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Technology for Obtaining Ethanol Extracts from Three *Nicotiana* Species Based on Their Tannin Content

*** Venelina Popova**
University of Food Technologies,
26 Maritza blvd.,
4002 Plovdiv, Bulgaria
vpopova2000@abv.bg

Stanislava Tasheva
University of Food Technologies,
26 Maritza blvd.,
4002 Plovdiv, Bulgaria
st_tasheva@abv.bg

Tanya Ivanova
University of Food Technologies,
26 Maritza blvd.,
4002 Plovdiv, Bulgaria
tantonieva@mail.bg

Albena Stoyanova
University of Food Technologies,
26 Maritza blvd.,
4002 Plovdiv, Bulgaria
aastst@abv.bg

***Abstract*—Common tobacco (*Nicotiana tabacum* L.) is the only wide-scale cultivated and economically important species among the over 70 members of genus *Nicotiana (*family Solanaceae). The rest of the species are less studied, but represent valuable plant sources for obtaining biologically active products. Therefore, the objective of this study was to create the technological backgrounds of obtaining extracts from three *Nicotiana* species – *N. alata* Link&Otto (jasmine tobacco), *N. rustica* L. (Aztec tobacco), and *N. tabacum* L., intended for medicinal and cosmetic use. The experimental scheme revealed the influence of the two main factors of plant materials extraction – temperature and duration, upon the accumulation of tannins in liquid bioactive extracts obtained with 30, 50, 70 and 95% ethanol from the leaves of the three tobacco species. The respective equations of extraction were derived. The optimal extraction conditions in terms of tannin content were: duration – 5 h, temperature – 60°C; solvent – 50% or 70% ethanol for *N. rustica* and *N. tabacum*, and 50% for *N. alata*.**

Keywords—Nicotiana ssp., ethanol extracts, equations of extraction, tannins

I. INTRODUCTION

Phytochemical studies devoted to the expansion of the collection of common or more exotic plant species available for obtaining secondary products that are rich in biologically active constituents has been an unambiguous focus of research in recent decades [1, 2]. A large number of traditional or novel techniques (such as microwave and ultrasound assisted extraction, accelerated and micro extraction, supercritical and subcritical extraction, etc.) have been employed in the obtaining of bioactive extracts from different morphological parts of the plants (leaves, stems, flowers, roots, etc.) [3-5]. The same plant material processed by different techniques or under different process conditions can yield phytoproducts with substantially different chemical composition, olfaction, functional properties and biological activity. When intending these extraction products for cosmetic purposes, there are some limitations on solvent selection due to possible issues with their direct on-skin application [6]. In this regard, ethanol remains the most popular and widely used solvent for obtaining liquid, concentrated or dry extracts used in food and cosmetic products [7, 8].

Tobacco, in particular, has been a subject of extensive research for centuries due to its important role in national economies and societies as the sole source for the manufacture of different smoking and smokeless products for human consumption. On the other hand, the history of tobacco ethnobotanical, medicinal, religious or ritual use, and its exploitation in biotechnology and plant engineering, as well as other alternative applications, have long granted a much more particular acknowledgment of the plant [9-11]. In this regard, many recent studies focus on tobacco as a source of biologically active secondary products [12, 13]. Most of the research explores *Nicotiana tabacum* L. (common tobacco), being the only wide-scale cultivated and economically important species of genus *Nicotiana* (family Solanaceae). However, each of the over 70 genetically and morphologically diverse *Nicotiana* species [14, 15] represents a plant material with highly specific composition and properties. Although less studied, all *Nicotiana* species represent valuable plant sources in terms of obtaining biologically active products with potential use in various areas. Many biologically active substances, belonging to different classes of chemicals, have been identified in the leaves of the tobacco species, types or grades – such as plant volatiles, terpenes, carotenoids, sterols, saponins, polyphenols, alkaloids, etc. [12, 16, 17].

Therefore, the objective of this study was to provide the technological backgrounds of obtaining extracts, on the basis of the biologically active tannins, from three *Nicotiana* species – *N. alata* Link&Otto (jasmine tobacco), *N. rustica* L. (Aztec tobacco), and *N. tabacum* L. (common tobacco), intended for medicinal and cosmetic use.

