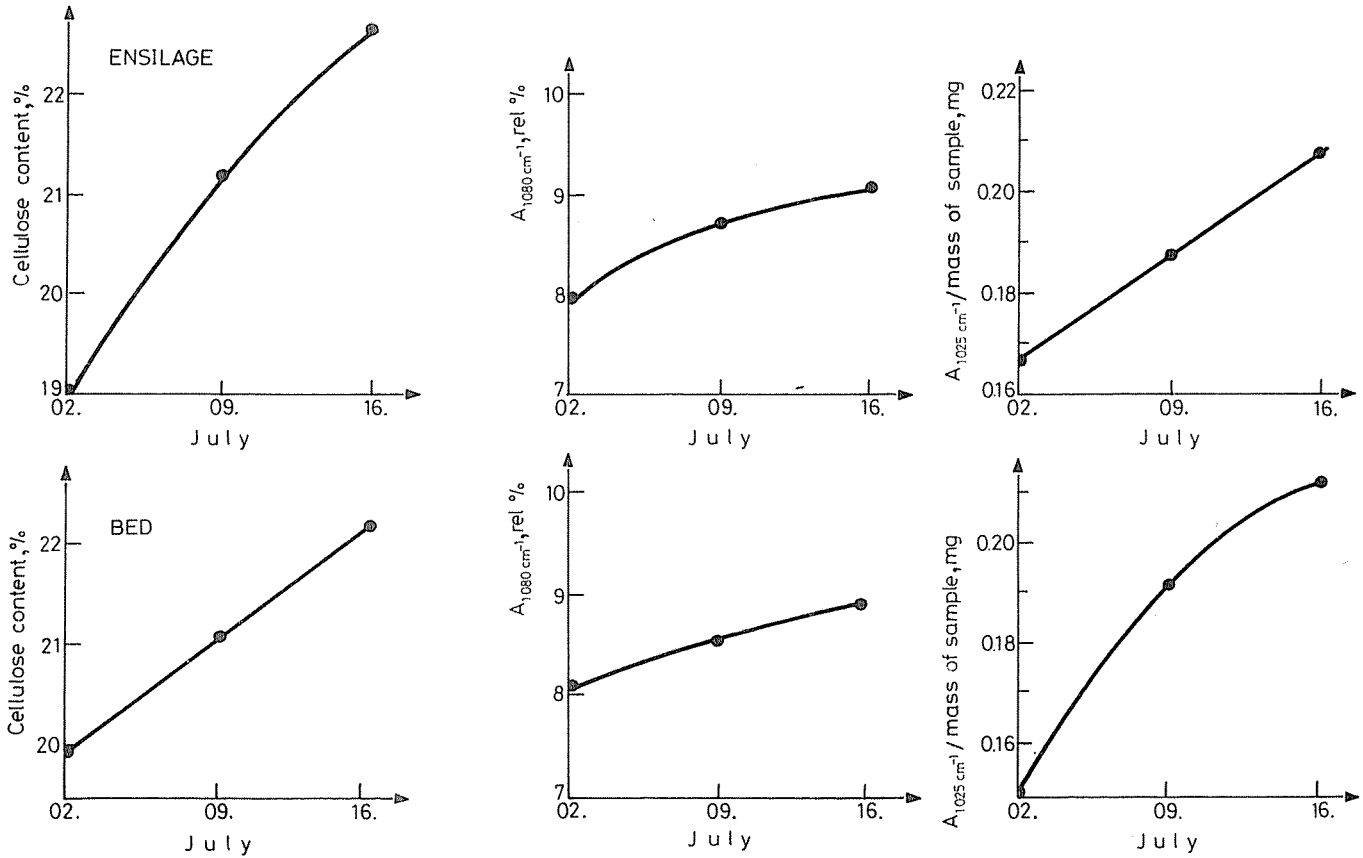

Fig. 5. Behaviour of data characteristic of the cellulose content of tobacco leaves

