



Fragrant bioethanol: A valued bioproduct from orange juice and essential oil extraction

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ABSTRACT

We identify the composition of a less known, yet important bioproduct obtained in relatively high amount during orange fruit processing for juice and essential oil extraction comprised of bioethanol dissolving valued terpenes. Green cosmetic, perfume and biosolvent applications are anticipated.

1. Introduction

The production of highly valued bioproducts beyond orange oil from orange waste is being increasingly practiced in most world's countries where significant orange crops are harvested (Boukroufa et al., 2015). Natural hydrocolloid pectin, (Ciriminna et al., 2015) for instance, is no longer obtained from lemon peel (and apple pomace) only, but also from waste orange peel, especially in the São Paulo State of Brazil (Oxenbøll Sørensen, 2015) where pectin manufacturers use orange peel abundant in an area where some 36% of the world's orange production (about 50 million tonnes in the 2016/2017 season) concentrates.

The overall objective of the orange biorefinery is to convert the rapidly degrading (thanks to endogenous enzymes and exogenous microbes) citrus peel waste into high value products (including pectin, flavonoids, carotenoids (Boukroufa et al., 2017) and cellulose) in zero-waste citrus processing plants (Boukroufa et al., 2015; Balu et al., 2012). Once low-energy and eco-friendly extraction technologies such as microwave- (Boukroufa et al., 2017; Balu et al., 2012; Ciriminna et al., 2016a) or ultrasonic-assisted (Boukroufa et al., 2015) extraction in water, which do not require neither peel drying nor the employment of mineral acids, will be up-scaled industrially, (Ciriminna et al., 2016b) this scenario will become an industrial reality.

With 87,859 ha of land hosting orange crops in the 2017/2018 season, (ISMEA, 2018) Sicily hosts about 60 per cent of Italy's orange

production. The yearly average output exceeds 1 million tonnes, even though significant variation can occur depending on the amount of irrigation water available. One bioproduct obtained by a citrus processing company (Agrumi-Gel) based in Sicily during orange juice extraction is a bioethanol distillate obtained from a production residue of high sugar content.

In further detail, the bioproduct is obtained via distillation of the centrifuges 'spari' originating both from the essential oil (EO) and juice residues and also from the depulped and other residues of citrus transformation. The emulsion produced as by-product of essential oil cold extraction via puncture of the fruits carried on by water prior to mechanical squeezing is comprised of small pieces of peel, water and essential oil. Said emulsion enters a tube with Florentine vases where the EO separates by floating after which, following a filtration step, it reaches a set of centrifuges to allow the fine separation of the mixture components. The centrifuges have three exits: water, EO and a "waste" solution generally colored in yellow and rather dense due to the solid particles from the original emulsion with a non-negligible amount of residual EO. This waste product accumulates with ongoing production, after which it is mixed with the product called "depulpazione" comprised of the high sugar solution of pulp residues separated via centrifugation during the orange juice production. The resulting high sugar mixture is added with commercial yeasts. Ethanol is abundantly formed via fermentation, after which the bioethanol solution is distilled along with residual EO. So far, this bioethanol has been mostly sold by the

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company as a biosolvent. We decided, therefore, to investigate its composition in closer detail.

2. Materials and methods

The alcohol volumic title, 91% v/v, was measured according to the internationally approved standard method for the assessment of alcohols in wines and spirits, (International Organisation of Vine and Wine, 2009) namely by densimetry with hydrostatic balance. A 100 mL aliquot was used in a distillation apparatus comprised of a 1 L flask with a standardized ground-glass joint, a Vigreux column 20 cm high, an elbow connector with an approximately 10 cm long straight-rimmed condenser (a West-type condenser) fitted vertically, cooling coil (40 cm long), and a drawn out tube taking the distillate to the bottom of a graduated collecting flask.

The terpenes were analyzed by two different but complementary techniques, namely solid-phase microextraction methodology (SPME) coupled with gas chromatographic separation and mass-spectrometry (GC/MS) to screen the orange flavour compounds, and GC-MS/MS direct injection analysis (Da Porto et al., 2012). In the former SPME-GC/MS method, a Focus GC coupled with a DSQ II MS (Thermo Scientific) was used. The capillary column used was a Equity-5 (Supelco, 30 m \times 0.25 mm I.D., film thickness 1.00 μ m).

For SPME extraction in immersion method, three different fibre coatings were used: divinylbenzene/carboxen/polydimethylsiloxane (DVB/Car/PDMS, 50/30 μ m), carbowax/divinylbenzene (CW/DVB, 70 μ m), and polydimethylsiloxane (PDMS, 100 μ m). All SPME fibers were supplied by Sigma-Aldrich/Supelco (Bellefonte, Pennsylvania). In order to assess the best extraction fibre and time several preliminary tests at increasing times (5, 10, 15 and 20 min) were evaluated (data not showed), it was determined that the best fibre had been PDMS and 5 min was suitable to obtain equilibrium and to reproduce the extraction procedure.

The sample was diluted in methanol 1:1000 v/v in a 8 mL vial. A calibration curve was obtained using commercial *d*-limonene (purchased by Aldrich) dissolved in methanol at different concentration. A sample aliquot (10 μ L) was inserted in vial sealed by a teflon septum and a plastic cap left under stirring for 10 min at 4000 rpm after which a PDMS 100 μ m fibre was immersed in the sample contained in the vial for 5 min.

