Composition of Trans-anethol and Other Aromatic Volatiles in Anisated Alcoholic Beverages by Head-Space GC-MS Chromatography

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Abstract: Aniseed distillates, like ouzo in Greece, raki in Turkey, pastis in France, and arak in Syria, are very popular in Mediterranean countries. This flavoured alcoholic beverage is produced by extraction and distillation of fermented grape pomaces with herbal seeds from aromatic plants like *Pimpinella anisum* L., *Foeniculum vulgare*, *Illicium verum* and other plants which are also characterized by the presence of congeners which arise during fermentation process. Trans-anethol and its isomer cis-anethol are the main volatiles and are responsible for aroma properties of these traditional aniseed distillates. The aim of this research work was to find the concentration of special aromatic agent, trans-anethol and cis-anethol and also the major aromatic substances which are responsible for the aromatic quality. The majority of their identified components are isolated by extraction and distillation from different commercial brands and homemade samples. The constituents were identified by GC-MS (gas chromatography-mass spectrometry) in this traditional spirit. The major constituents in distilled ouzo extract were also trans-anethol.

Key words: Trans-anethol, anis, distillates, ouzo.

1. Introduction

Anisated fermented distillates are alcoholic beverage, which served as aperitif as a short or long drink or are used in mixed drinks and cocktails [1]. All over the Mediterranean area, beverages of same family are drunk under the name “ouzo” in Greece, “raki” in Turkey, “pastis” in France, or “Sambuca” in Italy, etc..

Aniseed spirits are produced by distillation of pressed and fermented grapes after vinification. They flavoured using *Illicium verum* Hookerfil., *Pimpinella anisum* L., and *Foeniculum vulgare* seeds as an aromatic agents [2-4]. However, there are some differences between the production process of these spirits and their traditional use in Mediteranean culinary culture.

Raki and ouzo appear to be more similar than

others. Chemical studies have demonstrated that the extracts from *Pimpinella anisum*, *Illicium verum* and *Foeniculum vulgare* contain large amount of anethol (Fig. 1).

In Greece, local farmers developed small distilleries with topical character in order to produce anisated fermented grape pomace distillates [5]. Up to 1989, the year that the European legislation 110/2008, about distilled beverages, was issued, there were no legal restrictions concerning fruit distilled beverages in Greece. The National Law 971/1917 concerned only the production of industrial alcohol and distilled products of viticulture origin and alcoholic beverages from grape pomaces.

Fig. 1 The structural form of anethol molecule.

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However, there is few available information or data on anisated fermented grape pomace distillates [6]. Furthermore, it is very important to provide knowledge about the volatile composition, especially, the concentration of the main aromatic compound of *Pimpinella anisum*, *Foeniculum vulgare* and *Illicium verum* seeds which are used as an aromatic agent on the distillation procedures from the artisanal and commercial distilleries [7, 8].

This research is the third part of our researcher in anisated spirits. This knowledge should lead to a better quality and aromatic profile of this anisated spirit.

2. Material and Methods

2.1 Samples

Grape pomace samples were aromatised using the seeds of *Pimpinella anisum* (glykaniso), *Foeniculum vulgare* (marathon) and dry seeds of staranise (*Illicium verum*), and they were distilled in a small traditional distillation apparatus. The distillates were stored in vials at 4 °C until used.

2.2 Reagents

The chemicals used in this work were analytical grade, commercially available reagents (from Merck). Trans- and cis-anethol was obtained from Fluka Chemical Corp. They were supplied as pure grade chemicals with a purity > 98%. Water was distilled and deionised. The solution was stored in refrigerator (4 °C) in the dark.

2.3 Chemical Analysis of Volatile Compounds by Head Space GC-MS

The volatile components present in headspace fraction of anisated distillates were isolated and identified by using a balance pressure headspace system Perkin-Elmer HS40 (Perkin-Elmer Analytical Instruments, Uberlingen, Germany) coupled to a GC-MS (gas chromatography-mass spectrometry)-Q 5050 system (Shimadzu Co, Kyoto, Japan). 2 mL

sample from each sample was taken and introduced into a 22 mL round-bottomed vial with 1 mL aliquot of standard solution (2 mg pentanol-3 in ethanol-water solution 50:50 (v/v)); then, the vials were sealed with aluminium-rubber septa. The vials with samples were held at 50 °C for 25 min, purged and pressurised with helium at a flow rate of 40 mL·min⁻¹. The volatile compounds were driven through the transfer line which was held at 80 °C to the injector of the gas chromatograph. The volatile compounds were separated on an HP-INNOWAX capillary column (60 m length × 0.25 mm internal diameter, 0.25 µm film thickness) at the following conditions: injector temperature—200 °C; carrier gas helium—0.6 mL·min⁻¹; temperature program—45-100 °C at a rate of 4 °C·min⁻¹, held for 5 min and go to 200 °C at a rate of 8 °C·min⁻¹ and held for 12 min. The GC (gas chromatograph) column was directly connected without splitting to the ion source of QP 5050 quadrupole mass spectrometric detector which was operating in the scan mode within a mass range of m/z 30-350 at 2 scans·s⁻¹. The interface line to MS (mass spectrometric) was set at 250 °C. The MS was operating in an electron impact mode at electron energy of 70 eV and was calibrated by auto-tuning.

