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ANALYSIS OF CELLULOSE CONTENT IN STALKS AND LEAVES OF LARGE LEAF TOBACCO

Gordana J. Kulić* and Vesna B. Radojičić

University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Beograd-Zemun, Serbia

Abstract: Tobacco stalks are an under-utilized material that remains after the harvest of leaves. Given the share of large leaf tobacco in total production in Serbia (more than 85%) stalks could be easily available as well as payable raw material in the fabrication of cellulose. The aim of this study was to determine cellulose content in leaves and more importantly in stalks of large leaf tobacco and to provide necessary data to processors engaged in processing of biomass. Stalks and leaves of two types of tobacco, Burley and Virginia, reared in Srem, were used as experimental material samples. Two parallel methods were applied for determining cellulose content: method by Kürschner-Hanack and method by Updegraff. It can be concluded that both methods are applicable to tobacco, but the method by Kürschner-Hanack was found to be simpler, more convenient, faster and economically more cost-effective. Thus, it should be recommended for use. Results showed that stalks of large leaf tobacco contain on average 30.50% to 34.30% of cellulose. There was no statistically significant difference in the cellulose content between the two applied methods. Given the number of plants that lag behind in fields after harvesting leaves, 28,000 tons of cellulose could be used.

Key words: cellulose, tobacco leaf, tobacco stalk, large leaf tobacco.

Introduction

Rational production and processing of tobacco plant must include the entire biomass, both the main product leaves as well as stalks that remain after harvest. Residues (stalks and small leaves) and significant amounts of leaf scrap and waste generated during processing of tobacco, can serve as a very important secondary raw material from which, by final processing, a great number of products could be obtained in industry (Bandosz, 2007).

After the harvest of tobacco, a significant amount of tobacco stalks that remains in field should be used in some way. The stalk is unusable to manufacture products for enjoyment. Therefore, large amounts of stalks are mostly plowed

*Corresponding author: e-mail: gkulic@agrif.bg.ac.rs

under. Considering that these stalks contain a certain amount of nicotine, such manner of disposure is a problem (Wang et al., 2004). By categorization of tobacco waste (Radojičić et al., 2009) tobacco stalks are classified into green tobacco waste. If nicotine content in tobacco waste is higher than 500 ppm, by the regulations of the European Union, it is considered to be toxic. By pressing or briquetting plant alone or plant with waste fruit and crops, biomass could be obtained for further production of biogas (Özturk and Bayrakl, 2005; Peševski et al., 2010). By modern industrial processing of biomass building materials like variety of pressed boards could be produced. It is also possible to produce furniture parts (boards, plywood), agents used to clean metal surfaces as well as powder and other cosmetics (Peševski et al., 2010). Since this biomass contains high amounts of total sugars, it is suitable for production of alcohol and biogas by fermentations (Meher et al., 1995; Brohi and Karaman, 1998; Briski and Horgas, 2002).

In addition, because of the high nicotine content, tobacco stalk can be used to produce pesticides. This can be inferred from the research (Radojičić et al., 2008) regarding testing efficiency of nicotine-based insecticides, obtained from tobacco waste. Since stalk is biodegradable, it presents an attractive alternative not only to synthetic pesticides but also to fertilizers. This is a consequence of high content of organic matter and this was the subject of an extensive research done by Turkish researchers (Okur et al., 2008). It is also used to produce compost for growing mushrooms (Novotny and Zhao, 1999; Okur et al., 2008; Radojičić et al., 2008, 2009).

On the other hand, stalk is an important secondary raw material due to its high cellulose content. Cellulose is the main ingredient of paper, cardboard, textiles, cotton, flax and other vegetable fibers. Among esters of cellulose, cellulose acetate and cellulose nitrate are of the greatest significance. Cellulose acetate with degree of substitution of 2.0 to 3.0, with addition of softener, is used to produce fibers and films. Fibers of cellulose acetate are used in the textile industry ('silk acetate') and for making filters for cigarettes. There is a significant use of cellulose acetate foil for the production of the carrier of photo-sensitive layer of photo and movies, as replacement of highly flammable cellulose nitrate (Jovanović et al., 2002). Cellulose nitrate with the degree of substitution between 2.5 and 3 is used for the production of gunpowder, and with the degree of substitution of about 2.5 for production of fibers. Cellulose nitrate which is characterized with degree of substitution of about 2 is used as a thermoplastic polymer. Practically, the first thermoplastic polymer in the world is celluloid (Jovanović et al., 2002). Microcrystalline cellulose (E460) and cellulose powder (E460) are supplements in tablets and can also be used as thickeners and stabilizers in food production (Tehnologija hrane, 2010).

