Development of a technological procedure and selection of the corresponding optimal parameters of polysaccharides extraction from industrial wastes of tobacco production

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ABSTRACT

The optimum technological parameters of the extraction of a mixture of polysaccharides from tobacco wastes (plant stems, mouldy leaves, and tobacco dust) obtained from Gallaher Kazakhstan LLP are established. 50%-solution of ethanol is the optimal solvent. The ratio of the wastes and the solvent refers to 1:9 (v/v). Pre-infusion and continuous heating for 3 h at 80°C are required. Isopropanol is the optimal precipitator. The ratio of its application in respect to the wastes treated amounts to 1:5 (v/v). A process flow diagram of polysaccharide extraction from tobacco wastes is suggested. The results of this work can be applied to studies of comprehensive recycling in tobacco production.

Keywords: tobacco waste (Nicotiana tabacum L.), tobacco extraction, polysaccharides, a technological block diagram of polysaccharides extraction.

INTRODUCTION

Nicotiana tabacum L., also known as tobacco, is a significant economic product. Tobacco leaf production in China, for instance, is about 2.5 million tons in 2008 showing an increase of 22.9 % when compared with that in 2007 [1]. Nevertheless, over 20 % of tobacco resources are considered as processing waste, which causes a big issue in terms of its contribution to the pollution of the environment. The wasted tobacco leaves contain abundant bioactive compounds, such as polyphenols, proteins and aromatic compounds [1 - 3] making them economically valuable. Thus, it is relevant to study tobacco leaves to determine their chemical composition, and, hence, better utilize this resource.

Polysaccharides can often be found in plants and are composed of hundreds of monosaccharides. These natural high molecular weight polymers are highly valued for their multipurpose therapeutic properties, such as immune modulating, anti-pathogen, antitumor, antioxidant and anti-inflammatory activities [4].

Numerous innovative techniques are developed for the extraction of bioactive substances from plants. Mei et al. [5] study the effects of the extraction temperature, the extraction time, and the water/solid ratio on polysaccharides extraction from liriope roots. The effect of the same factors is studied by Samavati and Manoochehri-zade [6] in case of extraction of crude polysaccharides from the leaves of Mava Sylvestris.

Polysaccharides isolation from tobacco leaves is beneficial for using them as a nutritional supplement. The purpose of this study is to develop a technological procedure and select the optimal parameters of isolation of polysaccharides from industrial waste tobacco production. The further aim is to develop a waste-free production cycle.

EXPERIMENTAL

Sample description

The tobacco wastes (plant stems, mouldy leaves, and tobacco dust) used in this study were kindly provided by Gallaher Kazakhstan LLP (Almaty, Kazakhstan). They were dried at 45°C, sieved with a 40-mesh sieve (pore size, 0.42 mm) and stored in a dry room prior to the analysis.
Chemicals
Ethanol (96 %) was purchased from “Talgar-Spirt” (Talgar, Kazakhstan). Amyl alcohol and 2-butanol (≥ 99 %) were purchased from Sigma-Aldrich. Ethanol solutions of concentrations of 30 %, 50 %, 70 % and 80 % were prepared by diluting the initial ethanol solution (96 %) with distilled water. Dioxane-1,4 (≥ 99 %), ethyl acetate (≥ 99 %), chloroform, and isopropyl alcohol (≥ 99 %) were purchased from AppliChem GmbH (Darmstadt, Germany). Dimethylketone was purchased from Kazanorgsintez (Kazan, Russia). All chemicals were of an analytical grade.

Determination of the polysaccharides yield
The purpose of the research was to establish the total amount of the polysaccharides. The gravimetric method was chosen aiming this. Its advantage refers to its high selectivity to the carbohydrates polymeric forms. The four-fold excess of alcohol precipitates the polysaccharides and the proteins; mono-, oligosaccharides, and glycosides do not interfere with the analyses. The proteins were removed using the Sevag method [7].

The sample preparation
About 5.00 g of crushed tobacco wastes were placed into a 100 mL flask. Then, 50.0 mL of distilled water were added (the flask was connected to a reflux condenser), heated with stirring in a water bath for 1 h, and cooled. The extraction was repeated twice under identical conditions. The extracts were mixed and filtered. They were placed in a 250 mL volumetric flask together with the water used for the rinsing of the filter and the volume obtained was brought to the flask mark with additional distilled water.

