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REVIEW

The history of the ISEO: 1969-2019

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Abstract

Half of a decade of essential oil research was reviewed in a historical aspect starting from the first meeting in 1969, which was transformed to an international high scientific level named; Inteional Symposium on Essential Oils (ISEO).

Keywords: Essential oils, historical, meeting

Introduction

The International Symposium on Essential Oils (ISEO) was initiated by a few enthusiastic scientists in the heart of Europe a half Century ago. Our 50 ISEO meetings have been a great inspiration to scientists working in the field of essential oils and related areas. We have moved from the first applications of gas chromatography (GC) on the volatiles of plants separating an essential oil into a limited number of constituents to the fantastic separations described in publications during the last 5 years. With the much improved separations of the constituents during the 1970s new research areas within the study of essential oils opened up. Single constituents could be isolated, identified by structure elucidation, and by retention indices. The ISEO meetings could benefit from this by changing or better enlarging the research topics to attract more scientists to the meetings. The ISEO symposia became a meeting place where contact across scientific areas was made. Having essential oils as a main topic, but allowing the meetings to be held in places where the interest of essential oils had different goals and aims, allowed the meetings to become varied in the outlook of terpene and volatiles research.

Timelines and venues

Over the years, ISEOs were organized:

Table 1. Meetings to Symposia, Venue and Organizers

	Year	Venue	Host
initiation	1969	Leiden, The Netherlands	A. Baerheim-Svendsen
1	1970	Leiden, The Netherlands	A. Baerheim-Svendsen
2	1971	Freiburg, Germany	F.-W. Hefendehl
3	1972	Helsinki, Finland	M. von Schantz
4	1973	Freiburg, Germany	F.-W. Hefendehl
5	1974	Freiburg, Germany	F.-W. Hefendehl
6	1975	Leiden, The Netherlands	A. Baerheim-Svendsen
7	1976	Würzburg, Germany	K.-H. Kubeczka
8	1977	Freiburg, Germany	F.-W. Hefendehl
9	1978	Münster, Germany	H. Hörster
10	1979	Würzburg, Germany	K.-H. Kubeczka

11	1980	Groningen, The Netherlands	M.H. Boelens, H. Hendriks
12	1981	Marburg, Germany	K.-H. Kubezka
13	1982	Würzburg, Germany	K.-H. Kubeczka
14	1983	Freising-Weihenstephan, Germany	Ch. Franz
15	1984	Leiden The Netherlands	A. Baerheim-Svendsen
16	1985	Holzminden/Neuhaus, Germany	E.-J. Brunke
17	1986	Bad Bevensen, Germany	E. Stahl-Biskup
18	1987	Nordwijkerhout, The Netherlands	J.J.C. Scheffer
19	1988	Zürich-Greifensee, Switzerland	D. Lamparsky, R. Kaiser
20	1989	Würzburg, Germany	K.-H. Kubeczka
21	1990	Lahti, Finland	R. Hiltunen
22	1991	St.Vincent, Italy	C. Bicchi
23	1992	Auchincruive, UK	S. Deans, K. Svoboda
24	1993	Berlin, Germany	P. Weyerstahl, H. Schilcher
25	1994	Grasse, France	D. Joulain
26	1995	Hamburg, Germany	K.-H. Kubeczka
27	1996	Wien/Vienna, Austria	Ch. Franz, G. Buchbauer
28	1997	Eskisehir, Turkey	K.H.C. Baser, N. Kirimer
29	1998	Frankfurt, Germany	W. König, A. Mosandl
30	1999	Leipzig/Miltitz, Germany	Bell Flavours & Fragrances
31	2000	Hamburg, Germany	K.-H. Kubeczka, W. König
32	2001	Wroclaw, Poland	S. Lochynski,
33	2002	Lisbon, Portugal	A. Figueiredo
34	2003	Würzburg, Germany	K.-H. Kubeczka
35	2004	Giardini Naxos, Italy	L. Mondello, P. Dugo
36	2005	Budapest, Hungary	E. Nemeth
37	2006	Grasse, France	D. Joulain
38	2007	Graz, Austria	Ch. Franz, J. Novak, R. Bauer
39	2008	Quedlinburg, Germany	J. Schulz
40	2009	Savigliano, Italy	C. Bicchi, P. Rubiolo
41	2010	Wroclaw, Poland	S. Lochynski
42	2011	Antalya, Turkey	K.H.C. Baser, F. Demirci
43	2012	Lisboa, Portugal	A. Figueiredo
44	2013	Budapest, Hungary	E. Nemeth
45	2014	Istanbul, Turkey	K.H.C. Baser, F. Demirci
46	2015	Lublin, Poland	A. Ludwiczuk
47	2016	Nice, France	N. Baldovini
48	2017	Pecs, Hungary	G. Horvath
49	2018	Nis, Serbia	N. Radulovic
50	2019 (to be organized)	Vienna, Austria	J. Novak, I. Stappen, Ch. Franz

In 1965 two Norwegians came to De Rijksuniversiteit te Leiden, Leiden, The Netherlands: Professor Anders Baerheim Svendsen and me as this assistant started to reorganize the Department of Pharmacognosy from a descriptive, botanical research oriented department into a department for the study of interesting medical natural products with emphasis on chromatographic techniques, isolation of pure compounds and spectroscopic structure elucidation. Anders Baerheim Svendsen had already a group of friends as professors

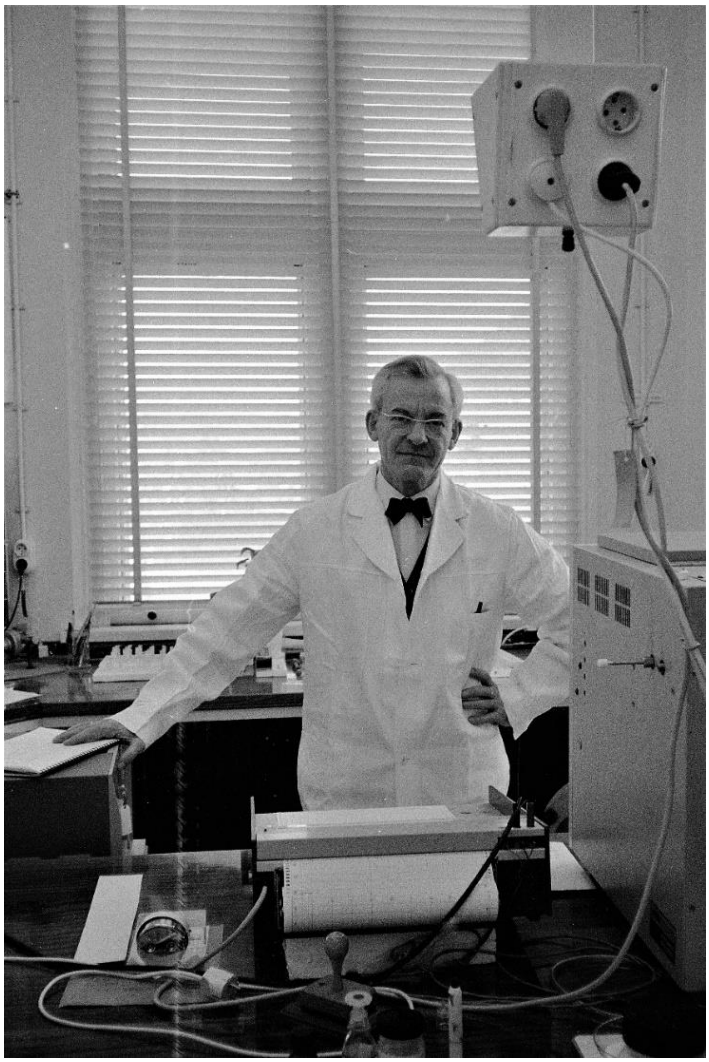
of Pharmacognosy in Germany, Switzerland and Brazil who wanted to change the emphasis of this university subject of pharmacognosy from botany into a study of natural products with interesting biological/medical effects following the ideas of Max von Schantz in Helsinki, Iconomou in Athens, Richard Wasicky in Sao Paolo. This was the time when the chromatographic techniques were in very active development, and especially gas chromatography. We have decided to choose essential oils as research topic as this was obviously the most complex, demanding and difficult natural occurring samples to study at that time using the available separation techniques. We were both fascinated by the separation power of the new gas chromatographic equipment as this obviously would lead to a better understanding of the complexity of volatiles in plant material. We were given a very nice budget by the director of the institute (Pharmaceutisch Laboratorium) and were able to purchase the first gas chromatographs within few weeks after arriving in Leiden in 1965).