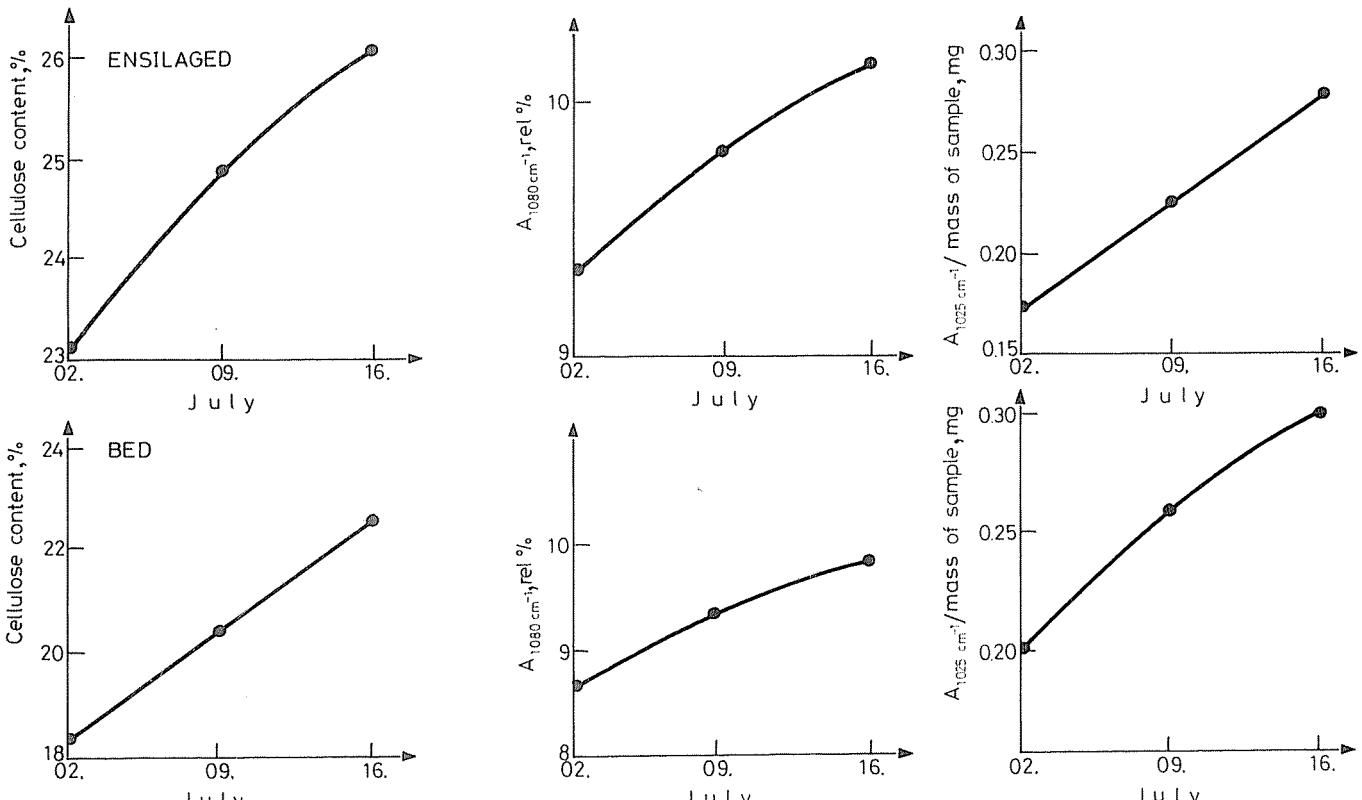

Fig. 6. Behaviour of data characteristic of the cellulose content of tobacco tendril-flower