In tobacco plant, cellulose does not serve as a reserve material, but contributes to mechanical strengthening as well as to the defense of plant tissues to protect them from the adverse environmental impacts. According to various authors, cellulose, as an indifferent substance which does not affect flavour, is present in different percentages (Tso, 1990). Thus, stalks contain 35% to 36% of cellulose, while the midrib has 10% to 15% of cellulose. In older leaves, cellulose is present in a crystalline form, while in younger leaves an amorphous form of cellulose prevails. Leaves contain on average 10% to 12% of cellulose (Davis and Nielsen, 1999). Cellulose content in leaves increases from bottom to the top of plant. According to earlier research (Tomić and Demin, 1977), stalks can contain up to 30% of cellulose. The midrib contains about 10% to 15%, leaves 7.20% to 14%, while the total cellulose content per plant is 6% to 13%. According to the data of the Ministry of Agriculture for the year of 2010, large leaf tobacco was grown in Serbia in the area of 5,345 hectares. Considering that the average number of stalks is 25,000 per hectare, a total number of large leaf tobacco plants produced in Serbia in 2010 were 133,625. This figure illustrates an enormous potential of tobacco stalks as raw materials for production of pure cellulose. Therefore, the aim of this work was to determine cellulose content in the stalks and leaves of tobacco plant that represent under-utilized tobacco waste, using two comparative methods. Since 1977 until now, no such research has been conducted in the Republic of Serbia. Therefore, the results of this study will be of great importance for the field of the fabrication of cellulose.

Materials and Methods

Samples were prepared from dried leaves and stalks remaining after the harvest of large leaf type of tobacco (Burley and Virginia) grown in the area of Srem.

Samples for this study were prepared as follows: homogenous mass of stalks of Burley tobacco type, 24.50 cm in length, 2 cm in diameter and of green color, was produced and used for analysis. In the first method, 2 g of these homogenous mass were used, while in the second method the initial mass of sample was 0.20 g. Stalks of Virginia tobacco type, which were 41 cm long, and 2.20 cm in diameter and dark green, were also homogenized and the same amount of samples was used. Leaves of Burley tobacco type were averagely 53.06 cm long, 21.06 cm wide and dark brown. Leaves of Virginia tobacco type were averagely 42.70 cm long, 16.02 cm wide and light yellow. Ten tobacco leaves of each type were milled and further analyzed.

Analysis of cellulose content was performed by two methods

Principle of the method by Kürschner-Hanack (cited by Šušterčić, 1979; Tehnologija hrane, 1987): This method is based on insolubility of cellulose in water and its resistance to action of dilute acids and bases. The sample was degraded with a mixture of nitric acid and acetic acid and boiled in apparatus that contained a Liebig's condenser. The solution was then filtered through a Büchner funnel. Then the filter paper containing an insoluble residue was dried in oven and measured. Analysis was done at the Faculty of Agriculture in Belgrade.

Principle of the method by Updegraff (1953): Plant fiber was soaked in acetic acid and nitric acid to remove lignin, hemicellulose and xylans. Cellulose in the sample was hydrolyzed to sugars. Total sugar content in solution was determined by anthron test (Bailey, 1957). Anthron solution was prepared by diluting 10 mg of anthron in 100 ml of 70% sulphuric acid. Calibration curve for determining concentration of sugars in the sample was constructed as follows: 1ml of standard glucose solution was boiled with 10 ml of anthron solution for 5 minutes. During heating glucose with sulphuric acid, colored product 5-hydroximetylfurfural was created, in reaction of aldehyde groups of glucose with anthron. The concentration of 5-hydroximetylfurfural was determined spectrophotometrically. The measurement was performed on spectrophotometer Cintra10e GBS, AGILEND 8453, at 625 nm. The concentration of glucose was calculated using standard curve. Based on the concentration of glucose, the content of cellulose was then calculated. The analysis was performed at the Faculty of Physical Chemistry in Belgrade.

