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Original Paper

COMPARATIVE EXTRACTION OF COMPONENTS FROM UKRAINIAN AND BALTIC AMBERS

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The research is devoted to the issue of processing substandard amber to enable the use of the processing products in food, cosmetics, and pharmaceutical industries. For the first time, comparison was made of the extractive characteristics of amber from the two largest world deposits – the Baltic and Ukrainian. Nine times more dry substances are transferred to the extract from Ukrainian than from Baltic amber, when extracting amber with ethanol under the same conditions. The antioxidant activity of the obtained extracts was determined by ABTS$^+$ and DPPH methods. The values of these indicators were higher in the extract of Ukrainian amber and also differed in extracts from different fractions. Analysis of the volatile components composition did not indicate a significant difference between the extracts of different amber, but clear differences were seen between the fractions from which the extract was obtained. The main component, representing more than a half of the volatile components, was o–Cymene. Most of the identified components are characterised by camphor odour. Amber from different places of origin have different extraction characteristics, which was confirmed by the research results.

Keywords: *extracts, succinite, fossil resin, volatile compounds, antioxidant activity.*

INTRODUCTION

Traditional technologies of chemical processing of substandard amber are energy-intensive, as they provide high-temperature processing of the material (pyrolysis) and subsequent chemical purification in order to isolate the target components (Vavra 2009; Ragazzi, 2016). However, the obtained products of such processing do not have the full range of valuable components contained in amber. In order to obtain a complex of biologically active components of amber for use in various industries, in particular food, cosmetics, and pharmaceuticals (Synoradzki, 2012), it is advisable to extract such a complex in the system liquid-solid phase.

Currently, scientific information on the extraction processing of amber is absent, but on the world market there are cosmetics and foods with amber extracts. Scientific literature on amber extracts are focused on the preparation of amber samples for analysis in gemology, and not in the context of industry. However, the selectivity of solvents for the extraction of components from amber has been studied (Chornyi *et al.*, 2020), which proves the effectiveness of solvents with low polarity, in particular ethanol, the use of which is acceptable in the production technology of products consumed by humans.

Due to its chemical composition, amber can be considered as a biologically valuable raw material (Tumiłowicz *et al.*, 2016; Mironov *et al.*, 2017). It contains natural succinic acid, which is much better absorbed by the body than synthetic, essential oils, which give raw materials and subsequent products a characteristic pleasant aroma and macro- and micro-elements that also carry biological value. To use all these advantages of raw materials in industrial products, it is advisable to obtain extracts of these raw materials, which are then introduced into the final product. Since such extracts contain essential oils, they should be investigated to determine the functional value of such extracts.

Previous litereraturе (Matuszewska and Czaja, 2002; Vīrgolici *et al.*, 2010; Jongseo, 2013) present the chemical composition of amber, in particular volatile substances, and a list of identified components. However, since most studies concern Baltic amber, it is worthwhile to determine also the composition of volatile components in Ukrainian amber, namely of their extracts. Since the chemical composition of amber, and consequently its extraction properties, may differ in different places of its origin (Virgolici *et al.*, 2010; Wagner-Wysiecka and Wicikowski, 2015; Wolfe *et al.*, 2016), it would be interesting to compare the extraction properties and chemical composition of Baltic and Ukrainian amber, which are part of the single Batlic–Dnieper amber province and ranks first and second, respectively, in terms of reserves and volume of mined amber in the world.

The purpose of this study is to compare the characteristics of dry matter obtained from ethanol extraction of Ukrainian and Baltic amber.

MATERIALS AND METHODS

Extraction of amber. Ukrainian amber mined in the Volyn region of Ukraine and Baltic amber collected on the Baltic coast near Liepāja, Latvia, were used in the study. Amber was crushed by hammer and sieved through sieves OLISLAB 1100 (OLIS Ltd., Odesa, Ukraine) with mesh diameter 2.5, 1.5, 0.5 mm to obtain two fractions — 2.0 mm (U2 and B2) and 1.0 mm (U1 and B1).

To study the extractive characteristics of amber, extraction was performed with ethanol 96% vol. by the method of dynamic maceration under the same process conditions: the boiling point of the extractant — 72 °Ń; the ratio of liquid and solid phases — 15 : 1; the duration of the process — 180 min, as described in previous studies (Synoradzki, 2012). The employed time is sufficient to saturate the extract with dry substances from amber, after which the system acquires close to equilibrium concentration and the diffusion process slows down and stops. To avoid escape of the reaction volume of extractant that evaporates continuously at the boiling point (Fig. 1), a reflux condenser was mounted above the reactor, which was placed in a water bath to maintain the system temperature.

