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Assessing the authenticity of absinthe using sensory evaluation and HPTLC analysis of the bitter principle absinthin

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Abstract

Absinthe is a spirit drink that owes its bitter taste to substances found in the wormwood plant (*Artemisia absinthium* L.). The prohibition against absinthe has recently been annulled, giving rise to numerous inferior products that lack the organoleptic characteristics of wormwood. The monoterpene thujone, which occurs in the essential oil fraction of wormwood, has previously been used as a marker substance to confirm the authenticity of absinthe. However, thujone possesses adverse toxicological properties; thus modern procedures have been developed to remove this substance from absinthe. In addition, thujone-free wormwood is also available from certain cultivation areas.

This study is the first to use sensory evaluation of wormwood taste, louche effect, and bitterness in order to classify absinthes. This study also introduces a simple, fast, and sensitive procedure using high-performance thin-layer chromatography (HPTLC) to assess the levels of absinthin, a characteristic bitter substance found in wormwood. Results of this study demonstrate a strong correlation between the concentration of wormwood and the organoleptically determined bitterness value ($R = 0.75$).

Keywords: Absinthe; Wormwood; *Artemisia absinthium* L.; Bitterness; Thujone; Absinthin; Spirit drink; High-performance thin-layer chromatography (HPTLC)

1. Introduction

Absinthe is a spirit drink with strong bitter taste and characteristic green colour that is currently experiencing a resurgence in popularity after nearly 70 years of prohibition. Today, absinthe is not only available in specialty shops or on the internet, but also in supermarkets. There are currently many different kinds of absinthe that are commercially available. Authentic absinthe is made from the wormwood plant (*Artemisia absinthium* L.) from the family of composites (Asteraceae). However, numerous inferior products have recently appeared in the market that lack the organoleptic characteristics of wormwood (Lachenmeier et al., 2004; Lachenmeier, Emmert, & Sartor, 2005; Lachenmeier, Walch, Padosch, & Kröner, 2006). Therefore, official testing and validation is needed to verify the presence of wormwood in order to protect the consumer from being deceived by inferior products.

Wormwood contains a pungent and exceptionally bitter essential oil (0.2–1.5%) that is dark-green to brown or blue in colour. One of the primary constituents of this oil is the bicyclic monoterpene thujone (Sacco & Chialva, 1988; Vostrowsky, Brosche, Ihm, Zintl, & Knobloch, 1981). Despite thujone's toxicity (Arnold, 1989; Bonkovsky et al., 1992; Höld, Sirisoma, & Casida, 2001; Höld, Sirisoma, Ikeda, Narahashi, & Casida, 2000; Hutton, 2002; Lachenmeier, Emmert, Kuballa, & Sartor, 2006; Olsen, 2000; Padosch, Lachenmeier, & Kröner, 2006; Strang, Arnold, & Peters, 1999), this compound has previously been exploited as a marker to assess the authenticity of absinthes (Manuel suisse des denrées alimentaires, 2000). However, the percentage of thujone can vary widely (0–42%), depending on

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the origin of the wormwood plant (Chialva, Liddle, & Doglia, 1983). Recently, thujone-free chemotypes of wormwood were found in the Spanish Pyrenees (Ariño, Arberas, Renobales, Arriaga, & Dominguez, 1999) and in Tuscany (Nin, Arfaioli, & Bosetto, 1995). These plants may be used by the beverage industries to produce absinthe that complies with the legal limitations on thujone content that were imposed along with the annulment of absinthe prohibition in the European Union (European Council, 1988). Another method to produce thujone-free absinthes involves detoxification of wormwood using supercritical fluid extraction (Stahl & Gerard, 1983) or the use of mild percolation of wormwood with water or ethanol (30%vol), which does not extract thujone (Tegtmeier & Harnischfeger, 1994). For all these reasons, thujone is unsuitable as an unambiguous marker of authenticity for absinthe.

