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PRODUCTION OF PRIMARY BIOMOLECULES IN DIFFERENT VARIETIES OF ORIENTAL TOBACCO (*NICOTIANA TABACUM* L.)

SUMMARY

During 2011 and 2012, on the experienced field of Scientific Tobacco Institute in Prilep experimental plots were set with oriental tobacco type Prilep (variety P-66), type Jaka (variety Jk-125/3) and type Basma (variety Basma-82).

Nicotiana tabacum L. which is the subject of biochemical - technological analysis is an important crop spread widely in the world. The research results indicate negative correlation of the fermentation processes and the proportion of carbohydrates, which contributes the decrease of tobacco quality. Increased hydrolysis due to prolonged fermentation causes significant reduction in the catalase activity. Nevertheless, the concentration of H₂O₂ converted into non-toxic products (H₂O and oxygen) is depended upon the duration and the conditions of the fermentation processes. Proteins and nitrogen in fermented material increased over prolonged fermentation. This study confirms that prolonged period of fermentation of tobacco raw reduces the quality of oriental tobacco.

Keywords: oriental tobacco, primary biomolecules, varieties.

INTRODUCTION

In the last decade most of the contemporary researchers has taken the tobacco (*Nicotiana tabacum* L.) as a central object because of its agro-economic meaning and its significance in phytochemical industry (Miceski and Petreski, 2006). It is also used as a model to study the effect of plant viruses, the physiology of mineral nutrition, genetic research, the negative effect of various types of pollution as well as research on alkaloid metabolism (Hoek et al., 2012). The tobacco as a culture provides opportunities for numerous genetic modifications including interesting attempts of a group of Russian scientists to clone species resistant to the impact of heavy metals (Kolodiazhnaya, 2007).

When it comes to the parameters which describe the quality of the tobacco plants they are not strictly defined, though often in this context, considering the concentration of sugars (especially soluble carbohydrates), proteins, organic

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acids, phenol and nicotine components (Drachev and Nikolova, 2005). The largest concentration of nicotine is contained in the organs with the highest vitality (leaves), where it is in the form of salts of citric and apple acid.

For these reasons, we approach to research the oxidative status of raw tobacco, on the one hand and parameters accurately define the quality of the raw material, on the other hand.

MATERIAL AND METHODS

As an object of analysis in this research are varieties of most exploited types of tobacco in the Republic of Macedonia: type Prilep variety P-66, type Jaka variety JK-125/3 and type Basma.

The leaves of different vintages and belts in tobacco mature simultaneously, but gradually, picking starts from the lowest leaves when they have reached their so-called “technological maturity”, where they have the most favourable terms of the components of the chemical composition, the greatest dry matter content and most favourable physical properties. For each variety of tobacco were performed a total of eight harvests or insertions, which were sorted in three groups or bands: lower, middle and upper belt. The extraction of the essential oil of tobacco raw material is performed in the laboratory by hydrodistillation method using Clevenger apparatus. The amount of the separated essential oil was determined by subtracting the volume of the extracted oil in the granulated part of the apparatus for hydrodestillation from the total volume of xylol and oil, reduced on dry matter.

As material for analysis, was taken fermented raw material from two harvests of tobacco crops in the year of 2011 and 2012 including three insertions: lower, middle and upper.

In the experiment are made the following analyzes: quantitative determination of the content of total and soluble carbohydrates (Dubois *et al.*, 1962), quantitative analysis of total nitrogen and proteins (Kjeldahl, 1883) and testing of the oxidoreductase activity (by testing the activity of the enzyme catalase which is extremely effective in terms of stressful influences from the external environment).

The content of total and soluble sugars is determined by the method of Dubois *et al.* (1962) by preparing a mixture of phenol and sulphuric acid and spectrophotometric analysis of the extracts at a wavelength of 485 nm.

The activity of catalase was determined through the method of Bach and Oparin (1923), through the neutralization of H₂O₂ with KMnO₄. This method is used today for routine analysis of this parameter, since it provides reliable and accurate results.

RESULTS AND DISCUSSION

The results from this research provide a clear picture of the most significant individual primary biomolecules which directly affect the quality

characteristics of fermented tobacco and they alter the physical and features flavors of tobacco products (Talhout et al., 2006).

