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Long-Term Stability of Thujone, Fenchone, and Pinocamphone in Vintage Preban Absinthe

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Research was conducted to ascertain whether analyses of vintage absinthe samples represent their original composition in the early 1900s. Absinthe stored in traditional green glass bottles and irradiated with ultraviolet light for up to 200 h exhibited unchanged composition. Samples stored in clear glass exhibited an 18% reduction in β-thujone content and a concurrent decoloration. These experiments indicate the stability of thujone in vintage absinthes, as these were stored in green glass bottles. The preserved color of the preban absinthes subjected to analysis indicates that no significant light exposure occurred throughout the duration of storage, and therefore provides indirect proof that no loss of terpenes occurred. The stability of absinthe was further demonstrated through the reanalysis of samples from 2001−2005, which exhibited no changes in thujone content as of 2008. A previous evaluation of preban absinthe was therefore valid and not confounded by significant thujone deterioration over time.

KEYWORDS: Alcoholic beverages; absinthe; thujone; fenchone; pinocamphone; *Artemisia absinthium* L.; wormwood; UV irradiation; long-term storage; accelerated aging

INTRODUCTION

We recently reported (*1*) the analytical results of 13 absinthes manufactured during the so-called “preban” era (i.e., the period of time that ranges from ca. 1805 until the banning of absinthe in France, in 1915). The absinthes were analyzed for thujone content as well as for further parameters that have been hypothesized as contributing to the toxicity of preban absinthe, including naturally occurring herbal essences (e.g., pinocamphone, fenchone), alcohols, and metals. The major (and perhaps surprising) outcome of the study was the finding that the thujone concentration of preban absinthe had been grossly overestimated in the past. Papers published in the 1990s postulated thujone concentrations as high as 260 mg/L on the basis of purely theoretical calculations and not actual analysis (*2, 3*), whereas the direct analysis of total thujone content in actual vintage samples was found to range between 0.5 and 48.3 mg/L. The average thujone content of 25.4 ± 20.3 mg/L fell within the modern European Union (EU) limit of 35 mg/L. The concentrations of pinocamphone and fenchone were similarly below toxic ranges (*1*).

After the online publication of this research (*1*), followed by a press release of the American Chemical Society (*4*) and an article in *Chemical & Engineering News* (*5*), the results of the study were mentioned in over 100 media publications throughout Europe and the United States. Certain press inquiries and some comments by the public were centered around the single question of the stability of thujone in the period between the time of manufacture around a century ago and the present day. An example is a letter to the editor (*6*), in which we were criticized for having dismissed the fact that thujone might have been degraded in 100-year-old bottles. Whereas most of the allegations could be easily rebutted due to a lack of scientific foundation [e.g., the erroneous claim of a low boiling point of thujone that might lead to evaporation (*7*)], we acknowledged that there are few research papers on the stability of thujone in alcoholic solutions.

The current study therefore investigates the long-term stability of absinthe using two different approaches: UV irradiation of absinthe until complete decoloration to simulate aging, and reanalysis of samples of commercial absinthes obtained from 2001 to 2005 to check stability over periods of up to 7 years. Additionally, we have analyzed some further preban absinthes, which became available since the publication of the previous study. We were also frequently asked about the thujone content of the vintage absinthes that have not met the strict authenticity criteria (*1*) with the speculation that those might have contained higher thujone contents. We therefore decided to report the results of two additional samples, which could be clearly

