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# SUBSTANTIATION OF TECHNOLOGICAL PARAMETERS OF THE OIL EXTRACT OBTAINED FROM URTICA DIOICA ROOTS BASED ON THE YIELD DETERMINATION OF PHYTOSTEROLS USING CAPILLARY GAS CHROMATOGRAPHY METHOD

**Mariana Fedorovska, Inna Yarema, Natalia Polovko, Iryna Ivanchuk**

*Due to the content of phytosterols, extractive preparations of Urtica dioica roots are able to show antiandrogenic effect in the case of external therapy of men and women with androgenic alopecia. Oil extracts (OE) are characterized by several advantages when applied to the skin of the scalp compared to water-alcohol extracts. For the development of OE technology from Urtica dioica roots, it is important to choose the optimal extraction parameters, which are based on the quantitative determination of phytosterols in the extractant and the studied samples of extracts.*

***The aim of the work*** *is to choose the optimal parameters for obtaining OE from Urtica dioica roots based on quantitative determination of phytosterols content in experimental samples of OE by gas capillary chromatography.*

***Materials and methods.*** *Objects of the research – Urtica dioica root, refined corn oil, refined sunflower oil, samples of oil extracts. Determination of phytosterol content in experimental samples was carried out by gas capillary chromatography (chromatograph "Crystal 2000", manufacturer – research and production company "Analytics").*

***Results.*** *5 different compounds of steroid structure (stigmasterol, β-sitosterol, etc.) were identified in sunflower oil by gas liquid chromatography, and 10 (campesterol, 2-α stigmasterol, β-sitosterol, Δ5-avenosterol, etc.) were identified in sunflower oil. The quantitative content of β-sitosterol in the sum of sterols of corn oil was significantly higher compared to the content of this substance in sunflower oil and amounted to 59.33 %. Optimal technological parameters were established considering the peculiarities of extraction with oil extractant and quantitative determination of the amount of phytosterols and β-sitosterol in experimental samples of OE. The total content of plant sterols in OE, including considering their amount in the extractant, was in the range of 7880 mg/kg; the amount of β-sitosterol was 4638 mg/kg.*

***Conclusion.*** *The choice of optimal parameters for obtaining OE from UDR based on determination of phytosterol yield by gas capillary chromatography was experimentally substantiated, namely: extractant – corn oil, raw material-extract ratio – 1:5, extraction time – 6 h, extraction method – maceration*

***Keywords:*** *Urtica dioica root, androgenic alopecia, oil extract, extraction parameters, phytosterols, gas capillary chromatography*

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## 1. Introduction

The group of herbal extracts includes oil extracts (Extracta oleosa) or medicinal oils (Olea medicata). Refined and cold pressed vegetable oils such as sunflower, corn, apricot, peach, almond, olive, soybean, peanut, sesame, coconut oil and others are used for extraction of medicinal plant raw materials (MPRM) [1].

Oil extracts (OE) are obtained by various technological methods: maceration, remaceration, extraction countercurrent and circulating, etc. OE technology in some cases could be complicated by the low intensity of mass metabolism between lipophilic components of plant cells and the extractant. However, the techniques used to intensify the extraction of BAS should not cause their destruction [2].

OEs, which are used in cosmetics for hair care, are called hair oils (HO) or hair tonics. Along with other hygienic hair care cosmetics such as lotions, shampoos, balms, masks, etc., HO has a number of benefits, namely: soften, give shine, promote easy combing, moisturize hair and scalp, regulate sebaceous glands, improve the nutrition of hair follicles due to the content in the base-carriers of unsaturated fatty acids, phospholipids, sterols and other biologically active substances (BAS) [3, 4].

The literature describes various methods of obtaining vegetable HO, namely: the cloth method, paste method, direct boiling method [5].

Many types of MPRM are used to obtain HO, including traditional (arnica flowers, birch leaves, burdock roots, mustard seeds) and raw materials of tropical and subtropical climates (white verbena grass (Eclipta alba L.), Asiatic pennywort grass (Centella asiatica (L.) Urb), henna leaves (Lawsōnia inērmis L.), flowers and leaves of Chinese hibiscus (Hibiscus rosa-sinensis L.), seeds of hawthorn (Trigonella oenumgraecum L.), etc.) [3, 4, 6]. There is known HO from the roots of burdock called "Burdock oil" (manufacturers: PJSC CPP "Chervona Zirka", Ukraine; Elfa Pharm, Poland). This

compound stimulates metabolism in the skin of the scalp, eliminates dandruff, prevents baldness [7].

HO technology uses a wide range of vegetable oils that have a fatty acid composition and, accordingly, are characterized by different properties. As a rule, the composition of oils combines saturated (palmitic, stearic), monounsaturated (oleic, palmitoleic) and polyunsaturated (linoleic, linolenic, arachidonic) fatty acids.

According to the literature in Table 1 shows the fatty acid composition of triglycerides of vegetable oils, which are used as MPRM extractants in the technology of NO [8, 9].

Table 1

The composition of fatty acids in vegetable oils

| Fatty oils | Fatty acid content, % | | |
|---|---|---|---|
| | Saturated | Monoun-saturated | Polyunsatu-rated |
| Apricot | 5.93 | 73.43 | 20.64 |
| Peanut | 18.20 | 48.50 | 33.30 |
| Corn | 11.06 | 27.87 | 61.07 |
| Flax | 11.32 | 15.02 | 73.66 |
| Almond | 7.27 | 71.02 | 21.71 |
| Olive | 15.57 | 73.39 | 11.04 |
| Sesame | 15.33 | 39.27 | 45.40 |
| Soybeans | 15.55 | 21.44 | 63.01 |
| Sunflower | 11.37 | 25.93 | 62.70 |

The composition of corn and sunflower oil (Table 1) is dominated by polyunsaturated fatty acids (more than 60 %) and a sufficiently high content of monounsaturated fatty acids (more than 25 %). Monounsaturated oleic acids are characterized by high resistance to self-oxidation, thermal stability and, accordingly, long shelf life. Polyunsaturated acids (α-linolenic, linoleic) are actively involved in the regeneration of cell membranes [8, 9]. Sunflower and corn oils are traditional domestic products produced on an industrial scale, as Ukraine has a significant raw material base. Therefore, considering the peculiarities of the composition of the FA, as well as the availability of raw materials, in experimental studies, it is advisable to use these extractants.