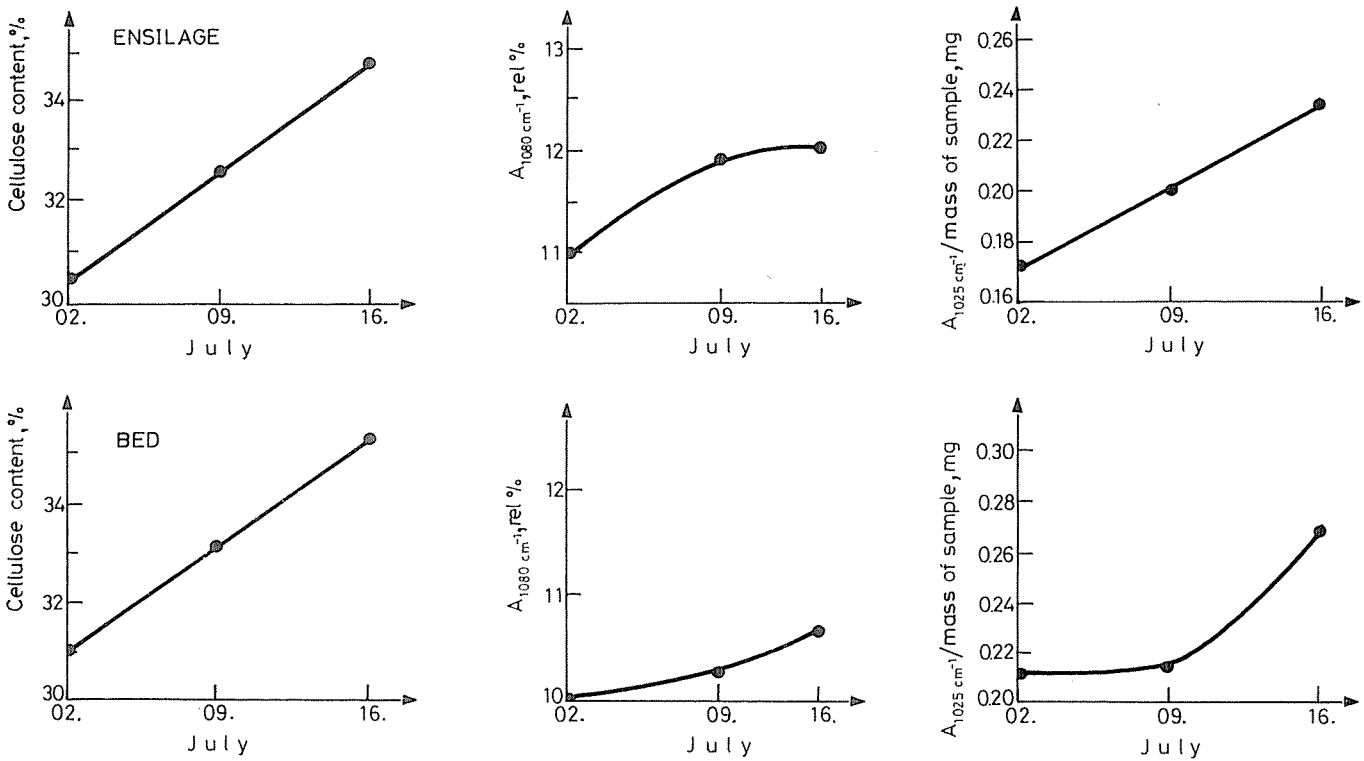

Fig. 7. Behaviour of data characteristic of the cellulose content of the stem

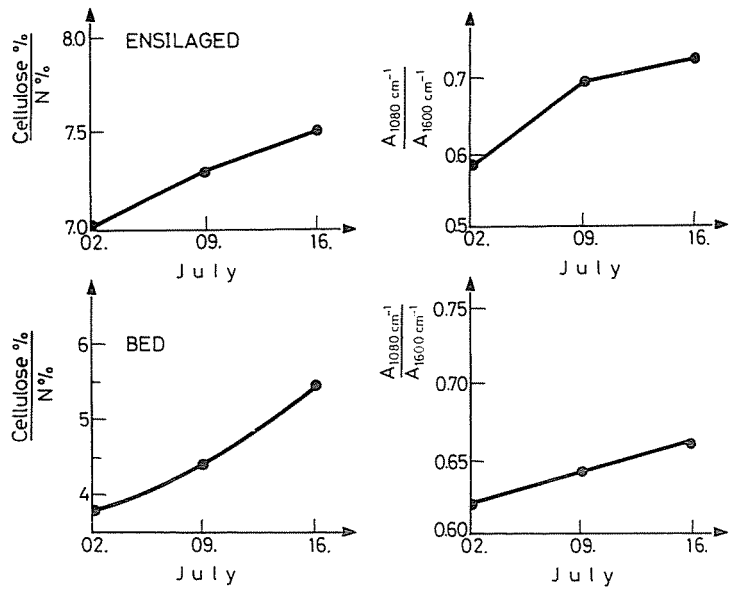

Fig. 8. Behaviour of data characteristic of the cellulose—protein ratio of the whole tobacco stock during ripening