In order to study the kinetics of extraction, sampling was performed every 20 min to determine the dry matter concentration by refractometric method.

Determination of pH value. The pH was recorded using a Jenway 3510 pH metre (Barloworld Scientific Ltd., Staffordshire, UK), with a glass electrode calibrated at pH 4 and 7. Triplicate measurements were completed for each sample.

Determination of volatile components. To determine the volatile components contained in the amber extracts, 5 ml

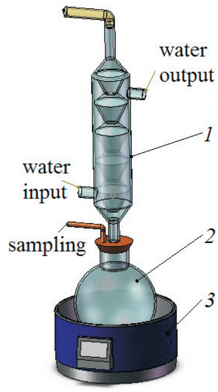

Fig 1. Extractor: 1, reflux condenser; 2, reactor; 3, water bath.

of the sample was taken and transferred to a 20 ml vial. Using a magnetic stirrer, the sample was stirred for 10 min at room temperature 25 °C, and then inserted into a vial of bipolar SMPE-coated fibre Carboxen/Polymethylsiloxane (CAR/PDMS) with thickness of 85 μm (Supelco Inc., Bellefonte, PA, USA) and kept for another ten minutes. During this time, the volatile substances released during stirring were adsorbed on the fibre SPME.

Volatile compounds from fibre were thermally desorbed in an injector on a PerkinElmer 500 gas chromatograph/mass spectrometer (GC/MS) (PerkinElmer, Inc., Shelton, CT, USA) with a capillary column Elite–Wax ETR (60 m × 0.25 mm i.d.; DF 0.25 μm). Analysis using the GC/MS was performed according to the following parameters: initial temperature of 40 °C maintained for 7 min, then increased from 40 °C to 160 °C at a rate of 6 °C min^{-1} followed by an increase from 160° C to 210 °C at a rate of 10 °C min^{-1} with final holding time of 15 minutes. Electron impact ionisation mode was set at 70 eV, and the ion source and inlet temperature were both set at 250 °C. The carrier gas (helium) was supplied with a constant flow of 1 ml/min. The obtained parameters were scanned at m/z 40–400 (Galoburda *et al.*, 2020). Components were identified by comparing their mass spectra with the Nist98 mass spectral library. In addition, linear retention indexes were calculated based on the retention times of alkanes (C8 – C20) and ware compared with literature data.

Determination of antioxidant activity. Antioxidant activity of the studied solutions was measured based on the scavenging capacity of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Absorbance was measured on a JENWAY 6300 spectrophotometer (Barloworld Scientific Ltd., Staffordshire, UK) at 517 nm. The radical scavenging activity (RSA) of the solutions was also measured by cation-radical analysis of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$). The $ABTS^+$ solution was diluted with phosphate buffer solution to obtain an absorbance of 0.800±0.030 at 734 nm. Ąntioxidant activity was expressed as Trolox-eq. mM/100 ml of solution. The equivalent antioxidant capacity of the Trolox sample is positively related to the antioxidant activity (Zarins *et al.*, 2018).

Determination of succinic acid. The concentration of succinic acid (mg/l) was determined with high-performance liquid chromatography (HPLC) (Schimadzu LC–20 Prominence, Shimadzu USA Manufacturing Inc, Canby, USA), detector DAD SPD-M20A, Solvent Delivery Unit LC-20AD, Column Oven CTO-20A, Autosampler SIL-20A, System Controller CBM-20A and data system LCsolution software.

The chromatography parameters used for separation of organic acids were: analytical column PerkinElmer C18, 4.6 mm × 250 mm, 5 µm, column temperature +35 °C, wavelength 210 nm, injection volume 10 µL, mobile phase: A (acetonitrile): B 0.05 M KH_2PO_4 (1:99) in gradient conditions and start flow rate 1.25 ml·min^{-1}.

RESULTS

Extraction curves for the amber samples are presented in Figure 2.