Other characteristic substances in wormwood include terpene lactone bitter substances such as absinthin (0.20–0.28%) and artabsin (0.04–0.16%) (Frohne, 1984). Of these two compounds, the dimeric sesquiterpene lactone absinthin is the predominant bitter substance (Fig. 1) (Schneider & Mielke, 1979). The bitterness of wormwood is not affected by the thujone extraction procedures described above (Stahl & Gerard, 1983; Tegtmeier & Harnischfeger, 1994). Therefore, this study attempted to use the bitter substances of wormwood as novel markers to confirm the authenticity of absinthe.

Thin-layer chromatography (TLC) is a traditional method used to analyse sesquiterpene lactones, which are easily visualized using spray reagents like vanillin/*o*-phosphoric acid, anisaldehyde or *p*-dimethylaminobenzaldehyde-sulphuric acid, sulphuric acid, resorcin-sulphuric or phosphoric acid, aluminium chloride or hydroxylamine (Merfort, 2002). Several TLC methods for the determination of bitter substances in wormwood were reported for pharmaceutical purposes (Schneider & Mielke, 1978; Schneider & Mielke, 1979; Wagner, Bladt, & Münzing-Vasirian, 1975; Wagner, Bladt, & Zgainski, 1983; Wormwood, 2005). By comparing TLC and HPLC profiles, Yashiro, Sugimoto, Sato, Yamazaki, and Tanamoto (2004) were able to determine the origin of wormwood extracts as the aerial parts of *Artemisia absinthium*.

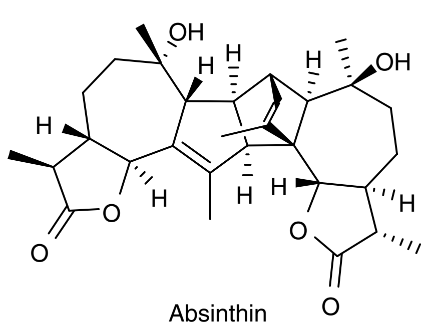

Fig. 1. Chemical structure of absinthin, the main bitter substance of *Artemisia absinthium* L.

In this study, a TLC method based on European pharmacopoeia (Wormwood, 2005) was adapted and a simple and economical HPTLC assay was developed in order to determine absinthin content in alcoholic beverages. Additionally, the bitter principle of wormwood is used for the first time to assess the authenticity of absinthe.

2. Materials and methods

2.1. Absinthe samples

Twenty-three absinthe samples submitted by local authorities to the CVUA Karlsruhe have been analysed for absinthin using HPTLC. In all cases, there was a full organoleptical and chemical examination, which included the determination of relative density and ethanol using steam distillation and densitometry (Lachenmeier, Burri, Fauser, Frank, & Walch, 2005; Lachenmeier, Sviridov, Frank, & Athanasakis, 2003), the determination of artificial food dyes using a separate TLC method (Frank, 2002) (the dyes are not separated using the absinthin method described in this paper), the determination of higher alcohols and other volatile substances using gas-chromatography with flame ionisation detector (Frank, 2002), as well as the determination of α- and β-thujone using gas-chromatography/mass spectrometry (GC/MS) (Lachenmeier et al., 2006).

2.2. Sensory evaluation of bitterness

The bitterness value of all absinthes was determined using the procedure given in the European pharmacopoeia (Bitterness Value, 2005; Wormwood, 2005). The bitterness value was calculated as reciprocal of the dilution that still has a bitter taste. It was determined by comparison with quinine hydrochloride, the bitterness value of which was set at 200,000. A trained taste panel comprising five persons was used and the bitterness value was calculated as average value for all panel members.

In addition, the taste panel qualitatively examined the absinthes for the existence of the typical wormwood aroma (reference: wormwood herb extracted in 60%vol ethanol) and the louche effect.

