Obtaining Anthocyanidin Chlorides from Larch and Fir Bark

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Abstract

The effect of the concentration of hydrochloric acid and exposure time on the yield of anthocyanidin dyes obtained from larch and fir bark is investigated by means of photometry. Total mass fraction of anthocyanidin compounds and the fraction of these compounds present in bark in the bound state are determined. Kinetic parameters of the process of obtaining anthocyanidin chlorides from larch and fir bark are determined.

INTRODUCTION

In order to broaden the raw material basis and ensure reasonable consumption of plant raw material, much attention is paid within the recent years to processing of various plant wastes. One of large-scale wastes of wood processing is bark. Bark is rich in valuable biologically active compounds. Coniferous bark contains large amount of phenolic compounds of various classes possessing biological activity. The best studied and most widely used in Pharmacia, perfume and cosmetics manufacture, food and other industrial branches are flavonoid compounds and flavilium salts. Anthocyanidin dyes, one of the groups of flavilium salts, are widely used in food industry. Anthocyanidin dyes for food industry are obtained from flowers, seeds, berries, vegetables, and wastes of their processing, the so-called mill cakes [1]. Each type of raw material gives both anthocyanes (dyes containing saccharide residues) and anthocyanidins (aglycones, or pigments containing no saccharides).

Anthocyanidins occur in nature usually as glycosides with one or two glucose residues or groups of other simple saccharides attached to the hydroxyl in 3, or 3 and 5 positions, as well as in the form of leucoanthocyanidins (reduced anthocyanidins) [2]. Oxidation of catechins gives Anthocyanidins, too [3]. Because of this, it is possible to obtain anthocyanidin dyes from plant raw material by several methods, including acid hydrolysis of the corresponding glycosides, treatment of colourless leucoanthocyanidins with acids, and oxidation of catechins in acid medium.

At present, the conditions and methods of quantitative determination of anthocyanidin dyes in fruit and vegetable raw materials are mastered best of all [3]. The most widely used method of obtaining anthocyanidins from the fruit and vegetable raw material is hydrolysis of anthocyanes (glycosides) by hydrochloric acid (2 M) at 100 °C for 40 min, or by 6 M hydrochloric acid at the same temperature for several minutes.

However, the same compounds can be obtained by the appropriate treatment of coniferous bark or its extracts. Anthocyanidins can be present in bark of different species both in the free state and bound in lignocarbohydrate complex. For example, the major part of leucoanthocyanidins is present in the free state in the bark of leaf-bearing trees, for example, in birch [4]. Leucoanthocyanidins of birch bark are analyzed by transferring them into the corresponding coloured anthocyanidin chlorides; the concentration of hydrochloric acid is 6–7 %, and exposure is 30 min. The processes involved in obtaining anthocyanidin dyes from the bark of Siberian coniferous trees are insufficiently investigated.

In the present work, the optimal conditions for obtaining anthocyanidins from the larch and fir bark were selected. Kinetic parameters of the process involving of bark tanning substances transformation into anthocyanidins were determing.

EXPERIMENTAL

The initial raw material was the bark of freshly cut larch selected in the vicinity of Krasnoyarsk (October, 1999). The larch bark was sampled at a distance of 1–2 m from the base; fir bark was sampled after the bark was removed at the Krasnoyarsk Paper and Pulp Plant (October, 1999).

Bark was dried in a drying chamber at a temperature of 104–105 °C for 5–6 h, ground, sieved; the fraction with particle size less than 1 mm was collected and dried further on in a vacuum desiccator. The composition of the used larch bark was: extractives, 22.6 %; easily hydrolyzed polysaccharides, 13.8 %; difficultly hydrolyzed polysaccharides, 19.5 %; cellulose, 20.3 %; lignin, 21.2 %; ash matter, 2.3 %. The composition of fir bark was: extractives, 20.4 %; easily hydrolyzed polysaccharides, 14.5 %; difficultly hydrolyzed polysaccharides, 17.0 %; cellulose, 17.2 %; lignin, 26.8 %; ash matter, 1.9 %; humidity, 28 %.