The results of extraction of dry matter from amber indicated nine times higher yield from Ukrainian amber than from the Baltic amber for the 1.0 mm fraction. It was also possible to determine the effect of amber size on dry matter yield, which has a similar effect for both types of amber. Extraction from the 1.0 mm fraction allowed to obtain 40–50 % higher concentration of dry matter in the extract than from the 2.0 mm fraction. We also determined the ash concentration in the extracts, but when 5 g extract was combusted at 500 °C for two hours, the ash did not reach the detection limit of 0.0001 g of ash. This indicates that the basis of dry matter extracted from amber are organic components, main of which is abietic acid (Jongseo, 2013).

The obtained extracts were analysed for antioxidant activity by two methods based on the scavenging effect of antioxidant components of amber extract against radicals — $ABTS^+$ cation and the DPPH chromogenic radical (Fig. 3).

pH value, which indicates the saturation of the extract with acids, was: U1 – 2.96; U2 – 2.81; B1 – 5.22; and B2 – 5.09.

The concentration of the target succinic acid in the extracts, estimated by gas chromatography, was: U1 – 70 mg/l; U2 – 77 mg/l; B1 – 201 mg/l; and B2 – 164 mg/l.

Amber contains essential oils that give it a characteristic odour. In order to study the yield of these essential oils in the process of ethanol extraction, the composition of volatile components of amber extracts obtained from different types of amber and different fractions was studied. Ethanol was not added to the results of the analysis as a component of the volatile composition of the extract, as it was used as a solvent.

Twenty-four major volatile components were identified in extracts of amber (Table 1). Regarding qualitative differences between the types of amber, acetal was not found in extracts from Baltic amber. Regarding quantitative differences in volatile components, eucalyptol, isoborneol, and naphthalene had higher concentration in extracts of Baltic amber, while the extract of Ukrainian amber had a relatively higher concentration of humulene, fenchone, romanal, isocaryophyllene, and neoclovene.

The detected volatile components can be divided into chemical classes that form the aroma of amber extract. Among the identified components, the main chemical classes were: alkanes, alcohols, ketones and aldehydes. Figure 4 shows that the extracts were dominated by alkanes, which contributed 71.7–87.8% of the total volatile components of the samples — 77.5±0.3 % among the extracts from the large fraction and 85±1% among the extracts from

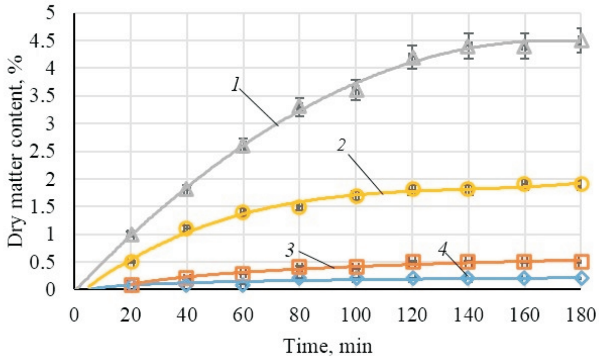

Fig. 2. Extraction curves of the extraction process of dry matter from amber: 1, Ukrainian amber with 1.0 mm fraction of; 2, Ukrainian amber with 2.0 mm fraction; 3, Baltic amber with 1.0 mm fraction of; 4, Baltic amber with a 2.0 mm fraction.

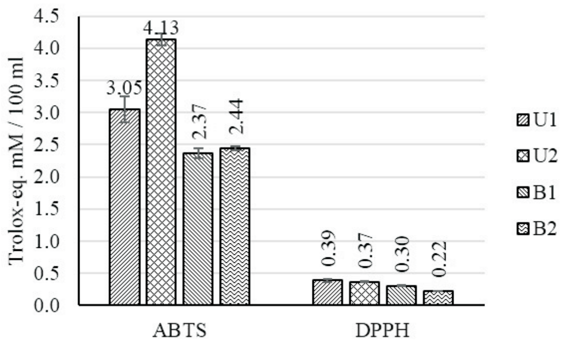

Fig. 3. Antioxidant activity of ambers ($p < 0.05$, $n = 3$): U1, Ukrainian amber with 1.0 mm fraction; ; U2, Ukrainian amber with 2.0 mm fraction ; B1, Baltic amber with 1.0 mm fraction; B2, Baltic amber with 2.0 mm fraction.