Figure 1. Prof. Dr. Anders Baerheim-Svendsen walking on the streets of Leiden taking photos of our "new" city.

These were the then excellent Varian GC's. However, very soon we discovered that the GC instrumentation available needed improvements for very volatile compounds analysis. We also had to develop preparative GC equipment to isolate pure compounds for the NMR, MS or FT-IR. An active collaboration with the company Becker Delft BV, Delft was very practical for this project. We had a lot of fun modifying a water bath to be used as the GC-oven allowing us to do GC-analyses down to and below zero degrees. The spectroscopic instrumentations were also in a very active industrial development (improving the sensitivity) following the possibility to isolate pure natural products from plants by the new chromatographic instrumentation available. It was period of hectic instrumentation development and something of a paradise for them in Leiden as we had a modern instrument workshop at their disposal who could help them in the improvement of the instrumentation and by making it possible to build new equipment. Parallel with the development of

gas chromatography we were building equipment in 1966 for column chromatography under pressure, an activity which started the instrumentation development of HPLC!

Figure 2. A very satisfied Prof. A. Baerheim Svendsen, when he and myself managed to run the first terpene analysis from *Juniperus communis* L. in August 1965 in the new laboratory



During the next years, we were occupied developing and improving the chromatographic instruments and to establish the Department of Pharmacognosy as a centre for natural products research. Working in a very active area of natural products it was natural to seek other young scientists interested in essential oil analysis for regular discussions. This was thoroughly discussed between Anders and me planning for the future as we wanted to make a discussion group, which would hopefully last for many years. This led them to approach K.-H. Kubeczka who was using GC instrumentation and interested in method development and F.W. Hefendehl, who had just published some interesting papers on the essential oil in *Mentha* leaves by analysing the essential oil in single oil glands /cells in leaves. Another interested young scientist was Simo Juvonen from Helsinki, who did some outstanding research on the needle of *Pinus*-species in Finland.

Figure 3. Anders and myself were testing the newly made brandy of cloudberries to observe if we had managed to keep the taste and smell during the production. Many of the flavouring agents naturally occurring in the plant material disappeared without a trace during the production procedures and posed a big problem for the product development. This was often the driving force behind our interest in the natural volatiles and initiated also contact with flavour and fragrance industry in The Netherlands and abroad.

Anders and myself approached Karl-Heinz Kubeczka and Friedrich Wilhelm Hefendehl at the GA meeting in Würzburg, in 1968 with a proposal to make a discussion group for all aspects of essential oil research and we agreed to participate. One could say that our meeting in Bürgerspital in Würzburg with many bottles of "Eschendorfer Lump" started the ISEO idea.

In The Netherlands, I was a member of a chromatography discussion group since 1966, and found this kind of arrangement very suitable for a discussion group in pharmacognosy and specifically on the applications of chromatography of the terpenes. The driving force behind the development was the change of research in pharmacognosy from emphasis on botany to the chemical study of natural occurring compounds. To make this practical we decided to meet a day after the annual GA meetings to start the discussion group with any questions regarding the essential oils (separation of terpenes, chemistry, chemotaxonomy or other relevant topics regarding the constituents of essential oils). Even then we discussed to enlarge the topics to other volatiles in nature in general but left this question to future meetings. It was a very active time in instrumentation development (end of 1960 and far into the 1970) where we introduced standard capillary GC, enantiomeric stationary phases, micromanipulators used in medicine to pick out single oil cells for analysis, solid sampling GC to improve the sensitivity, and micro cells for IR analyses. It was the time also in ISEO for discussing instrument improvements as main topic for the meetings. This was the start of the ISEO meetings.

In 1969, we had for disposal capillary GC instrumentation, and the first meeting with invited speakers took place in Leiden with two main speakers on capillary GC applications. The heads of analytical research from British Petroleum (BP) and from Shell gave their experiences from the petrochemical analysis by capillary GC. We also had nice collaboration with a company in Delft (Becker Delft BV), on the development of preparative GC equipment and a representative from this company was giving a lecture, too. It was a time when the instrumental development fitted perfectly with the complexity of the essential oil composition. I also especially remember Dr. Herout from Prague, who was one of the authors of a large number of publications on the structure of sesquiterpenes from essential oils together with Sorm and co-workers. Herout visited him several times in Leiden to discuss instrumental developments. I can never forget how he described the continuously distillation to isolate 50 Litres of *Mentha*-oil and the fractionated distillation to isolate enough of single sesquiterpenes to be able to do NMR analysis and structure elucidation. Whether the compounds isolated were artefacts formed during hours of distillation or not, was not a topic of discussion! The additional knowledge gathered on the composition of the essential oil compounds, developing NMR as well as hyphenated GC-MS techniques matched very well anyone interested in essential oils. Gradually the instrumental techniques became better and much more sensitive. For many years, the discussion on terpenes in plants focussed upon why the plants accumulated these lipophilic constituents in special "containers" in the living plant. What was the biochemical, and what was the biological reason of these compounds?

We focussed on the "essential oils" because this was a well-known product of pharmaceutical interest and because the analytical techniques at that time (around 1970) was very suitable for essential oil characterization. However, the scientific community consisted of a conservative group of scientists, and it took many years before a more thorough discussion on the biological aspects of terpenes in plants, enzymology of terpene formation and biological effects of volatiles took place. It was said that we have spent the best part of the last 20 years for these discussions. Separations techniques are no longer a big problem, structure elucidation of isolated volatiles likewise relatively easy and solvable.

Very soon the one-day meeting in connection with the GA meetings grew in popularity and we decided to set up a separate meeting for anyone interested in essential oils /terpenes, the *Symposium Ätherische Öle,* which was changed around 1980 into *International Symposium on Essential Oils* (ISEO). Young scientists from many areas found the ISEO meetings very instructive and we became like a family of scientists with different backgrounds but with the same goal to gain better knowledge about the terpenes and the essential oils. We had discussed how to promote the meetings and decided upon inviting young scientists from every area interested in essential oils and not to limit the participation to specific groups. Some of these former young scientists are still members of the scientific committee of the ISEO.