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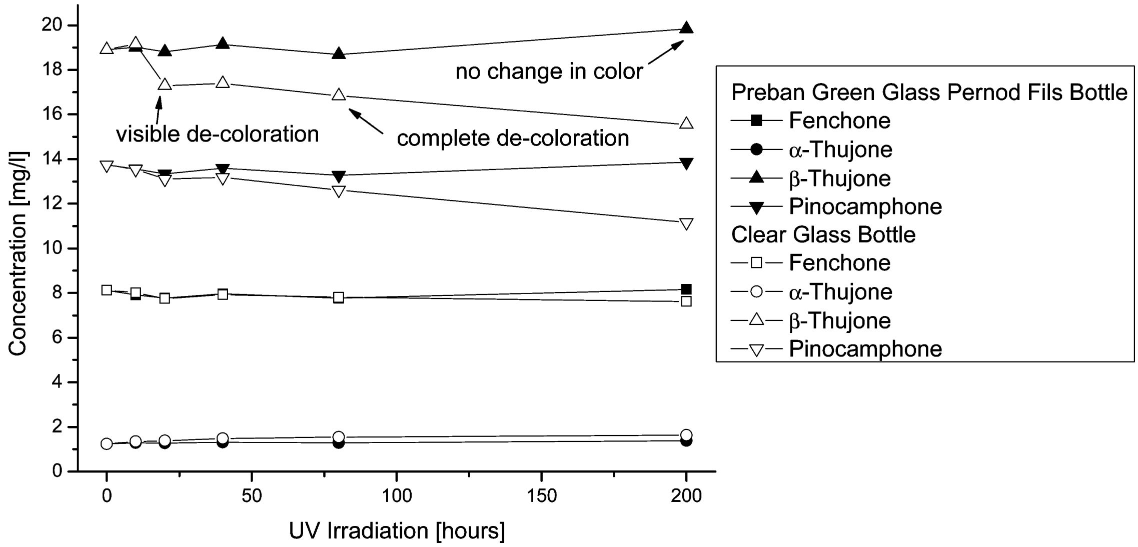

Figure 1. Influence of accelerated aging using UV irradiation on the thujone, fenchone, and pinocamphone contents of absinthe. No significant changes occurred in the green glass bottle, whereas the product in the clear glass bottle became completely decolored and some losses of the terpene compounds occurred.

assigned to the preban era but were previously excluded due to minor flaws (i.e., missing complete bottle labels).

MATERIALS AND METHODS

Samples and Sample Storage. The preban absinthes were sampled and authenticated according to the methodology previously described (*1*). The UV irradiation experiment was conducted with an absinthe manufactured by one of the authors (D.N.-M.) according to historic principles using antique Egrot alembics.

The modern commercial absinthes were sampled in the context of official food control by governmental food inspectors in the German Federal State Baden-Württemberg in the years between 2001 and 2005. The samples had been analyzed at the time of submission to the laboratory. The opened bottles (rest volumes approximately 50−200 mL) were then stored on an open laboratory shelf at ambient temperatures directly opposite a window. During storage, the bottles were exposed to full daylight, as no sun screens were available in the storage room. All commercial samples were reanalyzed in August 2008.

UV Irradiation Experiment To Simulate Aging. The experiment was conducted using two different types of bottles, an authentic preban Pernod Fils green glass bottle from ca. 1910 (1 L, glass thickness approximately 0.4 cm) and a modern clear glass spirits bottle (0.7 L, glass thickness approximately 0.3 cm) from one of the commercial absinthes. Both bottles were filled with 100 mL of the same absinthe, and six 25 W ultraviolet (UV) fluorescent tubes type "excellent E" built into a facial tanner type "NT 446 U" (Dr. Kern GmbH, Mademühlen, Germany) were placed 15 cm from the surface of the bottles. The bottles were exposed to the light for 10, 20, 40, 80, and 200 h.

The statistical evaluation of the experiments was done using the software package Design Expert V6 (Stat-Ease Inc., Minneapolis, MN). Analysis of variance (ANOVA) was used to determine the significance of the models. The models were checked for consistency by looking at the lack of fit and possible outliers.

Analysis and Analytical Quality Assurance of Thujone, Pinocamphone, and Fenchone in Absinthe. The analysis was conducted according to the method detailed in ref *1* without changes. The analytes were extracted using liquid−liquid extraction with 1,1,2-trichloro-1,2,2-trifluoroethane followed by gas chromatography with mass spectrometric detection (GC-MS). The limit of detection was 0.08 mg/L.