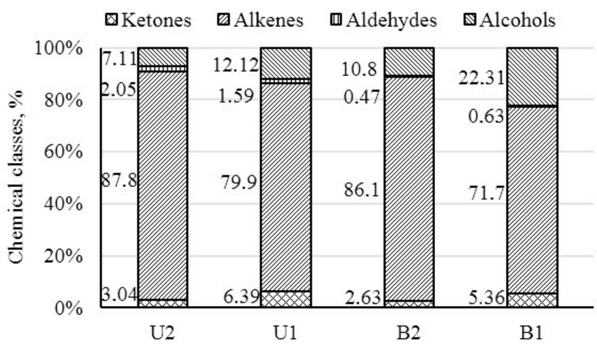

Fig. 4. Distribution of volatile components of amber extracts by chemical classes: U1, Ukrainian amber with 1.0 mm fraction; U2, Ukrainian amber with 2.0 mm fraction; B1, Baltic amber with 1.0 mm fraction; B2, Baltic amber with 2.0 mm fraction.

Table 1. Distribution of volatile substances of amber extracts and their organoleptic characteristics

Volatile component	Sample								Organoleptic characteristics
	Ukrainian amber with 1.0 mm fraction		Ukrainian amber with 2.0 mm fraction		Baltic amber with 1.0 mm fraction		Baltic amber with 2.0 mm fraction		
	peak area, $\times 10^5$	part, %	peak area, $\times 10^5$	part, %	peak area, $\times 10^5$	part, %	peak area, $\times 10^5$	part, %	
o–Cemene	1769	67.8	1352	67.1	1846	67.1	1206	61.5	camphoric
Toluene	162	6.2	60	2.99	80	2.9	13	0.68	paint
Camphene	104	3.98	32	1.6	215	7.81	29	1.49	camphoric
Pseudomunene	88	3.38	82	4.05	82	2.97	86	4.4	plastic
Ledol	71	2.71	2	0.08	4	0.13	4	0.22	sweet
Camphor	51	1.96	84	4.18	55	2.97	79	4.04	camphoric, mint, wood
Isocamphane	40	1.52	15	0.73	58	2.11	6	0.31	camphoric
Borneol	41	1.57	71	3.55	84	3.06	141	7.21	camphoric
Fenchol	39	1.49	133	6.62	118	4.29	179	9.16	camphoric, wood
Isocaryophyllene	38	1.45	23	1.13	11	0.42	14	0.69	wood, spicy
Romanal	34	1.3	22	1.12	13	0.47	12	0.63	wood, amber, herbal
Tricyclene	21	0.82	8	0.38	18	0.66	1	0.06	herbal, wood
Acetal	19	0.75	10	0.47	n.d.*	n.d.*	n.d.*	n.d.*	herbal, nut, sweet
Fenchone	18	0.7	29	1.46	10	0.37	14	0.72	camphoric
Humulen	13	0.49	9	0.45	5	0.18	6	0.3	wood
Naphthalene	5	0.21	4	0.19	13	0.48	19	0.99	tar
Epiglobulol	16	0.62	12	0.6	12	0.43	18	0.91	sweet, fruity
Decane	15	0.56	4	0.22	13	0.48	1	0.06	alkane
Cumene	14	0.53	9	0.47	13	0.49	10	0.53	petrol
Isoborneol	12	0.44	15	0.76	22	0.8	33	1.7	camphoric, herbal, wood
Mesitylene	10	0.39	8	0.4	14	0.51	11	0.56	sweet
Tetrahydrocarvone	10	0.38	15	0.75	7	0.25	12	0.59	mint, herbal
Eucalyptol	16	0.26	12	0.53	12	2.05	18	3.13	mint, spicy
Neoclovene	12	0.45	4	0.21	2	0.08	1	0.07	herbal
Total	3246	100	2775	100	3470	100	2661	100	?

n.d. – not detected.

the small fraction. Among the alkanes, *o*–cemene had the highest concentration, followed by camphene, pseudocumen, toluene, and others. The class of alcohols are represented by fenchol and borneol. Ketones in the extracts are represented in descending order by camphor, fenchon and tetrahydrocarvone, the latter of which had concentration 11±1.5%. The concentration of camphor and fenchon in the ketone fraction the fractions similar, but concentration of camphor is higher in Ukrainian and fenchon in Baltic amber extract. The lowest concentrations were for the aldehyde class, which was represented only by romanal in Baltic and Ukrainian amber and also acetal in extracts of Ukrainian amber.