Vegetable oils, in addition to fats and free fatty acids, contain BAS of hydrophobic nature, among which there are phytosterols (β-sitosterol, campesterol, stigmasterol, etc.). According to literature sources, sunflower oil contains (3–7) g/kg of phytosterols, and corn oil – (6–15) g/kg [10, 11]. The presence of phytosterols in the oils gives them additional benefits, as they combine the functions of both auxiliary and active ingredient.

For the production of OE with antiandrogenic action for scalp skin care, we chose nettle root (UDR). UDR contains about 1 % of phytosterols (β-sitosterol, campesterol, ergosterol, stigmasterol, etc.), fatty acids, lectins, lignans, polysaccharides, amino acids, trace elements, etc. [12, 13]. Due to the content of phytosterols, UDR extracts have antiandrogenic action (inhibit 5-α-reductase, block androgen receptors), so their effectiveness in the treatment of androgenic alopecia in cutaneous use has been confirmed [14–18].

From a chemical point of view, phytosterols are 4-des methyl sterols and stanols, which contain hydroxyl groups in position 3 and are characterized by a similar structural structure to cholesterol. Phytosterols, especially conjugated forms with fatty acids (FA), are hydrophobic substances that are insoluble in water, soluble in (90–96) % ethanol, chloroform, vegetable oils and other non-polar solvents. Accordingly, non-aqueous hydrophobic extractants should be used to extract BAS from UDR [10, 19].

To remove BAS from MPRM, you could use high concentration ethanol (70–96) % in the manufacture of tincture or liquid extract. However, strong ethanol will dry out the scalp skin and hair. Circulating extraction of chloroforms with subsequent extraction with ethanol in the liquid-liquid system can be used to obtain a thick extract. However, for topical use, the thick extract must be introduced into a suitable carrier (soft or liquid dispersion medium). Vegetable oil as an extractant has a number of advantages for extracts used in trichology, namely: well dissolves free and bound phytosterols, to accelerate and complete the extraction, you can use different temperatures, the oil penetrates the skin and is an active carrier of BAS, safe for long-term dermal application, has an emollient and nourishing effect on the skin of the scalp [20].

Maceration is a traditional static method of extraction, which consists in infusing MPRM with heated vegetable oil for a certain period of time, which depends on the type of MPRM, temperature regime, features of MPRM processing. The main disadvantages of the method are the duration of the process and the relatively low percentage of MPRM depletion. On the other hand, OE is insisted at an elevated temperature, which significantly reduces the extraction time (compared to tincture technology).

Various modifications of the maceration method are used to increase the BAS yield and reduce the extraction time, such as fine maceration (remaceration), maceration with forced circulation of the extractant, etc. [21, 22]. Remaceration is the division of the extractant into several parts and the gradual infusion of MPRM with each part. The use of a fresh portion of the extractant creates a difference in BAS concentrations in the raw material and extract, which allows you to effectively deplete MPRM in a shorter period of time. This method is possible when the ratio between the volume of MPRM and the liquid is significant, as it is necessary to pour the raw material into the "mirror" each time.

**The aim of the work** – selection of optimal parameters for obtaining OE from nettle roots on the basis of quantitative determination of phytosterols content in experimental samples of OE by gas capillary chromatography.

**2. Research planning (methodology)**

Substantiation of the choice of extraction parameters during the development of OE technology with UDR was carried out in 6 stages (Table 2).

Table 2

Experiment planning

| No. | Technological parameters | Selection options: |
|---|---|---|
| 1 | Extractant | – literature data on the content of fatty acids and phytosterols in vegetable fatty oils, the availability of raw materials on the domestic market [1]; – identification and quantification of phytosterols and β-sitosterol; |
| 2 | Temperature | – choice of extraction temperature, concentration and amount of humidifier according to the literature [2, 20, 21, 23, 24]; |
| 3 | Moisturizer | |
| 4 | "Raw material-extract" ratio | – identification and quantification of phytosterols and β-sitosterol; |
| 5 | Extraction time | |
| 6 | Extraction method | – theoretical analysis of literature data on extraction methods [21, 25]; – identification and quantification of phytosterols and β-sitosterol. |

## 3. Materials and methods

### 3. 1. Plant materials

**Nettle roots** (Urticae dioica Radix) (SPhU 2.1). Raw materials were harvested in Western part of Ukraine (Ivano-Frankivsk region) in the autumn period (end of September–October) in the phase when there is no vegetation of the aboveground part of the plant. Dried ground MPRM with particle sizes in the range of 1–5 mm was used for the study. Raw materials on morphological and microscopic characteristics met the requirements of SPhU 2.1 [26].

### 3. 2. Chemicals and standards

Extractives: refined corn oil (*Maydis oleum raffinatum*, *Refined Maize Oil*) and refined sunflower oil (*Helianthi annui oleum raffinatum,* EP; *Refined Sunflower Oil,* BP) [27], ethanol 96 % (SPhU 2.0, Vol 2. p. 233–238) [26].