From the start, we did not want to make a new society of scientists with rules and regulations. We wanted to keep it at the informal level without any other guiding group than a permanent scientific committee (also known as 'Monday Evening Group'). We also discussed the issue of reaching a broader variety of scientists such as those interested in synthetic chemistry, separation techniques, structure elucidation, traditional pharmacognosy, biodiversity, agronomy and biological effects of terpenes, all could attend these future meetings. The main issue for the ISEO Scientific Committee was to decide upon the next venue so naturally we had a discussion on the suitable places. The person in charge of the next meeting should be or become member of the scientific committee. This system has worked now for 50 years without any trouble.

With the passing of the years, we moved on in their careers and changed positions. Some of them stayed on at the university and some joined other governmental laboratories or industries and this was very healthy for new recruitment of scientists for the ISEO.

Friedrich Wilhelm Hefendehl joined the governmental laboratory in Berlin and disappeared from the ISEO meetings. Karl-Heinz Kubeczka wanted to enlarge the ISEO meetings and the number of participants and proposed more contact with industry. He did a lot of promotion of the ISEO and attracted the interest (and participants) from industry. I went back to Norway and joined the Department of Drug formulation at the University of Oslo. Simo Juvonen bought a pharmacy in Finland and became the traditional retail pharmacy owner/apotheker. Karlsen kept his subscription of the journal "Perfumer and Flavorist" to follow the development of flavour and fragrance chemistry and participated in the world-wide meetings of the Flavour and Fragrance industry (*International Congresses on Essential Oils, Fragrances and Flavours*). In this way, he kept contact with the research area of terpenes and essential oils although his research area now had focus upon drug formulation, encapsulation and stability problems of natural products.

Many new members of the ISEO committee joined in the study of the terpenes and volatiles. After a spell of 5-6 years, working with pharmaceutical industry and drug formulation I joined again the ISEO meetings, since he missed his old scientist friends from the terpene research area.

The topics of the ISEO has changed according to discussion of the scientific committee. The flavour and fragrance industry with their representatives joined in the years 70, hosted meetings, supporting the annual venue of the ISEO, and made it possible to keep this annual meeting running for many years. Emphasis of the meeting changed from purely analytical meetings via agricultural problems of the essential oil plants standardization up to microbiological applications - then back again to hyphenated techniques and their application to essential oils. I was particularly happy that we could invite Rodney Croteau and some of his collaborators to discuss the biosynthesis of the terpenes, a topic that we should again focus upon, as well as biotransformation of the terpenes (which terpenes are really present in the living plant). In the 1980s more investigations on the biological effects of the volatile terpenes started to emerge from the poster sessions. We even had an aromatherapist (Maria Lis-Balchin) giving lectures some years at the ISEO.

Katerina Svoboda published some fantastic pictures of essential oil cells in the living plant as a book and Alexander Pauli collected an enormous data base on the antimicrobial effect of specific essential oils. We had Roman Kaiser showing how plants (especially Orchids!) emitted complex scents at different times of the day/night to attract specific insects, and his transportable laboratory with a hot-air balloon with head-space collections of emitted fragrance mixtures will not easily be forgotten. We still see the pictures of the laboratory moving between the treetops in the jungle of the Amazons by the help of a hot-air balloon. His book "The Scent of Orchids" has always been one of the favourite books of scents and perfumery.

During these years all aspects of essential oil research has been covered. It was quite natural that Gerhard Buchbauer and K. Hüsnü Can Başer invited authors to join them in making a "Bible" for essential oil research resulting in "The Handbook of Essential Oils ", which has become a great success. Not to forget the books edited by and also written/collected by Karl-Heinz Kubeczka and other colleagues on the analysis of essential oils (see Figures 4-11).

There are many scientists who have contributed to the success of the ISEOs: Anders Baerheim Svendsen, Karl-Heinz Kubeczka, Friedrich Wilhelm Hefendehl, Chlodwig Franz, Heinz Schilcher, Peter Weyerstahl, Carlo Bicchi, Roman Kaiser, Wilfried König, Brian Lawrence, Daniel Joulain, Eva Nemeth, Gerhard Buchbauer, Stanislaw Lochynski, K. Hüsnü Can Baser, Fatih Demirci, Hans Scheffer, Luigi Mondello, Yoshinori Asakawa,

Yoshiaki Noma, Nicolas Baldovini, Armin Mosandl, Elisabeth Stahl, Katerina P. Svoboda, Patricia Rubiolo, Agnieszka Ludwiczuk, Heinz Hörster, Danuta Kalemba, Otto Sticher, Ana Cristina Figueiredo, Niko Radulovic…..and many others.

Gradually, more emphasis on the biological effects of volatile terpenes became the main issue of many of the later meetings on the ISEO. Even though there were more development in automation of the chromatographic equipment, new two-dimensional methods of GC and HPLC were applied to terpene analyses, terpene encapsulation became an interesting research area and the use of terpene-containing plants as additives to animal feed could become an interesting field for the future *etc*. In this way, the topics of the ISEO changed according to the interest from the applied field of volatile oils.

Changes of topics/Topic development

We started out with long discussions on the separation techniques for the terpenes and were trying to improve the methods during the first 10 years of the ISEO. Many of the ISEO participants were also interested in the biochemistry of the lower terpenes, and in the biosynthesis of these compounds. It was a great pleasure that Rodney Croteau agreed to come and give an overview and the latest results of his work on this very interesting group of natural products. He clearly enabled to understand the complexity of the biosynthesis of the terpenes, the complexity of enzymes active in the plant and the difficulties met when trying to manipulate the enzymatic system. His talk gave many of them ideas for future research. However, one should not forget another very important development in the chromatographic separation of the terpenes, the development of the enantiomeric column, allowing to differentiate between (+) and (-)-enantiomers of the monoterpenes. Instrumental in this area were Armin Mosandl and Wilfried König, who both gave several and interesting talks on the importance of enantiomeric forms of the volatiles. This could easily be applied to the verification of the oils in the market as well as giving them new ideas (and problems) in the field of the formation of the terpenes. Meanwhile, the separation of the terpenes had been brought so far that the publication of lists of constituents in an essential oil made little sense since many of the compounds detected could not possibly be present and the components identified on the basis of retention time on a single column and mass spectrum/library search became dubious. What was really present in the plant and what was purely artefacts originating in the isolation process of the essential oil was an important topic of discussion in the papers. This topic still needs further evaluation Two-dimensional GC efficiently reported on by Carlo Bicchi and Luigi Mondello clearly showed that many of the so-called baseline separated single peaks contained two or often more compounds. A proposal to enlarge the topics of the ISEO also to include other natural volatiles seemed “natural” as both analytical techniques, biosynthesis and biological effect studies will have to cover both groups. Essential oils are, after all, an artificial name of a group of natural products originally isolated by distillation and being lipophilic. In his opinion, this is a too narrow definition for the interest of the ISEO, and we needed to cover all natural occurring volatiles in the future! The group “essential oils” is a commercial product well known to pharmacists for several hundreds of years and has been kept by the ISEO maybe with a kind of respect to history. Volatiles in general requires special isolation techniques as well as typical formulation like encapsulation, which will enable us to study their biological effect without the compounds disappearing before the experimental endpoint! In our opinion, a natural development would be to enlarge our interest to volatiles in general as the industrial interest in volatiles is growing.