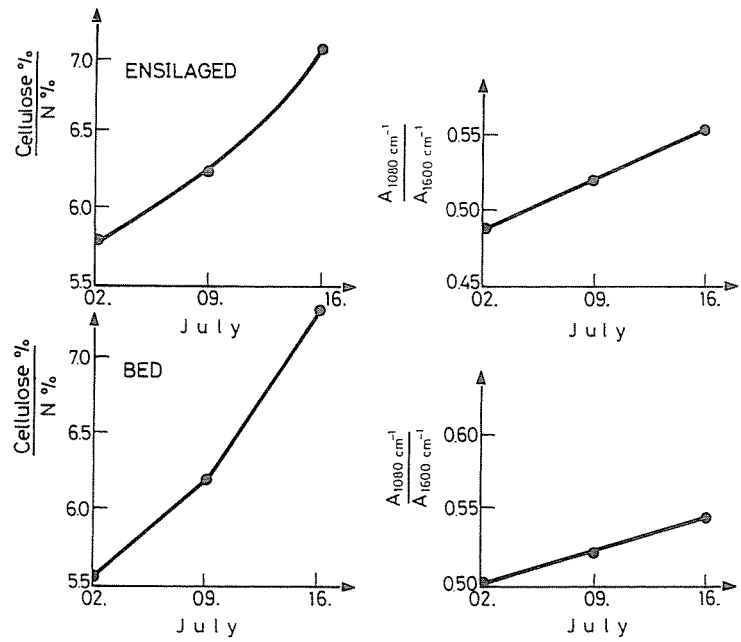

Fig. 9. Behaviour of data characteristic of the cellulose—protein ratio of the tobacco leaves during ripening

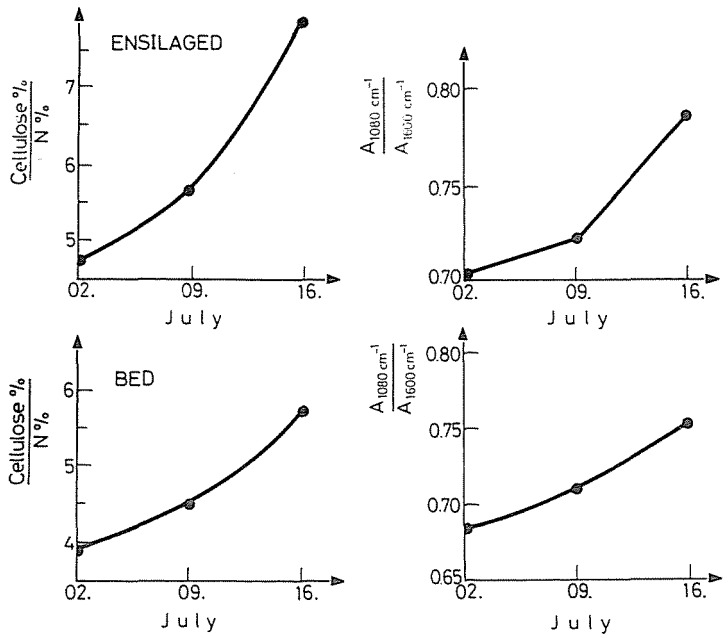

Fig. 10. Behaviour of data characteristic of the cellulose—protein ratio of tendril-flower during ripening