Reagents: 95 % ethanol; chloroform; hexane; ethyl acetate; petroleum ether; diethyl ether; pyridine; rhodamine W; cholesterol; hexamethyldisilosane; trimethylchlorosilane (TRI-SIL); potassium hydroxide – 60 % solution; sodium chloride; anhydrous sodium sulfate; β-sitosterol 1 g (purity not less than 90 % (P), HWI-Pharmaservices GmbH); stigmasterol 1 g (purity not less than 90 % (P), HWIPharmaservices GmbH) [28].

### 3. 3. Preparation of OE samples

Various factors were taken into account in the manufacturing process: MPRM to extract ratio, time and extraction method. The required portion of crushed MPRM was weighed on electronic scales, placed in glass containers, mixed with 90 % ethanol (ethanol to MPRM ratio – 0.6:1) and left to soak for 2 h [23]. Then 100 g of refined corn oil was added to obtain a "mirror" (if necessary, rammed MPRM), the mixture was heated to a temperature of (45±5) °C and left in a hot water bath to infuse for 6 hours or other duration if the optimal time extraction). The temperature was maintained at the required level, periodically heating the water bath. The cooled samples of OE were squeezed out, filtered through several layers of gauze; if necessary, the oil was adjusted to a mass of 100 g

### 3. 4. Identification and quantification of phytosterols in vegetable oils and OE samples by gas capillary chromatography [28]

The method is based on saponification of the oil with a solution of potassium hydroxide in ethyl alcohol, and the residue that cannot be saponified is removed with diethyl ether. Sterols are converted to trimethylsilyl esters and analyzed by gas capillary chromatography against a standard β-sitosterol.

*Devices:* saponification flask with reflux condenser and glass sections with a capacity of 150 ml; dividing funnel with a capacity of 500 ml; flasks with a capacity of 250 ml; pressure equalization funnels, 250 ml or similar in size, to collect diethyl ether residues; glass column, 350×20 mm, equipped with a stopper made of porous glass; water bath; reaction tubes, 2 ml.

*Methods of sample preparation*

The samples are heated to a temperature not exceeding 35 °C, mixed thoroughly with shaking. Weigh, to the nearest 0,001 g, approximately 1 g ($m_2$) of oil (OE) and transfer to a saponification flask with a reflux condenser and 150 ml glass sections. Add 50 ml of ethanol and 10 ml of 60 % potassium hydroxide solution. Connect the reflux condenser and heat at about 75 °C for 30 minutes. Disconnect the reflux condenser and cool the flask to about ambient temperature. Pour the contents of the flask into a separatory funnel, rinse the flask with 50 ml of water and 250 ml of diethyl ether. Thoroughly shake the separatory funnel for 2 minutes and allow the phases to separate. Drain the lower aqueous layer and wash the diethyl ether layer by shaking it with 100 ml of water 4 times in a row. To prevent the formation of an emulsion, it is necessary that the first two washes with water were carried out quietly (10 rotations). The third wash can be more thorough within 30 seconds. If the emulsion is formed, it can be destroyed by adding (5–10) ml of ethanol. In the case of adding ethanol, it is important to do a thorough wash twice more, because the presence of ethanol at the stage of rape affects the process of obtaining trimethylsilyl derivatives. Pass a clean layer without soap through a glass column containing 30 g of anhydrous sodium sulfate. Collect the ether in a 250 ml flask. Ceramic granules are added to prevent boiling, and evaporated almost to dryness in a water bath, trying to collect spent solvents.

*Method of preparation of trimethylsilyl esters.*

Pour the ether solution remaining in the flask into a 2 ml reaction tube containing 2 ml of diethyl ether and remove the ether with a stream of nitrogen. Rinse the flask twice with 2 ml of diethyl ether, each time pouring the solution into a test tube and removing the ether with a stream of nitrogen. Strengthen the sample by adding 1 ml of TRI-SIL reagent. Close the tube and shake thoroughly to dissolve. If dissolution is not complete, heat to (65–70) °C. Leave for at least 5 minutes before introduc-

tion into the gas chromatograph. Silylation should be carried out in a dehydrated environment.

*Standard solution of β-sitosterol:* prepare a solution of β-sitosterol in diethyl ether with a concentration of approximately 0.5 mg/ml (m1); the sample is taken to the nearest 0.001 g. A standard sample of β-sitosterol (HWIPharmaservices GmbH) was used for the study.

*Standard chromatographic solution of β-sitosterol*: add 1 ml of standard solution of β-sitosterol to the reaction tube and remove diethyl ether with a stream of nitrogen. Strengthen the sample by adding 1 ml of TRI-SIL reagent. Close the tube and shake thoroughly to dissolve. Leave for at least 5 minutes before introduction to the gas chromatograph.

*Chromatography conditions:* device – chromatograph "Crystal 2000"; column – DB 5Æ0.32 mm ´ 30 m, 0.25 μm; column thermostat temperature – 220 °C (holding for 2 min), temperature increase with a speed of 7 °C/min to 290 °C (holding for 15 min); injector temperature – 320 °C; detector temperature – 320 °C; carrier gas – helium; volumetric velocity – 30 ml/min; additional gases: nitrogen – 10 ml/min, air – 300 ml/min, hydrogen – 30 ml/min; flame ionization detector; flow separation – 1:5; injection volume – 1 μl.

The system is preliminarily tested by internal normalization by chromatographing a standard mixture of β-sitosterol and stigmasterol and lanosterol and confirming that the total peak area is 100 % for sterols and that sterols caused the same detector response.

*Analytical procedure.*

Inject 1 μl of sylated standard β-sitosterol solution and adjust the integrator settings. Inject another 1 μl of sylated standard β-sitosterol solution and determine the peak area (S1) for the standard β-sitosterol sample. Inject 1 μl of the sylated analyte solution and calculate the areas of all peaks.