Carbohydrates as primary products of photosynthetic activity, for the tobacco are extremely important because their precise determination is necessary for the proper classification of tobacco according to its quality (Troje et al., 1997). Carbohydrates show importance of maintaining the structure of cells, but have a significant role in the physical quality of oriental varieties of tobacco. In mature leaves of tobacco, carbohydrates can be represented up to 40-45% of dry matter.

Carbohydrates as primary metabolites are inversely proportional according to the content of protein, while with the alkaloids they show proportionality (Clarke et al., 2006).

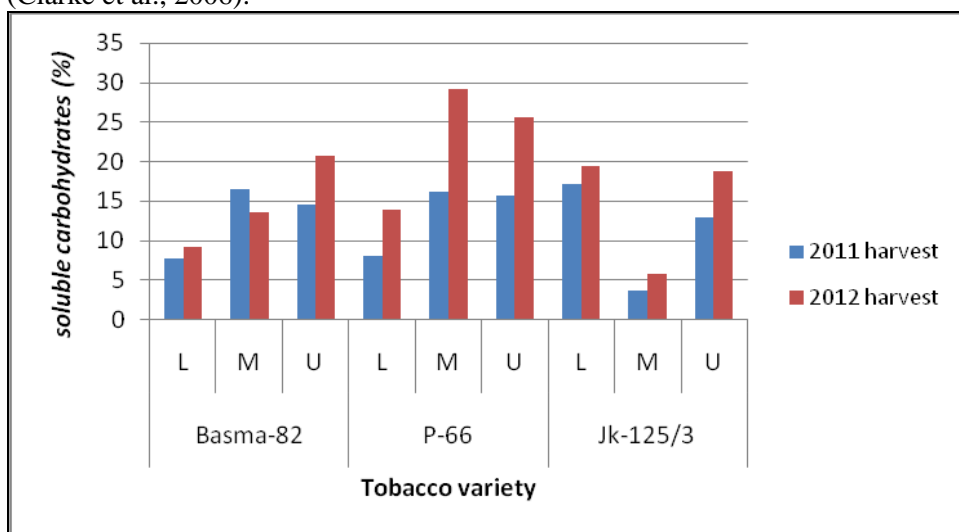


Figure 1. Concentration of soluble carbohydrates (%) in tobacco (*Nicotiana tabacum* L.) in three varieties (Basma-82, P-66 and Jk-125/3) in the three insertions of the 2011 and 2012 harvest (L-lower insertion, M-middle insertion, U-upper insertion)

The research results indicate an existence of a close relationship between the course of the process of fermentation and its duration with the proportion of carbohydrates in the raw material. Within the 2012 harvest, the content of total soluble sugars is significantly higher in all tested varieties, compared to samples from the harvest of 2011. It confirms that with prolonged fermentation of the raw material, the content of carbohydrates decreases, analogous to lowering the quality of tobacco. Delayed period of fermentation for several years is described in a study by Noguchi et al. (1971), which states significantly reducing of the content of reducing sugars, which is correlated with the results from this research. Among the variety P-66 shows the highest concentration of total carbohydrates (17 to 36%) and soluble carbohydrates (14 to 29%). After drying and fermentation most of the starch is converted into small molecules of

carbohydrates that have a role in the formation of the aroma of tobacco smoke by determining the acid-base balance (Yang *et al.*, 2003). Increased hydrolysis due to increased activity of enzymes (such as amylase), which is induced by the temperature and moisture content in tobacco leaves during fermentation.

The content of soluble carbohydrates is highest in the middle insertion in the varieties P-66 and Basma, which justifies that oriental types of tobacco fall into the class of quality tobaccos. Deviation from these results shows the variety JK-125/3, where the middle insertion is characterized by the lowest carbohydrate content (total and soluble). We assume that this deviation is due to the physiological characteristics of type Jaka, which in terms of the concentration of primary biomolecules has seen significant differences with other oriental types and varieties of tobacco (Boceski, 2003).