One must not forget Chlodwig Franz who generously hosted special scientific committee meetings at his house in Austria, to discuss the future of the ISEO meetings, namely “the Bach Meetings”. A very laudable initiative for important discussions between the most active members of our “essential oil family/ scientific

committee". These meetings have resulted in recommendations for the scientists studying terpenes and essential oils. These recommendations are guidelines for younger scientists to ensure repeatability of the experiments carried out on volatiles (Franz, 2002; .

Meanwhile, the perfumery industry partly moved on to synthetic compounds and away from the essential oils as raw material for their products. Interest in the essential oils seemed to diminish. However, biotransformation of terpenes, terpenes as antibacterial, importance of the terpenes for the functioning of the plant and not to forget the renewed interest in the essential oils from other parts of the world seemed to give the ISEO new energy. The biological effects of the terpenes/essential oils were taking over the chemistry and separation techniques as main interest for the participants, as *e.g*. the use of essential oils in animal health and feeding. This was regarded by me to be an important direction of the essential oil research. Instead of only investigating the essential oils for the benefit of perfumery, now we could really study the essentials of the terpene formation and functioning. ISEO had moved from the area of academic interest into separation techniques, to industrial products like perfumery, and then back to the biological effect in the plant and in medical applications. For the biological effects of the volatiles in the plants we would like to emphasize the work of Roman Kaiser on the scent of orchids and their diurnal variation. Interestingly, if one look at the research speciality of the participants, it covers a very broad aspect of essential oil research from agricultural problems, governmental regulations and separation techniques to biological effects, formulation of terpene products, synthesis of olfactory important copies of natural occurring compounds and stability of the volatile terpenes. In our opinion this is the key to the success we have had with the ISEO meetings and kept it alive for 50 years.

Figure 4. Publication of the workshops edited by K.-H. Kubeczka (1976-1978).

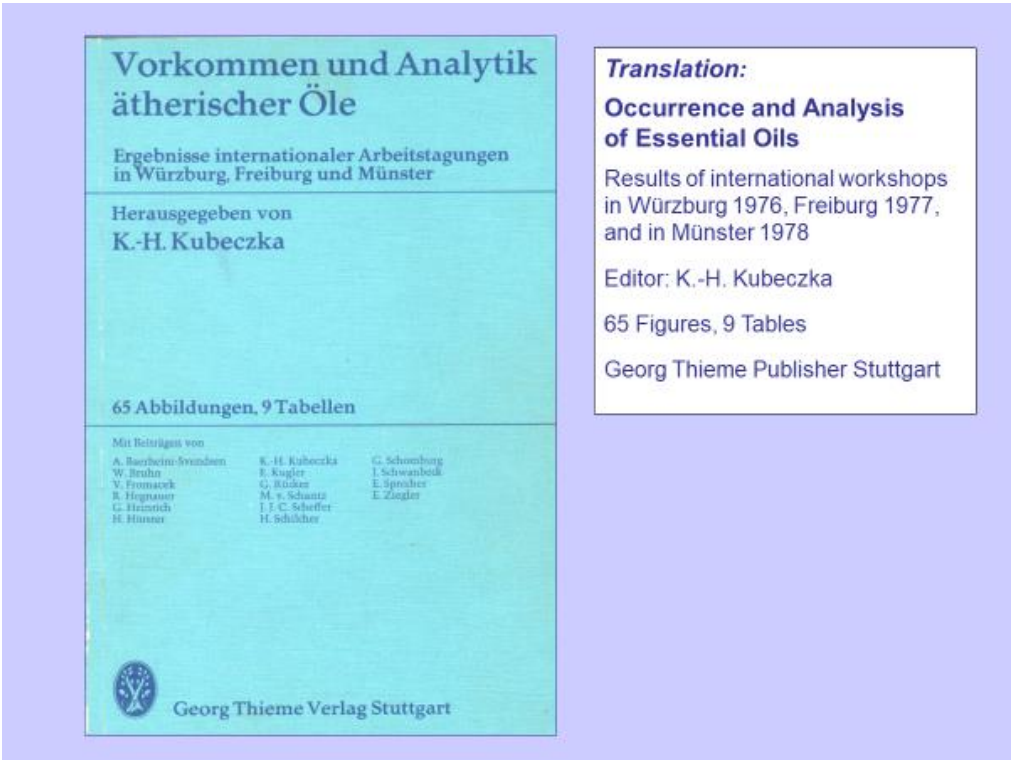


Figure 5. Proceedings of the 15th ISEO, 1984, released by M. Nijhoff & Dr. W. Junk Publishers (1985).

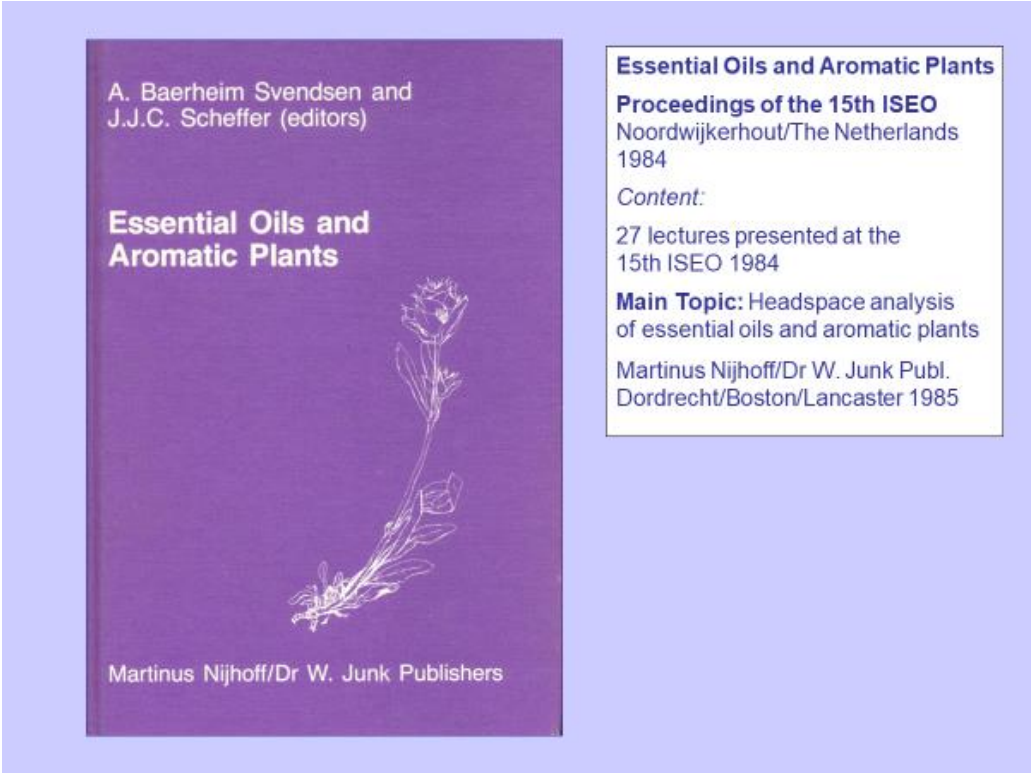


Figure 6. Special issue published in FFJ, 1987 by K.-H. Kubeczka.