2.3. Reagents and materials

Wormwood (*Artemisia absinthium* L., Asteraceae) was obtained from Caesar and Loretz (Hilden, Germany), Bombastus-Werke (Freital, Germany) and Heinrich Klenk (Schwebheim, Germany). For the use as standard, 100 g of wormwood from all three different suppliers, respectively, were blended and homogenized in a standard mixer. α-Thujone was purchased from Fluka (Buchs, Switzerland). Chemicals were purchased from Merck (Darmstadt, Germany) and Sigma–Aldrich Chemie (Taufkirchen, Germany).

2.4. Thin-layer chromatography apparatus and procedure

Thin-layer chromatography was performed on precoated 10 cm × 10 cm HPTLC glass plates (sorbent: silica gel; pore size: 60 ; fluorescence indicator: F254; Merck, Darmstadt, Germany). Sample volumes of 20 μl were applied to the plates as bands with a width of 6 mm using a TLC applicator (Automatic TLC Sampler III, Camag, Berlin, Germany). The plates were developed using a freshly prepared mobile phase of acetone/acetic acid (98%)/toluene/dichloromethane (10:10:30:50, v/v). After drying at room temperature, the spots were stained with a solution of acetic anhydride/sulphuric acid/ethanol (10:10:100, v/v) by dipping, followed by heating for 5 min at 104 °C. In the presence of absinthin, brown coloured spots can be detected by densitometry at 554 nm with slit dimensions of 3.00 mm × 0.45 mm, and a scanning speed of 20 mm/s (TLC-Scanner 3, Camag). Spectra of the identified spots were recorded in the range of 400–700 nm with a scanning speed of 100 nm/s. Using external standards of wormwood extracts, the concentration of wormwood in the absinthe samples could be determined semiquantitatively. For calibration, the concentration of wormwood was calculated as a function of the peak area of absinthin.

2.5. Identification of absinthin

For the identification of absinthin, the problem has arisen that this substance is not commercially available. The isolation from the wormwood plant after Herout, Novotný, and Šorm (1956) or total synthesis after Zhang et al. (2005) was not feasible in our laboratory. Therefore, identification of the bands under study was made with the aid of authentic wormwood extracts and the reference resorcinol, which were all applied onto each HPTLC plate. According to the European pharmacopoeia (Wormwood, 2005), absinthin has similar Rf values to resorcinol. In addition, the VIS spectra of the analytes were measured and compared to reference spectra of the authentic wormwood extract.

2.6. Sample preparation

Twenty-five milliliter of the absinthe sample were extracted with 50 ml of dichloromethane. The organic phase was separated, dried with anhydrous sodium sulphate and the filtrate was evaporated to dryness. The residue was dissolved with 0.5 ml of ethanol (96%). For the preparation of the wormwood external references after the European pharmacopoeia (Wormwood, 2005), 2 g of pulverized wormwood were extracted with 50 ml of boiling water for 5 min. After cooling, 5 ml of a solution of lead acetate (100 g/l) were added for clarification. The filtrate was extracted with 50 ml of dichloromethane and treated by the same procedure as the absinthe samples. For calibration, different dilutions of the wormwood extracts were used.

2.7. Validation studies

For the validation of the method, samples (60%vol ethanol) were spiked with wormwood extracts. The linearity of the procedure was evaluated between 0.1 and 10 g/l wormwood. For the determination of the limits of detection (LOD) and quantitation (LOQ), separate calibration curves in the range of LOD (0.01–0.2 mg/l) were established (DIN 32 645, 1994; Meier & Zünd, 2000). Repeated analyses of two authentic absinthe sample and wormwood reference were used to examine the precision of the method. The interference of typical ingredients of absinthes was assessed by analysing aniseed-flavoured spirit drinks spiked with thujone, anethole, yellow and blue food dyes (E102, E104, E124, E131, E133) and sugars (glucose, fructose and sucrose). The stability of the analytes before and after chromatography was determined by comparison between directly developed plates and plates developed after 24 h, as well as direct densitometry and scanning 24 h after development.