Tar substances were extracted from the larch and fir bark using the Soxhlet apparatus. A weighed portion of bark (50 g, humidity up to 1 %) was extracted with hexane for 8 h in a 250 ml Soxhlet apparatus. The yield of tar substances from larch bark was 2.3 g (4.6 %), from fir bark 4.5 g (9.0 %). Total mass fraction of anthocyanidins present in larch and fir bark was determined using the following procedure. A weighed portion of bark of 0.0500 g, extracted preliminarily with hexane (to remove tar), was added to each of three volumetric flasks of 25 ml; 45 ml of ethanol was added, then 0.75 ml of 33.3 % hydrochloric acid (which corresponds to a 0.5 % solution of hydrochloric acid in ethanol). Then volumetric flasks were filled till the volume mark with ethanol, closed with reflux condensers and heated in thermostat at (75 ± 0.2) °C. After the necessary time interval, the flasks were cooled to 20 °C, the solution was filtered through the Schott filter filled with a polyamide sorbent (the height of the sorbent layer being 2 cm). Optical density was measured with a KFK-3 photometer at 550 nm. The mean optical density was calculated using the three parallel experiments; anthocyanidin chloride concentration was determined using the calibration plot.

A series of experiments was made with the concentrations of hydrochloric acid 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 %, and exposure 1, 2, 3, and 4 h. To determine the mass fraction of anthocyanidin dyes present in the bound state, we used larch or fir bark extracted sequentially in Soxhlet apparatus with hexane (to remove tar) and with 96 % ethanol (to remove the substances present in the free state and able to form anthocyanidin chlorides in the presence of hydrochloric acid. The analysis of anthocyanidins present in the bark in the bound state was performed in a similar manner. The calibration plot (Fig. 1) was drawn for standard solutions of cyanidin chloride obtained from quercetin [5].

The kinetics of transformation of tanning substances in larch and fir bark into anthocyanidin chlorides was investigated by sampling the solution in ethanol. A flask with a volume of 250 ml was filled with 100 ml of ethanol,

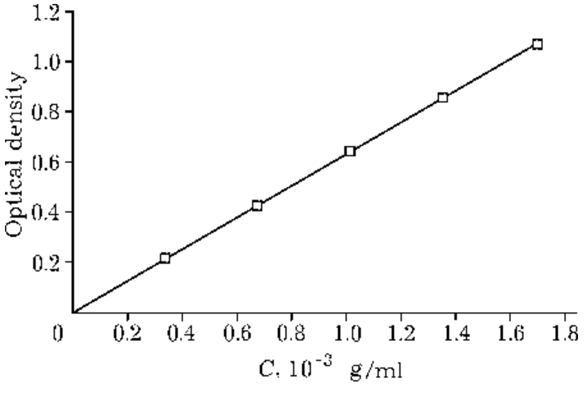

Fig. 1. Calibration plot for the standard solutions of cyanidin chloride.

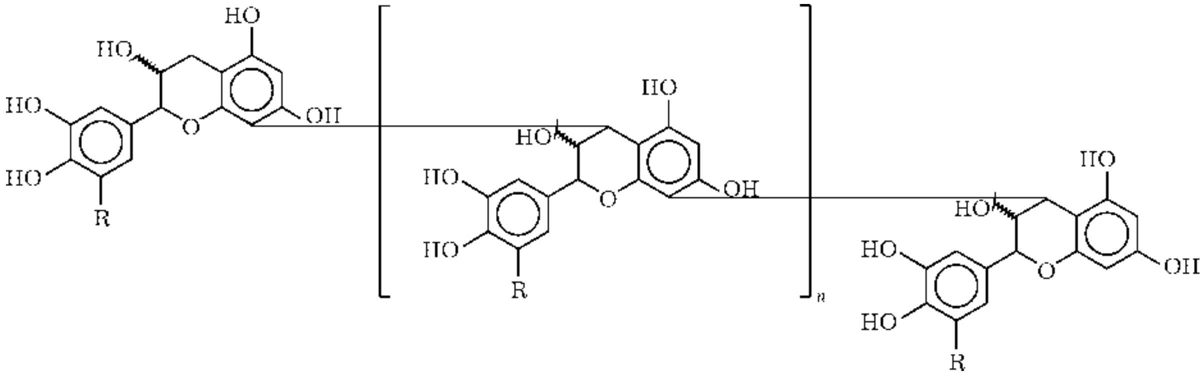

Fig. 2. Structure of condensed tanning substances. R = H − polymeric procyanidin; R = OH − polymeric prodelphinidin.

10 ml of tanning extract obtained by extraction of 15 g of the bark with 200 ml of ethanol in Soxhlet apparatus, and placed in a thermostat. After the necessary temperature was reached, we added 9 ml of concentrated hydrochloric acid; 1 ml of the reaction solution was samples each 5 min; the sample was added to 10 ml of ethanol, and the optical density was measured. The required temperature was maintained with the help of LP-207 ultrathermostat with an accuracy of ±0.2 °C. The rate of the transformation of tanning substances into anthocyanidin chlorides was calculated from the changes in optical density at 550 nm, where only the anthocyanidin dye absorbs light. The first-order rate constant (k_{ef}) was calculated from the obtained values using the least squares method [6]. The kinetic parameters of the reaction were calculated with the help of Arrhenius equation [7].