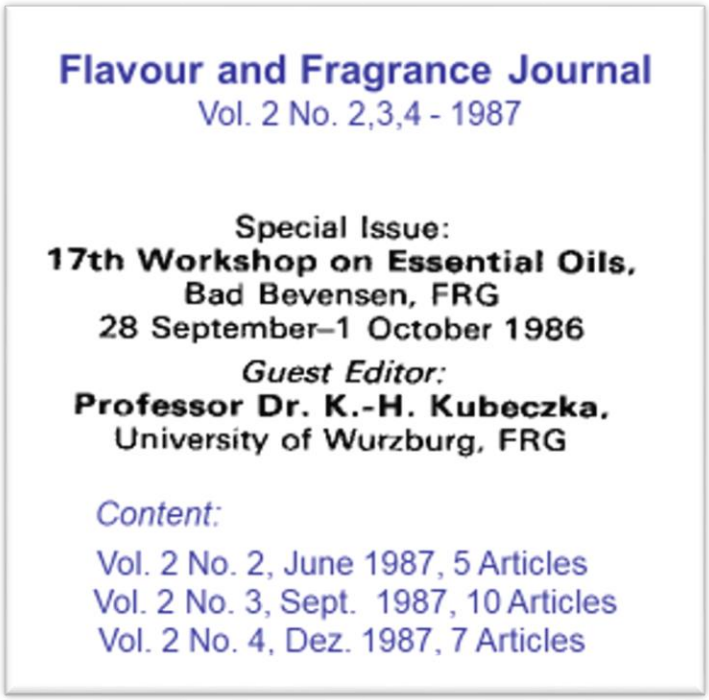


Figure 7. Proceedings of the 27th ISEO, edited by Ch. Franz, A. Mathe, G. Buchbauer (1997).

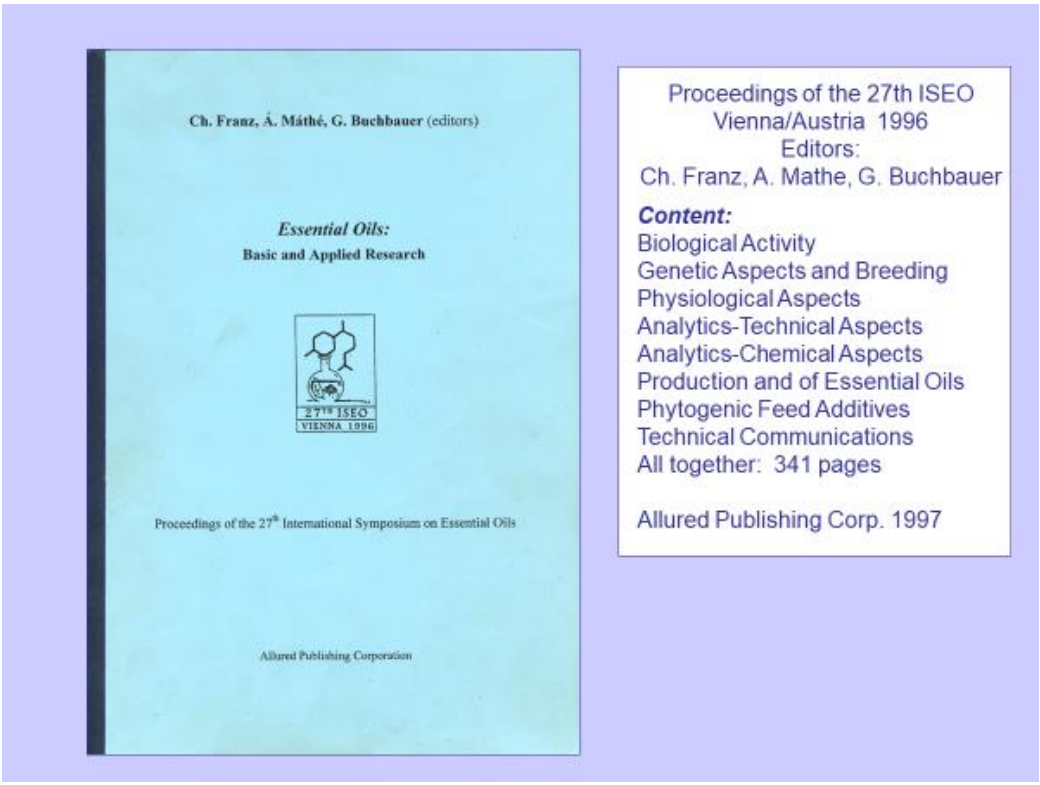
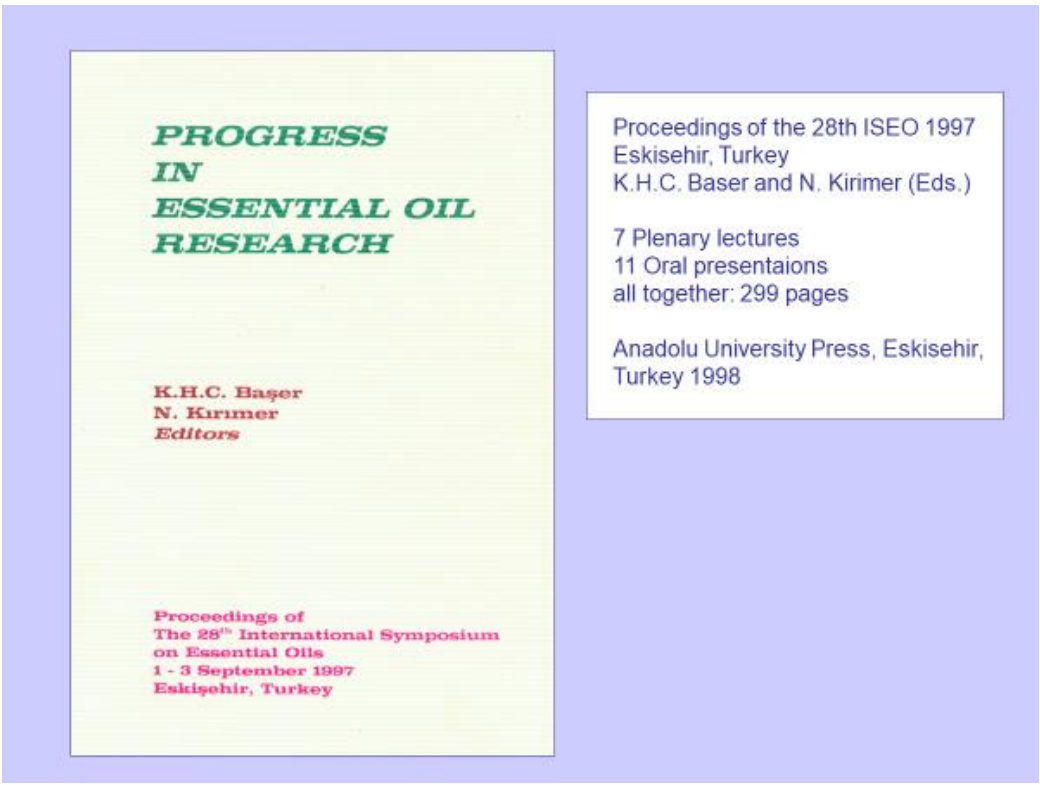


Figure 8. Proceedings of the 28th ISEO, edited by K. H. C. Başer & N. Kirimer (1998).



