Journal of Electroanalytical Chemistry 776 (2016) 114–119

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

journal homepage: www.elsevier.com/locate/jelechem

# Voltammetric profiling of absinthes

Mateusz Kowalcze, Małgorzata Jakubowska *

*AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Mickiewicza 30, 30-059 Kraków, Poland*

ARTICLE INFO

*Article history:*
Received 29 March 2016
Received in revised form 10 June 2016
Accepted 6 July 2016
Available online 07 July 2016

*Keywords:*
Absinthe
Voltammetry
CGMDE
PCA
CA
k-Means clustering

ABSTRACT

The paper demonstrates that differential pulse voltammetry with a controlled growth mercury drop electrode, supported by the unsupervised multivariate chemometric methods enables distinguishing of absinthes brands. Signals recorded for the unpretreated samples are repetitive for the same absinthe producer. Unprocessed complex voltammetric signals recorded by different accumulation time were an input data to PCA, HCA and k-means modeling. Homogeneous clusters were formed which reflected the natural order in the set of samples.



## 1. Introduction

Absinthe is a bitter, highly alcoholic (55%–70% v/v) beverage produced by the maceration and distillation of mixed herbs whose main ingredient is wormwood (*Artemisia absinthium*) and anise (*Pimpinella anisum*) [1–4]. Hyssop (*Hyssopus officinalis*), peppermint (*Mentha piperita*) and lemon balm (*Melissa officinalis*) are also often added. The most common color is green, although products in red or blue color can be found, as well. Each manufacturer protects the composition and proportions used in their herbal mixtures. Formally, absinthe is a type of a flavored vodka. Plants from the genus *Artemisia* have long been used to flavour alcoholic beverages, giving them a bitter taste and alleged tonic properties. Several different types of extracts (in water, water: methanol and methanol) and essential oil were obtained from leaves of wormwood collected in various natural habitats.

An interesting component of absinthe is dicyclic monoterpene - thujone, present in the form of two isomers: (−)-α i (+)-β. The percentage of thujone in wormwood oil is 0.53–1.22% of α-thujone and 17.5–42.3% of β-thujone [6,7]. α-Thujone is a neurotoxin, easily absorbed from the gastrointestinal tract and rapidly penetrating from the blood into the brain. It is used as a medicinal remedy worldwide, therefore numerous scientific studies have been reported on antiprotozoal, cytotoxic, antimalarial, antipyretic, antiradical and antioxidant, antimicrobial, anthelmintic properties of wormwood herb extracts [8–11]. In small doses, it stimulates the cerebral cortex, causing hyperreflexia and restlessness. In larger doses it causes muscle tremors, clonic convulsions and mental irritability [7]. It is hallucinogenic, what is caused by the α-isomer which exhibits higher psychoactive properties [12,13]. Since 2003, according to the EU standards, the maximum concentration of thujone in alcoholic beverages has been allowed at a level not exceeding 10 mg/kg of the beverage with an alcohol content greater than 25% (v/v) [13].

Another interesting ingredient is anise oil coming from the maceration of the fruit of anise (*P. anisum*). The main ingredient is methyl derivative of *p*-alkylphenyl - anethole, which exists as two geometric isomers: *cis* and *trans*. The percentage of anethole in aniseed oil is 0.1–0.4% of *cis*-isomer and 87–94% of *trans*-isomer. Anethole is a weak bactericidal substance [7,14]. Due to the highly hydrophobic nature of anethole, when diluted with water, the ouzo effect can be observed in absinthe - characteristic cloudiness of the solution.

### 1.1. History of the beverage

Medical use of wormwood in the treatment of parasitic infections has already been mentioned in the Ebers Papyrus, written around 1550 BCE [12]. Absinthe as we know it was presumably created by a French doctor Pierre Ordinaire and its composition was probably based on the mixture of wormwood, anise, hyssop, mint, chamomile, sweet flag, lemon balm, coriander and parsley. After the doctor's death, his recipe came into the possession of Henri-Louis Pernod who began advertising the beverage in 1797. The factor which had an unquestionable impact on the popularity of absinthe was the wars in Europe - absinthe accounted for soldiers as a tasty substitute for medicines [12].

Outstanding career of absinthe began in the late nineteenth and early twentieth century, becoming the primary beverage for the Parisian artistic bohemia where it gained an affectionate name *la Fée Verte* – Green Fairy. Psychoactive properties contained in Green Fairy's thujone together with a high content of alcohol contributed to the

* Corresponding author.
*E-mail address:* jakubows@agh.edu.pl (M. Jakubowska).

http://dx.doi.org/10.1016/j.jelechem.2016.07.014
1572-6657/

increase of an addiction – absinthism – and it was reason of a later prohibition of absinthe in Europe and the United States in the early twentieth century. However, no modern studies have proved that absinthe is a greater danger than other drinks with similar alcohol content. This enabled the restoration of its production at the end of the twentieth century [7,12].

### 1.2. Methods of production

The first stage [12,15], lasting between one week and one year, is maceration of the herbal ingredients: composition of herbs, where the largest share is that of wormwood (*A. absinthium*) and anise (*P. anisum*), flooded with high-percentage ethanol solution. Macerate is then separated from herbs and subjected to the rectification process. Substances which are highly soluble in ethanol, including thujone and anethole, remain in the colorless distillate.

