Thujone content in wormwood extracts at different conditions for extraction[1]

B. Krumm, R. Kölling, T. Senn

University of Hohenheim, Institute for Food Technology, Department of Fermentation Technology
Garbenstraße 23, 70599 Stuttgart-Hohenheim (Germany)

Introduction

The bicyclic monoterpen thujone is an essential component of wormwood oil, making up 40 to 90 per cent. The total thujone content is compounded of about 10% (-)-α-thujone, and of about 70 to 90% (+)-β-thujone (Fig. 1). These contents may, however, vary according to type, growing areas and drying procedures [1, 2]. The steam-volatile thujone is being dissolved out of the chopped up plant material through the alcoholic extraction of wormwood (*Artemisia absinthium* L.). The use of absinth wormwood for the production of spirits was forbidden for nearly one hundred years by national absinth laws of many European countries. This was due to the fact that thujone is being suspected of having a neurotoxic effect and of being the trigger for the disease absinthism, which is characterized by physical and mental disintegration. The maximum quantity of thujone in spirits has been regulated since 1991 by the guideline 88/3888/EWG from 22 June 1988. The maximum permissible quantities for thujone in alcoholic drinks were set in a new formulation of the flavouring directive to 5mg/kg in alcoholic drinks with an alcohol content of up to 25%vol, to 10mg/kg in drinks with an alcohol content of more than 25%vol, and to 35mg/kg in bitters, in accordance with a decision by the German federal council (*Bundesrat*) from 29 October 1991 [3]. Research by Lachenmeier et al. [4], however, demonstrated that around 50% of the spirits under investigation, all of which can be purchased on the market under the name “absinth”, contained less than 2mg/l of thujone. This indicates that those spirits were produced without using wormwood extracts. The sensory impression of this sample is not authentic with the traditionally produced absinth (according to personal research), as these spirits produced without wormwood extracts are lacking apart from the thujone also the sesquiterpene lactone absinthin, which is responsible for the bitter aroma that is typical for absinth [5]. In the course of this study, various extraction methods have been applied to different raw materials in order to explain how the use of wormwood will affect the thujone content of spirits.

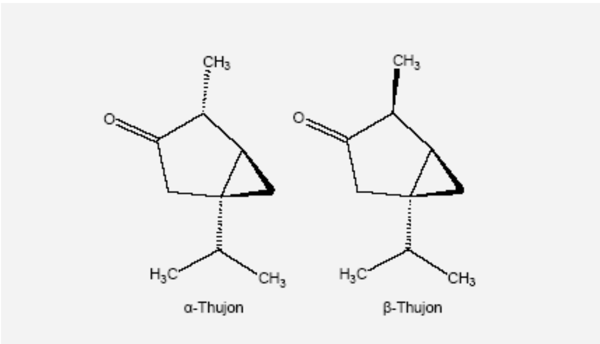

Fig. 1: Structural formula of (-)-α-thujone and (+)-β-thujone (isothujone)

Material and methods

The impact of the extraction method on the thujone content in the extract was tested on the basis of three different wormwood samples [Fig. 3]. Alpine wormwood harvested in July and September in Italy, and a collection of wild wormwood from Eastern Europe were macerated (soaked in cold solvent), digested (extraction using hot solvent, in this case under reflux), and percolated (extraction using continuously replaced solvent). The semi-automatic extraction device Timatic made by Tecnolab was used for the percolation (Fig. 4 and 5). All processes were carried out with a ratio of 1 to 10 (w/w) between drug and solvent. Ethanol (96%vol) was used as solvent. The wormwood extracts that were obtained in this way were mixed with the internal standard ester methyl nonanoate, and went through a sample preparation by means

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of a solid phase extraction (SPE) (DSC-18 column, 1 ml). First, the SPE material was activated with 1 ml methanol, and subsequently conditioned with 1 ml deionised water. 1 ml of the sample was then washed with 1 ml deionised water, and dried in a vacuum by means of suction. The elution occurred directly in a GC-vial containing 1 ml methanol. The GC analysis was carried out with a gas chromatograph of the type 17-A (Shimadzu) that contains an autosampler and autoinjector. A VF-5MS capillary column made by Varian (5% Biphenyl-, 95% Polydimethylsiloxane; 30 m; 0.25 mm ID; 0.25 µm DF) acted as separation column. A temperature programme of 50 to 150°C was employed with a heating rate of 5°C/min. Subsequently, the sample was baked out at 25°C/min up to 250°C. 1 µl was injected at a split ratio of 1:20. Helium was used as gas carrier [6]. The quantification occurred with the help of the peak area ratio of thujone at internal standards. The basis for this was a calibration with α-thujone standard 96% pure. (Fluka).


Fig. 2 (left): Different wormwood extracts; absinth made from historic original recipe (left), collection of wild wormwood (Eastern Europe) after the percolation (centre), Alpine wormwood (harvested in July, Italy) after percolation.

Fig. 3 (right): Chopped up wormwood; collection of wild wormwood (left), Alpine wormwood harvested in July (centre), Alpine wormwood harvested in September (right).

Results

All samples under investigation contained concentrations of β-thujone far below the permissible maximum value (Tab. 1). Every absinth under investigation that had been produced according to the French original recipe contained less than 1.5 mg/l of thujone. The α-Isomer [7], which is classified to be five times more toxic than the β-thujone, could not be detected in any of the samples.

Fig. 6 demonstrates the impact of the extraction procedure on the respective amount of β-thujone that had been extracted from the respective plant material. The highest contents arose from digestion; up to 50% lower values were obtained through percolation and maceration. Significantly bigger differences can be found when observing the individual chemotypes. Alpine wormwood harvested in July shows up to five times higher thujone contents compared to that harvested in September, and three to four times higher values in comparison to the collection of wild wormwood from Eastern Europe.