RESULTS AND DISCUSSION

Optimization of obtaining anthocyanidin chlorides from bark

We demonstrated in our previously published work [8], dedicated to the investigation of chemical composition of the anthocyanidin dye of larch bark, that the dye is formed as a mixture composed of different anthocyanidin chlorides (83−85 %); the major part of them is represented by cyanidin chloride and delphinidin chloride, while tanning substances account for 15−17 %. The anthocyanidin dye from the larch bark is composed mainly of cyanidin chloride, delphinidin chloride and peonidin chloride [9]. It was stated by separating and identifying the dyes with the help of column chromatography on polyamide sorbent that the tanning substances remain at the starting point. Because of this, in order to separate tanning substances for the purpose of optimizing the process of obtaining anthocyanidin dye from larch and fir bark, all the alcohol solutions were passed through a layer of polyamide sorbent after thermostating and before measuring optical density.

It is known that larch and fir bark is rich in catechins; their condensation in plants results in the formation of high-molecular compounds belonging to the class of tanning substances [10]. It is demonstrated in [11, 12] that many condensed tanning substances are mixed polymers composed on the basis of catechins and leucoanthocyanidins (flavan-3,4-diols). Prototypes of these polymers are proanthocyanidins (Fig. 2). The content of condensed catechins in larch bark can reach 20−22 %; in fir bark, 15−16 % [10, 13]. The acidic hydrolysis of condensed catechins results in the formation of monomolecular compounds of the catechin group; in turn, their oxidation in acidic medium results in the formation of anthocyanidin compounds. High yield of anthocyanidin dye from larch and fir bark can be explained by the fact that catechins comprise the major part of tanning substances. Oxidation reactions, establishing a close connection between catechins and anthocyanidins, are rather well investigated [2].

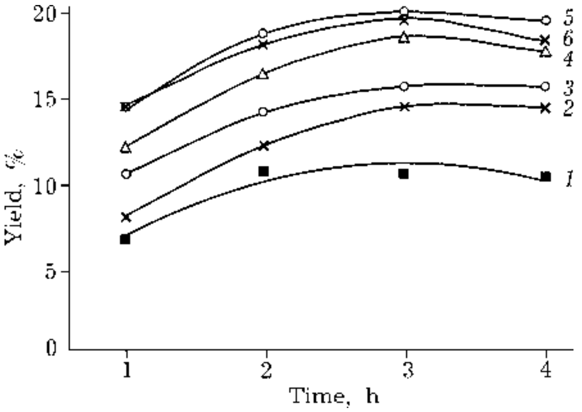

Fig. 3. Effect of the concentration of hydrochloric acid and exposure time on the total yield of anthocyanidin chlorides from larch bark. HCl content, %: 0.5 (*1*), 1.0 (*2*), 1.5 (*3*), 2.0 (*4*), 3.0 (*5*), 4.0 (*6*).

Because of high content of condensed catechins in larch and fir bark, one can assume that their depolymerization and oxidation of the formed catechins into anthocyanidin dye will require higher concentration of hydrochloric acid than that necessary for obtaining anthocyanidin chlorides from fruit and vegetable raw material. However, it was stated that the maximal yield of anthocyanidin dye (up to 20 % of the mass of absolutely dry larch bark (Fig. 3) and 16–17 % of the mass of fir bark) was achieved with the hydrochloric acid of about 3 % concentration.

In order to determine the fraction of substances that are able to form anthocyanidins and are included in the lignocarbohydrate complex of bark, we used the bark extracted preliminarily with ethanol. The treatment of the bark with ethanol helps extracting almost all the flavonoids present in the free state that are able to form anthocyanidins. One can see in Fig. 4 that the optimal conditions for the extraction of flavonoids present in the larch bark in bound state, and for transforming them into anthocyanidin chlorides, are 4 % concentration of hydrochloric acid and a 3 h duration of the process.

The 3–4 % concentration of hydrochloric acid and time of treatment with ethanol of about 3 h are optimal conditions for obtaining anthocyanidin dye from the fir bark; the yield of the dye is 16–17 %. The fraction of substances present in the fir bark in bound state, able to form anthocyanidin dyes, is only 13–15 %. It should be noted that a small amount (about 3 %) of the bound flavonoids (tanning substances, *etc.*) do not have any substantial effect on the whole process of obtaining anthocyanidin chlorides from larch and fir bark.