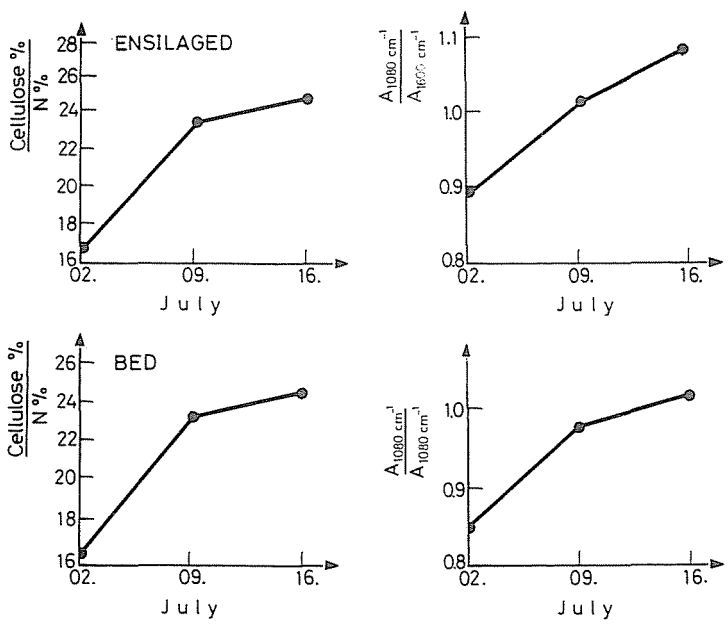

Fig. 11. Behaviour of data characteristic of the cellulose—protein ratio of tobacco stalk during ripening

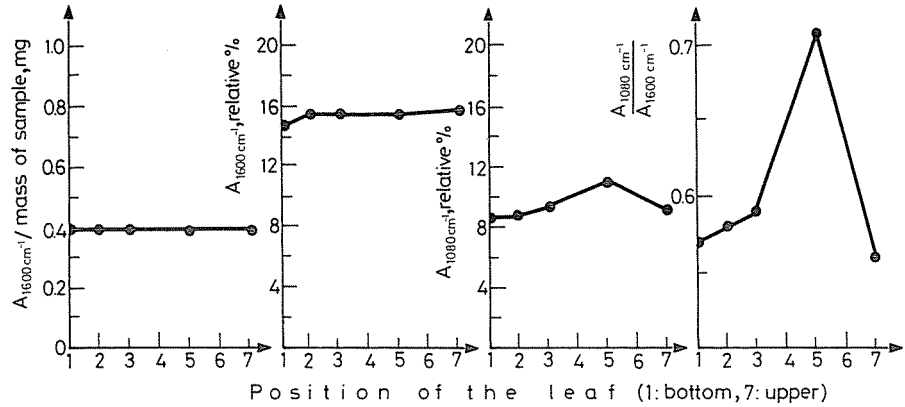

Fig. 12. Changes of some IR spectral data of the leaves of tobacco from Heves according to the position of the leaf on the plant

development period of the plant or during tobacco processing changes in chemical composition, caused by different parameters.

