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Real time aroma reconstruction using odour primaries

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ABSTRACT

A methodology was developed to derive odour primaries based on the actual composition of a set of target aromas to be reconstructed. These odour primaries are mixtures of pure aroma components. The methodology was applied to reconstruct the aroma of five *Citrus* species (lime, lemon, orange, grapefruit and mandarin) in real time using an aroma synthesiser with four nozzles dispersing odour primaries blended using 12 pure odorants that was built for this purpose. The composition of the actual and reconstructed *Citrus* aromas was analysed using gas chromatography–mass spectrometry (GC–MS). *Citrus* species could be discriminated based on their headspace volatile composition. The aroma synthesiser was shown to be able to blend aromas in real time. The theoretical and actual correct classification accuracies of the reconstructed aromas were 80% and 73%, respectively. The required number of primaries and also pure odourants to construct these primaries was higher than anticipated, suggesting that further research with respect to the dimensionality of aroma spaces is required.

1. Introduction

Aroma synthesis is an ancient art that has been practiced by mankind since ages through cooking and the creation of perfumes and fragrances for a wide range of applications. Cooking aims, amongst others, at combining raw plant or animal based raw ingredients and transforming them through various processes into a product that has a pleasant aroma. Perfume design is a complex process that integrates natural essential oils and fragrances with artificial ones and needs input from both perfumers and chemists.

Aromas are often very complex and may consist of many odourants—volatile compounds that interact with the odour receptors in the olfactory epithelium inside the nose cavity. The coffee aroma, for example, comprises more than 800 odourants [1] with a wide range of functional groups. Of these, 29 components were identified as being responsible for most of the roast and ground coffee aroma, and only 13 of them proved to have a key contribution [2–4]. The process of creating novel aromas is complicated by the enormous number of volatile odourants but also by the fact that the human olfactory system is synthetic rather than analytical. The large number of signals transmitted by olfactory receptor neurons in the olfactory epithelium upon stimulation by hundreds of odourants are integrated to one distinct aroma impression [5]. Studies have shown that humans typically can only identify up to four single components in an odourant mixture [6,7] and that the sensitivity varies widely from non-detection to parts per billion (ppb), depending on the odourant, panelists and method of threshold determination. For example, limonene—the volatile responsible for the typical aroma of *Citrus* fruit—has an odour threshold of 4–3000 ppb in water, while 2-isobutyl-3-methoxypyrazin that has a green pepper-like aroma can even be detected at concentrations as low as 0.001–10 ppb in water [8]. In the remainder of this manuscript we will use the term 'aroma' to both describe the human sensation caused by a mixture of odourants emanating from a food, as well as the mixture of odorants that evokes an aroma perception.

Recently there is an increased interest in real time aroma synthesis. Such a controlled delivery of odours has a great potential in the film industry, gaming, advertisement, healthcare and even to art. The smell of mud and gunpowder during a war scene or the smell of gasoline and burned rubber during a pursuit scene would definitely enhance the realistic experience of the storyline. Also, several companies provide pleasant and distinct smells to

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help sell products and to strengthen brand strategies (Ambius, Aartselaar, Belgium; ScentAir, Taplow, UK; Mood Media, Naarden, Netherlands). Aroma synthesis has also inspired art through aroma concerts on an Olfactiano (Peter De Cuypere, http://www.scentconcerts.com/) and the design of odour emitting clothing or jewellery (Jenny Tillotson, http://www.ceb.cam.ac.uk/directory/jenny-tillotson). Hundreds of patents exist on aroma evaporators and sprays, but only a few companies have addressed real time aroma synthesis, including MicroScent (Wilmington, DE); Aerome (Cologne, Germany); Aromajet (Plano, TX); Osmooze (Loriol-sur-Drome, France). They employ diverse physical mechanisms like capillarity, evaporation, nebulisation, spraying and piezo electric evaporation in their systems. The Nakamoto lab investigated odour recording with an electronic nose, followed by its reproduction by means of a mixing device using liquid basic aroma samples [9–11]. Kim et al. [12] described an array of tiny reservoirs constructed in PDMS, each filled with a different odourant. A 2D grid of electrical wires was used to heat individual reservoirs and expel the odourants through a tiny hole at the top of the reservoir. An excellent overview of aroma synthesisers, also called olfactory displays, is given in reference [13].