2.8. Statistics

All data were evaluated using standard statistical packages for Windows. Statistical significance was assumed at below the 0.05 probability level. Pearson's test was used to evaluate the significance of correlations.

3. Results

Table 1 summarizes the method validation data. The analyte exhibited good linearity with regression coefficients greater than 0.99. The detection limit for wormwood in absinthe was 0.05 g/l. No interferences were observed during the analysis of typical ingredients of absinthes or during

Table 1
Validation results of the HPTLC procedure

		Absinthin
Rf value		0.64 ± 0.03
LOD[a] (g/l)		0.05
LOQ[a] (g/l)		0.11
Intraday-Precision[b] (%) ($n = 5$)	Absinthe 1	13.5
	Absinthe 2	7.8
	Wormwood	11.6
Interday-Precision[b] (%) ($n = 16$)	Absinthe 1	15.8
	Absinthe 2	8.1
	Wormwood	11.9
Regression line	Linear range[a] (g/l)	0.1–10
	Correlation coefficient	0.999

[a] Expressed as g/l wormwood in sample. Limit of detection and quantitation were determined by establishing a specific calibration curve from samples containing the analyte in the range of LOQ. The limits were calculated from the residual standard deviation of the regression line [27,28].

[b] Precisions are expressed as RSD (%): precision = standard deviation/mean value 100 (%).

routine analyses of 23 authentic samples. The precisions never exceeded 13.5% RSD (intraday) and 15.8% RSD (interday). During the assessment of the stability, no significant loss or degradation of analytes was detected 24 h before and after chromatography.

The analysis results of 23 absinthe samples are listed in Table 2. The sensory evaluation yielded bitterness values upto 313. Using HPTLC, the wormwood content ranged from 0.1 to 7.8 g/l. In 10 samples, absinthin could not be detected.

4. Discussion

4.1. HPTLC procedure

The separation of the wormwood standard and absinthe samples is shown in Fig. 2. Fig. 3 shows typical VIS spectra of the samples after densitometry of HPTLC plates. As one can see, a very successful separation was achieved under the specified conditions. The principal advantages of HPTLC as a screening technique for wormwood are its low cost, methodological simplicity, high sample throughput, and minimal requirements for sample cleanup. Using a TLC-Scanner, it is possible to obtain a semi-quantitative estimation of the wormwood content in absinthe samples by assessing the content of the bitter substance, absinthin. As evidence by the validation data, this procedure is sensitive, selective, and reproducible. The applicability of the developed method was demonstrated by the investigation of 23 commercial food samples. All results were satisfactory according to the requirements of official food control.

4.2. Authenticity assessment of absinthe

Evaluation of absinthe authenticity presents a challenge for official food control because a legal definition of this spirit drink is not provided in either the German definitions (Begriffsbestimmungen für Spirituosen in der Fassung vom 24.6.1971, 1971) or in the rules of the European Union on the definition, description, and presentation of spirit drinks (European Council, 1989). Only recently were customs of trade and minimum standards for absinthe defined on the basis of a market review (Lachenmeier et al., 2004; Lachenmeier et al., 2006). A wormwood flavour, bitter taste, and turbidity when diluting with water (louche effect) should all be ascertainable for authentic absinthe. Moreover, products advertised as being of higher quality (e.g. with advertising claims like "according to historical recipes") should be produced by distillation, contain no artificial dyes, and have a minimum alcohol content of 45%vol. Therefore, all absinthe samples were examined for these minimum standards.

