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Headspace-solid phase microextraction coupled to gas chromatography–combustion-isotope ratio mass spectrometer and to enantioselective gas chromatography for strawberry flavoured food quality control

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ABSTRACT

Authenticity assessment of flavoured strawberry foods was performed using headspace-solid phase microextraction (HS-SPME) coupled to gas chromatography–combustion-isotope ratio mass spectrometry (GC–C-IRMS). An authenticity range was achieved, investigating on the carbon isotope ratio of numerous selected aroma active volatile components (methyl butanoate, ethyl butanoate, hex-(2E)-enal, methyl hexanoate, buthyl butanoate, ethyl hexanoate, hexyl acetate, linalool, hexyl butanoate, octyl isovalerate, γ-decalactone and octyl hexanoate) of organic Italian fresh strawberries. To the author's knowledge, this is the first time that all these components were investigated simultaneously by GC–C-IRMS on the same sample. The results were compared, when applicable, with those obtained by analyzing the HS-SPME extracts of commercial flavoured food matrices. In addition, one Kenyan pineapple and one fresh Italian peach were analyzed to determine the $\delta^{13}C_{VPDB}$ of the volatile components common to strawberry. The $\delta^{13}C_{VPDB}$ values are allowed to differentiate between different biogenetic pathways (C_3 and CAM plants) and more interestingly between plants of the same CO_2 fixation group (C_3 plants). Additional analyses were performed on all the samples by means of Enantioselective Gas Chromatography (Es-GC), measuring the enantiomeric distribution of linalool and γ-decalactone. It was found that GC–C-IRMS and Es-GC measurements were in agreement to detect the presence of non-natural strawberry aromas in the food matrices studied.

1. Introduction

Foods may contain more than a thousand chemical compounds that contribute to their flavour. Many of these naturally occurring compounds may be too unstable to be used in commercial flavourings because of the required shelf life of the final industrial product. For this reason, and also for commercial and economical advantages, "copies" of the natural flavour are often developed.

Flavours are the food additives that specifically aim to improve the taste of foods and to make them more appetizing. These are complex molecules linked together cleverly to boost the fragrance of the food containing them and to make pleasant beverages, yogurts, confectionery and many other industrial food products. Flavours can be divided into three categories, under definitions agreed in the E.U., depending on the origin and the characteristics of the molecules that compose it: *natural flavouring substances* (obtained from plant or animal raw materials by physical, microbiological or enzymatic processes); *nature-identical flavouring substances* (obtained by synthesis or isolated through chemical processes, which are chemically and organoleptically identical to flavouring substances naturally present in products intended for human consumption); and *artificial flavouring substances* (not identified in a natural product intended for human consumption, whether or not the product is processed). According to the actual European regulations on labelling for flavours added to foodstuffs, the word "flavouring" must be present in the ingredient list on the packaging of food products when they contain artificial flavours. The expression "natural flavouring" may only be used for flavour substances or flavour preparations which are extracted from vegetables or animal materials [1]. The aim of the present work is to provide new parameters useful to investigate on the nature of the aroma present in strawberry flavoured foodstuff.

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Many techniques exist to investigate the origin of the aroma of the food matrices and the most accredited one is the chiral investigation of volatile compounds [2–4]. Enzyme-catalysed reactions usually proceed with high selectivity thus, analyzing flavours and fragrances, enantio-cGC has proved to be highly efficient in origin-specific analysis. To obtain accurate information with respect to chirality, analytical procedures of the highest selectivity which employ chiral separation without racemisation must be utilised [5]. In addition, references of definite chirality are essential. Enantio-gas chromatography (Es-GC) can detect chiral flavoured compounds, defining their characteristic enantiomeric distributions. In 2001, Mosandl's group [6] analyzed by enantio GC–MS and extracted the volatiles by SBSE from strawberries. In this study the authors successfully developed a rapid screening method to determine the enantiomeric distribution on seven chiral volatiles (esters, acids and etherocyclic compounds) applying the newly commercialized at that time stir bar sorptive extraction. This sampling technique is somewhat similar to the well-known SPME, being a solvent-free technique, but allows higher enrichment factors. The same research group published in 2008 an article on the enantioselective determination of heptan-2-ol, cis- and trans-linalool oxides in blackberry extracts. The same components were also analyzed to determine multielement isotope ratios (C, H and O) [7].

As asserted by Mosandl in his recent review on flavour authentication [5], the elucidation of stable isotope distributions is highly desirable in view of fundamental studies in biochemistry, nutrition, drug research and also in the authenticity assessment of food ingredients.

With the introduction of gas chromatography coupled to isotope ratio mass spectrometer (GC–IRMS), the authentication of flavoured food has gained further scientific contribution, investigating on the plants' different metabolisms (C3, C4 or CAM) [5], on their geographic origins, and/or illicit production methods, such as sophistication by the addition of synthetic or natural compounds [5–13]. In particular, Kahale et al. [13] studied the major volatiles in Pear extracts determining $\delta^{13}C_{VPDB}$ and $\delta^{2}H_{VSMOW}$ values compared to those obtained with synthetic standards. These authors determined seven compounds in the SDE and LLE extracts, identified by GC–MS.

