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CHARACTERISATION OF ODORANTS IN WOOD AND RELATED PRODUCTS: STRATEGIES, METHODOLOGIES AND ACHIEVEMENTS

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Abstract:
Wood is a ubiquitous material that has been used by humans for building construction, furniture, products of daily use, or in the form of derived products such as paper and cardboard, for generations. Extensive studies have been made on the emissions of volatile organic compounds (VOCs) from diverse types of wood, yet only limited information is available on their related odour-active constituents. This article reviews the current state of knowledge on wood odours, covering studies targeting odorants from different woods and investigations on the physiological impact of wood odours on human beings. Further, modern strategies towards elucidating odorants in wood and wood-derived products using analytical tools in combination with human sensory evaluations are introduced and discussed. Specifically, these methods are based on well-established odorant analytical methodologies that are routinely used in the field of food science, namely gas chromatography-olfactometry (GC-O) in combination with sensory dilution approaches such as aroma extract dilution analysis (AEDA). The implementation of such methods allows the relative odour potency of individual odorants within a sample to be ascertained and their identities to be determined via gas chromatography mass spectrometry/olfactometry (GC-MS/O) and two-dimensional GC-MS/O (2D-GC-MS/O). The successful identification of potent odorant constituents of wood offers insights into the molecular basis of wood odour profiles, which can be used to understand the transition of possible off-odours or woody smells in wood-related products.

Key words: *gas chromatography-olfactometry; mass spectrometry; odour-active; physiology.*

INTRODUCTION

Olfaction is one of the oldest human senses from an evolutionary perspective (Albrecht&Wiesmann 2006). The action of smelling proceeds when scent molecules that are present in inhaled air are transported to the receptor sites at the nasal olfactory cleft. Individual odorant molecules then interact with the array of >350 different olfactory receptors at the epithelium to trigger neurological signals that elicit an odour impression in the brain (Buck 2004). Olfactory perception exhibits a high level of complexity that results from manifold activation and inhibition patterns. On the one hand, certain odorants can act on more than one receptor, yet conversely there are specific receptors that become activated by more than one type of odorous molecules. To add to this, specific odorants can undergo metabolism within the nose prior to reaching the receptors, thereby increasing the complexity of interactions (Schilling 2017). When an odour-active molecule binds to an olfactory receptor, the intra-cellular enzyme adenylyl cyclase becomes activated in a process that is mediated via G-proteins. This enzyme catalyses the transformation of adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP), which acts as a secondary messenger and leads to the opening of ion channels. Consequently, the resulting change in the cellular potential of the olfactory cell triggers a signal transmission to the brain, which is relayed in a complex pattern to different regions of the brain (Menini et al. 2004).

Human olfaction is generally underestimated, yet our sense of smell has many functions (McGann 2017). At the individual level, olfaction serves in subconscious chemo-communication between humans, whereas at the cellular level it facilitates signalling between individual cells within the human body. Notably, olfaction is an implicit aspect of our interaction with food, not only in providing a hedonistic parameter for food enjoyment, but also in relation to the detection and avoidance of spoilt food (Solov'yov et al. 2012). Besides food, humans regularly interact with many products that exhibit odour; wood is a raw material that can be included in this category. Humans are exposed to wood and related items on a daily basis, including furniture and construction materials, or derived products such as pencils, paper and packaging goods, to name but a few.

The odour of wood is typically described as pleasant with positive associations, and many consumers would preferably choose wood-based articles over those made out of synthetic materials. Despite its general positive appreciation, however, the exact nature of wood odour is largely unresolved, to date. The present study aimed to address this knowledge gap by reviewing wood odour-related studies and exploring the odorous constituents of wood and wood-based products by applying state-of-the-art odorant analytical tools. Specifically, several different wood species and diverse wood-based products such as cellulose fibres, pencils or paper and cardboard materials were investigated with respect to their odour and its composition. New insights arising from these studies can aid in understanding the formation pathways of individual odorous constituents in wood and help in implementing avoidance strategies of potential off-odour development in wood-based products. Furthermore, these data add to the scientific repository of the constituent odorants of wood and wood-based products.