Table 3 demonstrates a statistically significant linear correlation between the levels of absinthin and the sensory perception of bitter taste ($p < 0.0001$). In contrast, no correlation exists between bitter value and thujone. This proves that absinthe's bitter taste mainly originates from the sesquiterpene lactone bitter substances and not from

Table 2
Results of sensory and analytical evaluation of commercial absinthe samples

Sample no.	Origin	Sensory evaluation			Analytical evaluation		
		Wormwood taste	Louche effect	Bitterness value	Absinthin[a] (g/l)	α-Thujone (mg/l)	β-Thujone (mg/l)
1	Austria	+	+	14	n.d.[b]	n.d.	39.3
2	Austria	+	+	28	n.d.	n.d.	13.5
3	Czech Republic	–	–	38	n.d.	n.d.	n.d.
4	Czech Republic	+	–	313	3.4	10.8	n.d.
5	Czech Republic	+	–	100	1.3	29.9	6.8
6	France	+	++	14	0.5	6.3	7.1
7	France	+	–	33	n.d.	2.5	0.9
8	France	–	+	23	n.d.	6.3	4.8
9	France	+	+	6	1.9	0.6	0.5
10	Germany	+	++	28	1.5	n.d.	n.d.
11	Germany	–	+	n.d.	n.d.	5.9	4.4
12	Germany	++	+	140	3.5	2.8	67.3
13	Germany	–	+	28	1.8	0.1	0.1
14	Germany	++	–	274	6.4	n.d.	0.7
15	Germany	+	–	139	7.8	n.d.	n.d.
16	Germany	–	+	n.d.	n.d.	7.4	3.8
17	Germany	–	++	n.d.	n.d.	n.d.	n.d.
18	Germany	–	+	11	n.d.	1.2	0.2
19	Germany	–	++	11	0.5	0.1	4.8
20	Germany	–	+	60	0.3	2.8	3.0
21	Germany	+	+	55	1.4	1.7	26
22	Spain	–	+	12	n.d.	0.1	1.5
23	Spain	+	+	40	0.1	1	21

[a] Semi-quantitative result calculated as wormwood content in comparison to reference wormwood.
[b] Not detected.