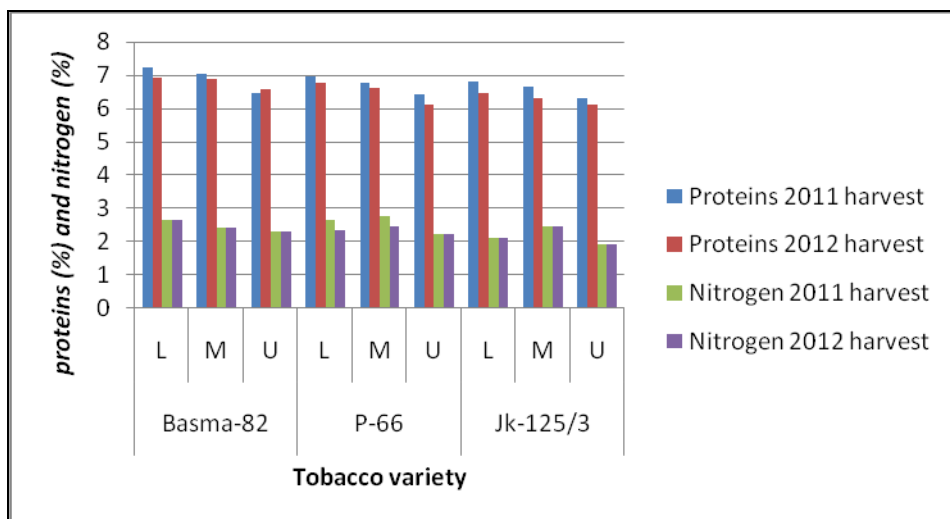


Figure 2. Concentrations of proteins (%) and nitrogen (%) in tobacco (*Nicotiana tabacum* L.) in three varieties (Basma, P-66 and JK-125/3) in the three insertions of the 2011 and 2012 harvest (L-lower insertion, M-middle insertion, U-upper insertion)

The synthesis of nitrogen by plants is intensified in the early stages of development (Wang *et al.*, 2008), so we have a high concentration of it in the lower insertion of tobacco. High values of the protein concentration indicate low quality of the tobacco plants (Johnson and Knowlton, 1970). In account for the reduction of other parameters that were examined in this study, there was an increase in protein content and nitrogen over the fermentation processes. Notably, although the differences are not significant, the concentration of protein and nitrogen in the 2011 harvest is higher than that at harvest 2012.

To study and validate the optimal conditions for fermentation of the tobacco is necessary to take into account the variation of catalyse as an enzyme that plays an important role in the processes of fermentation.

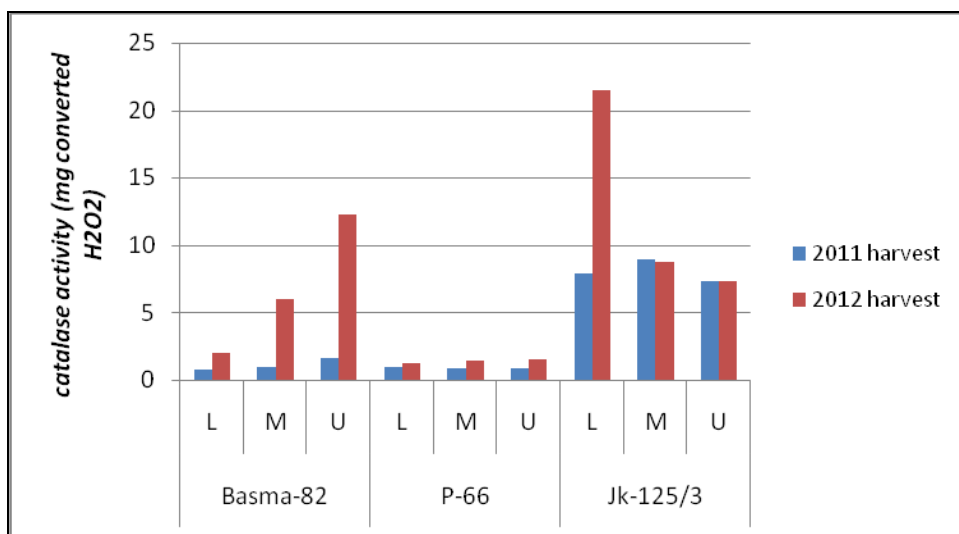


Figure 3. Catalase activity (mg converted H₂O₂) in tobacco (*Nicotiana tabacum* L.) in three varieties (Basma-82, P-66 and Jk-125/3) in the three insertions of the 2011 and 2012 harvest (L-lower insertion, M-middle insertion, U-upper insertion)

It's high activity inhibits the activity of certain microorganisms that are essential for fermentation (Di Giacomo et al., 2007). During fermentation the content of catalase is reduced, and in the context of these data and the results of this study, where the highest activity of catalase show leaves harvested in 2012 for all tested varieties. The activity of catalase in the tested samples recorded significantly higher activity of catalase in variety Jaka, which is stated in the literature as a species which is characterized by high resistance to tobacco mosaic virus (TMV), the low temperature during the cultivation and invertebrate pathogenic organisms (Uzunoska, 1985).