*Calculation of results.*

Determine the content of β-sitosterol in the analyzed sample according to the following formula:

$$X, \text{mg/kg} = \frac{S_2}{S_1} \times \frac{m_1}{m_2} \times P \times 10, \qquad (1)$$

where $m_1$ – mass of β-sitosterol contained in 1 ml of standard solution, mg;

$m_2$ – sample weight, g;

$S_1$ – the peak area of β-sitosterol for the standard sample;

$S_2$ – the peak area of β-sitosterol for the analyzed sample;

$P$ – the content of β-sitosterol in the standard sample, %.

Determine the content of the amount of phytosterols in the analyzed sample according to the following formula:

$$\sum X, \text{mg/kg} = \frac{\sum S}{S_1} \times \frac{m_1}{m_2} \times P \times 10, \quad (2)$$

where $m_1$ – mass of β-sitosterol contained in 1 ml of standard solution, mg;

$m_2$ – sample weight, g;

$S_1$ – the peak area of β-sitosterol for the standard sample;

$\Sigma S$ – the total area of the peaks for the analyzed sample;

$P$ – the content of β-sitosterol in the standard sample, %.

### 3. 5. Statistical analysis

Statistical analysis of the results was performed according to the methods described in SPhU 2.0, Vol. 1, § 5.3, using Statistica 6.0 program and Microsoft Excel 2016. All determinations were performed in five samples ($n$=5), the results are presented as the mean±confidence interval (CI); $P$=95 %.

## 4. Results

The first stage of the experiment was to confirm the qualitative and quantitative content of phytosterols in sunflower and corn oil by gas capillary chromatography. The results of the research are given in the form of chromatograms (Fig. 1, 2) and the characteristics of the peaks and the percentage of phytosterols in the studied vegetable oils (Tables 3, 4).

After theoretical justification of the choice of extractant samples of OE were obtained in refined corn oil in the ratio from 1: 2 to 1: 8 with a multiplicity of 1. The required portions of crushed MPRM were weighed on laboratory electronic scales AXIS BTU-210 (Table 5) and samples of OE were prepared according to paragraph 3. 3.

The choice of the optimal ratio of MPRM-extract was justified by the results of determining the sum of phytosterols and β-sitosterol by gas capillary chromatography in OE samples. The research results are shown in Fig. 3, 4.

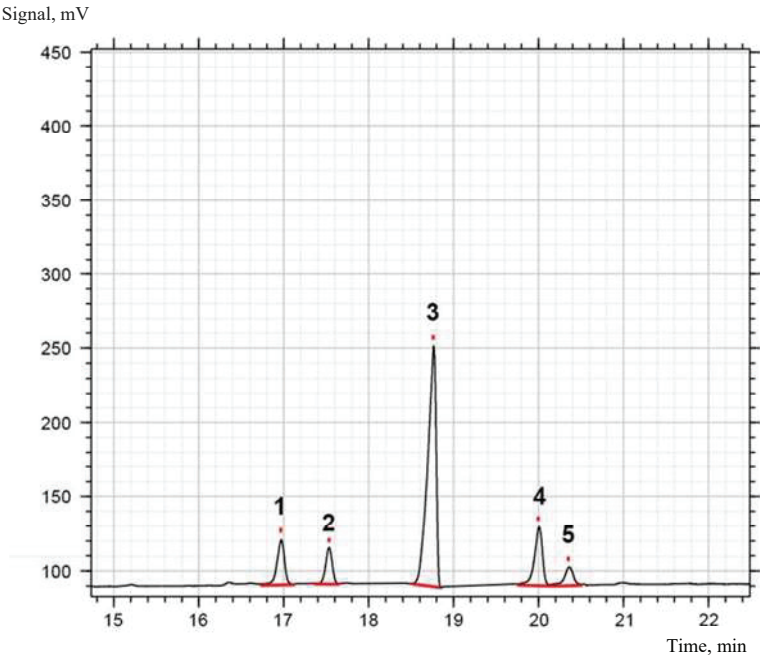


Fig. 1. Chromatogram of phytosterols of sunflower oil

Table 3

Characteristics of peaks and percentage of phytosterols in sunflower oil

| No. | Substances | Time, min | Height | Area | Concentration, mg/kg | Concentration, % |
|---|---|---|---|---|---|---|
| 1 | Campesterol | 16.966 | 57.226 | 3.2509 | 263.25 | 9.75 |
| 2 | Stigmasterol | 17.532 | 46.548 | 1.8400 | 149.04 | 5.52 |
| 3 | β-sitosterol | 18.762 | 324.13 | 21.591 | 1748.25 | 64.75 |
| 4 | Δ7-avenosterol | 19.997 | 74.040 | 5.3236 | 431.19 | 15.97 |
| 5 | Unidentified | 20.350 | 22.127 | 1.3399 | 108.27 | 4.01 |
| Sum | | | | 33.3454 | 2700 | 100 |

Table 4

Characteristics of peaks and percentage of phytosterols in corn oil

| No. | Substances | Time, min | Height | Area | Concentration, mg/kg | Concentration, % |
|---|---|---|---|---|---|---|
| 1 | Campesterol | 16.951 | 81.744 | 7.3500 | 593.856 | 10.31 |
| 2 | Stigmasterol | 17.511 | 59.874 | 5.2830 | 426.816 | 7.41 |
| 3 | Unidentified | 17.970 | 27.214 | 2.6127 | 211.392 | 3.67 |
| 4 | Unidentified | 18.036 | 14.522 | 1.0795 | 86.976 | 1.51 |
| 5 | Unidentified | 18.305 | 6.7841 | 0.77058 | 62.208 | 1.08 |
| 6 | β-sitosterol | 18.755 | 364.73 | 42.283 | 3417.408 | 59.33 |
| 7 | Δ5-avenosterol | 18.992 | 20.671 | 3.1926 | 258.048 | 4.48 |
| 8 | Δ7-stigmastenol | 19.544 | 7.7622 | 1.4478 | 116.928 | 2.03 |
| 9 | Δ7-avenosterin | 19.944 | 59.015 | 5.3740 | 434.304 | 7.54 |
| 10 | Unidentified | 20.311 | 18.764 | 1.8783 | 152.064 | 2.64 |
| Sum | | | | 71.27148 | 5760 | 100 |

