Methods of Intensifying Extraction of Colorants from the Pericarp of Manchurian Walnut

Tamara Viktorovna Levchuk, Nikita Sergeevich Polonik, Natalia Yurievna Chesnokova, Lyudmila Vladimirovna Levochkina, Alla Alekseyevna Kuznetsova
School of Biomedicine, Far Eastern Federal University
690000, Russia, Vladivostok, Russky Island, Ajax St. 10

Abstract
The article determines the methods, conditions and parameters of extracting colorants from the pericarp of Manchurian walnut. The highest yield of dry matter was observed in ultrasonic extraction from the pericarp of walnut in milky and consumer maturity stages, respectively. Boiling a sample of Manchurian walnut pericarp in consumer maturity stage also contributed to increasing the yield of dry matter; its content was 16.7%. The least productive was soaking samples in ethanol – the yield of dry matter was 3.5% for the consumer maturity stage, and 2.4% for the milky maturity stage. The studied extracts were characterized by the presence of absorption maxima in the UV range (250-260 nm) and the visible spectral range (410-420 nm). The absorption maxima in these spectral ranges indicate the presence of quinonoid compounds, such as juglone, derivatives from 1,4-naphthoquinones and their glycosides in the extracts.

Keywords: Manchurian walnut pericarp, Manchurian walnut pericarp extracts, ultrasonic extraction, juglone, 1,4-naphthoquinone.

INTRODUCTION
Quinones represent a large and rather heterogeneous group of compounds. Their color varies from pale yellow through orange, red, purple and brown to almost black. They are important pigments in a number of fungi, lichens and certain groups of invertebrates. Quinones have also been extracted from the bark of trunk wood, roots, fruit, and pericarp of higher plants [1].

The main quinoid structure is cyclohexanedione, which is a derivative from monocyclic or polycyclic aromatic hydrocarbon. In natural quinonoid pigments, the main carbon skeleton usually contains substitutes, among which methyl, hydroxy- and methoxy-groups are met most frequently [2-5]. The most interesting representatives of pigments of the quinoid group are juglone (5-hydroxy-1,4-naphthoquinone), lawsone (2-hydroxy-1,4-naphthoquinone), 1,4-naphthoquinones and their derivatives.

One of the most available sources of quinonoid plant pigments is Manchurian walnut (Juglans manshurica Maxim). Manchurian walnut is widely distributed in Manchuria (Northern China), the Korean Peninsula, and in the Far East of Russia, in the Primorski Krai in particular [6-8].

By the morphological structure, the fruit of Manchurian walnut is composed of the pericarp and the walnut itself with a seed. The pericarp has the richest chemical composition. It contains quinones (juglone) up to 0.03%, alkaloids, 12-14% of tannin and colorants, 2.6% of cellulose, 18.4% of pectin, up to 12% of minerals, 0.8% of vitamin C, as well as quercetin, rutin and their derivatives [9]. The chemical composition of the Manchurian walnut pericarp explains its antioxidant, antibacterial, antiparasitic and anticancer properties [10-12].

There are various methods of extracting colorants [13-15] from plant material. However, the issue of intensifying their extraction still remains relevant. This research is aimed at studying the methods of intensifying and the conditions of extracting colorants contained in the pericarp of Manchurian walnut, which will allow obtaining extracts with high share of colorants and biologically active substances.

MATERIALS AND METHODS
The objects of the study were pericarp of Manchurian walnut (Juglans manshurica Maxim) in the milky (harvested in June) and consumer (collected in October) maturity stages and extracts based on it. The source samples were pericarp of Manchurian walnut in the milky maturity stage frozen at -18°C, and pericarp of walnut in the consumer maturity stage dried at 80°C. For extracting colorants from the pericarp of Manchurian walnut, the most common methods of extraction were chosen, such as 1) boiling of crushed pericarp samples in water for 5 min (samples 1 and 5); 2) soaking of crushed samples in 98% ethanol for 24 hours (samples 2 and 6); 3) extraction of crushed samples in water and 98% ethanol with the use of ultrasonic bath Crystal-2.5 (design and experimental production engineering office DEPEO) Kristall, Russia) for 30 min (samples 3, 4, 7, 8). The samples were ultrasonically treated with the exposure frequency of 44 kHz and the power of 0.3 kW.

For imaging of UV/Vis spectra, samples 2, 4-8 were diluted ten times with distilled water, and samples 1 and 3 with the highest content of dry matter were diluted one hundred times with distilled water. UV/Vis spectra were recorded with a Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan). Before recording UV/Vis spectra, all extract samples were centrifuged at 2,000 g at laboratory centrifuge Eppendorf-5810R and filtered through the Millipore filter nozzle (25 μm, polyethersulfone). Thickness of the light-absorbing layer in recording spectra was 1 cm. UV/Vis spectra were recorded in the wavelengths range between 800 and 250 nm.

To determine the content of dry matter, samples of extracts had been evaporated until dry, and then finally dried in vacuum until constant weight was reached.

RESULTS AND DISCUSSION
The studies have shown that the method of extraction and the medium used for extraction had a significant influence on the matter transition from the pericarp into the extract. The mass fractions of dry matter in samples of extracts from Manchurian walnut pericarp are shown in Table 1.

As shown in Table 1, the highest yield of dry matter (3.4-5.8% and 6.5-33.4%) was observed in ultrasonic extraction from the pericarp of Manchurian walnut in milky and consumer maturity stages, respectively. Using water as an extractant for ultrasonic extraction from the pericarp of Manchurian walnut of consumer maturity resulted in the maximum dry matter yield (33.4%). On the contrary, ultrasonic extraction of soluble substances from the walnut pericarp in the milky maturity stage went better in ethanol (5.8%).