The next step, lasting several days, is the refinement of the distillate by re-coloration, addition of sweeteners and possible preservatives. A natural colorant is chlorophyll which may be prepared by adding peppermint (*M. piperita*) or lemon balm (*M. officinalis*) leaves to the distillate. Often, instead of using plant extracts, coloring agents such as E140 or others are applied. At this stage, sweeteners are added to the distillate, such as sugar, its synthetic substitutes or glycosides (e.g. from liquorice root (*Glycyrrhiza glabra*)).

The last step is the dilution of the distillate up to the desired ethanol content - usually 55% or 70% ethanol v/v, the determination of thujone concentration and packing the product.

### 1.3. Profiling of food by electrochemical data

In recent years, several reviews, tutorials, and overviews have focused their attention on different aspects of chemometric applications in electroanalytical chemistry, illustrating the great interest in the subject and the potential for future applications [16–19]. The combination of modern electroanalytical techniques and chemometric pattern recognition is a powerful strategy which greatly contributes to promotion and quality assurance in the food industry. They consist of authentication of raw materials and verification of the origin, or the specific parameters of the product. The combined use of modern electroanalytical instrumentation with chemometric data modeling was envisaged as a significant improvement in the capabilities of electrochemical experiments. Many papers were published that illustrate the use of electrochemical data and multivariate methods in the investigation and evaluation of food. Research related products with a complex biological matrices such as, for example, wine [20,21], beer [22], honey [23] or teas [24,25].

In this work we propose the methodology of distinguishing of the absinthe samples based on chemometric analysis of differential pulse (DP) voltammograms recorded on the a controlled growth mercury drop electrode. Unsupervised multivariate methods, utilizing recorded voltammograms are used in order to characterize absinthes on the basis of their brand.

## 2. Experimental

### 2.1. Measuring apparatus and software

A multipurpose Electrochemical Analyzer M161 with the electrode stand M164 (both MTM-ANKO, Poland) was used for all voltammetric measurements. The classical three-electrode quartz cell of 20 mL volume was applied. It consisted of a controlled growth mercury drop electrode (CGMDE) as the working electrode, a double-junction reference electrode Ag/AgCl/KCl (3 M) with replaceable outer junction (3 M KCl), and a platinum wire as an auxiliary electrode. The ambient temperature was ca. 22 °C. The MTM-Anko EALab 2.0 software enabled electrochemical measurements, data acquisition and processing of the results.

### 2.2. Chemicals and glassware

Potassium nitrate (pure p.a., POCh, Poland) was used for the preparation of the supporting electrolyte. The other chemical was HPLC grade methanol (POCh, Poland). All reagents used were prepared using quadruply distilled water (two last stages from quartz). Glassware was first immersed in 6 M nitric acid, and then rinsed repeatedly with distilled water.

### 2.3. Samples

The studied samples were 6 different brands of absinthes, produced in European countries, which were purchased in local markets. The basic information about measured absinthes were given in Table 1. Three independent samples of the same brand were tested.

### 2.4. Standard procedure of measurements

Measurements were performed using differential pulse stripping voltammetry (DP SV). The procedure of the mercury drop electrode refreshing was carried out before each measurement. The investigation of absinthes was performed in the supporting electrolyte: 0.1 M $KNO_3$ (total volume 5 mL) filling the quartz voltammetric cell. The volume of added absinthe was 50 μL. The potential of the electrode was changed in the following sequence: accumulation potential, $E_{acc} = 0.15$ V accumulation time, $t_{acc} = 0$, 20 or 60 s (3 variants). During the accumulation step, the solution was being stirred (ca. 500 rpm) using a magnetic stirring bar. Then, after a rest period of 5 s, a differential pulse voltammogram was recorded from 0.15 to −1.4 V. The other experimental parameters were as follows: step potential, $E_s = 4$ mV; pulse potential, $E = 30$ mV; time of potential step, 40 ms (20 ms waiting + 20 ms sampling time).

**Table 1**
Summary information about measured absinthes.

| Name | Short name | Origin | Alcohol content % (v/v) | Color |
|---|---|---|---|---|
| Absinthe Absente[a] | abt | Distillerie de Provence, France | 55 | Emerald, naturally colored |
| Absynthion Polski Absinthe | ath | Toorank Distilleries, Poland | 55 | Emerald, colored by E140 |
| Absinth Mr Jekyll | mrj | Pabst & Richarz Vertriebs, Germany | 55 | Emerald, naturally colored |
| Absinth Originál | org | GAS Familia, Slovakia | 70 | Brilliant blue, colored by E133 |
| Rodnik's Absinthe Red | rod | Beveland Distillers, Spain | 70 | Red, naturally colored |
| Absinthium Helvetica Facito[b] | hel | Poland | 55 | Light brown, naturally colored |

[a] Instead *A. absinthium* in the composition of herbals *A. abrotanum* was added.
[b] According to a Swiss recipe from the 19th century.