Table 5

The amount of MPRM for the preparation of 100 g of OE

| The ratio of MPRM – extract | | | | | | | |
|---|---|---|---|---|---|---|---|
| The amount of MPRM per 100 g of extract, g | 1:2 | 1:3 | 1:4 | 1:5 | 1:6 | 1:7 | 1:8 |
| | 50.0 | 33.3 | 25.0 | 20.0 | 16.7 | 14.3 | 12.5 |

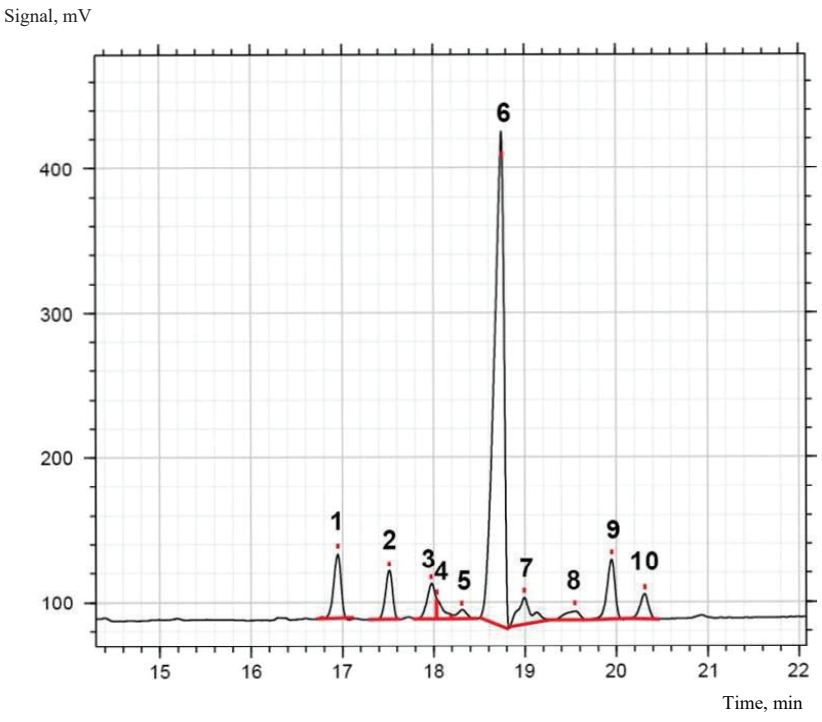


Fig. 2. Chromatogram of phytosterols of corn oil

Table 6

The results of determining the amount of phytosterols depending on the method of extraction

| No. | Method of extraction | Quantitative content of the amount of phytosterols, mg/kg |
|---|---|---|
| 1 | Maceration, infusion time – 6 hours | 2120±6.55 |
| 2 | Remaceration (bismaceration), infusion – 3 hours | 1994±4.40 |
| 3 | Remaceration (bismaceration), infusion – 6 hours | 2315±5.04 |

The next step was to study the effect of extraction time on the efficiency of extraction of phytosterols (mg/kg) from UDR. The study used samples of OE, which were prepared in a ratio of 1:5, insisted with different time intervals: for 1, 3, 6, 9, 12 hours. The results of the dependence of the extraction of the amount of

phytosterols on the time of extraction are shown in Fig. 5.

The final stage of the study was to justify the choice of extraction method in the process of OE technology. The study used the classical method of maceration, as well as the method of remaceration (bismaceration) with the division of corn oil into two equal parts (Table 6).

At the last stage of the experiment, we made OE according to the developed extraction parameters and determined the amount of phytosterols in it (Fig. 6, Table 7).

Table 7

Characteristics of peaks and the percentage of phytosterols in OE (extractant – corn oil; MPRM-extract ratio – 1: 5)

| No. | Substances | Time, min | Height | Area | Concentration, mg/kg | Concentration, % |
|---|---|---|---|---|---|---|
| 1 | Cholesterol | 15.209 | 1.4667 | 0.12138 | 9.456 | 0.12 |
| 2 | Brasycosterol | 16.359 | 3.6456 | 0.32 | 26.004 | 0.33 |
| 3 | Campesterol | 16.984 | 103.17 | 10.097 | 816.368 | 10.36 |
| 4 | Stigmasterol | 17.551 | 79.698 | 7.2561 | 586.272 | 7.44 |
| 5 | Unidentified | 18.005 | 24.902 | 2.3471 | 189.908 | 2.41 |
| 6 | Unidentified | 18.070 | 18.964 | 1.8427 | 148.932 | 1.89 |
| 7 | Unidentified | 18.359 | 6.5120 | 0.61046 | 49.644 | 0.63 |
| 8 | β-sitosterol | 18.812 | 461.24 | 57.392 | 4638.168 | 58.86 |
| 9 | Δ5-avenosterol | 19.044 | 28.111 | 4.4619 | 360.904 | 4.58 |
| 10 | Δ7-stigmastenol | 19.593 | 9.719 | 1.7378 | 140.264 | 1.78 |
| 11 | Δ7-avenosterin | 20.027 | 76.404 | 7.9657 | 643.796 | 8.17 |
| 12 | Unidentified | 20.382 | 26.558 | 2.8198 | 227.732 | 2.89 |
| 13 | Cystrostadinol | 21.695 | 4.7976 | 0.52932 | 42.552 | 0.54 |
| Sum | | | | 97.50126 | 7880 | 100 |