The participants

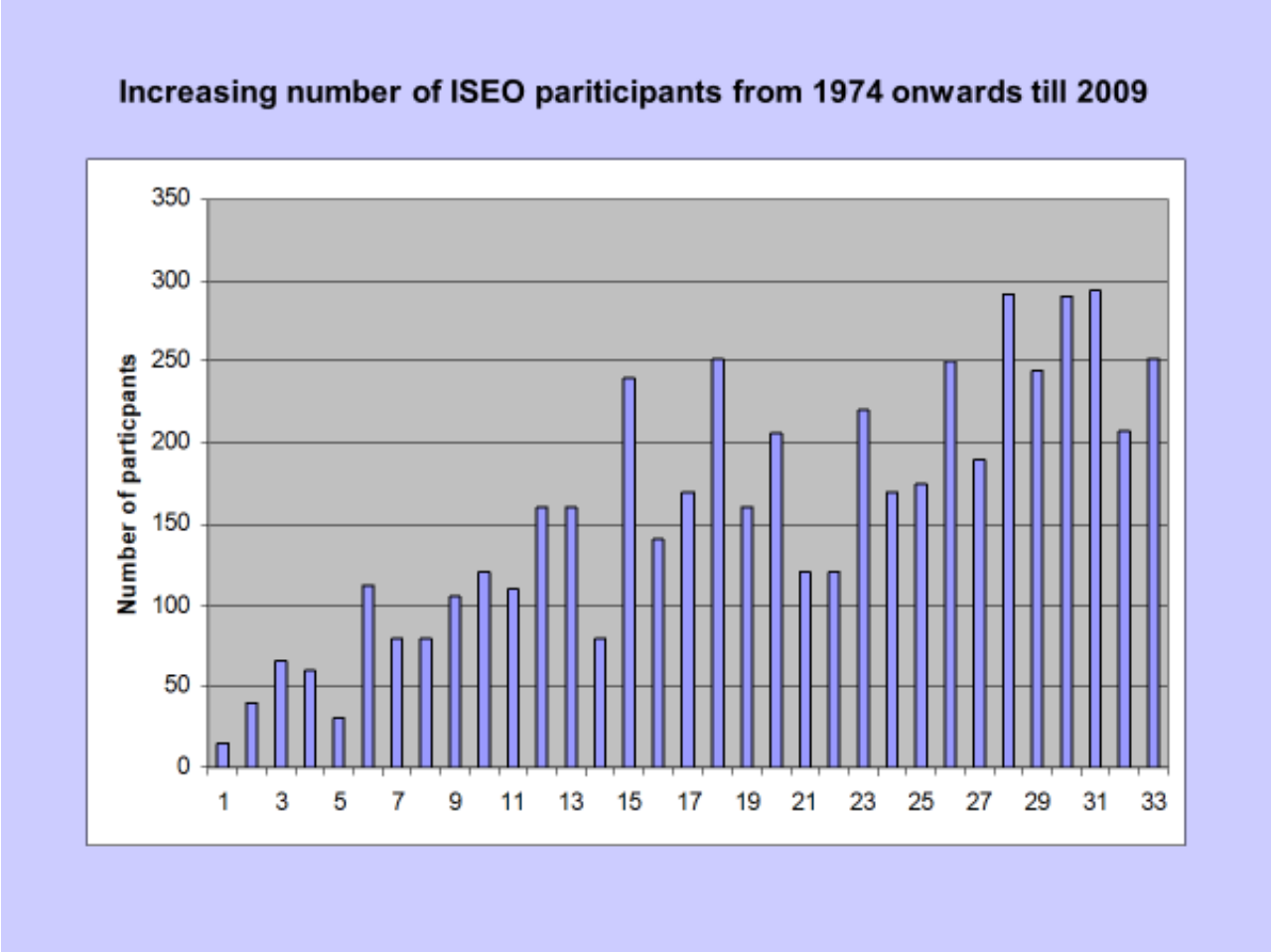
The most important task of an informal organization is the attraction of younger scientists as new regular participants. There is a permanent slow change in the Scientific Committee due to the fact that no official 'elections of the board' are necessary but elder members of the committee retire and by that way younger scientists are able to influence and define ISEO anew. So far, it seems that this gradual change is continuous.

The future and status 2019:

Actually, we have at our disposal

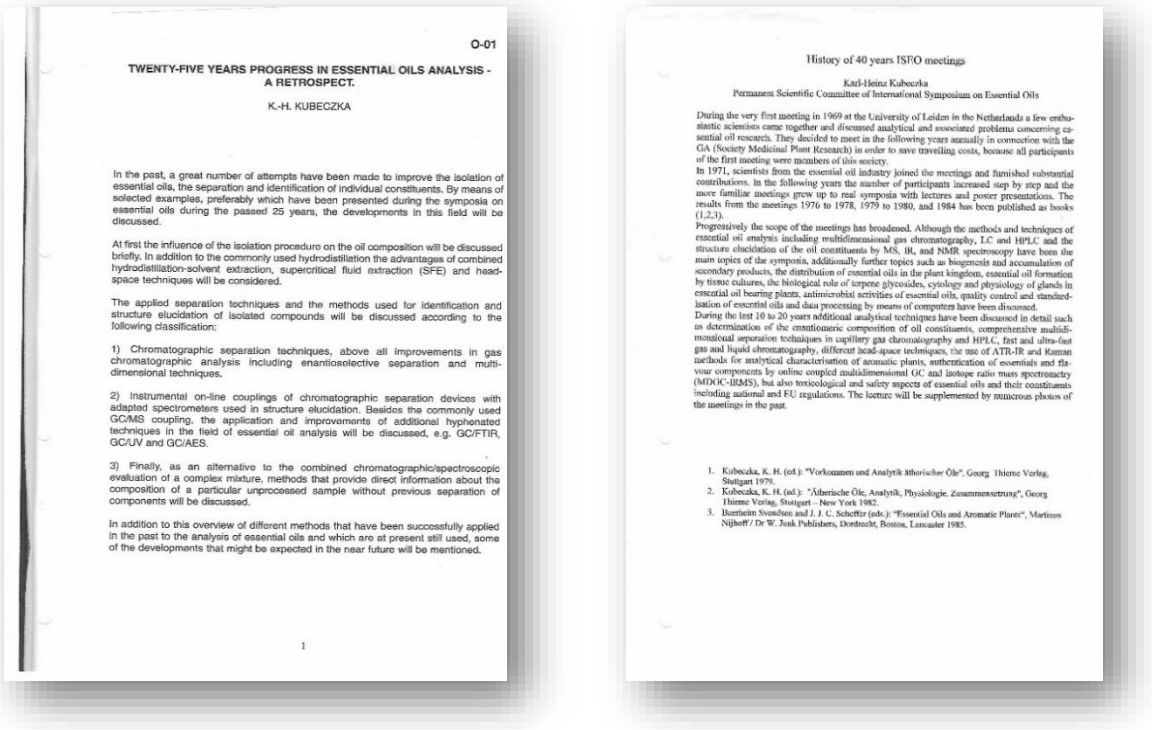
- an annual meeting (ISEO)
- a web site: http://iseo-pc.org
- journals (JEOR, FFJ and recently NVEO)
- a regular number of participants
- a scientific committee that works
- and after 50 years a definite place in the scientific community with a number of younger scientists participating in the organization and the meetings.