Identification of the compounds was carried out by computer-matching of their mass spectral data with those of known compounds in the Shimadzu NIST62 Mass Spectral Database and by comparing their retention times and mass spectra to 3-pentanol as internal standards solution.

Quantification was performed by integrating the peak areas of TIC (total ion chromatograms) by the Shimadzu Class 500 software.

Oven temperature programme—50-260 °C at a rate of 4 °C·min⁻¹; transfer line temperature—270 °C; carrier gas helium—at a linear velocity of 31.5 cm·s⁻¹; inlet split ratio—1:60; MS source ionization energy—70 eV; scan time—1 s, covering a mass range of 40-300 amu.

The constituents were identified by comparison of

their mass spectra with those in a computer library (LIBR-TR and Wiley 5 Library) or with authentic compounds. The identifications were confirmed by comparison of their retention indices of volatiles either with those of authentic compounds or with data in the literature.

Quantitative results were obtained by calculating the average value of three samples.

3. Results and Discussion

The results of the quantitative determination of trans-anethol, cis-anethol and major volatiles in *Pimpinella anisum* (glykaniso), *Foeniculum vulgare* (marathon) and *Illicium verum* (star anise) samples are presented in Tables 1 and 2.

In total, 10 major aromatic constituents were identified.

The chemical composition of volatiles in *Pimpinella anisum* (glykaniso) samples shows that the major volatile in seeds extract is trans-anethol (92.4%), and cis-anethol levels is 0.5%. *Foeniculum vulgare* (marathon)—trans-anethol concentration is 89.1% and

cis-anethol is 0.7%. *Illicium verum* (star anise)—trans-anethol concentration is 95.3% and cis-anethol is 1.1%.

Hopefully the results of the chemical composition of those seeds, which are used in traditional and commercial distilleries [8], will bring useful information associated with trans- and cis-anethol concentration and other aromatic agents.

4. Conclusion

In *Pimpinella anisum* (glykaniso), *Foeniculum vulgare* (marathon) and *Illicium verum* (star anise) seeds, which are used as an aromatic agent in anisated distillate, trans- and cis-anethol and 10 different aromatic compounds were identified. Trans-anethol, the compound which gives the anisated taste in anisated alcoholic beverages, exists in all samples in satisfactory levels. Trans-anethol exists in higher levels in *illicium verum* (star anise), but with not important differentiation from the other aromatic seeds. It was noticed that limonene and myrcene were in satisfactory levels to all samples.

Table 1 Chemical percentage composition (%) of major aromatic volatiles from *Pimpinella anisum* (glykaniso), *Foeniculum vulgare* (marathon), and *Illicium verum* (star anise) in anisated alcoholic beverages.

Compounds	RI*	<i>Pimpinella anisum</i> (glykaniso) (%)	<i>Foeniculum vulgare</i> (marathon) (%)	<i>Illicium verum</i> (star anise) (%)
Sabinene	955	0.7	ND	0.3
Myrcene	979	0.9	1.4	0.5
p-cymene	1,006	0.4	1.4	0.2
d-Limonene	1,010	0.9	0.5	2.4
n-Nonanal	1,075	0.2	0.3	0.5
Anisaldehyde	1,203	0.1	2	ND
Carvacrol	1,286	0.1	1.1	ND
a-bergamotene	1,436	0.3	1.2	2.1
Phellandrene	1,511	0.2	0.4	1.4
Caryophellene	1,568	0.2	0.2	0.3

*RI: retention index;

ND: not detected.

Table 2 Chemical percentage composition (%) of trans- and cis-anethol from *Pimpinella anisum* (glykaniso), *Foeniculum vulgare* (marathon), and *Illicium verum* (star anise) in anisated alcoholic beverages.

Compounds	RI*	<i>Pimpinella anisum</i> (glykaniso) (%)	<i>Foeniculum vulgare</i> (marathon) (%)	<i>Illicium verum</i> (star anise) (%)
Trans-anethol	1,234	92.4	89.1	95.3
Cis-anethol	1,221	0.5	0.7	1.1

*RI: retention index.

Also, it is important to continue this research to have better knowledge for the chemical variability of those seeds, which was used from the ancient years as an aromatic and medicinal herb in food and beverages.

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