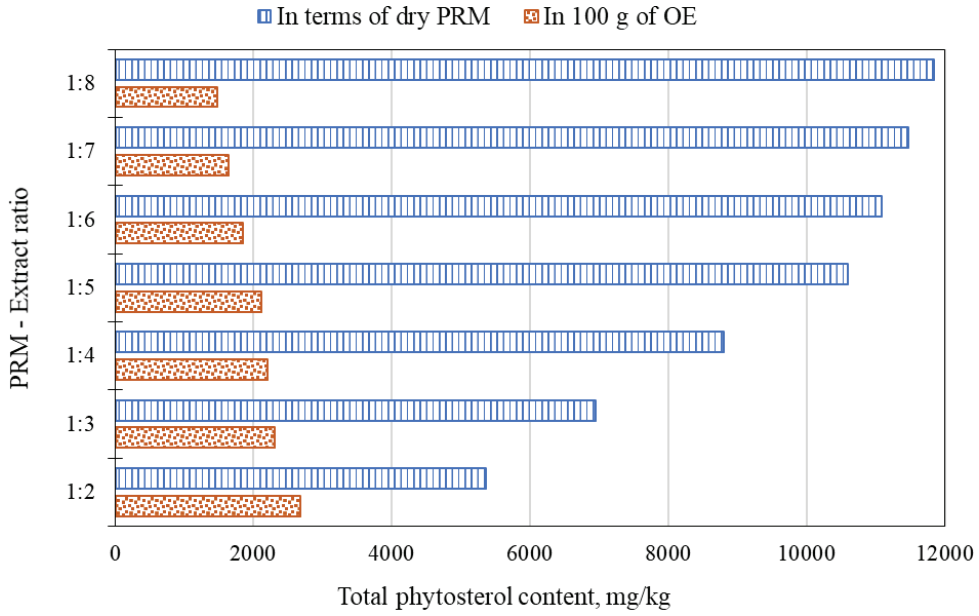


Fig. 3. The content of phytosterols in OE depending on the ratio of MPRM – extract

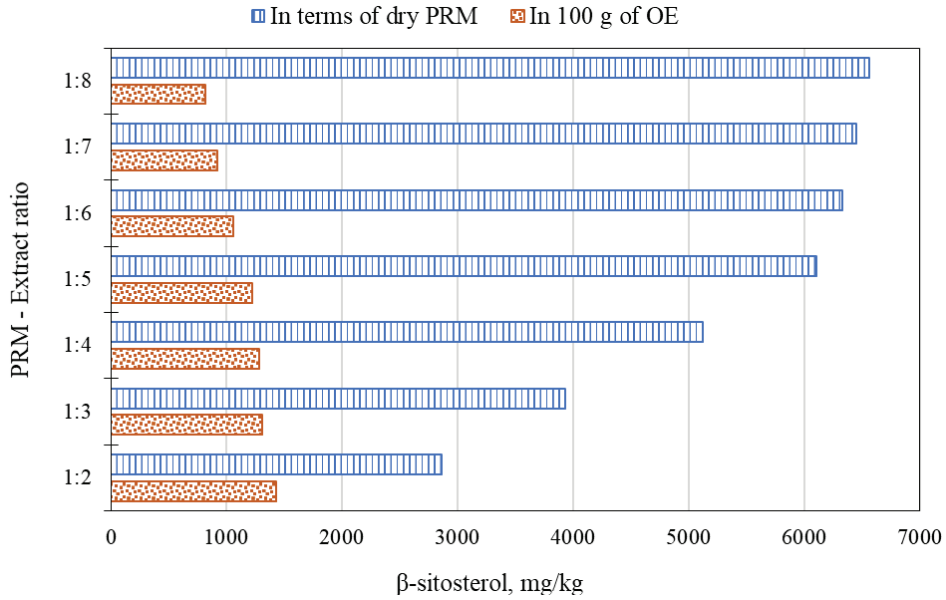


Fig. 4. The content of β-sitosterol in OE depending on the ratio of MPRM – extract