Boiling samples of consumer maturity stage Manchurian walnut pericarp ensured increased extraction of dry matter, and was 16.7%. However, the use of this method of extracting dry substances from Manchurian walnut pericarp in the milky maturity stage reduced their yield 5.5 times.

The least productive was soaking samples in ethanol – the yield of dry matter was 3.5% for the consumer maturity stage, and 2.4% for the milky maturity stage.
Table 1. The mass fractions of dry matter in samples of extracts from Manchurian walnut pericarp

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Initial walnut sample</th>
<th>Extraction method</th>
<th>Dry matter content in the extract, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dry walnut, consumer stage, 5 g</td>
<td>Boiling in water, 5 minutes</td>
<td>16.7±0.5</td>
</tr>
<tr>
<td>2</td>
<td>Dry walnut, consumer stage, 5 g</td>
<td>Soaking in ethanol (98%), 1 day</td>
<td>3.5±0.2</td>
</tr>
<tr>
<td>3</td>
<td>Dry walnut, consumer stage, 5 g</td>
<td>Ultrasonic extraction, 30 min, water</td>
<td>33.4±1</td>
</tr>
<tr>
<td>4</td>
<td>Dry walnut, consumer stage, 5 g</td>
<td>Ultrasonic extraction, 30 min, ethanol</td>
<td>6.5±0.1</td>
</tr>
<tr>
<td>5</td>
<td>Frozen walnut, milky stage, 5 g</td>
<td>Boiling in water, 5 minutes</td>
<td>3±0.3</td>
</tr>
<tr>
<td>6</td>
<td>Frozen walnut, milky stage, 5 g</td>
<td>Soaking in ethanol (98%), 1 day</td>
<td>2.4±0.1</td>
</tr>
<tr>
<td>7</td>
<td>Frozen walnut, milky stage, 5 g</td>
<td>Ultrasonic extraction, 30 min, ethanol</td>
<td>3.4±0.1</td>
</tr>
<tr>
<td>8</td>
<td>Frozen walnut, milky stage, 5 g</td>
<td>Ultrasonic extraction, 30 min, water</td>
<td>5.8±0.4</td>
</tr>
</tbody>
</table>

In general, it was found that walnut in the consumer maturity stage is characterized by the significantly higher dry matter content in the extracts obtained by boiling in water and by ultrasonic extraction. Insufficient extraction of dry matter in samples of Manchurian walnut pericarp in the milky maturity stage is most likely due to the more dense structure of its cell wall and lower content of colorants in this period of walnut ripening.

The qualitative composition of the obtained extracts of Manchurian walnut pericarp in various maturity stages was studied through UV/Vis-spectra. UV/Vis spectra of extracts from Manchurian walnut pericarp in the consumer and milky maturity stage are shown in Figures 1 and 2.

![Figure 1. UV/Vis spectra of the extracts from Manchurian walnut pericarp in the consumer maturity stage. Digits indicate the UV/Vis spectra of respective samples.](image1)

As follows from the recorded UV/Vis spectra, most studied extracts are characterized by the presence of absorption maxima in the UV range (250-260 nm) and in the visible spectral range (410-420 nm) (Figure 1 and 2). The absorption maxima in these spectral ranges indicate the presence of quinonoid compounds, such as juglone, derivatives from 1,4-naphthoquinones and their glycosides in the extracts (Fig. 3).

The results show that the absorption maximum in the visible range (410-420 nm) for samples of extracts from pericarp in the consumer maturity stage is expressed more weakly (Fig 2) than in the spectra of Manchurian walnut pericarp samples in the milky maturity stage. This may be explained by decay of unstable glycosides of 1,4-naphthoquinones in the pericarp of Manchurian walnut during ripening from the milky to the consumer maturity stage, which results in decreasing the content of quinoid connections.

To confirm the presence of juglone and 1,4-naphthoquinone derivatives in pericarp extracts, UV/Vis spectra of some individual quinonoid compounds were additionally recorded: lawsone – the main pigment of henna, isonaphtazarine, 2-hydroxy-juglone and 3-hydroxy-juglone (Fig. 3). All the studied quinones are characterized by the presence of a pronounced absorption band in the UV range (260-290 nm). The absorption maximum in the visible range (410-420 nm) is observed only for two isomers of hydroxy-juglone – 2-hydroxy-juglone and 3-hydroxy-juglone. Thus, the presence of juglone derivatives in the extracts of Manchurian walnut pericarp has been verified by comparing the UV/Vis spectra of the extracts and samples of quinonoid compounds.

![Figure 2. UV/Vis spectra of the extracts from Manchurian walnut pericarp in the milky maturity stage. Digits indicate the UV/Vis spectra of respective samples.](image2)

![Figure 3. UV/Vis spectra of some quinonoid compounds (1 - isonaphtazarine, 2 - lawsone, 3 - 3-hydroxy-juglone, 4 - 2-hydroxy-juglone).](image3)
CONCLUSION
The use of ultrasound at the frequency of 44 kHz and at the power of 0.3 kW for 30 minutes for extracting colorants from Manchurian walnut pericarp is the most efficient method. With that, the degree of dry matter extraction increases more than twice, compared to the traditional methods. The chosen modes allow obtaining extracts with high content of colorants and biologically active substances from plants.

REFERENCES