The use of GC–IRMS enhanced by enantioselective measurements permits to attain a more complete characterization of the matrices investigated. In addition, where Es-GC investigations fail, for the lack of flavour chiral compounds, the isotope ratios provide information useful for quality control. GC–IRMS and Es-GC measurements, used in combination, have already shown their efficiency on the determination of fruit aromatized food, giving useful references for food quality control [4,14].

In this study, the aroma of strawberry flavoured foods was investigated, measuring the carbon stable isotope ratio and the enantiomeric distribution of some volatile components. Measurements were initially carried out on samples of organic strawberries to acquire the reference authenticity range. Subsequently some commercial flavoured strawberry food products were analyzed. In particular, twelve different volatiles were analyzed by GC–C-IRMS and the $\delta^{13}C$ values were used to build the range of authenticity. The components investigated were never simultaneously determined in the studies previously carried out on strawberry.

Additionally, other fruit samples were investigated by GC–C-IRMS and Es-GC: extracts from fresh strawberries, Kenyan pineapple and fresh Italian peaches all obtained from a local market. HS-SPME was used as sample preparation technique for the extraction of volatiles from all the analyzed samples [15–17].

The results obtained can provide valuable tools for quality assessment of fruit flavoured food.

2. Materials and methods

2.1. Samples and sample preparation

Fresh organic Italian strawberries were harvested in the 2010 season in three different locations in Messina, Sicily. These samples, not treated with fertilizers, were homogenized and immediately analyzed, to attain the authenticity range reference. Another sample was obtained homogenizing some commercial fresh strawberries of unknown origin, purchased in a local market. Moreover, two yogurts (without fruit pulp, of different brands), a box of candies and a lolly ice, all flavoured with strawberry aroma were analyzed. To determine a blank reference for the yogurts, two plain yogurts were also analyzed (not fruit aromatized, of the same brands as the flavoured ones).

A commercial natural Italian peach and a commercial Kenyan pineapple, also purchased in a local market, were homogenized and analyzed to compare the isotope ratios and the enantiomeric distribution of the components in common with strawberry (γ-decalactone and linalool).

All the mentioned fruits, the lolly ice and yogurt samples were homogenized and blended with NaCl saturated water and the volatile components were extracted from 10 ml half filled SPME vial (with silicone/PTFE septa). The candies aroma (head space) was extracted in a 10 ml SPME vial, filled with 4 grams of product.

2.2. (HS-SPME) extraction parameters

HS-SPME time and method of extraction, applied in this work, followed the parameters reported by Jetti et al. [17], selecting the aroma active compounds employing the HS-SPME technique. The period of manual extraction from a 10 ml half filled SPME vial (with silicone/PTFE septa) was 60 min at agitation of 2000 rpm. Equilibration time was 15 min at 50 °C, at the same agitation speed. The fibre desorption was made at 250 °C for 1 min in the gas chromatograph injector of GC–C-IRMS, Es-GC and GC–MS devices.

The fibre employed was chosen in order to collect a range as wide as possible of aroma compounds, and particularly to ensure the extraction of esters, lactones and terpenoids responsible for the characteristic fruit flavour. The best expectations from the analyses were given by divinylbenzene–polydimethylsiloxane–Carboxen SPME fibre (DVB–PDMS–Carboxen) (Supelco) 50/30 μm thickness and with a length of 1 cm that was conditioned prior to use at 270 °C for 1 h.

2.3. Chemicals

Chemicals used for identification were: methyl butanoate, ethyl butanoate, hex-(2E)-enal, methyl hexanoate, buthyl butanoate, ethyl hexanoate, hexyl acetate, linalool, hexyl butanoate, octyl isovalerate, γ-decalactone and octyl hexanoate (Aldrich). For the enantioselective retention time determination, enantiomerically pure standards were used: (+) and (−) γ-decalactone and (+) and (−) linalool (Aldrich).

2.4. GC–C-IRMS device and analyses

The system consists of a Trace GC Ultra equipped with a TriPlus autosampler (disabled for these measurements), retrofitted to the combustion interface GC/CIII and hyphenated to the isotope ratio mass spectrometer Delta V Advantage (all purchased from Thermo Fisher Scientific, Milan, Italy). Data are collected in triplicate by the Isodat 2.5 software (Thermo Fisher Scientific).

GC: column: SLB-5ms (silphenylene polymer) 30 m × 0.25 mm i.d., 0.25 μm d_f (Supelco, Milan, Italy); temperature program: 40 °C held 2.5 min, 3 °C/min to 250 °C held 2 min; splitless injector