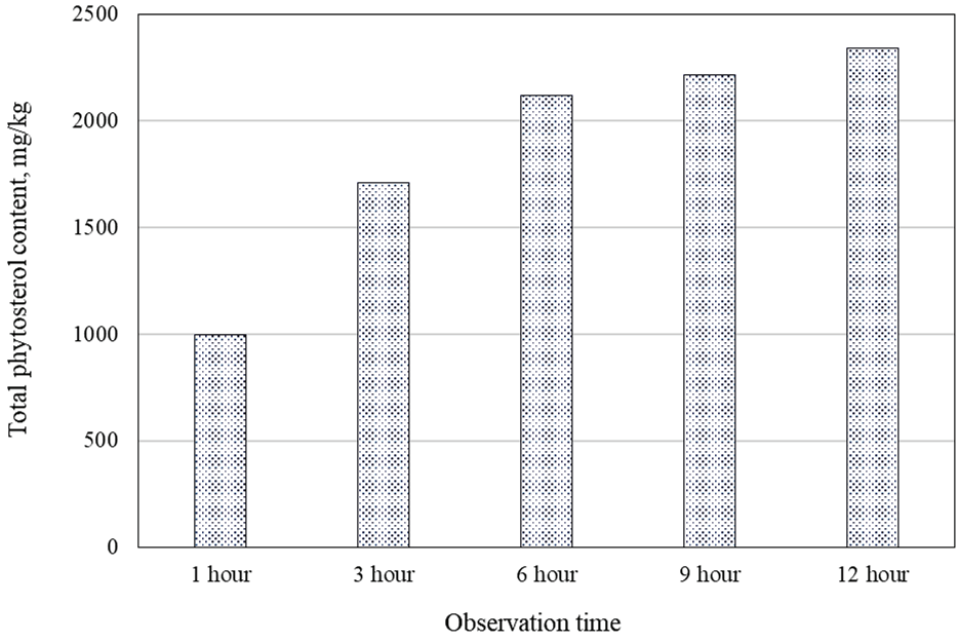


Fig. 5. Dependence of the amount of phytosterols on the time of extraction

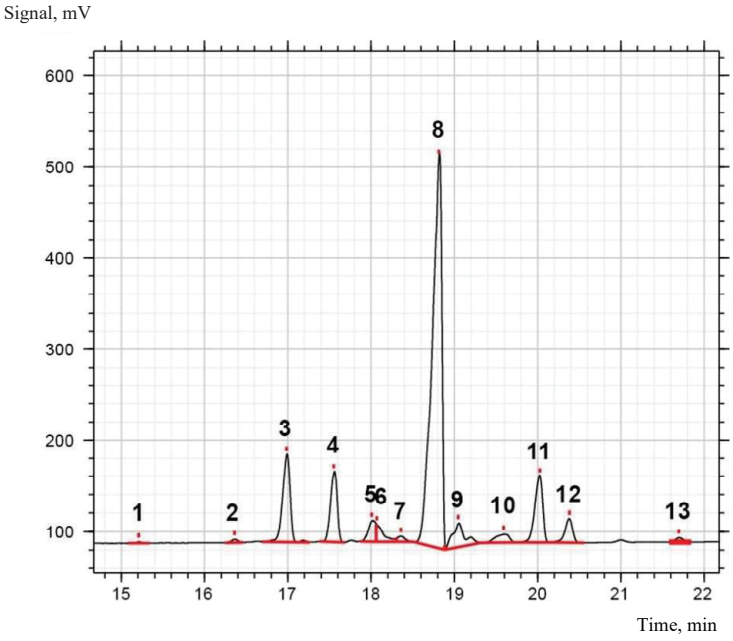


Fig. 6. Chromatogram of phytosterols in OE of nettle roots; extractant – corn oil; MPRM-extract ratio – 1:5

**5. Discussion of research results**

On the chromatogram of phytosterols of sunflower oil (Fig. 1) there are peaks of 5 different substances (1 – campesterol, 2 – stigmasterol, 3 – β-sitosterol, 4 – Δ7-avenosterol, 5 – unidentified compound of steroid structure). The amount of sterols was in the range of 2700 mg/kg, with a concentration of β-sitosterol of 1745 mg/kg. As can be seen from Table 3, the percentage of β-sitosterol in the amount of sterols was the highest and amounted to 64.75 %.

On the chromatogram of phytosterols of corn oil (Fig. 2) there are peaks of as many as 10 different substances: 1 – campesterol, 2-α stigmasterol, 3–5, 10 – unidentified steroid compounds, 6 – β-sitosterol, 7 – Δ5-avenosterol, 8 – Δ7-stigmastenol, 9 – Δ7-avenosterin. The quantitative content of the sum of sterols was higher compared to sunflower oil and was 5760 mg/kg, while the concentration of β-sitosterol was 3417 mg/kg; the amount of β-sitosterol in the sum of sterols of corn oil was also the highest and amounted to 59.33 % (Table 4).

Therefore, taking into account the profile of phytosterols and their quantitative content in both extractants, corn oil was chosen for the production of OE.

OE is obtained by infusing crushed MPRM oil with heating. In the process of manufacturing OE, such technological parameters as temperature regime, raw material-extract ratio, extraction time, etc. are taken into account.

In the production of OE, there is often a problem associated with low mass transfer between the hydrophobic components of MPRM cells and the oil extractant. In addition, the oils are characterized by relatively high viscosity, which also slows down the extraction of BAS from plant material. Therefore, increasing the temperature is used to enhance the extraction processes. However, the increase in temperature causes not only an increase in the extraction rate, but also the destruction of thermolabile BAS plant material and extractant. Accordingly, it is advisable to carry out the extraction at a temperature of (45±5) °C [22].

Enhancement of diffusion processes between MPRM and oil extractant is possible by pre-wetting the raw material with a solvent that is immiscible with oil. According to the literature, it has been experimentally established that pre-moistening of MPRM with ethanol contributes to the destruction of intermolecular bonds in plant cell structures. As a result, the penetration of the oil extractant into the cells of MPRM is facilitated, the desorption of both hydrophilic and lipophilic BAS plant raw materials is enhanced [2, 12, 24].

Ethanol of different concentrations – 40, 70, 90, 95 % is used for humidification [24]. The choice of solvent concentration depends on the physicochemical properties of the BAS to be removed. Since free and esterified phytosterols are non-polar compounds and have hydrophobic properties, it is advisable to use higher concentrations of ethanol. We chose 90 % ethanol because the liquid is well wetted by MPRM, dissolves phytosterols and to a lesser extent (than 95 % ethanol) causes denaturation of ballast substances. The choice of the ratio of ethanol and MPRM was carried out based on data from the literature, which states that the amount of liquid for soaking should be 0.5–1.0 hours relative to the mass of MPRM. The amount of humidifier depends on the porosity of the raw material – for dense MPRM (seeds, bark, roots) use 0.5–0.7 hours of liquid relative to the mass of MPRM. The swelling time of MPRM is from 1.5 to 2 hours [20, 21, 23–25]. Accordingly, to moisten the UDR, we used 90 % ethanol in the amount of 0.6 hours by weight MPRM; swelling time was 2 hours.

