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Identification of aroma-active volatiles in banana *Terra* spirit using multidimensional gas chromatography with simultaneous mass spectrometry and olfactometry detection☆

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ABSTRACT

Fruit spirits have been produced and consumed throughout the world for centuries. However, the aroma composition of banana spirits is still poorly characterised. We have investigated the aroma-impact compounds of the banana *Terra* spirit for the first time, using multidimensional gas chromatography (MDGC and GC × GC) in a multi-hyphenated system – i.e., coupled to flame ionisation detection (FID), mass spectrometry (MS), and olfactometry (O). Solid-phase microextraction (SPME) was used to isolate the headspace aroma compounds of the banana spirit. The detection frequency (DF) technique was applied and aroma regions, detected in the first column separation at >60% Nasal Impact Frequency (NIF), were screened as target potent odour regions in the sample. Using a polar/non-polar phase column set, the potent odour regions were further subjected to MDGC separation with simultaneous O and MS detection for correlation of the aroma perception with MS data for individual resolved aroma-impact compounds. GC–O analysis enabled 18 aroma-impact regions to be located as providing volatiles of interest for further study; for example, those comprising perceptions of flower, whisky, green, amongst others. Compounds were tentatively identified through MS data matching and retention indices in both first and second dimensions. The principal volatile compounds identified in this work, which are responsible for the characteristic aroma of the banana spirit, are 3-methylbutan-1-ol, 3-methylbutan-1-ol acetate, 2-phenylethyl acetate and phenylethyl alcohol. This is the first such study to reveal the major aroma compounds that contribute to banana spirit aroma.

1. Introduction

Banana (*Musa* spp.) is an important food crop which grows extensively in tropical and subtropical regions and is widely consumed throughout the world. However, loss and waste in fruit production represent a significant cost in the market; developing alternative products for banana is imperative. The distillates industry has demonstrated interest in producing novel alcoholic beverages from residue or unusual raw materials [1]. Considering its excellent sensory properties, the manufacture of banana-based beverages is of interest to the industry, especially because of the pleasant flavour and amount of sugar. Many studies have shown the aroma profile of banana [2–5]. The highest rated compounds contributing to the aroma of this fruit, were esters, followed by aldehydes. The main esters were isoamyl acetate and 2-pentanol acetate, that contribute to fruit notes; aldehydes n-hexanal and 3-methylbutanal were important odour-active compounds in banana, providing herbal-green-grassy aroma [3,4]. The abundant sugar content in banana fruit suits the preparation of fermented-distilled beverages (spirits). Thus, banana spirit has been produced here using the banana residue, which comprises banana in the last stage of maturation with little commercial value, but which enables the acquisition of different flavours and, potentially, may attract new markets [1].

Spirit beverages are characterised by their alcohol content in addition to a variety of volatile organic compounds (VOCs),

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including those that may impart specific aroma properties. These are affected by many factors, such as the choice of raw materials; the fermentation conditions, including the yeast strain; the distillation process; the aging of the product; and, the type of wood used for storage [6]. The composition and concentration levels of these compounds vary widely for each spirit. The complexity of the molecular composition arises from the factors outlined above. The large number of compounds, comprising a wide range of concentrations, requires analytical methods having good performance, i.e., separation power and sensitivity [7]. Frequently, compounds at trace levels have greater influence on the sensory properties of alcoholic products than those present in high concentrations, according to relative odour impact [8]. However, in order to understand the contribution of each VOC to odour quality, it is not sufficient just to know whether these compounds are present or absent, one also must have knowledge of how they are perceived at given concentrations [9]. In addition, the identification of these compounds may be used to determine the flavour characteristics of the spirit, to detect illicit compounds and to identify inconsistent manufacturing practices [1].

Despite the fact that distillate spirits, especially fruit spirits, have been produced for centuries, there are few published studies about their characteristic aroma composition across the many different products. Furthermore, no study has, until now, focused on identifying key aroma compounds of banana spirit by gas chromatography–olfactometry (GC–O).

Use of the human nose as a detector is necessary because perception thresholds may be far lower than those of instrumental detectors [10]. It is also able to sense aroma attributes in the detected odour [9]. In complex samples, the identification of odour compounds using conventional GC–O in conjunction with flame ionisation detectors (FID) or mass spectrometry (MS) can be limited due to peak co-elution in the odour regions. Incorrect identification may result in a trace odorant being masked by a large odourless or weak odour active compound [9,11].