Statistical analysis

Experimental results are presented statistically, giving mean and its standard error, median, intervals of variations, standard deviation and coefficient of variation, using statistical package STATISTICA for Windows V.8 (Maletić, 2005). Equality of variances of analyzed treatments was tested by Levene's test. Given that homogeneity of variance was confirmed, testing of statistical significance of average values for each tested parameter between the two applied methods for measuring cellulose content was done by t-test with significance threshold of 5% and the corresponding number of degrees of freedom (df).

Results and Discussion

Test results of cellulose content, using two methods, are shown in Table 1.

Selected samples had very low variability ($Cv < 6.5\%$) which indicates the presence of homogeneous samples, and the advantage of using the mean relative to the median as characteristic of average ratio.

The results presented in Table 1 and Figure 1 showed that the cellulose content varied both by type of tobacco and by plant organ that is being analyzed, regardless of the applied method.

Tobacco stalks contained more cellulose than the leaves, which was also confirmed in previous studies (Tomić and Demin, 1977; Brohi and Karaman, 1998). Based on the results obtained by the Kürschner-Hanack method, the average content of cellulose in the stalk of Virginia is 30.50%. This result is consistent with the values specified by Tomić and Demin (1977). The average content of cellulose in the Burley stalk was 34.00%, and was higher by 10.29% compared to Virginia and also higher than cellulose content in the stalks as referred to in previous studies (Tomić and Demin, 1977).

Table 1. Main indicators of descriptive statistics of fiber content (%) obtained by the Kürschner-Hanack and Updegraff methods.

Cellulose content	$\bar{x} \pm S\bar{x}$	Median	X max.-X min.	Stand. dev.	Cv (%)
	Kürschner-Hanack method				
Burley stalks	34.00 ± 0.537	34.15	35.10-32.60	1.074	3.16
Virginia stalks	30.50 ± 0.838	30.35	32.40-28.90	1.675	5.49
Burley leaves	17.00 ± 0.212	17.05	17.40-16.50	0.424	2.50
Virginia leaves	16.00 ± 0.234	19.59	16.60-15.50	0.469	2.93
	Updegraff method				
Burley stalks	34.30 ± 0.582	34.15	35.70-33.20	1.163	3.39
Virginia stalks	31.60 ± 1.014	32.00	33.30-29.10	2.028	6.42
Burley leaves	17.20 ± 0.342	17.30	17.90-16.30	0.683	3.97
Virginia leaves	16.40 ± 0.372	16.55	17.10-15.40	0.744	4.54

Differences in values of cellulose content in the leaves of examined tobacco, obtained by the Kürschner-Hanack method, are not too pronounced. Leaves of Virginia tobacco type contain on average 16.00% of cellulose. Cellulose content in Burley leaves is higher and amounts to 17.00%, which is only 5.88% more than the cellulose content in the leaves of Virginia. These results were not in accordance with previous studies (Brohi and Karaman, 1998); these values were on average higher by 23.50%. These differences are probably due to different growing conditions and usage of different cultural practices, in what we did not have an insight, because it was not provided by the experiment.

Table 1 also shows the values of cellulose content in the stalks and leaves of tobacco, obtained by the Updegraff method. In general, both types of tobacco contain greater amounts of cellulose both in tobacco stalks and leaves, compared with the results obtained by the method of Kürschner-Hanack.

The mean value of cellulose content in the Burley stalks amounts to 34.30%, which is 0.73% higher than the value obtained by the Kürschner-Hanack method. Cellulose content in Virginia stalks is 31.60%, which is 3.48% higher than the value obtained by the first method.

Using the Updegraff method for analysis of cellulose content in the leaves of examined tobacco, obtained values were from 16.40% for Virginia to 17.20% for Burley. Therefore, compared to the values obtained by the Kürschner-Hanack method, the differences are much less pronounced than is the case with the amount of cellulose in the stalks of tobacco.

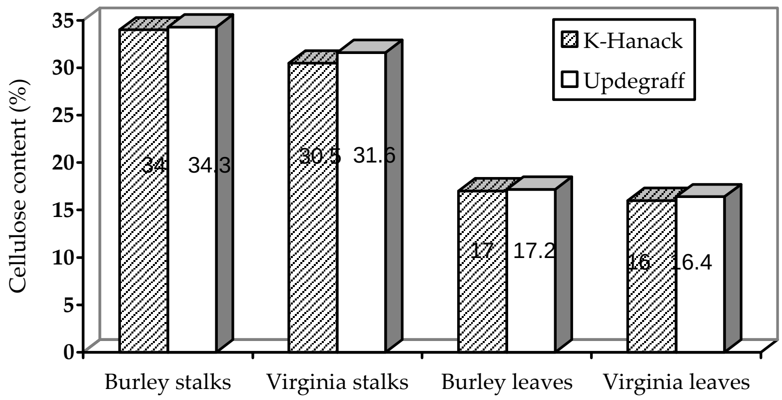

Figure 1. Comparative analysis of average values of cellulose content (%) obtained by the Kürschner-Hanack and Updegraff methods.