The results of the experiment confirmed (Fig. 3) that with a decrease in the mass of MPRM per 100 g of OE also decreased the total content of phytosterols. The concentration of the studied BAS in OE at a raw material-extract ratio of 1:2 was in the range of 2680±9.6 mg/kg, at 1:5 – 2120±8.4 mg/kg, at 1:8 – 1480±9.5 mg/kg. However, when converting the amount of BAS to dry MPRM, the extraction efficiency increased, namely: at a ratio of MPRM – extract (1:2) the content of the amount of extracted phytosterols was 5360±9.2 mg/kg, at 1:5 – 10600±10.5 mg/kg, at 1:8 – 11840±9.6 mg/kg MPRM, respectively. As can be seen from Fig. 3, the efficiency of extraction of the sum of BAS increased intensively from a ratio of 1:2 to 1:5. With a further decrease in the amount of MPRM per unit weight of extractant (ratio 1:6–1:8), the extraction efficiency slowed down, as there was a slight increase in the amount of phytosterols in terms of dry MPRM.

Analysis of the results of determining the content of β-sitosterol in OE samples with different MPRM-extract ratio (Fig. 4) also confirmed the correlation between the increase in extraction efficiency in terms of dry MPRM with increasing amount of extractant. Thus, the content of β-sitosterol at a raw material-extract ratio of 1:2 was 2860±3.6 mg/kg MPRM; with subsequent increases in multiplicity (from 1:3 to 1:5), the intensity of BAS extraction increased sharply and at a ratio of 1:5 showed the result of 6105±2.2 mg of β-sitosterol per kg of MPRM. Increasing the multiplicity to 1:8 did not significantly increase the efficiency of BAS extraction, as the content of β-sitosterol was 6560±2.8 mg/kg (the obtained data correlate with the results of extraction of phytosterols).

Therefore, taking into account the content of the sum of phytosterols and β-sitosterol in the OE itself and the efficiency of BAS extraction from MPRM, the raw material-extract ratio was chosen: 1:5.

The results of the dependence of the extraction of phytosterols on the extraction time showed (Fig. 5) that it is advisable to infuse for 6 hours, because during this time the dynamics of extraction of extracted phytosterols increased rapidly. Extending the extraction period to 12 h did not significantly increase the recovery of the amount of BAS.

The selected optimal ratio of MPRM and extract 1:5 using the maceration method allowed to obtain OE with a high content of phytosterols. Because nettle roots are fibrous, the amount of free space between the raw material particles is large with a UDR of 0.7848±0.0054. Appropriately weighed and moistened with ethanol MPRM, required some compaction to fill the required amount of extractant to the "mirror".

In an experiment in the method of remaceration (bismaceration) with the separation of corn oil (1:5) into two equal parts, MPRM required intensive compaction to completely cover it with the appropriate amount of fluid. The volume of extractant was at the limit. The yield of phytosterols under the conditions of extraction for 3 h was slightly lower compared to maceration (2120±6.55 mg/kg) and had a value at the level of 1994±4.40 mg/kg (Table 6). In the next experiment, with increasing extraction time (6 h each portion), the yield of the sum of BAS was higher and was 2315±5.04 mg/kg. The obtained results can be substantiated by the fact that diffusion processes are slowed down in a highly compacted MPRM, since the change in the thickness of the saturated diffusion layer is slow, and the extraction time increases accordingly. Given the yield of the sum of BAS, the lack of additional technological operations and the duration of extraction, for the manufacture of OE, we chose the classic method of maceration.

Taking into account the results of theoretical and experimental studies, we chose the following parameters of extraction of OE: extractant – corn oil, humidifier – 90 % ethanol, raw material – extract ratio – 1:5, temperature – 45±5 °C, time – 6 h, method – maceration.

In Fig. 6 shows the chromatogram of phytosterols of OE, which was produced under selected conditions. There are peaks of 13 substances on the chromatogram. Compared with the pure extractant, other phytosterols were additionally detected in the extract, namely: traces of cholesterol (found in minimal amounts in plants), brasycosterol, campesterol, cystrostadinol. The distribution of phytosterols in the mixture had a similar profile, where the largest percentage was occupied by β-sitosterol (58, 86 %), in second place were stigmasterol Δ7-avenosterol, the content of which in the mixture was in the range (7–8) % (Table 7). The total content of plant sterols in OE, including taking into account the BAS extractant, had a value in the range of 7,880±9.4 mg/kg; the amount of β-sitosterol is 4,638±4.0 mg/kg.

Therefore, the proposed technology makes it possible to obtain OE with a high content of phytosterols in a minimum of time and using simple equipment. To intensify the extraction of BAS from plant material in further studies, it is advisable to use maceration with forced circulation of the extractant, with the connection of ultrasound, with the grinding of raw materials in a liquid medium.

**Study limitations.** The concentration of ethanol and the duration of the stage of soaking the raw material was chosen according to the literature. In this work, we used only static extraction methods, such as maceration and remaceration, which are used in the technology of oil extracts.

**Prospects for further research.** Study of the influence of ethanol concentration and soaking time of raw materials on the yield of phytosterols in OE; study of the influence of ultrasound or other methods of extraction intensification on the efficiency of extraction of phytosterols from UDR.

**6. Conclusions**

1. Taking into account the composition of fatty acids of vegetable oils, as well as the results of determining the qualitative composition and quantitative content of phytosterols by gas capillary chromatography, the feasibility of using corn oil as an extractant OE is substantiated.

2. The choice of optimal parameters for obtaining OE from UDR based on determining the yield of phytosterols by gas capillary chromatography is experimentally substantiated, namely: extractant – corn oil, raw material-extract ratio – 1:5, extraction time – 6 h, extraction method – maceration.

**Conflict of interests**

The authors declare that they have no conflicts of interest.

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