II. MATERIALS AND METHODS

A. Plant Material

Leaves of three tobacco species – *N. alata* Link&Otto (jasmine tobacco), *N. rustica* L. (Aztec

tobacco), and *N. tabacum* L. (common tobacco) were investigated. Common tobacco was represented by the local Oriental variety “Plovdiv 7”. All tobaccos were grown side-by-side in the fields of the Tobacco and Tobacco Products Institute, located in the region of Plovdiv, South Bulgaria. Mature leaves were handpicked and sun cured, by applying the classical technology for oriental tobacco. Until processing, leaves were stored in an air-conditioned environment, packed in plastic bags and cardboard boxes. The average leaf samples were oven-dried (40°C, 6 h), then ground and fractionated to the particle size required by the respective analysis.

The plant material was analyzed to determine the initial moisture content (by drying at 105°C to constant weight [18] and the content of tannins (by titration of a hot water extract with potassium permanganate solution [18].

B. Obtaining and Evaluation of Extracts

Laboratory extraction was conducted in a static batch mode, by maceration in the solvent. The hydro module (raw material to solvent) was 1:10 (w/v). Four solvent concentrations were used in the extraction of tobacco leaves: 30, 50, 70 and 95% (v/v) ethanol. The solvent and its concentrations, as well as plant material weight ratio were chosen on the basis of authors’ previous published data [19]. The impact of the technological factors, i.e. temperature and duration, on extraction dynamics was examined by mathematical modeling of the experiment as a two-factor analysis on three levels. Temperature levels were 20, 40 and 60°C, and process duration levels were 1, 3, 5 and 7 h, respectively. The effectiveness of the process was evaluated in terms of tannin content in the obtained extracts [18].

Experimental data from each of the variants in the experimental matrix were processed in order to derive the respective equations of tannin extraction. Equation coefficients were assessed for significance (by the standard *t*-test procedure) and for adequacy (by *F*-test).

C. Statistical Analysis

All experiments were done in at least three repetitions. Statistical significance of data was assessed by Student’s-test or ANOVA.

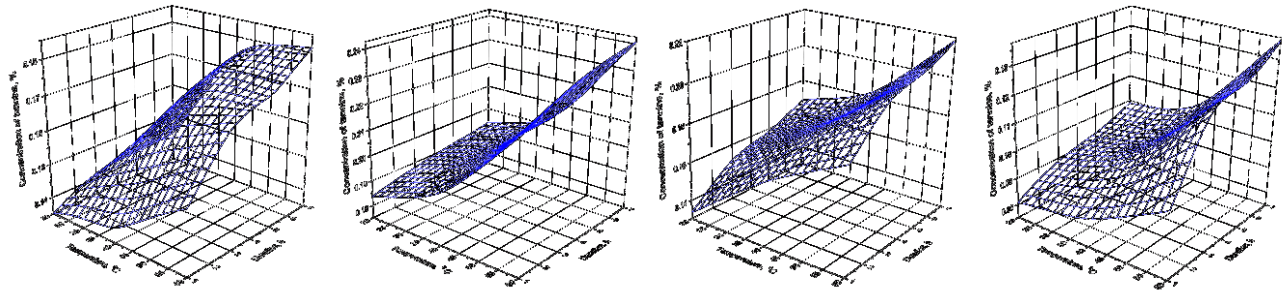
III. Results and Discussion

The initial moisture content of the analyzed tobacco leaf samples were as follows: *N. alata* – 10.17%, *N. rustica* – 9.17%, and *N. tabacum* – 7.84%. The content of tannins in the plant materials (determined by exhaustive extraction) was 6.18%, 11.94%, and 10.33%, respectively for the three species. There are no available data about tannin content in the leaves of *N. alata* and *N. rustica*, but the parallel to another Oriental tobacco variety (Krumovgrad 90) reveals about twice as higher tannin concentration in Plovdiv 7 variety leaves (10.33% vs 5.32%) [13, 19].

The extracts were clear liquids and their color varied upon solvent concentration: brown (for the extracts obtained with 95% ethanol), light-brown (with 70% ethanol), dark-yellow (with 50% ethanol), and yellow (with 30% ethanol).