Figure 9: Number of participants at ISEO Meetings from 1974 (Freiburg) until 2009 (Savigliano).



It is first and foremost this last point that has kept ISEO alive and active all these years. The running of the ISEO does not depend upon only “older, experienced” scientists but equally well upon new members of the ISEO family. The open organization of the ISEO is very special but it works with special arrangements for young scientists. Let us celebrate the 50th anniversary of the ISEO as it is!

Figure 10. Two abstracts by the lectures made by Karl-Heinz Kubeczka at the 25th and the 40th ISEO meeting covering the historical developments



O-01

TWENTY-FIVE YEARS PROGRESS IN ESSENTIAL OILS ANALYSIS - A RETROSPECT.

K.-H. KUBECZKA

In the past, a great number of attempts have been made to improve the isolation of essential oils, the separation and identification of individual constituents. By means of selected examples, preferably which have been presented during the symposia on essential oils during the passed 25 years, the developments in this field will be discussed.

At first the influence of the isolation procedure on the oil composition will be discussed briefly. In addition to the commonly used hydrodistillation the advantages of combined hydrodistillation-solvent extraction, supercritical fluid extraction (SFE) and head-space techniques will be considered.

The applied separation techniques and the methods used for identification and structure elucidation of isolated compounds will be discussed according to the following classification:

1) Chromatographic separation techniques, above all improvements in gas chromatographic analysis including enantioselective separation and multi-dimensional techniques.

2) Instrumental on-line couplings of chromatographic separation devices with adapted spectrometers used in structure elucidation. Besides the commonly used GC/MS coupling, the application and improvements of additional hyphenated techniques in the field of essential oil analysis will be discussed, e.g. GC/FTIR, GC/UV and GC/AES.

3) Finally, as an alternative to the combined chromatographic/spectroscopic evaluation of a complex mixture, methods that provide direct information about the composition of a particular unprocessed sample without previous separation of components will be discussed.

In addition to this overview of different methods that have been successfully applied in the past to the analysis of essential oils and which are at present still used, some of the developments that might be expected in the near future will be mentioned.

1

History of 40 years ISEO meetings

Karl-Heinz Kubeczka
Permanent Scientific Committee of International Symposium on Essential Oils

During the very first meeting in 1969 at the University of Leiden in the Netherlands a few enthusiastic scientists came together and discussed analytical and associated problems concerning essential oil research. They decided to meet in the following years annually in connection with the GA (Society Medicinal Plant Research) in order to save travelling costs, because all participants of the first meeting were members of this society.
In 1971, scientists from the essential oil industry joined the meetings and furnished substantial contributions. In the following years the number of participants increased step by step and the more familiar meetings grew up to real symposia with lectures and poster presentations. The results from the meetings 1976 to 1978, 1979 to 1980, and 1984 has been published as books (1,2,3).
Progressively the scope of the meetings has broadened. Although the methods and techniques of essential oil analysis including multidimensional gas chromatography, LC and HPLC and the structure elucidation of the oil constituents by MS, IR, and NMR spectroscopy have been the main topics of the symposia, additionally further topics such as biogenesis and accumulation of secondary products, the distribution of essential oils in the plant kingdom, essential oil formation by tissue cultures, the biological role of terpene glycosides, cytology and physiology of glands in essential oil bearing plants, antimicrobial activities of essential oils, quality control and standardisation of essential oils and data processing by means of computers have been discussed.
During the last 10 to 20 years additional analytical techniques have been discussed in detail such as determination of the enantiomeric composition of oil constituents, comprehensive multidimensional separation techniques in capillary gas chromatography and HPLC, fast and ultra-fast gas and liquid chromatography, different head-space techniques, the use of ATR-IR and Raman methods for analytical characterisation of aromatic plants, authentication of essentials and flavour components by online coupled multidimensional GC and isotope ratio mass spectrometry (MDGC-IRMS), but also toxicological and safety aspects of essential oils and their constituents including national and EU regulations. The lecture will be supplemented by numerous photos of the meetings in the past.

1. Kubeczka, K. H. (ed.): "Vorkommen und Analytik ätherischer Öle", Georg Thieme Verlag, Stuttgart 1979.
2. Kubeczka, K. H. (ed.): "Ätherische Öle, Analytik, Physiologie, Zusammensetzung", Georg Thieme Verlag, Stuttgart – New York 1982.
3. Baerheim Svendsen and J. J. C. Scheffer (eds.): "Essential Oils and Aromatic Plants", Martinus Nijhoff / Dr W. Junk Publishers, Dordrecht, Boston, Lancaster 1985.

Figure 11. Progress in Essential Oil Research, edited by E.-J. Brunke (1986)

Progress in Essential Oil Research

Proceedings of the International Symposium on Essential Oils Holzminden/Neuhaus, Federal Republic of Germany September 18–21, 1985

Editor
Ernst-Joachim Brunke

W DE G

Walter de Gruyter · Berlin · New York 1986

Important guidelines

"*for good practices of carrying out the study of essential oils and natural volatiles*"

These guidelines are intended to be of assistance to researchers working in the field of essential oils (EO) and natural volatiles, and set the basis of good practice in experimental research and communication of results. These guidelines are not all mandatory, as depending on the specific goals of the research considered*, i.e.* some recommendations may not be essential if we are not directly related to the main goal of the study.

There are still many scientific papers, poster presentations and lectures presented during symposia in which the term of EO is used incorrectly. Because of this, at the beginning of these guidelines, it is necessary to define what is the 'Essential Oil'. EO are complex mixtures of volatile compounds produced by living organisms and isolated by physical means only (pressing and distillation) from a whole plant or plant part of known taxonomic origin. In contrast, extracts obtained by solvent extraction with different organic solvents or by supercritical fluid extraction (SFE) may not be considered as essential oils.

These guidelines are formally divided into those dealing with chemical and biological issues, but the biological part cannot be considered separately from the chemical one, as both are generally dependent one on another.

1. The chemical issues span from sampling of the material to identification and quantification of the volatile constituents.

The sampling of the material has to be representative and in accordance with the goal of the study. Researchers should define if characterization of a species, catching of intraspecific variability, comparison of different populations, accessions or detecting the role of special factors on the EO variability is the focus of the study. And, for example, a study demonstrating an infraspecific variability of EO composition should be based on irreproachable and statistically significant sampling procedures, while a description of the volatiles of a rare and endangered new species may rely on the analysis of a single sample. In parallel with this, care should be taken to sampling the appropriate plant part, at the appropriate time and developmental phase each of which should be defined. Besides, geographical coordinates of the collection place are mandatory. The valid botanical identification of plant material has to be backed up by a deposition of voucher specimens in a herbarium or a collection, freely available to other interested researchers. Good quality photographs or scans of the sample specimen should be provided as supplementary material, to facilitate the confirmation of the correct botanical identification.