Wood extractives – important fractions of wood constituents

Wood primarily comprises of the biopolymers cellulose, hemicelluloses, and lignin, with minor amounts of inorganic compounds and extractives. The latter consist of terpenoids, steroids, fats and waxes, and phenolic constituents (Sjöström 1981). They contribute significantly to the odour of wood by being precursors of the odour-active compounds, thus the specific class of extractive under investigation is of high relevance when studying wood odorants. One study on extractives from the heartwood of five different Scots pine (*Pinus sylvestris* L.) trees, which were processed via Soxhlet extraction and subsequently analysed using gas chromatography-mass spectrometry (GC-MS), led to the detection of primarily fatty acids and resin acids (Ekeberg et al. 2006). In another study on this substance class, proteins, amino acids, fatty acids, terpenes, resin acids, steroids, and phenols were detected (Rowell 2005). By comparison, the main lipid classes identified in lipophilic extractives from pine wood by GC-MS were fatty acids, resin acids, triglycerides, sitosterol, waxes, and sterol esters (Gutiérrez et al. 1998).

Wood essential oils

Wood essential oils, which are derived from extractives via steam distillation or hydro-distillation, are rich in wood odorants and have been the subject of several studies in relation to their odour composition. The essential oil of cedar wood (*Calocedrus decurrens (*Torr.) Florin) heartwood, for example, was found to be mainly composed of p-cymene and p-menthane derivatives (Veluthoor et al. 2011), sesquiterpene hydrocarbons, oxygenated derivatives and monoterpenes (Dai et al. 2013). Other studies focussing on the composition of essential oils from Pinaceae woods indicated that monoterpenes (hydrocarbons and oxygenated), sesquiterpenes, and diterpenes were the major constituents, thereby offering first insights into the relevance of terpenoid compounds to the wood odour (Garcia Vallejo et al. 1994; Radulescu et al. 2011; Salem et al. 2015).

Wood volatiles

Wood extractives are rich in volatiles, typically with several hundred different constituent VOCs in individual species. Although many of these are not odorous in nature, a broad overview of the volatiles hitherto detected in diverse woods offers insights into prospective main odour-active candidates that contribute to wood odour.

GC-MS analyses on wood extractives (distilled wood chips) of black pine wood and juniper species (Uçar&Balaban 2002) revealed a total of 140 VOCs in juniper (Uçar&Balaban 2002) and over 200 in black pine tree (Uçar&Balaban, 2001), most of which were monoterpenes, sesquiterpenes or other terpenoids. Other studies on Scots pine (*Pinus silvestris* L.) confirmed monoterpenes as being the major group of wood volatiles (Flodin&Andersson 1977), but have also revealed the presence of many aliphatic aldehydes and alcohols (Weissbecker et al. 2004).

Volatiles of oak and comparable woods have been investigated extensively due to their common and widespread use in cooperage for the maturation of alcoholic beverages (Cadahía et al. 2003; Fernández de Simón et al. 2009; Vichi et al. 2007). Most related studies have focused on the volatiles in naturally-seasoned and toasted wood, which represent the common treatment conditions of woods used in barrel-making. As a result many of the reported substances are formed by degradation of wood lignin, carbohydrates and lipids and Maillard processes of the original wood constituents during these heat treatments. Compounds included a range of phenolic compounds, furan derivatives, lactones, alkyl aldehydes and ketones.

Despite the wealth of information from studies on volatiles in wood, in most cases these do not allow for the odorous character of the wood to be predicted.

Physiological effects of wood odour

The physiological influence of wood odour on animals and humans has been the focus of several investigations. In one study, the essential oil from Sakhalin fir (*Abies sachalinensis* (Mast.)) was observed to have an anxiolytic effect on mice (Satou et al. 2011). In another study, α-pinene, an important wood odorant, was observed to have an alleviating effect by suppressing stress-induced hypothermia in rats (Akutsu et al. 2002).