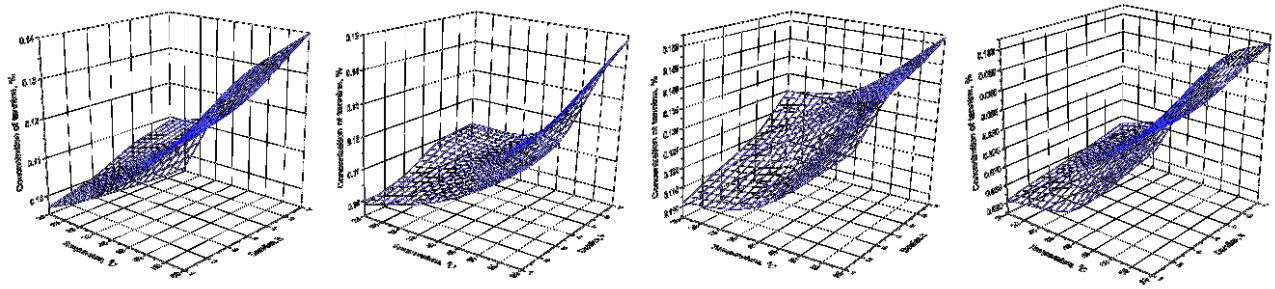
Fig. 1, 2 and 3 present the patterns of tannin accumulation in the extracts obtained by the adopted experimental schemes, for each of the tobacco species. The results show that the increase of temperature from 20 to 60°C and the increase of process duration from 1 to 5 h intensify tannin extraction, regardless of ethanol concentration and plant material origin. Extraction process duration was additionally prolonged to 7 h, which resulted only in an insignificant increase in the tannin content of the extracts, supporting therefore an assumption that the process was subsiding and there were no rational grounds for its continuation over 5 hours.

The maximal amount of tannins was accumulated in the extracts obtained at 60°C for 5 hours, and for the studied species, it was as follows: *N. tabacum* (0.27%), *N. alata* (0.24%), and *N. rustica* (0.15%).



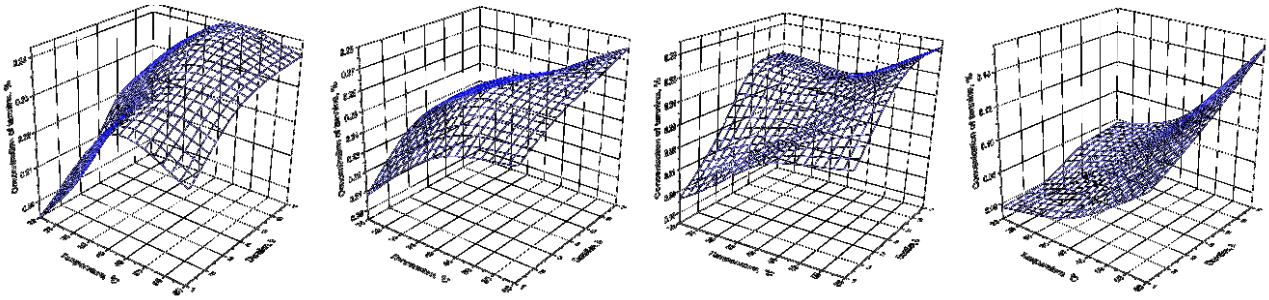
a) with 30% ethanol b) with 50% ethanol c) with 70% ethanol d) with 95% ethanol

Fig. 1. *Tannin content vs temperature and duration, in extracts from N. alata leaves*



a) with 30% ethanol b) with 50% ethanol c) with 70% ethanol d) with 95% ethanol

Fig. 2. *Tannin content vs temperature and duration, in extracts from N. rustica leaves*



a) with 30% ethanol b) with 50% ethanol c) with 70% ethanol d) with 95% ethanol

Fig. 3. *Tannin content vs temperature and duration, in extracts from N. tabacum ("Plovdiv 7" variety) leaves*

As the experimental data presented on the figures above suggest, the main two factors of the extraction process, i.e. duration (x_1) and temperature (x_2), have both decisive influence on the transfer and accumulation of tannins in the extracts. The strong relationship between the tannin content in the ethanol extracts and the conditions of the process was confirmed by the derived equations of tannin extraction, which were validated as adequate and with significant coefficients. The respective equations for the extraction of tannins from the three tobacco species are presented in Table 1.