As the credibility of this method was questioned (*6*), we wish to comment on method validation and quality assurance conducted in the institute. The CVUA Karlsruhe works according to the principles outlined in ISO 17025 and is externally accredited. The accreditation includes the alcohol and GC-MS laboratories including the method for thujone determination in spirits. A full method validation was previously reported (*8*). This method is based on the liquid−liquid extraction methodology established and validated by Rapp et al. in 1994 (*9*). The method performed successfully in an interlaboratory trial (*10*). The sample preparation using liquid−liquid extraction gave results not statistically different from other methods such as distillation or solid-phase microextration (*11*). Never has a deterioration of thujone in calibration standards been observed as objected in ref *6*. On the contrary, a calibration curve was reanalyzed from stock solutions prepared in 2007 against a freshly prepared calibration curve in 2008, and no significant differences were found between the calibrations. The analysts have always used freshly prepared calibration standards (in this study and in all previous ones), and due to internal and external quality assurance measures, the supposition that thujone deterioration in standard solutions led to under-reporting of thujone contents in preban absinthe can be wholly excluded.

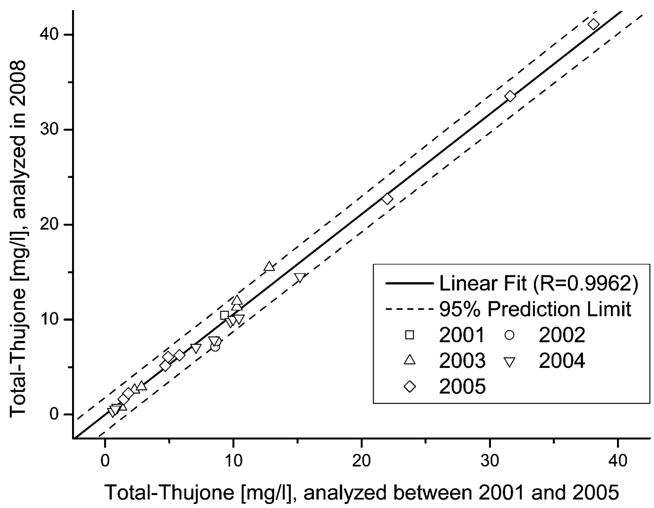

Figure 2. Comparison of samples ($n = 26$) analyzed between 2001 and 2005 with current analyses. In between, the samples were stored in daylight at ambient temperatures.

RESULTS AND DISCUSSION

Stability of Thujone, Pinocamphone, and Fenchone in Artificially Aged Absinthe. The results of the accelerated aging experiment using UV irradiation are shown in **Figure 1**. The

Table 1. Analysis Results of Preban Absinthes for Thujone, Fenchone, and Pinocamphone

sample no.	sample name	authenticity criteria fulfilled[a]	α-thujone (mg/L)	β-thujone (mg/L)	total thujone (mg/L)	fenchone (mg/L)	total pinocamphone (mg/L)
P14	Edouard Pernod, Switzerland, ca. 1900	yes	3.5	18.5	22.0	2.2	7.5
P15	Pernod Fils, France, ca. 1910	yes	6.5	40.5	47.0	30.9	2.0
P16	Jules Pernod, France, ca. 1910	yes	6.6	29.0	35.6	0.2	0.8
P17	Grand Distillerie Lyonnaise, France, ca. 1895	yes	1.2	15.9	17.1	3.2	12.1
P18	Duval-Dubied, France	yes	6.6	20.4	27.0	5.5	2.1
P19	Pernod Fils, France, ca. 1910	yes	9.5	28.0	37.5	0.5	0.7
P20	Pernod Fils, France, ca. 1914	no (missing label)	2.6	24.0	26.6	11.5	2.6
P21	Pernod Fils, France, ca. 1914	no (missing label)	3.3	36.5	39.8	25.4	0.9

[a] Authenticity criteria and results from samples P1−P13 were reported previously (*1*).

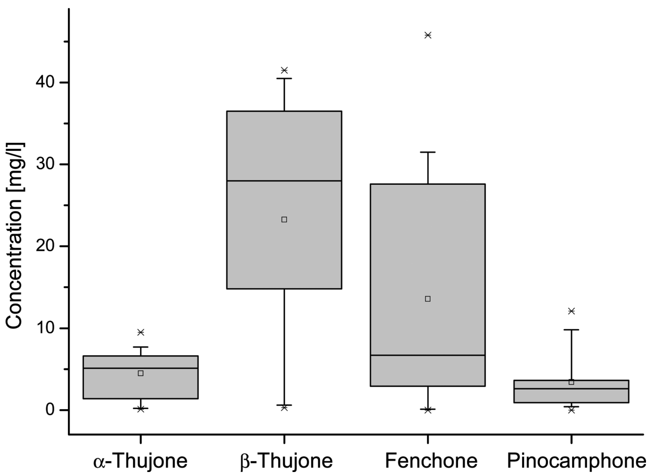

Figure 3. Box chart of the thujone, fenchone, and pinocamphone concentrations in 21 samples of preban vintage absinthe.

