The Value of Naturals, DNA Barcoding, Alternative Solvents and Beyond

Highlights from the 33rd International Days of Essential Oils & Extracts.

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The 33rd International Days of Essential Oils & Extracts (*www.appam.fr*) was held last autumn in Digne, France, and focused on a range of current issues impacting the flavor and fragrance industry. Meanwhile, the "Best Poster" award went to Alexander Wollinger, Theresa Höss, Didier Touraud and Werner Kunz (all University of Regensburg, Germany) for "Towards a selective extraction of irones of *Iris germanica* L. involving green ionic liquids."

Alternative Solvents

The practical application of alternative solvents in odorant extraction was the topic of Aurore Filly's (Green Extraction Team; Avignon, France) talk. The current requirements to spare energy and take care of the environment invite the industry to question the use of conventional solvents (hexane and its derivatives, ethanol, etc.), she said. The management of these solvents is delicate—for instance, hexane is classified CMR 3[a]—as they remain in vegetal residues once the extraction process is finished. The purpose of Filly and her colleagues' work has been to find new solvents with properties that can be considered less "toxic" and more "ecological" by using software such as Hansen and COSMO RS. For example, ethyl acetate has been used for the extraction of caraway (*Carum carvi*) seeds; using this process, carvone contents of the extract were found to be nearly identical to those obtained by classical methods.

A Perfumer on the Value of Naturals

Today, there are fears in the fragrance industry that have been induced by anticipated new regulatory requirements such as the European Commission's targeting of so-called fragrance allergens; as a result, perfumers work in a disturbing and anxious environment—"the perfumery world is in danger." This was the milieu described by noted perfumer Jacques Cavallier (Louis Vuitton), who, along with François Demachy (Dior) and Thierry Wasser (Guerlain), is an LVMH internal perfumer. Despite anxieties, said Cavallier, it is necessary not to forget the fundamentals of the creation of perfumes: what really exists between the "claims" and the "material data," what stands between words and facts, and between claims and acts.

First, he said, it would be necessary to put an end to the questionable issue of natural against synthetic: chemical synthesis is not an enemy of natural chemistry, and it is now necessary to dismiss this perception. It is also necessary to recognize that in past decades there were only a few investments in the natural field, with possibly the exception of supercritical carbon dioxide extraction, while the research in chemistry profited from important investments.

On the one hand, it is necessary to consider that the word "natural" is associated with the word "expensive," which discourages those materials' use. On the other hand, "natural" is attractive in the media and in product claims. It is consequently necessary to question a strategy in which the composition claims say "natural," without being submitted to regulatory

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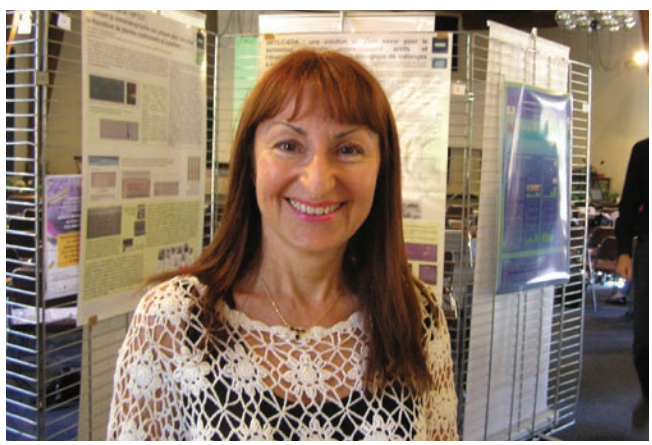

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[a]*http://echa.europa.eu/documents/10162/13562/cmr_report_en.pdf*

requirements. And, as far as the cost is concerned, there are many examples in which compounds rich in natural components are efficient at low concentrations (for example, less than 5%), far lower than the usual concentrations of 10–12%.

Cycle effects are also to be considered, Cavallier explained. In the years 1980–2000, synthetic materials arrived at an apex, while the present trend is to revert to a focus on natural components. He argued that the industry should benefit from the magic of naturals, while also speaking the truth of the role of synthetics.

Finally, Cavallier insisted strongly on the mutual need existing between all actors in the fragrance "channel." The perfumer, he said, is linked with the producer and the grower, the producer with the perfumer, the perfumer with the client. Beyond odorant molecules and essential oils, there is a shared and common heritage—a wellspring of many histories. This is not only a commercial relationship, Cavallier concluded: It is time to put actions in accordance with claims.