Results of Levene's test (Table 2) indicate that in all the samples the precondition was met for the use of parametric t-test, because of the homogeneity of variance.

Table 2. Results of Levene's test of homogeneity of variances and a comparative analysis of average values of cellulose content (%) using t-test.

Cellulose content	Method	$\overline{x}$	Levene's test		t-test	p-level
			F	p-level		
Burley stalks	K-Hanack Updegraff	34.00 34.30	0.200	0.670	0.379	0.718
Virginia stalks	K-Hanack Updegraff	30.50 31.60	0.369	0.566	0.836	0.435
Burley leaves	K-Hanack Updegraff	17.00 17.20	0.600	0.468	0.497	0.636
Virginia leaves	K-Hanack Updegraff	16.00 16.40	0.774	0.413	0.910	0.398

T-test for the investigated properties of cellulose, using two methods, showed no statistically significant differences between them ($p > 0.05$).

Results showed that leaves, particularly the stalks of large leaf tobacco, contained significant percentage of cellulose. These results confirmed the possibility of a rational use of the tobacco waste generated in the process of preparing and processing of tobacco, and especially the possibility of a rational utilization of tobacco stalks, that remain after the harvest in the fields. Namely, when the percentage of cellulose is multiplied by the number and weight of large leaf tobacco stalks, produced in Serbia in the year of 2010 (data provided by the Ministry of Agriculture), it becomes obvious that 28,000 tons of cellulose can be further utilized.

Conclusion

The investigation of cellulose content in the aboveground biomass of large leaf tobacco led to the following conclusions:

Large leaf tobacco stalks contain on average 30.50% to 34.30% cellulose. Given the number of plants that remains in the fields after the harvest of leaves, it is possible to use 28,000 tons of cellulose. Leaves contained significantly less cellulose (16.00-17.20%). In relation to stalk, this value was 50% lower. Burley tobacco type has higher cellulose content in stalks and leaves in comparison to the tobacco of Virginia type.

Differences in the values of cellulose content in stalks and leaves of tobacco were not statistically significant by application of two different methods. Both methods could be used for testing cellulose content in tobacco. However, the method by Kürschner-Hanack was found to be simpler, more convenient, faster and economically more cost-effective.

References

Bailey, R.W. (1957): The reaction of pentoses with anthrone. Biochem. J. 68:669-672.

Bandosz, T.J. (2007): Catalytic adsorbents obtained from municipal sludges, industrial sludges, compost and tobacco waste and process for their production. United States Patent Application, New York.

Briski, F., Horgas, N. (2002): Aerobic composting of tobacco solid waste. Book of Abstracts, Technological Choices for Sustainability, Maribor, Slovenija, pp. 36.

Brohi, A.R., Karaman, M.R. (1998): Determination of the utilization of nitrogen from tobacco waste by wheat crop with ^{15}N tracer technique. Turkish Journal of Agriculture and Forestry 22:593-600.

Davis, D.L., Nielsen, M.T. (1999): Tobacco-production, chemistry and technology. Oxon [etc.]. Blackwell Science.

Jovanović, S., Stojanović, Ž., Jeremić, K. (2002): Polimeri na bazi obnovljivih sirovina. Hemijska industrija 56(11):47-460.

Maletić, R. (2005): Statistika. Poljoprivredni fakultet, Beograd.

Meher, K.K., Panchwagh, A.M., Rangrass, S., Gollakota, G. (1995): Biomethanation of tobacco waste. Environmental Pollution 90(2):199-202.