Such relaxing effects have similarly been demonstrated in other trials. The essential oil of Siberian fir (*Abies sibirica (*Ledeb.))*,* for example, was observed to reduce the arousal level in the panellists when performing visual exercises, as indicated by electrocardiogram and electroencephalogram signals of the panellists (Matsubara et al. 2011). In another study, an experiment that assessed subjective differences in feelings of wellbeing in a room equipped with walls of Japanese cedar wood compared to a control conditioned room observed that liking and feeling responses of the panellists were significantly different between the two conditions (Matsubara&Kawai 2014).

Different feelings of comfort have also been reported between forest and city environments. In one particular study on the physiological influences of such environments it was reported that cerebral activity and salivary cortisol levels reflected a relaxing influence for panellists in a forest environment compared to that of a city (Park et al. 2007). This effect might relate to the observation that pine needles have a potent antioxidant activity and can increase cell viabilities (Ka et al. 2005).

Investigations on wood odour

As indicated above, despite a series of focussed studies on wood extractives and volatiles, and physiological effects of wood odours, few reported investigations on the odour-active compounds in wood exist. Indeed, the odour of native wood has hitherto not been fully characterised. One particular study on this topic focussed on the most odour-active substances in various wood extracts, in particular in woods used for barrels in the aging of wine and spirits (Culleré et al. 2013). In that study, wood chips of acacia, chestnut, cherry, ash or oak were toasted with medium intensity and the samples were then analysed by GC-O and multi-dimensional GC. Different odorants were detected, including various phenolic compounds, as well as compounds arising from the degradation of wood carbohydrates and lipids. Due to the toasting process, however, it is unclear whether or not the respective odorous compounds result from the fresh and native wood or were formed during thermal degradation. Similar studies using GC-O and GC-MS were performed on toasted and non-toasted American, French and Russian oak woods (Díaz-Maroto et al. 2008). Many short- and medium-chain alkyl aldehydes, acids and alcohols, as well as various compounds with fruity and floral notes like linalool oxide, phenylethanol and trans-cinnamaldehyde, were found to be present in the toasted and non-toasted oak wood samples.

Overall, woods used to make barrels for the aging and maturation of alcoholic beverages have been the subject of studies on their odour-active constituents in view of exploring how these might affect the final beverage product. By comparison, odorant investigations on other types of wood, especially those used for furniture or other products of daily use, or in the paper and cardboard industry, are very scarce. Knowledge on the odorous constituents of wood, however, can be essential when trying to ascertain the source of off-odours in wood-based products and in the development of associated avoidance strategies. Such wood-based products include pencils, fibre materials, or packaging materials. Cardboard packaging has been the subject of a targeted odorant investigation in which a sensory analysis revealed that the most potent odours were cardboard-like, woody, and musty (Czerny&Buettner 2009). Aroma extract dilution analysis (AEDA) (Grosch 2001) and two-dimensional GC-MS/O measurements indicated that these odours were caused by the presence of aldehydes and phenolic compounds (Czerny&Buettner 2009).

Release kinetics of volatile wood constituents

In terms of health and wellbeing, not only is the identification of the odour-active constituents of wood important, or an understanding of how these affect human physiology, but given their ubiquitous nature, it is also essential to ascertain their concentrations in different environments, especially in the indoor setting. Wood is a strong source of emissions in the indoor environment and a large contributor to overall indoor air quality (Weschler 2009), although most of the 'negatively' associated emissions from woods are from composite woods such as plywood, particleboard, fibreboard and oriented strand board (OSB), which utilise adhesive resins that can emit a mixture of aldehydes and terpenoids (Hodgson et al. 2002). In addition to classical VOC analysis using GC-MS, the use of on-line mass spectrometric approaches might in future offer insights into the release kinetics of individual odorants from wood and wood-based products. One such method is the chemical ionisation-based technique of proton-transfer-reaction mass spectrometry (PTR-MS), which is a quantitative, real-time analytical tool for characterising rapid concentration changes of VOCs (Hansel et al. 1995). The technique has been successfully implemented to investigate emissions from living trees (Williams et al. 2001), but studies on the kinetics of wood emissions are very limited. In one such investigation, emissions from OSB were comparatively studied using two types of PTR-MS instrument, namely a PTR-quadrupole-MS (PTR-QMS) and a PTR-time-of-flight-MS (PTR-TOFMS) (Schripp et al. 2014). The focus of those studies, however, was less on identifying individual compounds or characterising their emission kinetics, but rather to generate mass spectral fingerprints for use in quality control assessments. In another related study, the kinetics of the generation of secondary products of terpene oxidation – the latter being the primary compound class emitted from wood – under the influence of coexisting carbonyl compounds were investigated using PTR-MS (Ishizuka et al. 2010).