The precipitation step
Twenty-five milliliters of this solution were placed in a centrifuge tube. Then 75 mL of 96 % ethanol were added, mixed and heated in a water bath at 60ºC for 5 min. After 30 min, the content was centrifuged at a 5000 rpm for 30 min. The supernatant was vacuum-filtered through a glass filter with a porosity size of 16. It was then dried to a constant weight at 100ºC - 105ºC. Then the precipitate was quantitatively transferred on the same filter and rinsed with 15 mL of 96 % ethanol. The filter with the precipitate was dried at 100ºC - 105ºC to a constant weight.

The Sevag method for proteins removal
The precipitate was dissolved in 5 mL of distilled water. 25 mL of chloroform and 5 mL of 2-butanol were added to remove the protein content from the polysaccharide solution. The flask was put in a stirrer for 1 h. The emulsion obtained was centrifuged for 15 min at 10,000 rpm. The top aqueous layer containing the polysaccharides was left. The procedure was repeated 3 times. Then 140 mL of 96 % ethanol were added to precipitate the polysaccharides, while the further biotesting procedure was done in accordance with Section 3.7.

The polysaccharides yield calculation
The polysaccharides yield based on the absolutely dry raw sample was calculated in percentage (X) using the following formula:

\[
X = \left( \frac{m_1 - m_2}{m} \right) \cdot \frac{250 \cdot 100}{100 - W} \cdot \frac{1}{25} 
\]

where \(m_1\) was the mass of filter (g), \(m_2\) was the mass of the filter with the precipitate (g), \(m\) was the mass of the tobacco wastes (g), while \(W\) stood for the losses during the drying of the raw materials (%).

The selection of the optimal technological schemes for obtaining substances from the plant raw materials was based on the achievement of the highest possible extraction of the target compounds. The following parameters were optimized: the extractant, its optimal ratio with the plant raw materials, the extraction time, the temperature and the mode.

Selection of the optimal extraction solvent
Different solvents, such as distilled water, 30 %-ethanol, 50 %-ethanol, 70 %-ethanol, 80 %- ethanol, 50 %-dioxane, ethylacetate, dimethylketone, 2-butanol, and dioxane were selected for the extraction process.

The next step of the substance production optimization referred to the determination of the optimum ratio between the raw materials and the extractant selected in the previous experiment. Obviously, the greater amount of the solvent used in the extraction process resulted in a greater amount of the substances dissolved, but maintaining a constant amount of the plant raw material was required. However, the increase of the amount of the extractant might lead to a decrease in BAS concentration in the extract, so it could not be infinite. Therefore, ratios of 1:5, 1:7, 1:9 and 1:10 (v/v) were selected aiming to
determine the optimal values of the ratio between the extractant and the plant raw material.

Selection of the optimal ratio of the selected extractant and the plant raw materials

Four experimental samples were prepared. About 5.00 g of the sample were placed in each flask. Then 25.0 mL, 35.0 mL, 45.0 mL and 50.0 mL of the solvent were added. Each flask was put in a water bath and refluxed for 3 h. The polysaccharide content in the extract was determined using the method described in Section 2.3.4.

The next step was to examine the temperature effect on the polysaccharides yield in case of using the selected optimal ratio between the plant raw materials and the solvent (50 %-ethanol), i.e. that of 1:9 v/v.

Selection of the optimal extraction temperature

Six samples each weighing 5.00 g were extracted by 45 mL of 50 %-ethanol. The first sample was left at a room temperature to stand until the next day (24 h).

Other samples were refluxed for 3 h in a water bath at 40ºC, 50ºC, 60ºC, 70ºC and 80ºC. The polysaccharide content was determined using the method described in Section 2.3.4. The data obtained is presented in the form of a histogram (Fig. 2).

Optimization of the extraction time

To establish the optimal extraction temperature, five samples each weighing 5.00 g were refluxed in a water bath with 45 mL of 50 %-ethanol within 1 h, 2 h, 3 h, 4 h and 5 h at 80ºC. The polysaccharide content was determined using the method described in Section 2.3.4.

Determination of the optimal extraction mode

Four experimental samples were prepared. The second and fourth samples were mixed with 50 %-ethanol in a ratio of 1:9 v/v and left at a room temperature for 24 h. The first and third samples were mixed with 50 %-ethanol in a ratio of 1:9 v/v on the next day. Then all four samples were heated at the selected optimum temperature of 80ºC using two modes: the third and fourth sample were heated within the optimal time in the
previous experiment (3 h); the first and second samples’ solvent was decanted each hour, and a new portion of 50 %-ethanol was added (3 h in total) (Fig. 4).