The highest concentration of tannins in the extracts was achieved under the following conditions: duration – 5 h, temperature – 60°C, solvent concentration – 50% ethanol for the leaves of *N. alata,* and 50% and 70% ethanol for *N. rustica* and *N. tabacum*. Within the scope of study matrix, these could be considered the optimal set-up of process parameters in terms of maximal accumulation of tannins in the extracts obtained from tobacco leaves. The lowest content of tannins in the extracts was achieved with 30% ethanol, followed by 95% ethanol, independent of tobacco species. The differences in the tannin concentration in the extracts obtained with the four ethanol concentrations could be attributed to the change in solvent selectivity of the respective ethanol-water mixture. Our results agree well with previous findings about the influence of process duration, temperature and ethanol concentration on tannin extraction reported in studies on different essential oil bearing and medicinal plants and herbs, e.g. tobacco leaves (*N. tabacum* L., ecotype Krumovgrad) [13, 19], mint leaves (*Mentha piperita* L.) [20], thyme (*Thymus vulgaris* L.) [21], and rosemary (*Rosmarinus officinalis* L.) [22].

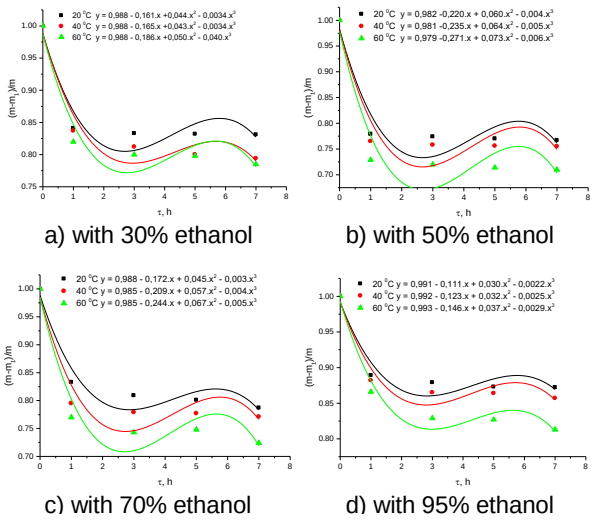
TABLE I. EQUATIONS OF TANNIN EXTRACTION FROM TOBACCO LEAVES

Extracts with	Tobacco	Equation of extraction[d]
30% ethanol	NA[a]	$y = 0.160 + 0.014x_1 + 0.008x_2 + 0.001x_1x_2 - 0.002x_1^2 - 0.003x_2^2$
	NR[b]	$y = 0.111 + 0.014x_1 + 0.006x_2 + 0.003x_1x_2 + 0.005x_1^2 + 0.002x_2^2$
	NT[c]	$y = 0.232 + 0.013x_1 + 0.009x_2 - 0.002x_1x_2 - 0.015x_1^2 + 0.020x_2^2$
50% ethanol	NA	$y = 0.200 + 0.022x_1 + 0.005x_2 + 0.002x_1x_2 + 0.010x_1^2 - 0.001x_2^2$
	NR	$y = 0.108 + 0.136x_1 + 0.006x_2 + 0.001x_1x_2 + 0.007x_1^2 + 0.002x_2^2$
	NT	$y = 0.258 + 0.017x_1 + 0.012x_2 - 0.003x_1x_2 - 0.012x_1^2 - 0.007x_2^2$
70% ethanol	NA	$y = 0.176 + 0.026x_1 + 0.011x_2 + 0.001x_1x_2 + 0.001x_1^2 - 0.007x_2^2$
	NR	$y = 0.122 + 0.013x_1 + 0.006x_2 + 0.001x_1x_2 + 0.006x_1^2 - 0.001x_2^2$
	NT	$y = 0.231 + 0.015x_1 + 0.017x_2 - 0.004x_1x_2 + 0.002x_1^2 + 0.001x_2^2$
95% ethanol	NA	$y = 0.099 + 0.015x_1 + 0.009x_2 + 0.004x_1x_2 + 0.007x_1^2 - 0.007x_2^2$
	NR	$y = 0.074 + 0.017x_1 + 0.005x_2 + 0.003x_1x_2 + 0.007x_1^2 - 0.003x_2^2$
	NT	$y = 0.078 + 0.026x_1 + 0.011x_2 + 0.003x_1x_2 + 0.001x_1^2 + 0.002x_2^2$

[a]*NA – N. alata Link&Otto;* [b]*NR – N. rustica L.;* [c]*NT – N. tabacum L. (Oriental type, variety Plodviv 7);* [d] *y – concentration of tannins in the extract (%); x_1 – duration of extraction (h); x_2 – temperature of extraction (ºC)*