Despite the aforementioned kinetics-related investigations, there are hitherto no studies using on-line analytical tools that solely target the emissions of wood odorant constituents in view of their implications for daily exposure or wellbeing.

Identification of wood and related products – a new approach

Whereas the general framework on odorous wood constituents, namely wood volatiles, essential oils, and physiological effects, have already been the subject of several studies, only limited information is available on the odour-active substances. In an attempt to close this current gap in knowledge on wood odorants, we carried out comprehensive targeted investigations to sensorially characterise and identify the main odorants of wood and wood-based products in a range of non-barrel woods like incense cedar (Schreiner et al. 2017). We applied modern-odorant analytical tools, as routinely applied for aroma analysis of food, to investigate several types of wood as well as cellulose fibres and products made from wood.

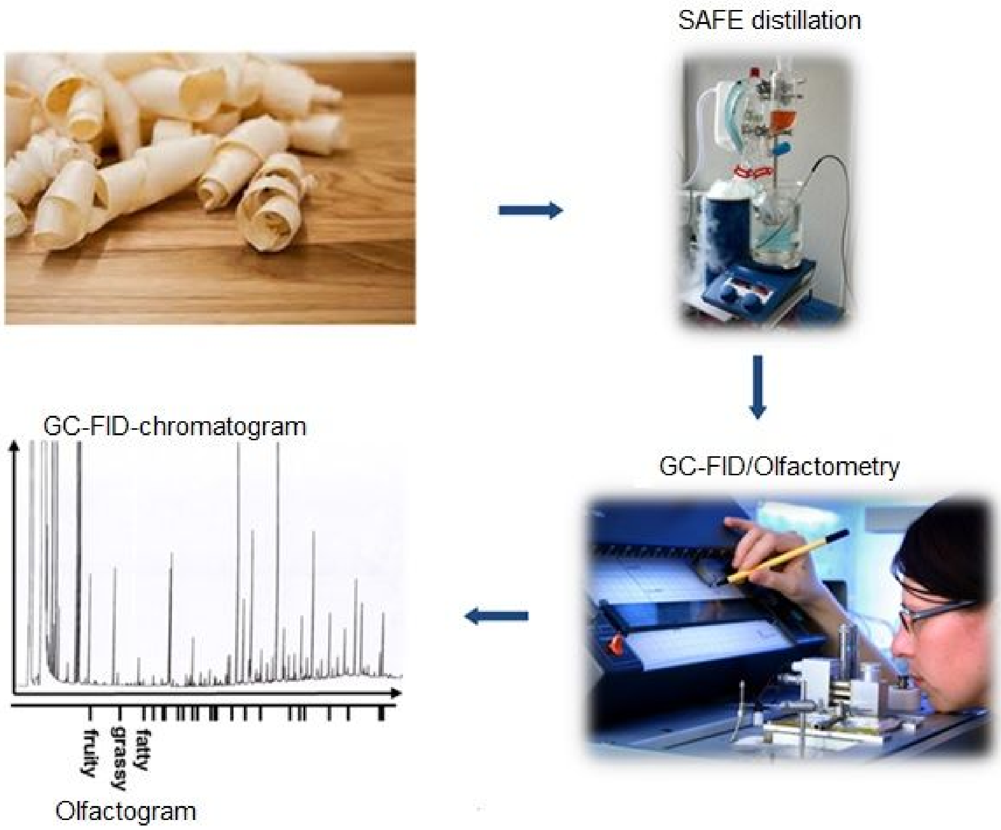

Fig. 1.
Typical steps in analysing wood odorants: solvent extraction and gentle distillation of the volatile fraction by solvent assisted flavour evaporation (SAFE) followed by gas chromatography-olfactometry (GC-O) (with flame ionisation detection; FID) to screen for the odorous compounds. The output of this analytical process is a chromatogram from the GC-FID, with signals relating to individual compounds eluting from the chromatographic column at different retention times, and an olfactogram, describing the odour impressions of individually eluting compounds, as perceived by the trained panellist at the odorant detection port. (© Fraunhofer IVV).