— *Change of the IR spectral characteristics of tobacco leaf, depending on the place occupied on the plant*

Of the tobacco leaves of Heves a sample series of 5 members was investigated, the leaves were positioned (upwards from below) in places 1, 2, 3, 5 and 7. We investigated of the IR spectral data the dependence of the intensity of the band at 1600 cm^{-1}—attributable mainly to protein components—on the position occupied on the plant (Fig. 12). Our measurements showed that the intensity of this band does not change virtually.

However, the measure of absorption at 1080 cm^{-1}—arising mainly from carbohydrates—increased up to the 5th leaf, to return then at the 7th leaf to the value measured for the 1st leaf. Thus, the change of the quotient ($A_{1080\,cm^{-1}}/A_{1600\,cm^{-1}}$), characteristic of the carbohydrate-protein ratio, reveals also at the 5th leaf a maximum.

— *Application of the PRIMA pattern recognition method for the evaluation of the IR spectra of tobacco plant parts*

After the description of our results, obtained by classical analysis and traditional spectroscopy, we report on the computer-aided evaluation of the IR spectra of tobacco plant parts listed in Table 1.

We outlined in the introduction aim and method of our investigations. In this chapter we give also in detail our working method and results.

The IR spectra of 31 samples, prepared in 3 parallel measurements were available for our investigations. These spectra did not show visible differences because of the reasons already mentioned.

We assumed that if we apply the PRIMA pattern recognition method, this will separate the samples of various origin on the basis of their IR spectral data. For the computerized evaluation the intensity of the bands was determined at 14 positions, given in Table 2. Their values were expressed in "absorbance, relative %", introduced by us (see investigation of IR spectra). In addition, 3 absorbance quotients were determined, which are characteristic of carbohydrate—protein ratio, and of the shape of the structured bands of carbohydrates and hydrocarbons. They were the following:

$$A_{1080\ cm^{-1}}/A_{1600\ cm^{-1}};\quad A_{1080\ cm^{-1}}/A_{1020\ cm^{-1}};$$

$$A_{1375\ cm^{-1}}/A_{1400\ cm^{-1}}.$$

The 17 data given above characterized each of a sample. Then we investigated according to the kind of the plant parts, ripeness, mode of cultivation and the position occupied on the plant, whether the pattern recognition method separates the samples.

First, 5 classes were formed according to the kind of the parts, and considering the available leaf, tenderil-flower, stem, stalk and whole plant samples, taken at the same point of time, as "teaching" samples, the "teaching" program was developed. Next followed recognition, where all the samples were considered as unknown, and the "recognition" program separated them with a certainty of 97—100%, according to whether they were leaves, tendril-flowers, etc.

The procedure was the same, when we formed according to the ripeness of the different plant parts 3 classes (in accordance with the point of time of sampling). Here, recognition power proved to be 100%.

Similarly, the program recognized with a certainty of 100%, whether the plant part derived from a plant, cultivated by ensilaged or bed mode of cultivation.

Finally, 5 classes were formed according to the place occupied on the plant (places 1, 2, 3, 5 and 7), and for this the "teaching" program was developed. Considering the leaf samples as unknown, the recognition program decided with a certainty of 100%, which place was occupied by which leaf on the plant.

Summarizing our experiences, it can be established that tobacco plant parts, apparently having completely identical IR spectra, are separated by the pattern recognition method PRIMA according to their origin. On the basis of knowledge yielded by this experimental series, we can recommend the application of the method above to research workers, who study the change in composition of natural subtances of complicated composition, caused by different parameters.