As we have mentioned above (see Fig. 2), the main sources from which anthocyanidin compounds are formed in the bark of coniferous trees are the tanning substances. The transformation of plant tanning substances of pyrocatechin nature into anthocyanidin dyes is only poorly investigated. Any kinetic data characterizing the transformation of tanning substances in fir and larch bark into anthocyanidins are absent from the literature. However, the kinetics of the transformation of natural polymers, i.e. cellulose and lignin, has been already investigated. In our opinion, the most similar type of transformations is involved in the hydrolysis of the amorphous part of cellulose; this process is described as the two sequential reactions of the first order [14, 15]:

$$\text{Cellulose} \xrightarrow{k_1} \text{Glucose} \xrightarrow{k_2} \longrightarrow \text{Hydroxymethylfurfural}$$

It is demonstrated that the rate constant of the transformation of cellulose into glucose is much less than the rate of glucose oxidation into hydrooxymethylfurfural.

Similar to cellulose transformation, the transformation of tanning substances (TS) into anthocyanidin dyes (AD) can be considered

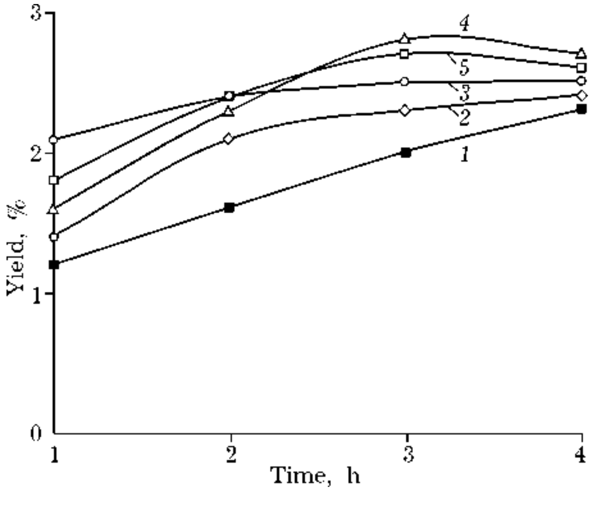

Fig. 4. Effect of the concentration of hydrochloric acid and exposure time on the yield of bound anthocyanidin chlorides from larch bark. HCl content, %: 1.5 (*1*), 2.0 (*2*), 3.0 (*3*), 4.0 (*4*), 5.0 (*5*).

TABLE 1

Rate constants for obtaining anthocyanidin chlorides from bark, k_{ef}, 10^{-4} s^{-1}

Bark	Temperature, °C				
	70	72	74	76	78
Larch	1.56	1.83	2.26	2.60	3.08
Fir	1.92	2.22	2.67	3.12	3.52

within the frames of the kinetics of a process including two sequential first-order reactions:

$$\text{TS} \xrightarrow{k_1} \text{Catechins} \xrightarrow{k_2} \text{AD}$$

Tanning substances and products of their transformations are well soluble in ethanol; because of this, the homogeneity of the process is evident. Polymeric tanning substances are depolymerized in acidic medium with the formation of catechins; they are oxidized to form anthocyanidin compounds. The slowest stage of the process is depolymerization of tanning substances, since otherwise catechin would be present in the reaction mixture during the transformation of tanning substances into anthocyanidin dyes; however, catechin was not detected in the system. This points to the fact that tanning substances are slowly depolymerized to form catechin ($k_1 << k_2$), which is rapidly transformed into anthocyanidin dye. This means that the whole process of obtaining anthocyanidin chloride is determined by the rate constant of depolymerization of tanning substances (k_1).

The kinetics of anthocyanidin chloride formation

The kinetics of the transformation of tanning substances into anthocyanidin compounds is investigated at different temperatures using the spectrophotometry. Since λ_{max} of tanning substances differs from that of anthocyanidin dyes, this method allows one to follow the transformations of tanning substances of fir and larch bark into anthocyanidin compounds. The presence of isosbestic points in the series of the recorded electronic spectra in the visible region depicting the transformation of tanning substances of fir and larch bark into anthocyanidin chlorides suggests that the process goes on without the formation of any by-products [16].