CONCLUSIONS

As a result of this research we can conclude that the first harvest of 2012 within the content of total and soluble sugars and the content of the catalase enzyme is significantly higher in all tested varieties, compared to samples from harvested 2011th. Also, the results indicate an increasing in protein content and nitrogen over the fermentation processes. Although the differences are not significant, the concentration of protein and nitrogen is higher in 2011 harvest than that at harvest 2012. It confirms that prolonged period of fermentation of the raw material reduces the quality of oriental tobacco plants.

REFERENCES

- Clarke, M. B., Bezabeh, D. Z., Howard, C. T. (2006) Determination of Carbohydrates in Tobacco Products by Liquid Chromatography–Mass Spectrometry/Mass

- Spectrometry: A Comparison with Ion Chromatography and Application to Product Discrimination. *J. Agric. Food Chem.* 54 (6): 1975–1981
- Di Giacomo, M., Paolino, M., Silvestro, D., Vigliotta, G., Imperi, F., Visca, P., Alifano, P., Parente, D. (2007). Microbial Community Structure and Dynamics of Dark Fire-Cured Tobacco Fermentation. *Applied and Environmental Microbiology* 73(3): 825–837
- Drachev, D., Nikolova, V. (2004). Study on the tobacco quality of Virginia group grown in Bulgaria Technological study on the quality of Virginia tobacco introduced varieties grown in the districts of North Bulgaria (Part II). *Biotechnol. & Biotechnol. Eq.* 19(1): 38-45
- Dubois, M., Gilles, M. A., Hamilton, J. K., Rebers, P. A., Smith, F. (1956). Calorimetric method for determination of sugars and related substances. *Anal. Chem.* 28: 350-356
- Hoek, J., Gendall, P., Gifford, H., Pirikahu, G. (2012). Tobacco branding, plain packaging, pictorial warnings and symbolic consumption” *Qualitative Health Research*, 22: 630-639
- Johnson A. D., Knowlton R. W. (1970). The Effect of Manganese on Tobacco-D Leaf Quality and on the Inorganic Cation Levels of Tobacco-D Leaves. *Australian Journal of Experimental Agriculture and Animal Husbandry* 10: 118-123
- Kjeldahl, J. (1883). A new method for the determination of nitrogen in organic matter. *Zeitschreft fur Analytische Chemie.* 22: 366-368
- Kolodiaznaya, Y. S. (2007): “Tobacco plant which is not afraid of heavy metals” Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Novosibirsk; http://www.healthstairs.com/russian_science_news.php?newsido=13
- Miceski, T., Taskoski, P. (2006). Agrochemical Management in Production of Tobacco” In: 70th Anniversary of Plant Protection Institute and Annual Balkan Week of Plant Health, May 28-31, 2006, Kostinbrod, Bulgaria
- Noguchi, M., Satoh, Y., Nishida, K., Andoh, S., Tamaki, E. (1971). Studies on storage and ageing of leaf tobacco. Part IX. Changes in the content of amino acid-sugar compounds during ageing. *Agricultural and Biological Chemistry* 35: 65–70
- Troje, Z. S., Frobe, Z., Perovic, D. (1997). Analysis of elected alkaloids and sugars in tobacco extract. *Journal of Chromatography A.* 775: 101-107
- Wang, J., Wang, S., Bi, Q., Yu, S., Yang, Y., Sun, F., Wang, A., Yan, Q., Wang, P. (2009). Effects of N,P,K Rates on Yield and Quality of Flue-cured Tobacco Honghuadajinyuan. *Chinese Tobacco Science* http://en.cnki.com.cn/Article_en/CJFDTotal-ZGYV200905009.htm
- Yang, S. S., Smetena, I., Huang, C. B. (2003). Determination of tobacco alkaloids by gas chromatography with nitrogen-phosphorus detection. *Anal. Bioanal. Chem.* 373(8): 839-843
- Tallhout, R., Opperhuizen, A., van Amsterdam, J. G. (2006): Sugars as tobacco ingredient: Effects on mainstream smoke composition. *Food Chem. Toxicol.* 44(11): 1789-1798