Novotny, T.E., Zhao, F. (1999): Consumption and production waste: another externality of tobacco use. Tobacco Control 8:75-80.
Okur, N., Kayikçioğlu, H., Okur, B., Delibacak, S. (2008): Organic amendment based on tobacco waste compost and farmyard manure: influence on soil biological properties and butter-head lettuce yield. Turkish Journal of Agriculture and Forestry 32:91-99.
Özturk, T., Bayrakl, M. (2005): The possibilities of using tobacco wastes in producing lightweight concrete. Agricultural Engineering International: the CIGR Ejournal. 7, Manuscript BC 05 006.
Peševski, M., Iliev, B., Živković, D., Jakimovska-Popovska, V., Srbinoska, M., Filiposki, B. (2010): Possibilities for utilization of tobacco stems for production of energetic briquettes. Journal of Agricultural Sciences 55(1):45-54.
Radojičić, V., Nikolić, M., Ićitović, S. (2008): Some possibilities of tobacco waste utilization. Ecological truth 08, Proceedings of the VIII Scientific Professional Conference, Ecological Truth, Soko Banja, pp. 481-484.
Radojičić, V., Milošević, M., Tomašević, B. (2009): Tobacco waste management in Serbia. Ecological truth 09, Proceedings of the IX Scientific Professional Conference, Ecological Truth, Kladovo, pp. 218-221.
Šušterčić, N. (1979): Ispitivanje materijala. Kemijsko-tehnološki obrazovni centar, Zagreb.
Tehnologija hrane (1987): Pravilnik o metodama analiza kakaa. "Sl. List SFRJ", br. 41/87.
Tehnologija hrane (2010): Pravilnik o kvalitetu i uslovima upotrebe aditiva u namirnicama i o drugim zahtevima za aditive i njihove mešavine. "Sl. List SCG", br. 56⁄2003, 4⁄2004 - dr. pravilnik, 5⁄2004 - ispr. i 16⁄2009.
Tomić, LJ., Demin, A. (1977): Tehnologija proizvodnje i poznavanje duvana. Minerva, Subotica-Beograd.
Tso, T.C. (1990): Production, physiology and biochemistry of tobacco plant. Ideals, Inc., Beltsville, MD.
Updegraff, N. (1953): Hydrogen cyanide. Petroleum Refiner 32(9):197.
Wang, S.N., Xu, P., Tang, H.Z., Meng, J., Liu, X.L., Huang, J., Chen, H., Du, Y., Blankespoor, H.D. (2004): Biodegradation and detoxification of nicotine in tobacco solid waste by a *Pseudomonas* sp. Biotechnology Letters 26(19):1493-1496.

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ANALIZA SADRŽAJA CELULOZE U STABLJIKAMA I LISTOVIMA KRUPNOLISNIH DUVANA

Gordana J. Kulić* i Vesna B. Radojičić

Univerzitet u Beogradu, Poljoprivredni fakultet, Nemanjina 6, 11080 Beograd-Zemun, Srbija

R e z i m e

Duvanska stabljika predstavlja nedovoljno iskorišćen materijal koji ostaje nakon berbe listova. Obzirom na udeo krupnolisnih duvana u ukupnoj proizvodnji duvana u Srbiji (preko 85%), stabljike mogu predstavljati lako dostupnu i isplatljivu sirovinu u fabrikaciji celuloze. Cilj ovih eksperimentalnih istraživanja bio je određivanje sadržaja celuloze u listovima i pre svega u stabljikama krupnolisnih duvana, radi pružanja neophodnih podataka prerađivačima koji se bave preradom biomase. Kao materijal za istraživanje korišćeni su uzorci stabljika i listova dva tipa duvana, Berlej i Virdžinija, gajenih na području Srema. Primenjene su dve uporedne metode za određivanje sadržaja celuloze: metoda po Kürschner-Hanack-u i metoda po Updegraff-u. Može se zaključiti da su korišćene metode primenljive na duvan, s tim što je metoda po Kürschner-Hanack-u bila jednostavnija, praktičnija, brža i ekonomski isplatljivija, tako da se može preporučiti za rad. Rezultati istraživanja su pokazali da stabljike krupnolisnih duvana sadrže prosečno 30,50% do 34,30% celuloze. Listovi su imali značajno manje celuloze (16,00-17,20%). U odnosu na stabljike ova vrednost je manja za preko 50%. Razlike u sadržaju celuloze u zavisnosti od primenjene metode, nisu bile statistički značajne. Obzirom na broj stabljika koji zaostaje na njivama nakon berbe listova, moguće je iskoristiti 28000 tona celuloze.

Ključne reči: celuloza, duvanski listovi, duvanska stabljika, krupnolisni duvani.

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*Autor za kontakt: e-mail: gkulic@agrif.bg.ac.rs