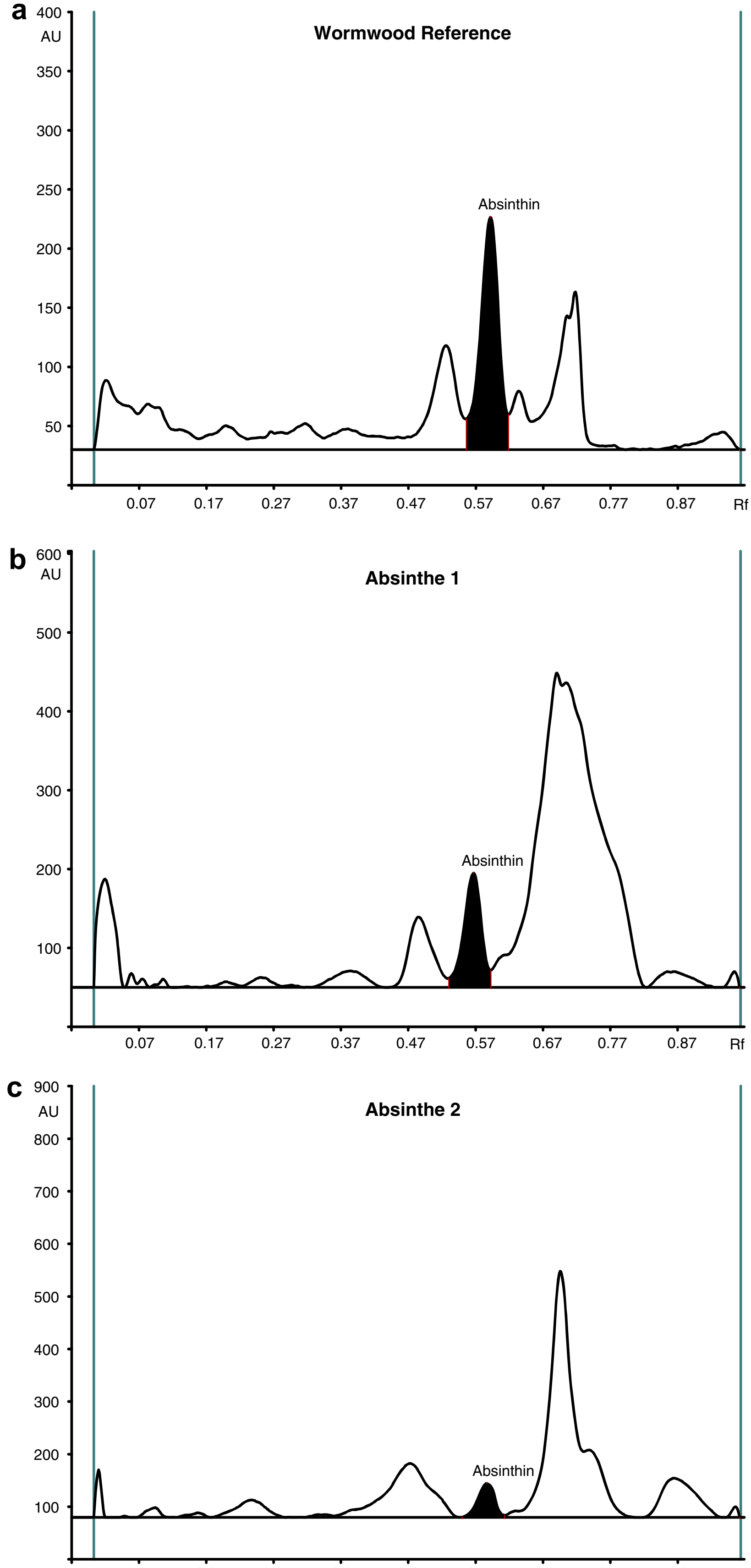

Fig. 2. Separation of wormwood (a) and authentic absinthe samples (b and c) on HPTLC silica plates detected at 554 nm using in situ densitometry. The spots of the marked peaks (absinthin) are dark brown in colour. The peak assignment can be verified using VIS spectra. (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

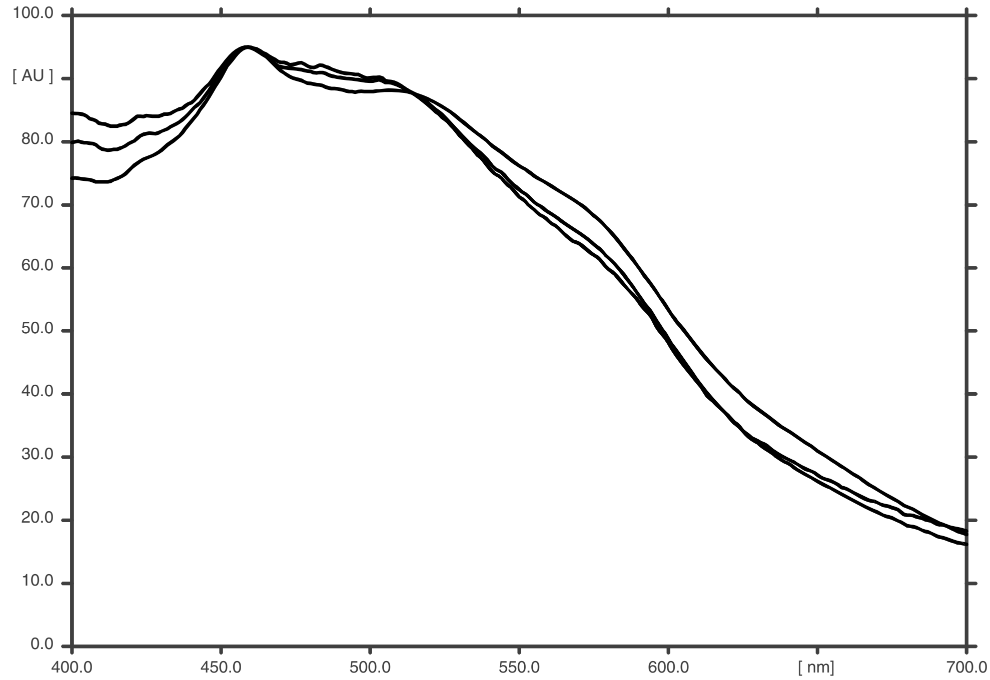

Fig. 3. HPTLC VIS spectra of absinthin acquired using in situ densitometry (Wormwood standard at three different concentrations).