Although any aroma can in theory be reconstructed by blending its constituent odourants in appropriate amounts using the systems described above, the often large number of odourants that produce a specific aroma and the diversity of aromas is a major hurdle. As much as 10,000 odours can be detected by the human nose [14]; a simplistic aroma reconstruction system would thus consist of a mixing systems with 10,000 channels, each dispensing one particular odourant, which practically is not feasible. However, the total number of aroma receptor genes and corresponding receptor proteins that humans use to smell is relatively small (about 400 [15,16]). Nonetheless this allows us to discriminate between a vast universe of odours as the individual receptors are not very specific and respond to various degrees to different odourants [16]. This is similar to colour vision, where only three colour receptors respond to a different but broad and overlapping wavelength range of the electromagnetic spectrum allowing humans to perceive millions of colours. Inversely, in theory almost every imaginable colour can be blended using three primary colours only, although in practice usually four (cyan, magenta, yellow and black) are used in commercial printing applications. It, therefore, seems feasible to conceive odour primaries – mixtures of odourants with different but possibly partially overlapping composition – that can reconstruct every imaginable smell [17]. While this is an exciting research question [18], it is beyond the scope of this manuscript which will be confined to the much smaller olfactory space generated by fruit odourants. Many aroma biosynthesis pathways are relatively well conserved amongst species and even beyond; the same odourants are often found in very different fruit, albeit in a different concentration and in combination with different other odourants. Within the *Citrus* genus, for example, the aroma is dominated by mostly the same terpenes and terpenoids but in different relatively concentrations [19]. Such confined aroma spaces are interesting because there are most likely no unique odourants per *Citrus* species; as a result trivial classifiers based on unique odourants can be excluded.

The hypothesis tested in this article is whether it would be possible to reconstruct fruit aromas by blending a relatively small number of odour primaries—essentially mixtures of pure odourants blended prior to the actual aroma synthesis. Nakamoto et al. [20] addressed this hypothesis before and attempted to reconstruct the aroma of 158 essential oils using 12 or 30 basis vectors using the non-negative least squares method. However, rather than the actual aroma composition, they reconstructed the mass spectral fingerprint. While fingerprinting based on mass spectra has been used successfully to discriminate odours [21], mass fragments are not unique, and pure odourant that evoke widely different odour perceptions may produce identical mass ions. For example, all monoterpenes have a molecular ion at m/z 136 and a typical fragmentation ion at m/z 93. Reconstructions with very similar mass spectrum but causing a completely different aroma perception are thus conceivable. A potentially better approach that we will investigate in this article is to derive primaries that allow to reconstruct the actual chemical composition of target aromas. We will adopt a data driven approach, using multivariate statistical techniques to project an aroma space onto a subspace of considerably lower dimension. The aroma reconstruction will be implemented in the latter subspace and aims to approximate the chemical composition of the target aromas rather than their overall mass spectrum as in [20]. To investigate this concept the relatively small aroma space spanned by *Citrus* species was targeted. Odour primaries were constructed and the original aroma space was projected on these odour primaries. Finally, the odour primaries were blended in real time in order to reconstruct the original aroma profiles and compare them later with the reference aroma profiles.

2. Materials and methods

2.1. Citrus *samples*

Five species of *Citrus* fruit were selected: 'Eureka' lemons (*Citrus* × *limon* (L.) Burm.f.) and 'Cambria' oranges (*Citrus* × *sinensis*(L.) Osbeck) from South-Africa, 'Tahiti' limes (*Citrus aurantifolia* (Christm.) Swingle), 'Ruby red' grapefruits (*Citrus* × *paradisi* Macfad.) from Mexico and 'Clementina Hoja' mandarins (*Citrus reticulata* Blanco) from Spain. The selection was based on commercial availability and diversity of their aromas. From each species, four different fruit were sampled for aroma analysis, resulting in a total of 20 samples. Fruit were cut to pieces, with the peels still attached as they contain most of the aroma compounds. These pieces were then mixed in a blender. Of each sample, 6.20 mL was diluted to 10 mL with 3.8 mL of a saturated KCl solution and mixed to stop the enzymatic reactions that would affect the aroma profile and to enhance the release of volatiles out of the matrix and into the headspace. All samples were collected in glass vials of 20 mL that were instantaneously frozen in liquid nitrogen and kept at −80 °C until further analysis. The vials were sealed airtight by means of a cap with septum.

2.2. Aroma analyses

Each *Citrus* sample was thawed in a water bath at 35 °C during 15 min, followed by incubation at 30 °C during 1 h to achieve equilibrium between the sample and its headspace. This was followed by an extraction step of 10 min using a StableFlex SPME fiber (df: 50/30 μm; measure: 24 gauge) coated with divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS) from Supelco (Bornem, Belgium). The volatiles were desorbed from the fibre at 250 °C into the a split/splitless injection port of an Agilent 6890 N gas chromatograph (GC) (Agilent Technologies, Diegem, Belgium) coupled to an Agilent 5973 Network Mass Selective Detector (MS). The split ratio was set to 16:1 and the samples were injected by means of an autosampler (MPS2, Multipurpose sampler, Gerstel, Germany). The separation was achieved on a 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness, Supelco Co., Bellefonte, USA). Helium was used as carrier gas at a constant flow rate (1.24 mL min^{-1} at 30 °C). The GC temperature program was as follows: 55 °C (2 min), 85 °C (1.5 °C min^{-1}), 145 °C (2.5 °C min^{-1}) and 240 °C (40 °C min^{-1}). The temperature was kept at 240 °C for two more minutes.