References

1. ERŐSS-KISS, K., VERESS, G., SZAKÁLAS, GY.: *Magy. Kém. Folyóirat* 94/5, 215 (1988)
2. ERŐSS-KISS, K., VERESS, G., SZAKÁLAS, GY.: *Periodica Polytechnica* 34/4, 273 (1990)
3. VERESS, G.: *Magy. Kém. Folyóirat* 92, 556 (1986)
4. JURICSKAY, J., VERESS, G.: *Anal. Chim. Acta* 171, 61 (1985)
5. JURICSKAY, J., VERESS, G.: *Magy. Kém. Folyóirat* 92, 255 (1986)
6. ERŐSS-KISS, K., MESZLÉNYI, G., VALTINYI, D., MEISEL, T.: *Periodica Polytechnica* 30, 185 (1986)
7. ERŐSS-KISS, K., SZAKÁLAS, GY., VERESS, G., VALTINYI, D.: *Acta Physica* 63/1—2, 119 (1988)
8. ARANY, S.: *Dohányipar* 3, 95 (1973)
9. LÁSZTITY, R., TÖRLEY, D.: Alkalmazott élelmiszeranalitika II. (Applied food analysis II.) Mezőgazdasági Könyvkiadó, Budapest, 1987.
10. FRANKENBURG, W. G.: *Southern Chemist*, March, 315 (1949)
11. GYŐRVÁRI, B.: Dohányipari Technológia (Technology of Tobacco Industry). Kertészeti Egyetem, 1981.
12. MÁNDY, GY.: *Dohánybiológia* (Tobacco biology), Kertészeti Egyetem, 1953.
13. RÉDEI, E.: Dohányipari vizsgálatok (Investigation of the tobacco industry), Kertészeti Egyetem, 1987.
14. STEDMAN, R. L.: *Chem. Rev.* 68, 153 (1968)
15. TSO, T. C.: Physiology and Biochemistry of Tobacco Plants, Dowen, Hutchinson and Ross Inc., Stroudsburg, Pa. 1972.
16. TSO, T. C., JEFFREY, R. N.: Arc. Biochem. Biophys. 80, 46 (1959)
17. TSO, T. C., GORI, G. B.: Beitr. Tabakforsch. 8/4, 167 (1975)
18. KABURAKI, Y., SATO, X.: J. Agr. Chem. Soc. Japan 36, 865 (1962)
19. BUSH, L. P.: *J. Chromatogr.* 73/1, 243 (1972)
20. EGRI, L.: Egyes dohánytípusok alkaloidjainak vizsgálata különös tekintettel a nornikotin-kialakulás mechanizmusára (Investigation of the alkoloids of some tobacco types, with particular view to the mechanism of nornicotine formation). Akadémiai Kiadó, Budapest, 1956.
21. JACOBSON, J. S.: Arch. Biochem. and Biophys. 93, 580 (1961)
22. WHITFIELD, D. M., ROWAN, R. S.: Phytochem. 13, 77 (1974)
23. WEYBREW, J. A.: Tobacco Science 1, 1 (1957)
24. GEORGE-KYOGOKU-YOSHIMASA: Pract. Spectrosc. 1, 717 (1977)
25. WETZEL, D. L.: *Anal. Chem. Acta* 55/12, 1165 (1983)
26. LONG, T. M.: *Anal. Proc.* 20/1, 35 (1983)
27. LONG, T. M.: *Anal. Proc.* 20/1, 69 (1983)
28. MC CLURE—NORRIS—WEEKS: Beitr. Tabakforsch. Int. 9/1, 13 (1977)
29. HAMID—MC CLURE—WEEKS: Beitr. Tabakforsch. Int. 9/5, 267 (1978)
30. MC CLURE—WILLIAMSON: Beitr. Tabakforsch. Int. 11, 219 (1982)
31. WILLIAMSON—MC. CLURE: Beitr. Tabakforsch, 19, 55 (1986)
32. MC CLURE—HAMID—GIESBRECHT—WEEKS: *Appl. Spectr.* 38/3, 322 (1984)
33. SOHÁR, P., HOLLY, S., VARSÁNYI, GY.: *Kémiai Közlemények* 31, 197 (1969)
34. ERŐSS-KISS, K.: Kutatási jelentés a beton-adalékszerek vizsgálatáról (Research report on the investigation of concrete additives), 1986. Department of General and Analytical Chemistry of the Technical University, H-1521 Budapest

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