DISCUSSION

To assess the extractive characteristics of raw materials, it is necessary to determine the process kinetics, which can enable to determine the duration of extraction and the time required to reach completion at the equilibrium concentration of the system.

The $ABTS^+$ method indicated higher values of antioxidant activity in the larger fraction, which may indicate concentration of antioxidant components of amber acting on cation radicals closer to the periphery of the particles and in the oxidising crust. A greater difference between fractions was observed in Ukrainian fossil amber (35%), which is more susceptible to surface oxidation than in Baltic amber (3%), which is transported by the sea. This also confirms the idea of the distribution of antioxidant components in amber particles (Pastorelli, 2012). It was observed that the pH level of the extracts differs both between the type of amber and fractions. The observed decrease in pH indicates enrichment of the extract with acidic substances such as abietic acid, which has high concentration and is the basis of the polymer skeleton of amber (Vavra 2009). Therefore, the pH index, similarly as antioxidant activity estimated by $ABTS^+$, differs between the amber fractions.

Antioxidant activity estimated by scavenging by DPPH chromogen radicals was lower than for $ABTS^+$. The activity estimated by DPPH was higher for Ukrainian compared to Baltic amber extracts, and higher in the smaller fraction. The antioxidant activity of the extracts were estimated based on dry matter concentration of extracts, but based on dry weight of amber the relative activity would increase for Baltic amber.

Succinic acid concentration in extracts was 2.5±0.3 times higher for Baltic compared to Ukrainian amber. It should be noted that the concentration of succinic acid is not proportional to the concentration of dry matter extracted from amber.

Regarding the composition of volatile substances of amber extracts, the main difference were between fractions and not between amber regions. In most cases, a higher concentration of volatile substances was observed in extracts obtained from the larger fraction of amber. Only eucalyptol, fenchone, camphor, tetrahydrocarvone, fenchol, isoborneol and borneol had higher concentration in extracts from the smaller fraction. Estimation of the total peak area showed higher concentration of most volatile components in the extracts obtained from the larger fraction of amber. There was a slight difference in the total peaks area < 7 % between the extracts of different amber types, the concentration of volatile substances in the samples is similar. This leads to the opinion that the concentration of volatile substances is not proportional to the concentration of dry matter, which was determined during the extraction process.

The list of detected volatile components in this study and the studies (Matuszewska and Czaja, 2002; Yamamoto *et al.*, 2006). The earlier studies were also carried out on ethanol extracts of Baltic amber, but the differences with our study results can be explained by different methods used for deetermination.

Since most volatile components of amber extracts are characterised by a camphor aroma, respectively, the obtained extracts hade inherent odour components.

CONCLUSIONS

The best extraction results were obtained by extracting dry matter from Ukrainian amber, which yielded nine times more dry matter than Baltic amber. The amber fractional composition affected the extraction. When extracting Ukrainian amber with the 1.0 mm fraction, it was possible to obtain 136% higher yield than for the 2.0 mm fraction. A similar result is obtained for Baltic amber, which yielded 150% more for the smaller fraction. However, according to the results of the antioxidant activity analysis of the extracts by $ABTS^+$ method, higher activity was observed for the larger fraction, which may indicate concentration of antioxidant components of amber acting on cation radicals closer to the particles periphery and in the oxidizing crust.

A total of 23 volatile components were detected in extracts of Baltic amber, and additionally acetal in Ukrainian amber. More than half of the composition of volatile components was represented by *o*–cemene, and a number of other components could also be characterised by the same camphor aroma, according to the concentration of organoleptic parameters that form the corresponding amber odour of the obtained alcohol extracts.

In conclusion, we found that the place of origin of amber significantly affects its extraction characteristics. According to the results of this study, for the industrial production of amber extracts it is advisable to use Ukrainian amber.

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NO UKRAINAS UN BALTIJAS DZINTARA EKSTRAHĒTO KOMPONENŠU SALĪDZINĀJUMS

Darbā salīdzināti ekstrakcijas produkti no divām lielākajām pasaules dzintara atradnēm — Ukrainas un Baltijas. Parādīts, ka tie būtiski atšķiras: ekstraktam no Ukrainas dzintara ir daudz vairāk sausnes nekā no Baltijas dzintara. Secināts, ka industriālai izmantošanai vairāk ir piemērots ekstrakts no Ukrainas dzintara.