The dynamics of tannin extraction in dependence with the technological parameters of the process was further studied, and the experiments were carried out under the above described conditions. The results from the individual experiments are presented in terms of residual (non-extracted) tannins in the plant material after the completion of the respective extraction period. It was calculated according to the equation: $y=(m - m_i)/m$, where m was the initial tannin content in the plant material (%) and m_i – the content of extracted tannins for the respective time period (%). The polynomial regression models of tannin extraction dynamics (all with R^2-values within the range 0.7-0.9, providing sufficient degree of determination) are shown on Fig. 4, 5 and 6.

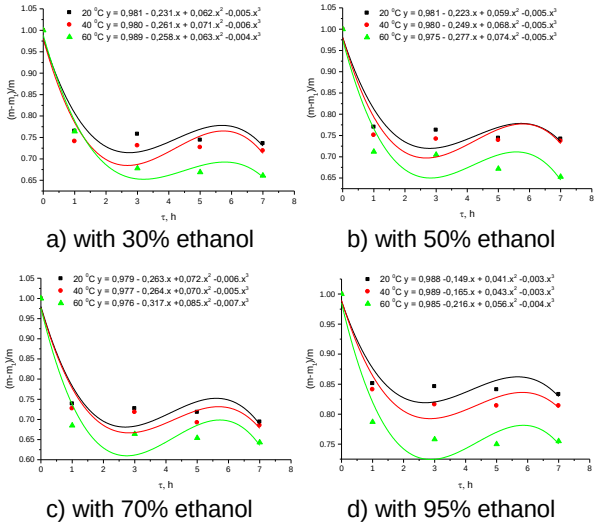
The analysis of the dynamics curves revealed that the extraction of tannins ran most intensively during the first 3 to 5 hours, after which the rate subsided and ceased until the 7th hour.



a) with 30% ethanol b) with 50% ethanol

c) with 70% ethanol d) with 95% ethanol

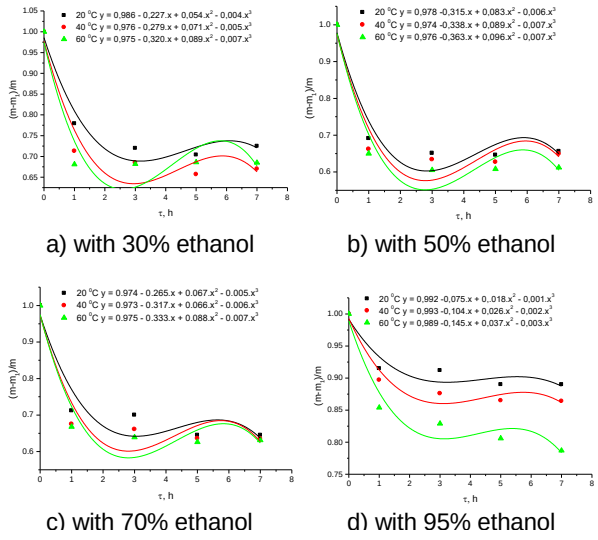
Fig. 4. *Regression models of tannin extraction from N. alata leaves*



a) with 30% ethanol b) with 50% ethanol

c) with 70% ethanol d) with 95% ethanol

Fig. 5. *Regression models of tannin extraction from N. rustica leaves*



a) with 30% ethanol b) with 50% ethanol

c) with 70% ethanol d) with 95% ethanol

Fig. 6. *Regression models of tannin extraction from N. tabacum ("Plovdiv 7" variety) leaves.*

IV. Conclusions

By applying the adopted model of two-factor experiment on three levels a technology of obtaining liquid ethanol extracts from the leaves of different tobacco species has been developed. The highest concentrations of tannins were obtained under the following combination of process parameters: duration of 5 hours, temperature of 60°C, and concentration of ethanol – 50% for the leaves of *N. alata,* and 50% or 70% for the leaves of *N. rustica* and *N. tabacum*. To the best of our knowledge, these are the first data about extraction dynamics reported for the two less popular *Nicotiana* species – *N. alata* and *N. rustica*. The outcomes from the study provide the technological grounds for the practical obtaining of tannin-containing phytoextracts from a new set of plants.

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