Repeatable and clearly described sample preparation methods should be used, including the biological and laboratory replicates. Contamination and artefact formation should be avoided/kept to a minimum as much as possible in the context of the main goal of the study. The yield of the volatiles should be expressed as a percentage based on dry material content of the plant sample.

The GC-MS-based identification of constituents has to rely upon repeatable and accurate analytical procedures. It should be based on the comparison with authentic mass spectra and retention index data. The use of columns of different polarity to provide a set of different retention indices is encouraged. Co-injection experiments of reference compounds are highly recommended. Other means of identification, such as chemical transformations, fractionations leading to structural analysis etc., significantly improve the quality of the work and are much appreciated. Determination of the enantiomeric ratio of the constituents by chiral GC is another highly welcomed addition. The raw GC-MS electronic files (.D, .MS, .MSF, .R##, .SMS *etc*. type files) of the main analyses described in the work should be provided as supplementary data.

Quantification of constituents has to be performed by internal standardization using GC-FID or GC-MS as recommended by IOFI and reliable recent publications (Cachet et al., 2016; Begnaud & Chaintreau, 2016).

2. The biological properties of EO samples and/or their components constitute the second main part of EO research.

Testing of biological activities can be done in vitro or in vivo, on 3 different forms of EO: on the entire oil as such, on the oil in a soluble form (to improve its bioavailability), or on encapsulated oil (to enhance its contact time with the targeted biological system). Here again, the experimental protocols should be validated and repeatable, in direct link with the goal of the study, and described in sufficient detail to be reproduced by other researchers.

Many types of biological activities can be explored to characterize the potential valorization of EOs for human, veterinary and agricultural applications (antimicrobial, antioxidant, various systems including central nervous system, upper respiratory tract, cardiovascular system, dermatological applications; insect repellent/attractant, pest management *etc*.). Many other types of biological activities deserve attention and should be studied to expand this non-exhaustive list of potential EO research axes. However, it should be considered that the effect of complex mixtures such as EOs on even more complex systems (living organisms) is far from simple and should not be restricted to the effect of a single substance on a specific target, but rather an intricate combination of synergistic/antagonistic interactions requiring advanced testing procedures. In this context, it must be stressed that studies without a sound chemical characterization of the EO sample tested are completely meaningless and even in some instances hazardous. In biological activity tests, both in vitro and in vivo, safety issues need to be followed and non-toxic concentrations need to be applied/considered according to current literature.

There are several methods to perform antimicrobial activity of different extracts, compounds. A review published in 2016 summarized the *in vitro* assays (Balouiri et al., 2016). The examination of antimicrobial activity of essential oils or components is recommended according to standards published by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The following data are recommended to indicate in the article:

- Source of essential oil and/or components (e.g. distilled or obtained from market)
- Solvent should be indicated
- Positive and negative controls are also recommended
- Type of strains (*e.g*. ATCC or from other culture collection)
- In case of strains isolated from clinical samples, ethical approval or certificate should be recommended
- Growth conditions of microorganisms
- If new method is applied, detailed description should be recommended
- In case of interaction/combination tests, intervals of FICI should be indicated
- Calculation of values of activity should be described (*e.g.* MIC, MBC)
- Statistical analysis should be recommended (*e.g.* in case of diameter of inhibition zones)

To study the antimicrobial activity of EOs the following methods are recommended (due to the volatility and hydrophobic character of EOs):

- broth dilution (macro and/or micro, anti-biofilm)
- direct bioautography
- vapour-phase-mediated antimicrobial (VMAA) (Feyaerts et al. 2017 and 2018)

Bach resolutions 2018

The 2nd respective "Brainstorming Workshop" was organized at Bach Castle, Carinthia, Austria on 2-4 March 2018. In 2001 the initial idea of the Workshop was raised by Prof. Chlodwig Franz, who invited 15 colleagues to Austria. In this year, 12 colleagues, who are the members of the Permanent Scientific Committee of ISEO (International Symposium on Essential Oils) met again to take part in the Workshop. After two days of open-space and fruitful discussion concerning the present state and future trends in essential oil (EO) research, the group elaborated and adopted the following résumé:

- In the scientific literature and research papers (manuscripts) quite often insufficient data concerning, *e.g*. description of plant samples, the parameters of GC experiments, as well as qualitative and quantitative analyses of EOs and/or natural volatiles can be found making the results doubtful. Therefore, there is a need to prepare guidelines, which are intended to be of assistance to researchers working in the field of EO and natural volatiles, and set the basis of good practice in experimental research and communication of results. It should be clearly defined as to what the EO and what the volatiles are.
- These guidelines consist of mandatory and recommended points depending upon the specific goals of the research considered, i.e. some recommendations may not be essential if we are not directly related to the main goal of the study.
- These guidelines are formally divided into two main parts: chemical part and biological testing. The biological part cannot be considered separately from the chemical one, as both are generally dependent on each other. The chemical part includes sampling, sample preparation, identification of constituents and quantification of constituents. Every step related to chemical or biological parts should be statistically appropriate. Repeatability, accuracy and validation are highly important characters in the studies of EOs.
- During ISEO Symposia, workshops are planned to be organized with the topic "How to conduct research into EOs and natural volatiles?" The above mentioned guidelines (standards) will be introduced to help researchers in their laboratory work. On the other hand, these workshops may give answer to the researchers' question "How to set up a biological activity test?"
- If commercial essential oils are used for safety, pharmacological or clinical studies, their identity and chemical composition (including chiral analysis) has to be ascertained.
- There are several scientific hot-topics associated with EOs and volatiles, e.g. their role in animal feeding, in nutrition and in food industry. Therefore, the issue of safety and toxicology of these natural chemicals should not be neglected.
- There are only a few numbers of human clinical studies involving EOs. More clinical trials and safety considerations are required.
- Synergism between Western and Eastern Medicine and the discovery of traditional use of EOs should be explored.
- Endophytes live in symbiosis with a plant, but most of the endophyte-plant relationships are not well understood. However, endophytes may often produce metabolites with diverse biological activities. The role of endophytes in aromatic plants should, therefore, be studied.
- Genetics, metabolomic approaches and bioinformatics are worth involving in EO research. In 2001, the 1st Bach Resolution concluded that "*Biodiversity of the chemical composition of aromatic plants, both genetically and environmentally induced, will provide a valuable background for the identification of a gene coding for individual terpene synthesis. The better understanding of the biosynthesis of terpenes leads the way to molecular tailoring of terpene synthesis*." This statement is still valid.

In the future, the ISEO Permanent Scientific Committee would like to inform researchers working in the field of EO and natural volatiles about the event of ISEO Symposia on the following official website: http://iseo-pc.org/.

The members of Permanent Scientific Committee of ISEO present at the 2nd "Brainstorming Workshop" at Bach Castle, Carinthia, Austria:

Nicolas Baldovini, K. Husnu Can Baser, Carlo Bicchi, Fatih Demirci, Chlodwig Franz, Gyorgyi Horvath, Jan Karlsen, Stanislaw Lochynski, Agnieszka Ludwiczuk, Eva Nemeth-Zamborinè, Niko Radulovic, Patrizia Rubiolo.

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