Fig. 1 shows the typical steps undertaken during the targeted analysis of wood odorants. Volatile constituents of individual wood samples are extracted by solvent assisted flavour evaporation (SAFE) (Engel et al. 1999) and this extract is then further enriched by means of Vigreux distillation and micro-distillation (Bemelmans 1979). The gentle conditions for distillation (SAFE and distillation columns held at 55°C) reduce the likelihood that the odorant substances are lost in the extraction process (e.g., through degradation or evaporation) and avoid the potential generation of new odorants during distillation. The odorous substances are then analysed by GC-O (cf. Fig. 2) and are ranked according to aroma extract dilution analysis (AEDA) (Grosch 1994). Compound identification is then made by GC-MS and two-dimensional GC-MS (2D-GC-MS/O).

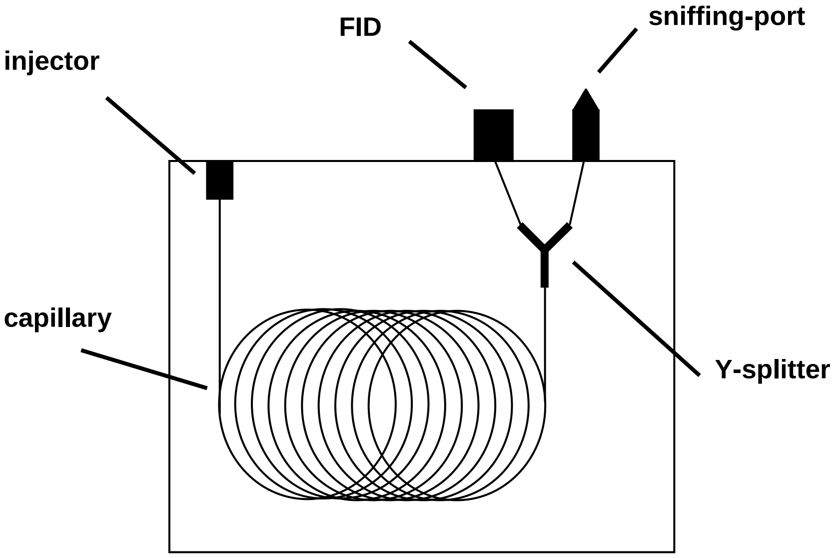

Fig. 2.
Schematic illustration of a gas chromatography-olfactory (GC-O) system with flame ionisation detection (FID) and simultaneous olfactory assessment via a sniffing port. (© Fraunhofer IVV)

After screening for odorants by GC-O, a more comprehensive analysis by 2D-GC-MS/O is carried out for purposes of compound identification and to target substances present at low concentrations or those that co-elute with other compounds (cf. Fig. 3). In the latter, the volatiles are pre-separated on a GC column and the odorants are detected via GC-O. Then, the specific odour compounds are transferred onto a second capillary of the 2D-GC-MS/O system. After separation, the odorants will be transferred after splitting the gas-stream between a detector (MS) and a sniffing port. The use of two chromatographs with analytical capillaries of different polarity leads to an increased separation efficiency and sensitivity.