ANOVA for response surface two-factor interaction models showed a lack of significance for fenchone ($p = 0.1107$), meaning that neither the bottle type nor the time of irradiation exerts any influence on the fenchone content. The model for pinocamphone was significant ($p < 0.0001$). Pinocamphone remained constant in the green glass bottle; only in the clear glass bottle at irradiation times above 20 h was a decrease evident. The models of both thujone isomers were also significant (α-thujone, $p < 0.0001$; β-thujone, $p = 0.0001$). Again, no significant changes occurred in the green glass bottle, but were evident in the clear glass bottle. Whereas the changes were marginal for α-thujone (below 0.2 mg/L), the β-isomer was reduced by approximately 18% (from 19.0 to 15.5 mg/L) during the 200 h irradiation in the clear glass bottle. During the experiments, the interesting visual observation was noted that the color of the absinthe in the clear glass bottle exhibited signs of fading beginning at 20 h of irradiation, and the product was completely decolored at 60 h. The fading of color appears to be correlated with the loss of β-thujone. This is proof for our previous hypothesis that natural antioxidants extracted from the plant material may be responsible for the relatively high stability of thujone in absinthe (*8*). Chlorophyll, mainly responsible for the color of absinthe, is an especially likely candidate for this antioxidant activity (*12*).

The long-term storage experiments of authentic food matrices are in complete agreement with the previous study on the stability of thujone in model solutions by Fröhlich and Shibamoto (*13*). Thujone remained stable during storage experiments in 100% ethanol and 30% ethanol at either pH 2.5 or 6.5. Only at pH 11.5 did a very rapid epimerization of α-thujone to β-thujone take place, reaching an equilibrium of approximately 1:2 α-thujone to β-thujone. At different temperatures (100, 20, and 0 °C), nearly no difference in reaction rate was observed. Our own group had previously confirmed the result reported by Fröhlich and Shibamoto that increased temperature (up to 50 °C) has no influence at all on thujone in authentic absinthes (*8*), and this is the reason we have not studied temperature influence again during the current study.

We had previously conducted an UV irradiation study of real absinthes in clear glass laboratory flasks with an irradiation time up to 25 h (*8*). In this experiment, authentic absinthe bottles were employed, as well as longer irradiation times (until complete decoloration of the product in the clear glass bottle). An important observation lies in the fact that green glass bottles effectively protect the spirit from color degradation and thujone degradation as well. This can lead to a recommendation even for current manufacturers to use the traditional green glass bottles to increase the color stability of naturally colored products.

We conclude from the experiments that thujone as well as fenchone and pinocamphone are completely stable during storage in green glass bottles and that only minor losses (up to 20%) may occur after high UV exposure in clear glass bottles.

Stability of Thujone in Commercial Absinthes and Vintage Absinthes. The accelerated aging experiments using unrealistically high UV irradiation leading to such high energy levels to cause decoloration appear to be far from the real situation of spirit storage. As this institute began analyzing absinthe in 2001 and has not changed the methodology since, the researchers exercised the opportunity to reanalyze catalogued samples. **Figure 2** shows the comparison between the analyses of samples obtained in the years 2001−2005 and the 2008 results for those same samples. There were no significant differences between the data sets ($p = 0.8829$). We conclude from these results that storage (even under disadvantageous conditions as in this case, room temperature and daylight) has no influence on the thujone concentration in absinthe.

The results are transferable to vintage preban absinthes via the following considerations:

(a) The vintage preban absinthes in this and the previous study were generally stored in dark cellars that provide relatively constant, subambient temperatures in the range of 10−15 °C.

(b) The vintage preban absinthes sampled were stored in dark green glass bottles (with the exception of sample P5, Berger absinthe, which was in a clear glass bottle).

