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Analytical Methods

Selection of a representative extraction method for the analysis of odourant volatile composition of French cider by GC–MS–O and GC × GC–TOF-MS

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ABSTRACT

The experimental conditions of a representative odour extraction method were determined after testing eight SPME, dynamic headspace and purge-and-trap procedures. Headspace SPME with a Car/PDMS fibre was evidenced to be the most suitable method to obtain representative extract of cider odour. This method was applied to extract the volatile compounds of two French ciders and gas chromatography–mass spectrometry–olfactometry (GC–MS–O) was used to analyse the odourant profile and the aroma-active compounds of these ciders. Thirty-six odourant zones were perceived in one cider and 24 in the other. Comprehensive two-dimensional gas chromatography combined with time-of-flight mass spectrometry was used to identify odour-active compounds which were undetermined after the first chromatographic separation. Ethyl 2-methylbutanoate, 2-phenylethanol, ethyl butanoate, ethyl 2-methylpropanoate, ethyl hexanoate, oct-1-en-3-one, 2-phenylethyl acetate, ethyl dodecanoate, 3-methyl-1-butanol and 2-methylbutanoic acid were among the most potent odourants in both ciders, as well as oct-1-en-3-one, which could be identified only by comprehensive GC. Thanks to the association of the two methods, 80% of the aroma-active compounds were identified, some of them being present at trace levels in ciders.

1. Introduction

Foods and beverages contain numerous volatile compounds. Among all the compounds identified by instrumental techniques, only a few are really involved in the perception of the product odour or aroma. Some volatiles are non-odourant while the odourants do not contribute equally to the overall aroma of the product, notably because of differences in their concentrations and their detection thresholds. Moreover, when studying very complex products, such as fermented foods or beverages, samples can have a much greater complexity than anticipated. GC–olfactometry has been used for many years to study food odour-active compounds. But in such complex products, peak overlap can occur in odour-active regions, leading to ambiguous or problematic peak identification. For instance, the peak corresponding to a trace odourant can be masked by that of a large odourless compound, leading to an incorrect identification. One-dimensional chromatography often lacks the resolution and separation power required to separate, identify and quantify all the compounds responsible for the odour-active regions highlighted by olfactometry judges.

Two-dimensional GC has thus been developed and applied to the separation of very complex food volatile compositions like in coffee (Ryan et al., 2004), essential oils (von Mühlen, Zini, Caramão, & Marriott, 2008) or smoked foods (Catanéo et al., 2010). The combination of olfactometry and 2D-GC seems to be a solution to overcome the problems of identification of compounds contributing to odourant areas of the chromatogram. Most of the studies combining 2D-GC and olfactometry have involved heart-cutting of co-eluted peaks from the first column then sending them to a second column connected to the olfaction port (Culleré, Escudero, Campo, Cacho, & Ferreira, 2009; Culleré, Escudero, Pérez-Trujillo, Cacho, & Ferreira, 2008; Sasamoto & Ochiai, 2010). The second column is of classical length but has a different polarity from the first one. This technique, referred to as MDGC-O, can be applied to resolve co-elution zones and identify the compound responsible for the perceived odour among the other co-eluted compounds. Its main drawback is that the number of cuts is limited in a run and a minimum amount of time is needed between two cuts to stabilize the system. As a result, the screening of the whole chromatogram on the second dimension would need several runs and would be extremely laborious and time-consuming. Another possibility is to use comprehensive GC (GC × GC). In this technique, a modulator placed at the end of the first column allows a rapid sampling of the fractions of the eluate that is sent to a second fast GC column with a different polarity. Thanks to this system, the whole first dimension effluent is

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transferred to the second column. Nevertheless, the combination of GC × GC and olfactometry is very complicated for judges. Indeed, GC × GC produces multiple peak slices for each compound and rapid eluting peaks. Kristenson et al. (2003) showed peak widths at half-height to be between 30 and 140 ms, depending on the type of modulator, while the human breathing cycle is about 3–4 s. Some researchers have applied GC × GC–O to the analysis of perfumes (d'Acampora Zellner, Casilli, Dugo, Dugo, & Mondello, 2007) but this study was conducted with only two judges, which has been shown to be insufficient to validate GC–O results (Pollien et al., 1997). GC × GC–TOF-MS and GC–O have more often been applied in parallel, the second dimension separation being used to resolve co-eluted portions and identify the compound responsible for the perceived odour in GC–O (G. Eyres, P.J. Marriott, & J.-P. Dufour, 2007; Rochat, de Saint Laumer, & Chaintreau, 2007).