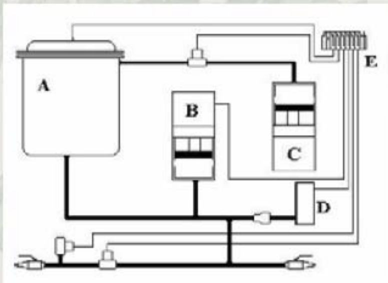
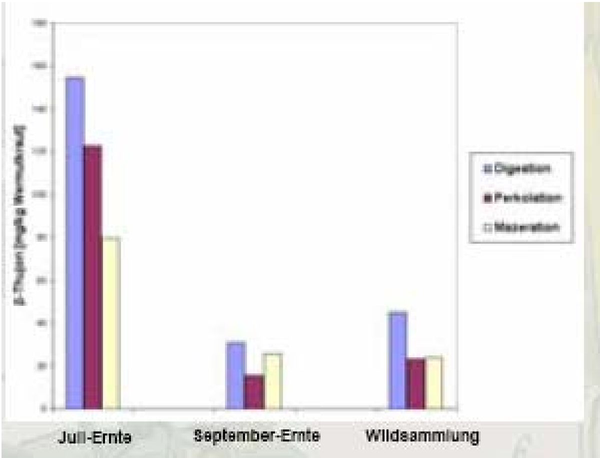



Fig. 4 (left): Semi-automatic extraction device for the simulation of a percolation of the type Timatic made by Tecnolab, Italy.
Fig. 5 (right): Depiction of the principle of function of the Timatic. A: extraction chamber; B: active pressure piston; C: secondary pressure piston; D: pump; E: pneumatic valve.

The Timatic is a solid-liquid extractor of the kind that is being used for the industrial production of herbal spirits. According to the manufacturer, the device simulates the effects of a percolation. This is achieved by a dynamic phase within which a pre-set pressure is being generated, and which during the extraction cycle within a programmable frequency is alternating in and out of the plant cells together with a static phase for the transfer of the solvent. The pressure phase prevents an effective seeping-in of the plant material. At the same time, this prevents the formation of selected channels as well as a partial oversaturation of the solvent.



July harvest September harvest Wild collection

Fig. 6: Depiction of the measured β-thujone amounts which were dissolved out of one kilogramme of each of the individual wormwood chemotypes by means of the employed extraction procedures.

Table 1. Average β-thujone concentrations of different wormwood extracts and β-thujone amounts obtained during the extraction of one kilogramme plant material each.

	Alpine wormwood						Wild collection		
	July harvest			September harvest					
	β-thujone (mg/l)		β-thujone / kg wormwood (mg)	β-thujone (mg/l)		β-thujone / kg wormwood (mg)	β-thujone (mg/l)		β-thujone / kg wormwood (mg)
Digestion	12.5	± 0.35	155	2.5	± 0.14	31	3.6	± 0.12	45
Percolation	10.4	± 0.65	123	1.5	± 0.24	16	2.6	± 0.40	23
Maceration	6.4	± 0.18	80	2.1	± 0.09	26	1.9	± 0.02	24

Conclusions

If one considers the β-thujone as a kind of lead compound for the product's positive wormwood flavour, matching our own experiences as well as the results by Lachenmeier et al. [4], correspondingly high-quality extracts may be produced via the percolation procedure without the disadvantages of the thermal strain during digestion. Nevertheless, statistically secured taste evaluation with final products gained from these extracts are still outstanding. It can, however, be expected that also the available, very different, wormwood samples will not contain final products with critical thujone concentrations. Further investigations are necessary in order to show in how far a distillative addition of thujone to wormwood products is possible, and whether this could possibly explain thujone contents of 35 mg/kg and higher.

Bibliography

[1] LANG, M., FAUHL C., WITTKOWSKI, R.: Belastungssituation von Absinth mit Thujon (BgVV-Heft 08/2002). Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin 2002.

[2] TATEO, F.: Influence of the drying process on the quality of essential oils in *Arthemisia absinthium*. *Mitt. Gebiete Lebensm. Hyg.* 1991, 82, 607-614.

[3] Aromen-Verordnung vom 22.12.1981: Änderung vom 29.10.1991, Bundesgesetzblatt (Federal Law Gazette) 1991 Teil I, S. 2045.

[4] LACHENMEIER, D. W., FRANK, W., ATHANASAKIS, C., PADOSCH, S.A., MADEA, B., ROTHSCHILD, M.A., KRÖNER, L. U.: Absinth - ein Getränk kommt wieder in Mode: toxikologisch-analytische und lebensmittelrechtliche Bewertungen. *Deutsche Lebensmittel-Rundschau* 2004, 4, 117-129.

[5] SCHNEIDER, G., MIELKE, B.: Zur Analytik der Bitterstoffe Absinthin, Artabsinthin und Matrizin aus *Arthemisia absinthium* L. Teil I: Isolierung und Gehaltsbestimmung. *Deutsch. Apoth. Ztg.* 1979, 119, 977-982.

[6] EMMERT, J., SARTOR, G., SPORER, F., GUMMERSBACH, J.: Determination of α-/β- thujone and related terpenes in absinthe using solid phase extraction and gas chromatography. *Deutsche Lebensmittel-Rundschau* 2004, 9, 352-356.

[7] BIELENBERG, J.: Die grüne Fee. Zentralnervöse Effekte durch Thujon. *Österr. Apoth. Ztg.* 2002, 56, 566-569.