Table 3
Linear correlation between bitterness value and absinthin, α-Thujone, and β-Thujone

Parameter	Bitterness value/ Absinthin	Bitterness value/ α-Thujone	Bitterness value/ β-Thujone
Correlation coefficient	0.75	0.22	0.06
Probability	<0.0001	0.31	0.79

Table 4
Minimum requirements of absinthe

Minimum requirements
Characteristic aromatic flavour and bitter taste caused by natural extracts or distillates of wormwood (*Artemisia absinthium* L.)
Colour: uncoloured or greenish
Characteristic clouding if diluted with water (“louche-effect”)
Detectable concentration of absinthin
Standard chemotypes: β-thujone > α-thujone
Further requirements for premium grade products
No artificial dye (colouring achieved only with wormwood and other herbs)
Distillative manufacturing
Minimum alcoholic strength 45%vol

monoterpenes that also might activate human bitter taste receptors and might explain the bitterness values of samples with not detectable absinthin concentrations (e.g. samples no. 7, 8, 18, and 22). As there are other possible sources for bitterness (e.g. falsification with bitter substances from citrus fruits), the sensory evaluation of bitterness can only be used as complement to the other criteria.

In two of the absinthe samples (no. 3 and 17), neither thujone nor absinthin could be identified. Furthermore, a wormwood taste was not detectable in these samples. These results indicate that the bitter substance, absinthin, is suitable as marker for the evaluation of absinthe and can be integrated as a new criterion into the proposed minimum standards (Table 4).

4.3. Quality classification of absinthe

Traditional recipes for absinthe, call for an initial maceration of wormwood and other dried herbs (e.g. anise, fennel, and lemon balm). The macerate is greenish-brown and aromatical, with the typical camphor-like flavour of *Artemisia* species. The flavour is reminiscent of chamomile and is strongly bitter and produces a slight burning sensation (Ströhmer, 2002). During the subsequent distillation of the macerate, the majority of the low-volatile bitter substances are separated. The typical volatile, spicy components of wormwood flavour occur in the first fractions between 80 and 60%vol. The flavour of the middle fractions is reminiscent of clove and cinnamon (Ströhmer, 2002). Traditionally, a type of steam-distillation was used as significant amounts of water were added to the alcoholic macerate prior to distillation. Due to the influence of this water-cum-steam distillation, higher concentrations of substances with lower volatility may have been distilled over. Distillation of absinthe should never be carried on to the end, as the taste of the product would be too strong, and less fine. Therefore, only the main fractions (heart) were used for the production of high-quality absinthe (Padosch et al., 2006). Wormwood and other herbs are again added to the clear distillate in order to extract chlorophyll for the typical greenish colour, as well as further flavour compounds that produce a mild bitter taste. Due to easy denaturing of the chlorophyll by light and heat, the typical colour of a traditionally manufactured absinthe is only pale-green. Finally, the beverage is attenuated with water to reduce it to bottling strength. None of the samples in this study fell below our proposed minimum value of 45%vol of alcoholic strength. The majority of products had alcoholic strengths above 55%vol.