Independently of the techniques used for analysis, sample pre-treatment can be an important source of errors in the characterisation of odourant compounds, mainly in food with a complex volatile composition. The success of food odour characterisation by GC–O depends largely on the nature of the extract (Plutowska & Wardencki, 2007). Some authors even consider that the extraction method is the factor that most contributes to the reliability of olfactory results (Mistry, Reineccius, & Olson, 1997). Indeed, the extraction step should produce extracts with aroma characteristics as close as possible to those of the corresponding food. The evaluation of the representativeness of extracts has already been applied to validate the efficiency of various extraction techniques, including solvent-free extraction methods (Mehinagic, Prost, & Demaimay, 2003; Sarrazin, Le Quéré, Gretsch, & Liardon, 2000). Solvent-free extraction methods avoid exposing judges to potentially toxic solvent when they were evaluating the extracts. Besides, the use of a solvent leads to other drawbacks. The solvent peak often masks very volatile compounds on chromatograms. The elimination of the solvent leads to the loss of very volatile odourant compounds and can also induce the formation of new compounds if the sample is heated. Moreover, organic solvents can provoke co-extraction of other components of the matrix, apart from the desired volatile fraction. Dynamic headspace, purge and trap and solid phase micro-extraction (SPME) have previously been applied to extract volatile compounds with a good odour representativeness (Mebazaa et al., 2009; Mebazaa, Mahmoudi, Rega, Ben Cheikh, & Camel, 2010; Poinot et al., 2007; Rega, Fournier, & Guichard, 2003). However, solvent-free extraction methods have rarely been compared for their representativeness.

Few studies have been carried out on the odourant compounds of hard ciders in general and French ciders in particular. Williams and collaborators were probably the firsts to study hard cider volatile composition (Williams, 1974; Williams, May, & Tucknott, 1978; Williams & Tucknott, 1978), by different extraction methods. Le Quere, Husson, Renard, and Primault (2006) studied the volatile compounds extracted from French ciders on a liquid/liquid extraction column. Chinese cider and cider from Asturias have also been investigated for their volatile components, isolated by SPME (Peng, Yue, & Yuan, 2009; Wang, Xu, Zhao, & Li, 2004), or purge-and-trap extraction (Rodríguez Madrera, García Hevia, Palacios García, & Suárez Valles, 2008, 2005). In these studies, all the compounds or the major volatile compounds (on a quantitative basis) were identified in ciders but those compounds responsible for the cider aroma were generally not searched for. Only Xu, Fan, and Qian (2007) and Williams and Tucknott (1978) used GC–O to study the aroma-active compounds of ciders obtained by different extraction methods. The composition of these extracts varied greatly depending on the technique but their representativeness was not studied. In these two studies, odours associated to gas chromatographic peaks were assessed by only two or three judges. To our knowledge, 2D-GC has never been applied to ciders. Yet, hard cider is a very complex product for which this technique could give interesting results.

The objective of this study was to develop a global methodology to identify the compounds responsible for the odourant properties of French ciders. First, the extracts obtained by eight extraction methods were compared for their representativeness. Only solvent-free extraction methods were chosen in this study, based on dynamic headspace, purge-and-trap, direct immersion and headspace SPME with three different fibre coatings (PDMS, Car/PDMS, DVB/Car/PDMS). The most representative extraction method was then applied to two French ciders and the obtained extracts were analysed by GC–MS–O and GC × GC–TOF-MS to identify the most potent odourants of these ciders.

2. Materials and methods

2.1. Materials

A commercial sweet French cider conditioned in 25 cL bottles was purchased in a local supermarket and used to set up a representative extraction method.

The two ciders (A and B) analysed by gas chromatography (GC)–mass spectrometry (MS)–olfactometry (O) and comprehensive GC (GC × GC–TOF-MS) were produced by the *Institut français des productions cidricoles* (Le Rheu, France). They were chosen for their different odour perception (confirmed by a triangular test, with 1% risk).

Chemicals: ethanol (99.9%) was obtained from VWR (Briare, France). All other standards were obtained from Sigma Aldrich (St. Quentin Fallavier, France) with purity ⩾97% except for ethyl 3-methylbutanoate (⩾90%), α-farnesene (mixture of isomers), oct-1-en-3-one (50% in 1-octen-3-ol).

2.2. Extraction methods

SPME was conducted either by exposure to the headspace of the sample (HS-SPME) or immersion in the cider (direct immersion SPME) using three types of fibre (Supelco, Bellefonte, PA, USA): DVB/Car/PDMS (10 mm long, 50/30 μm film thickness), Car/PDMS (10 mm long, 85 μm film thickness) and PDMS (10 mm long, 100 μm film thickness). The cider sample (20 mL for HS-SPME or 60 mL for direct immersion SPME) was placed in a 60 mL brown vial tightly capped with a Teflon/silicone septum under stirring for 30 min until equilibrium. Temperature and extraction time were selected according to a previous experimental design (data not shown). A temperature of 37.5 °C and 10 min of extraction were found to be the optimum conditions to have the highest similarity score and the better volatile composition (number of peaks). These conditions were then applied in the present study.

Dynamic headspace and purge-and-trap extractions were conducted with a concentrator (model LSC 2000; Teckmar Inc., Cincinnati, OH, USA), equipped with a capillary interface for cryofocusing, connected to the GC. For dynamic headspace, 20 mL of cider were introduced into a flask (60 mL) containing a stir bar. The temperature of the sample was maintained constant at 37.5 °C with a heating ring. The headspace of the sample was purged with helium for 5 min at 60 mL min^{-1}. Volatile compounds extracted were swept into a porous adsorbent polymer (Tenax trap) maintained at room temperature. For purge-and-trap, 60 mL of cider was introduced into the flask, maintained at 37.5 °C and bubbled with helium for 5 min. As for the SPME study, the time of purge was chosen after a previous study conducted on purges of 5, 10 and 30 min. Volatiles extracted were swept into the Tenax trap.