(c) The bottles were sealed using corks and in some cases additional wax seals.

(d) All vintage preban absinthes were still strongly colored at the time of analysis, so that the influence of light—if any—was below the levels in the artificial aging experiment.

For all of these reasons, we conclude that there is no proof to support the hypothesis that the results of our previous study were confounded by significant thujone degradation. On the contrary, thujone (and the other terpenes) appear to be relatively stable when stored in conditions approximating that of preban absinthe. We accordingly believe that the analytical results obtained are representative of the spirits' original content at the time of manufacture [this is also in accordance with the finding that absinthes made today from apparently authentic 19th century protocols tend to have thujone concentrations in the same range as found in vintage samples (*1*)]. Our studies have clearly indicated that absinthe tightly sealed in anaerobic green glass bottles, stored in the dark, does not change significantly with time. It should also be pointed out that even if the maximal loss of 20% was applied (e.g., if the products had been stored in clear glass bottles and subjected to considerable UV exposure), our prior interpretations that the concentrations of suspect compounds are below toxic levels would be upheld (*1*).

Further Insight into the Composition of Vintage Preban Absinthe and Its Variability. The analytical results of eight further preban absinthe samples are shown in **Table 1**, so that with the inclusion of the 13 samples analyzed previously (*1*), data on 21 vintage absinthe samples are currently provided. The recent samples were likewise within the previously reported concentration range for the compounds of interest, so that the inclusion does not cause significant changes of the distribution (**Figure 3**). It is noteworthy that despite the relatively low sample number, the data appear to reach a normal distribution. For example, the Kolmogorov–Smirnov test reported a significant normal distribution for all analytes (α-thujone, $p = 0.460$; β-thujone, $p = 0.729$; fenchone, $p = 0.240$; pinocamphone, $p = 0.130$), whereas the Shapiro–Wilk test proved normal distribution only for α-thujone ($p = 0.252$).

The total thujone content of the 21 samples falls in the range between 0.5 and 48.3 mg/L with an average concentration of 27.8 ± 17.1 mg/L and a median concentration of 33.3 mg/L. It is notable that a large variability in content is evident even in products of the same brand, produced and bottled in the same facility (e.g., Pernod Fils), and from the same period. As thujone deterioration has been excluded as an explanation for the variability in the thujone content, the following explanations can be offered: (a) variation in essential oil profile and content of the wormwood herb (e.g., regional and seasonal differences, herb chemotype) (*3*); (b) drying conditions of the herbs (drying between 6 and 12 months leads to losses of thujone) (*1*); (c) distillation equipment, as dependent on the mode of distillation and rectification of different thujone yields occur (*14*); and (d) standardization of product quality to maintain a consistent organoleptic profile by adjustment of recipes (*1*).

When all of the different influences are considered, it is not surprising that the thujone content of absinthe is normally distributed. In our view, the highest influence of the four possibilities is the inadvertent use of different wormwood chemotypes over the years or decades, which can contain radically different levels (0–70%) of thujone (*3*).

In conclusion, we restate the previous finding that no reasonable connection can be drawn between thujone concentration and the flavor or quality of absinthe and that thujone, as such, cannot be tasted (i.e., organoleptically isolated) in finished absinthe (*1*). However, the public focus around the findings of our previous paper was again centered almost exclusively on thujone. It has become evident that a significant percentage of consumers and potential consumers *want* the myths about the psychotropic properties of absinthe to be true, even in the face of compelling evidence that they are not. For these consumers, absinthe, without its drug-like allure, is no longer interesting. In this misapprehension they are encouraged by a handful of unscrupulous manufacturers who continue to hype the thujone myth (*15*). The potential degradation of thujone over time was seized on as a potential flaw of our original analysis. We believe that these new tests now conclusively refute the thujone degradation hypothesis and confirm that our initial results were not only valid at the time of testing but fairly indicate the chemical composition of the absinthes at the time of original manufacture.

Conflict of Interest. D.W.L. and T.K. declare no conflicts of interest. The CVUA Karlsruhe received no external funding. D.N.-M. and T.A.B. own companies dealing with absinthe; however, there are no financial or other interests that might be affected by publication of the results contained in this study.

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