High-quality products are still produced today that follow the original recipes and contain no artificial dyes or other additives. Therefore, a high wormwood fraction was proven in products no. 12, 14 and 15. Alternatively, some products are manufactured only from the herb distillates and are characterised by a particularly mild flavour. Since these products are colourless, they are sold under supplementary customary names like "Blanche" or "La Bleue". The sample no. 9 can be assigned to this group of high-quality products, which possess a pronounced wormwood flavour. Because of the distillation procedures, these products have lower levels of the low-volatile bitter substances relative to the products manufactured by maceration. Nevertheless, absinthin and thujone could be detected in this sample. However, varying distillation and rectification conditions could significantly influence the concentrations in the product and present a limitation of the method especially if the distillate is not coloured with wormwood. Therefore, the absence of absinthin cannot directly disprove the authenticity but the sample must be evaluated in consideration of a holistic view of all parameters. For example, samples no. 1 and 2 had not detectable absinthin concentrations but a noticeable wormwood taste and relatively high thujone concentrations. In these cases, a production using commercially available wormwood oil that contains no absinthin can be assumed. Sample no. 23 also has only a very low concentration of absinthin but due to the other criteria, this sample can be judged as authentic absinthe.

In contrast to the high-quality samples, many products are currently manufactured by mixing ethyl alcohol of agricultural origin with commercially available extracts of wormwood and other herbs. The macerated herbs are not distilled in this process, and the extracts are immediately reduced after filtration to bottling strength. These absinthes are characterized by a strongly pronounced wormwood taste and a very strong, nearly unpleasant bitter note. A typical example of this category is absinthe no. 4, which is characterized by the highest bitter value in the study.

In order to produce the typical green colour with few herbs, artificial dyes are added (in particular a mixture of tartrazin (E102, FD& C Yellow no. 5) with patent blue V (E131) or brilliant blue FCF (E133)). Such products include samples no. 10 and 21. These samples still possess an acceptable wormwood flavour, which can be confirmed by the analysis of absinthin. However the bottles containing these samples do not contain the proper labels denoting the presence of dyes, which is typical for absinthes produced in Spain and Czech Republic. In some cases, dyes other than those indicated on the label were detected in the product. 41% of all absinthes examined at the CVUA Karlsruhe were determined to have improper labelling for dyes (Lachenmeier et al., 2004).

4.4. Food chemical and food regulatory evaluation

Inferior products do not contain any herb extracts and are manufactured by artificial aromatizing and colouring of ethyl alcohol. In this study, two absinthes (samples 3 and 17) were determined to be inferior due to the absence of wormwood. Furthermore, the other minimum standards for absinthe (Table 4) were not fulfilled by these products, thus these products should not technically be marketed under the name "absinthe." The consumers who purchased these samples were deceived by these inferior and often expensive products.

In particular Czech absinthes deviated strongly from taste and composition standards relative to absinthes from other countries, such as Switzerland, France, or Germany. Many of these samples did not produce the louche effect and did not possess the typical bitter taste of wormwood. Instead, these samples tasted sweet and mint-like. Additionally, these beverages have an atypical turquoise colour caused by the use of artificial dyes. According to a statement issued by an importer, this composition is typical for Czech absinthes and represents the typical formulation consumed in the Czech Republic. However, this deviation should be marked on the label to ensure that the consumer is not deceived. Other products have all the typical criteria of absinthe besides the louche effect (e.g. sample no. 14). This is caused by the absence of anise and fennel that predominantly contribute to the louche effect with their essential oils. Such a deviation should also be clearly labelled on the bottle.

Based on the results presented in this report, we recommend a manufacturing control be placed on absinthe products that fail an organoleptical examination and do not contain detectable levels of thujone or absinthin, or if there are any inconsistencies between the different criteria. This control will allow authorities to examine the recipes used by these manufacturers and validate or disprove the analytical results. A minimal level of wormwood and a detectable louche effect should be mandatory if a beverage is to be marketed under the customary name "absinthe."

5. Conclusion

To our knowledge, the HPTLC assay is presently the only useful method to determine the wormwood proportion in absinthe.

The advantage over the use of thujone for authentification of absinthe is that the bitter substances are unique to wormwood and cannot be incorporated into the beverage from other sources. For consumer protection and in the interest of a consistent evaluation, absinthe should be included into the European rules on the definition, description and presentation of spirit drinks (European Council, 1989). Unfortunately the suggestion of Germany for such an European regulation recently failed (Lang, Fauhl, & Wittkowski, 2002).

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