Comprehensive two-dimensional gas chromatography ($GC \times GC$) has proven to be a valuable tool to characterise very complex food volatile compositions. However, the combination of olfactometry and $GC \times GC$ may be complicated for precise aroma adjudication, because of the slow breathing cycle of a human assessor compared to the rapidly eluting peaks from the system. Multidimensional GC (MDGC) is able to resolve a number of selected co-eluting compounds in aroma regions, whilst permitting olfactory assessment of the individual compounds. It employs heart-cutting (H/C) and cryo-trapping (CT) devices for isolation and transfer of target solute(s) from a first dimension column (^{1}D) to a second dimension column (^{2}D), comprising a different column phase and being equipped with a sniff port outlet [11–13].

To determine odour compounds, the method used for isolation of the analytes from the matrix is particularly important. The appearance of 'aromagrams' in GC–O depends largely upon the sample preparation procedure, which might affect the composition of the isolated compounds [8]. Several techniques have been applied to aroma extraction in food applications, particularly in alcoholic beverages. Headspace solid-phase microextraction (SPME) has been widely used as an effective technique to probe the headspace composition of a material, in combination with GC–O to study and characterise aroma-active compounds [14]. This technique avoids possible contamination of the extract by non-volatile sample components and yields useful qualitative data due to its simplicity and ease of sample preparation [15]. A mixed polydimethylsiloxane/carboxen/divinylbenzene (PDMS/CAR/DVB) fibre of 2 cm length has been used in most cases reported in the literature, which ensures satisfactory yield of the largest amounts of odour compounds from alcoholic beverages [8].

The purpose of this work is to identify the aroma-active compounds from banana *Terra* spirit by screening potent odours using GC–FID/O with the detection frequency (DF) technique. Odour regions were profiled by using GC–FID/O and targeted for complete separation and further identification using heart-cut MDGC with simultaneous olfactory and mass spectrometry (MS) detection. Headspace SPME is used as a sampling procedure to extract aroma compounds. Compounds were tentatively identified using GC–MS and retention index (*I*) matching in GC–FID/O and MDGC–MS/FID/O. Further identification was also performed using some standard odorant compounds.

2. Materials and methods

2.1. Sample and chemical standards

The banana *Terra* spirit (40% ethanol) was manufactured in the *Microbiology Industrial Laboratory* of *Faculdade de Farmácia* of *Universidade Federal de Minas Gerais* (Brazil). The alcoholic fermentation of banana must employed enzymatic treatment and wet commercial yeast of *Saccharomyces cerevisiae* (*S. cerevisiae*). The distillation was performed using traditional copper alembics of 5 L. The fermented banana must was transferred to the vessel to a maximum of 2/3 of its capacity. During the distillation, samples of approximately 50 mL were recovered in order to obtain three fractions of different ethanol content: "head" (>54% v/v), "heart" (36–54% v/v), and "tail" (<36% v/v). The "heart" of the banana *Terra* spirit was placed into glass bottles and stored in the dark until analysis.

Standard analytical reagents were used as follows: ethanol (J.T. Baker, Mexico), 3-methylbutan-1-ol (Vetec, Brazil), 1-hexanol, (Fluka, Germany), 3-methylbutan-1-ol-acetate, acetic acid, 2-phenylethyl acetate, phenylethyl alcohol (Aldrich, Germany) and sodium chloride (Merck Chemical Co., Merck KGaA, Germany). Ultrapure water was obtained from a Milli-Q purification system (Millipore, Milford, MA).

After preliminary screening, the SPME fibre selected was polydimethylsiloxane/carboxen/divinylbenzene (PDMS/CAR/DVB) 50/30 μm, 2 cm (Supelco, Bellefonte, PA, USA).

2.2. Sample preparation and SPME procedure

For sample preparation, a 4.0 mL aliquot of banana *Terra* spirit, diluted with ultrapure water and brought up to a volume of 50.0 mL in a volumetric flask, was used. The extraction of volatile compounds was carried out using headspace solid-phase microextraction (SPME) with a 2 cm PDMS/CAR/DVB fibre performed manually (Sigma–Aldrich, St. Louis, MO, USA). The fibre was previously conditioned following the manufacturer's instructions. For SPME, an 8.0 mL aliquot of banana spirit solution, with the addition of 2.0 g of sodium chloride, was used in a 20 mL Pyrex vial. The sample was equilibrated for 5 min at 60 °C, before starting the extraction by exposing the fibre to the sample headspace for 25 min at 60 °C. The total procedure was performed under magnetic stirring. SPME conditions followed previous studies. The fibre was thermally desorbed in the GC inlet port for 2 min at 240 °C under splitless conditions [7].

A C8–C20 n-alkane mixture (Sigma–Aldrich) was used to determine linear retention indices (*I*). The tentative identification of VOCs was performed by comparing the mass spectra against library database records (NIST 08 Mass Spectrum library) and retention index data.