The subsequent tasks referred to the determination of the optimal precipitant of 50 %-alcohol extract and the optimal ratio of the extract and the precipitator.

**Determination of the optimal precipitant**

Four samples of the extract obtained by the optimized technological procedure were mixed with alcohols whose volume was four times greater than that of the sample: ethyl alcohol was used in case of sample 1, isopropyl alcohol was used in case of sample 2, butyl alcohol was used in case of sample 3, while amyl alcohol was used in case of sample 4. The samples were mixed with the alcohols pointed above and cooled for 2 h at 0°C. The polysaccharide content was defined by a quantitative method. The data obtained is presented in the form of a histogram (Fig. 5).

**Determination of the optimal ratio of the extract and the precipitator**

Equal amounts of the extract obtained by the optimized technological procedure were mixed with the optimal alcohol amount at different solid-to-fluid ratios: the first extract – at a ratio of 1:2 v/v, the second extract - at a ratio of 1:3 v/v, the third extract - at a ratio of 1:4 v/v, while fourth extract - at a ratio of 1:5 v/v. The mixtures containing the samples and the alcohol were cooled for 2 h at 0°C. The polysaccharide content was cooled by a quantitative method. The data obtained is presented in the form of a histogram (Fig. 6).

**RESULTS AND DISCUSSION**

**Selection of the optimal extraction solvent**

A color change is observed in all vials during the selection of the optimal extraction solvent, but not all cases show a high polysaccharides yield. This is valid for solvents as dioxane, 50 %-dioxane, 2-butanol, dimethyl ketone, and ethyl acetate. The solvents which provide the highest polysaccharides extraction refer to water and the 30 %-, 50 %-, 70 %- and 80 %-aqueous ethanol solutions. 50 %-ethanol (Table 1) is the optimal solvent of polysaccharides extraction.

**Selection of the optimal ratio of the selected extractant and the plant raw materials**

The optimal ratio of the raw materials and the extractant (50 %-ethanol) is 1:9 v/v (Fig. 1).
Selection of the optimal extraction temperature

Based on the values obtained, it is clear that the maximum polysaccharides yield at a ratio of 1:9 v/v is observed at 80°C (Fig. 2).

Optimization of the extraction time

According to the polysaccharides yield at different extraction times, it can be concluded that the optimal extraction time is 3 h (Fig. 3).

Determination of the optimal extraction mode

The data obtained shows that extraction with pre-infusion and continuous heating for 3 h is the most effective (Fig. 4).

Determination of the optimal precipitant

The further research shows that isopropanol is the best polysaccharides precipitant (Fig. 5).

Determination of the optimal ratio of the extract and the precipitator

The optimal ratio of the extract and the precipitator (in this case isopropyl alcohol) is 1:5 v/v (Fig. 6). Thus, the optimal technological scheme for obtaining the desired amount of polysaccharides from tobacco wastes can be presented as a block diagram (Fig. 7).

Biotesting of the polysaccharides

The study on the hepatoprotective activity of the polysaccharide phytocomplex is carried out using 100 white outbred male rats with a body weight of 220 g - 250 g. The generally accepted method of experimental (preclinical) studies of new drugs [8] in accordance with the regulations of the European Convention for protection of vertebrates used for experimental and other research purposes is applied. The rats are injected with a phytocomplex of polysaccharides 1 h prior to hepatotoxin application - carbon tetrachloride. The screening preparations reveal that all tested phytocomplexes have a hepatoprotective effect against acute poisoning at a low concentration (100 mg/kg); whereas, high doses (200 mg/kg and 300 mg/kg) cause blood circulation disorders. It is well recognized that there is a significant consumption of ATP (adenosinetriphosphate) under the effect of stress factors on the living organism; therefore, glucose is the main substrate for the synthesis of energy-rich compounds [9]. Thus, the protective effect of the investigated polysaccharide preparations can be attributed to the possibility of maintaining the energy status of the liver cells.

CONCLUSIONS

The optimal conditions of the extraction of polysaccharides from tobacco wastes refer to a 3-hour single extraction with 50%-ethanol at a ratio of 1:9 at 80°C with preliminary infusion duration of 24 h, followed by precipitation (with isopropyl alcohol at a ratio of 1:5). The results obtained in this research can be used to study the comprehensive recycling of the tobacco production.

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REFERENCES

3. Z.Teng, Q.Wang, Extraction, identification and