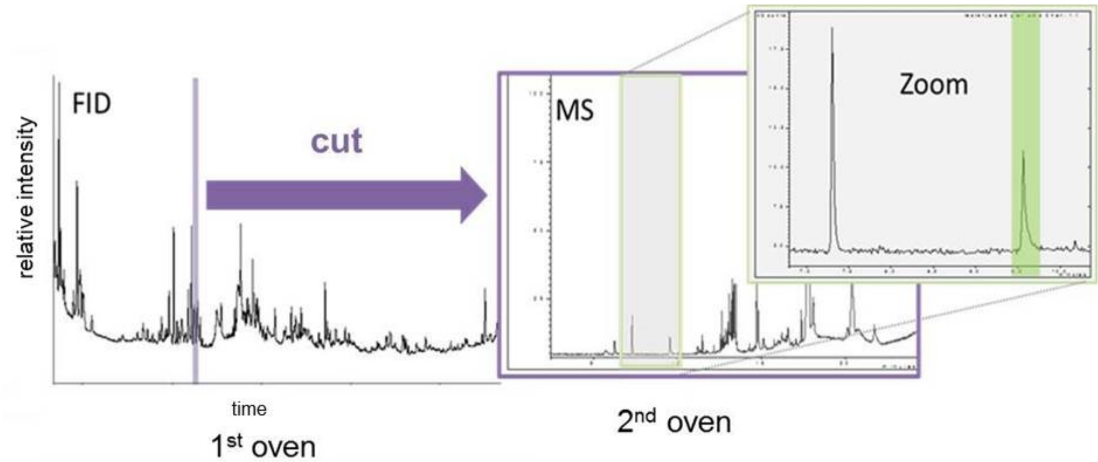

Fig. 3.
Separation and detection of odorants via two-dimensional gas chromatography-mass spectrometry/olfactometry (2D-GC-MS/O). A specific elution period from the first capillary (chromatograph oven) is cut and transferred to a second capillary of different polarity. (© Fraunhofer IVV)

If odorants remain unresolved after 2D-GC-MS/O analysis, structure-odour-relationships can be explored. This involves the synthesis of chemically-related substances of the known odorants and a subsequent investigation of their properties. Such structural effects on odour impressions have been comprehensively explored for fatty acid-derived compounds and terpenoids, as well as for aroma compounds that may contribute to the odour of wood and wood-based products (Elsharif&Buettner 2017; Lorber&Buettner 2015; Lorber et al. 2016; Schranz et al. 2017). Once the key parameters such as retention indices and odour quality are known, a prediction of an odorant's structure can be made, allowing for the synthesis of various related compounds and a directed investigation of the structure-odour relationships with a view to elucidating the identity of the target molecule.

In one study, we analysed the odour-active constituents of cedar wood, which revealed numerous odorous substances that represent various smells such as fatty notes, cheesy and fruity smells, as well as typical wood-like, green notes. Five compounds were detected for the first time as wood odorants in incense cedar, including the pencil-like smelling thymoquinone (Schreiner et al. 2017).

CONCLUSIONS

Wood is a natural product that is rich in VOCs, many of which are odorous, yet comprehensive knowledge on these odorants is limited, to date. We successfully applied combined human-sensory and odorant analytical methods to investigate the chemical structures of the main odorants in selected woods and wood-based products. After characterising the odour of the wood via sensory evaluations, samples are analysed by GC-O and are ranked according to odour potency via AEDA. The samples are then analysed using GC-MS and 2D-GC-MS/O to reveal the identities of the main constituent odorants. Although this analytical procedure is well-established in the field of food aroma analysis, it represents a new approach for elucidating wood odour, and indeed the use of SAFE distillation for wood products for a targeted odorant extraction is a new application in this field of research. The results of these studies can help to establish a wood substance library comprising data on substances identified in wood and wood-based products, their odour qualities, and their analytical properties such as linear retention indices and characteristic mass spectra. Such a library helps to create a better understanding of compounds that generate typical wood smells, and is an essential aid when assessing the potential daily impact of wood odorants on humans and their wellbeing.

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