Rate constants of the formation of anthocyanidin chlorides from larch and fir bark, calculated assuming the first-order reaction, are listed in Table 1. Apparent energy and entropy of activation were calculated for the reactions under investigation (Figs. 5 and 6). Negative entropy of activation for the formation of anthocyanidin chlorides from larch bark (−72.6 J/(K mol)) and fir bark (−102.6 J/(K mol)) can be evidence of the fact that the system passes from disordered state characteristic of tanning substances (*i. e.*, polymers with the polymerization degree from 200–300 to 2500–3000) into a more ordered state represented by an-

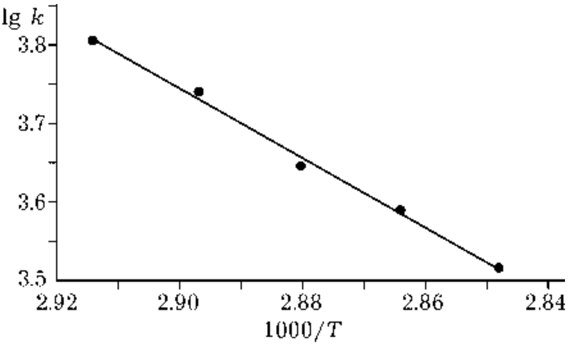

Fig. 5. Arrhenius plot for obtaining anthocyanidin chlorides from larch bark: E_{act} = (87.7 ± 5.4) kJ/mol, $\Delta H^{\#}$ = (84.8 ± 5.4) kJ/mol, $\Delta S^{\#}$ = −72.6 J/(K mol).

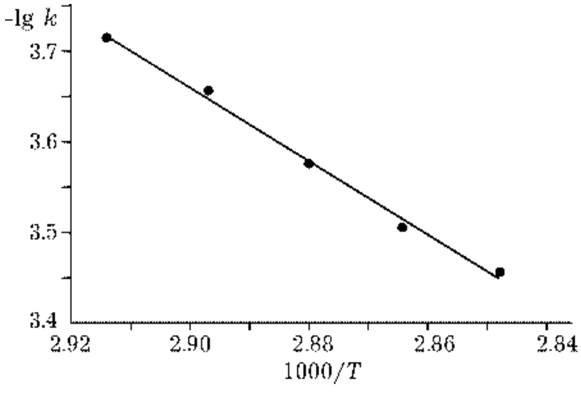

Fig. 6. Arrhenius plot for obtaining anthocyanidin chlorides from fir bark: E_{act} = (76.7 ± 4.6) kJ/mol, $\Delta H^{\#}$ = (73.8 ± 4.6) kJ/mol, $\Delta S^{\#}$ = −102.6 J/(K mol).

thocyanidin chlorides. This fact suggests that depolymerization of tanning substances into catechins proceeds slower than the oxidation of catechins into anthocyanidin chlorides, otherwise the ordering of the system would remain practically unchanged, since catechins and anthocyanidin chlorides should possess similar ordering degree; thus, entropy change would be insignificant.

It should be noted that higher energy of activation for obtaining anthocyanidin chlorides from larch bark (E_{act} = (87.7 ± 5.4) kJ/mol) in comparison with that for fir bark (E_{act} = (76.7 ± 4.6) kJ/mol) can be explained by the fact that tanning substances of larch bark have higher polymerization degree. Entropy of anthocyanidin chloride obtaining from larch bark is much less in its absolute value than that for fir bark (see Figs. 5, 6). One can assume that tanning substances of larch bark have a more uniform and ordered composition, which is in agreement with the literature data characterizing their factor of merit. For example, for the tanning extract obtained from larch bark, the factor of merit is 73 %, while that for fir bark is 53 %. Tanning agents possessing the best factor of merit have polymerization degree 300 to 3000. The factor of merit of the tanning extract decreases sharply when polymerization degree of the tanning compounds is below 300 or more than 3000 [13].

CONCLUSIONS

1. Optimal conditions for obtaining anthocyanidin dye from larch and fir bark are determined photometrically. It is stated that a maximal yield of anthocyanidin dye from larch bark (up to 20 %) and fir bark (16–17 %) is achieved using ethanol containing 3–4 % of hydrochloric acid; the duration of the process should be about 3 h.

2. It is stated that heating in an alcohol solution of hydrochloric acid leads to the formation of anthocyanidin chlorides from the major part of tanning substances of catechinic nature present in larch and fir bark. The fact that the tanning substances of larch and fir bark belong to the class of catechins is confirmed by their ability to be transformed into anthocyanidin dyes.

3. Kinetic data characterizing the transformation of tanning substances of fir and larch bark into anthocyanidin dyes are obtained.

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