The 2014–2015 scientific committee of "Journées Internationales des Huiles Essentielles & Extraits"; front row, from left: J.L. Matout (Aromax/COSMED cosmetics SME federation); J.C. Bayle (IFF-LMR); X. Fernandez, chairman of the committee (Nice University); J.J. Etienne, ATN conseil; and F. Badie (Payan Bertrand); back row, from left: M. Moisseeff (Asquali); H. Brevard (Robertet); P. Andre (Botanicos Ethic) and J. Ph. Paris (Payan Bertrand).

The Allergen Challenge

Pat Sandra (Research Institute for Chromatography, Belgium) discussed analytical methods for cutaneous allergens per European regulations. The introduction of regulatory requirements for allergen declarations (2003) necessitated the development of appropriate analytical methods. Sandra explained that the expected new requirements—about 80 new substances to declare, per an opinion from the Scientific Committee on Consumer Safety[b]—may necessitate an extension of these techniques and tools to new substances needing determination, for instance oxidized allergen derivatives.

The European Standard EN 16274 was published in 2012. This dealt with the GC analysis of samples "ready to be injected," which underscored the problems encountered for the analysis of allergens in finished products such as alcoholic-type fragrance, lotions, gels, emulsions, etc. The main challenge was the possible co-elution of the target substance with a matrix compound (coming from solvents, fatty acids, surfactants, etc.).The separation between the target substances and components of the matrix was not predictable.

Consequently, it was necessary to associate and combine several analytical techniques. For example, to run GC with two columns of different polarity, a temperature flux modulation could be substituted with a temperature modulation. Later in the process, the identification of effluents can be conducted by mass spectrometry combined with simple, double and even quadrupole determinations associated with Q-ToF (time-of-flight) analysis. To conclude, it appeared that the content of allergens was relatively easy to estimate in essential oils, more complex in perfumery products and requiring a sequenced approach for cosmetic products. The complete equipment necessary to conduct such analyses costs about €300,000, according to Sandra.

"Metabarcoding" Natural Extracts with DNA Analyses

A DNA fragment and sequence methodology for natural extract authentication was the subject of a talk by Nicole Giraud (DNA Gensee, Le Bourget du Lac, France). The identification and quality control of substances of natural origin (ex: extracts and

[b]*http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_073.pdf*

essential oils) is typically established by physical and chemical methods (GC, HPLC, HPTLC, NMR, MS, etc.) and, sometimes complementarily, based on a specific component which signals the origin of the analyzed matter.

Giraud explained that because the sequencing of DNA (usually that of chloroplasts) of plants is growing rapidly, it is now possible to associate DNA sequences to a given botanical species, establishing a well-defined genetic signature. This represents an effective "barcoding" of natural materials. Furthermore, following the treatments necessary to obtain extracts (in aqueous or oily media, by fractionation, etc.), the long sequences of DNA are cut and even altered, but these fragments nevertheless retain an identification that provides the plant-source identity. This is what Giraud called "metabarcoding."

Giraud described a large number of examples of application: for plants, elucidating extracts of *Lavandula stoechas*, *Lavandula latifolia* or *Lavandula angustifolia*; for hydro extracts, differentiating among three different species of roses found within a single rose water; and for oils (argan) or essential oils (petitgrain bigarade). These DNA fragments, which have an unquestionable signature of their plants despite treatment for their extraction, can be identified in different media—aqueous and oily. Consequently, these fragments stand for a highly reliable manner of identifying origin (traceability), quality and absence of adulteration(s). This represents a spectacular breakthrough in the analytical world.

Additional Highlights

Other presentations of note included Laure Saint-Lary's (Payan Bertrand) discussion of natural extract identification via the "metabolomics approach." This "fingerprinting" method, detailed on Page 20 of the February 2015 issue of *P&F*, selects and tracks precursors or products linked to specific reactions associated with the plant extracts being studied. This requires some statistical software. Saint-Lary presented an example showing the differentiation of absolutes of violet leaves ex France versus ex Egypt.

Eric Chaisse (CRIEPPAM) presented a general update on the last 10 years of development of Stolbur phytoplasma transmitted in young plants by *Hyalesthes obsoletus*, inducing the final decline and death of the plants. Intensive research has been undertaken by all stakeholders to better understand the situation and find solutions. Among improvements is the treatment of plants by kaolinite, which provides a screen around the plants, dissuading cicadelle/leafhopper contamination.

For information on future industry events, visit www.perfumerflavorist.com/events/calendar/.

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