The PDMS fibre loaded with the volatile components was then introduced into the GC injector in splitless mode (5 min) using the following GC ramp: column temperature at 40 °C for 4 min, and then heating at 10 °C/min up to 300 °C and hold for 5 min. The fibre desorbed for 10 min into the injector. The temperature of the transfer line was set at 280 °C. He (5.0) at a constant flow of 1 mL/min was used as gas carrier. All analyses were in triplicate. Mass spectrometry conditions were EI (+) with an ionization energy of 70 eV, mass range 40–400 Da, scan time 1/s, start time 1.0 min, temperature of the ion source 200 °C. The component assignment was based on computer matching with the WILEY 7 and NIST 02 mass spectral libraries, and on comparison with data retrieved from literature.

3. Results and discussion

Table 1 lists the compounds identified in the orange spirit with SPME-GC/MS. Along with ethanol and *d*-limonene, four other terpenes were detected in the PDMS fibre.

The amount of *d*-limonene in the orange spirit was assessed via direct injection after diluting 1:1000 the spirit in methanol. The sample was analyzed by GC Trace 1310 (Thermo Scientific) coupled with a TSQ 8000 coupled Triple Quadrupole GC-MS/MS instrument equipped with an autosampler (Triplus, Thermo Scientific). A 1 μ L sample was automatically inserted into injector by the autosampler.

The column was the same capillary column Equity-5-MS capillary column Equity-5 (Supelco, 30 m \times 0.25 mm I.D., film thickness

Table 1
Compounds identified in distillate with SPME-GC/MS.

Mixture component	Retention Time (min)	Relative abundance (%)
Ethanol	1.95	1.23
α -Pinene	12.33	10.38
β -Pinene	13.22	20.73
<i>d</i> -Limonene	14.12	56.76
Borneol	16.48	2.24
α -Terpineol	16.94	8.66

0.25 μ m). The injector was set in split mode 1:20 with a constant temperature of 250 °C. Again, He (5.0) at a constant flow of 1 mL/min was used as gas carrier. The oven temperature program was initially set at 40 °C (4 min isothermal) followed by heating at 10 °C/min up to 150 °C, after which the heating rate was raised to 40 °C/min until reaching 300 °C (hold 2 min). The mass spectra were acquired both in full scan mode (50–300 Da) and in SIM mode (59, 83, 93, 101, 121, 136 Da).

As expected, the chromatogram in Fig. 1 shows several signals beyond the five main terpenes identified in the PDMS fibre (1–5 signals in Fig. 1). The relative amounts of volatiles (semiquantitative analysis) were obtained by direct injection in GC-MS/MS using 1 hexanol d_{13} (Sigma-Aldrich/Supelco) as internal standard, multiplying the ratio of target compound/internal standard areas by the concentration (μ g/L) of the internal standard. Applying this method, the amount of *d*-limonene in the orange spirit was found to be 7175 ppm.

In brief, along with valued *d*-limonene, a valued fragrance and antimicrobial agent, (Ciriminna et al., 2014) the ethanol-based bioproduct obtained each year at the company in about 30–40 t/year amount (depending on the season), contains α - and β -pinene (10.38% + 20.73%), namely the structural isomers of the most abundant terpenoid in Nature whose α isomer has anti-inflammatory properties, (Bae et al., 2012) and powerful antimicrobial activities against bacterial and fungal cells (Rivas da Silva et al., 2012).

Significant amounts of borneol (2.24%) and α -terpineol (8.66%) were also found in the orange spirit. The former is a bicyclic monoterpene found as common ingredient in many traditional Chinese and Japanese herbal formulations as analgesic and anesthetic whose isomers have a highly efficacious positive modulating action at GABA_A receptors (Granger et al., 2005). Abundant in orange juice, the alcohol terpenoid α -terpineol is well known for its anti-inflammatory effect by inhibiting formation of pro-inflammatory cytokine IL-6, (Held et al., 2007) and exhibits also anticancer activity against human cell lung carcinoma through suppression of NF- κ B signalling (Hassan et al., 2010).

4. Conclusions

In conclusion, the manuscript describes the approach to obtain flavoured ethanol from citrus waste used in a modern citrus processing plant. The extraction of this valuable product based on the biorefinery concept is relevant, especially in the context of the emerging citrus biorefinery in large scale in citrus producing countries (Boukroufa et al., 2015; Ciriminna et al., 2015; Balu et al., 2012). The analyses first reported in this account show that the bioproduct obtained fermenting the orange EO extraction and juice extraction aqueous residues is a highly valued bioproduct comprised of bioethanol and citrus terpenes with plentiful potential applications. Adding relevance to these new results is the high content of *d*-limonene in the orange spirit (7175 ppm), which in simultaneous saccharification and fermentation (SSF) of citrus peel waste by *Saccharomyces cerevisiae* was found capable to inhibit yeast growth and ethanol formation at initial limonene concentrations equal (or higher) to 0.33% (v/v); (Wilkins et al., 2007) with scholars subsequently showing that in order to observe 12 times higher ethanol concentrations (14.4–29.5 g/L) and yields (90.2–93.1%) from

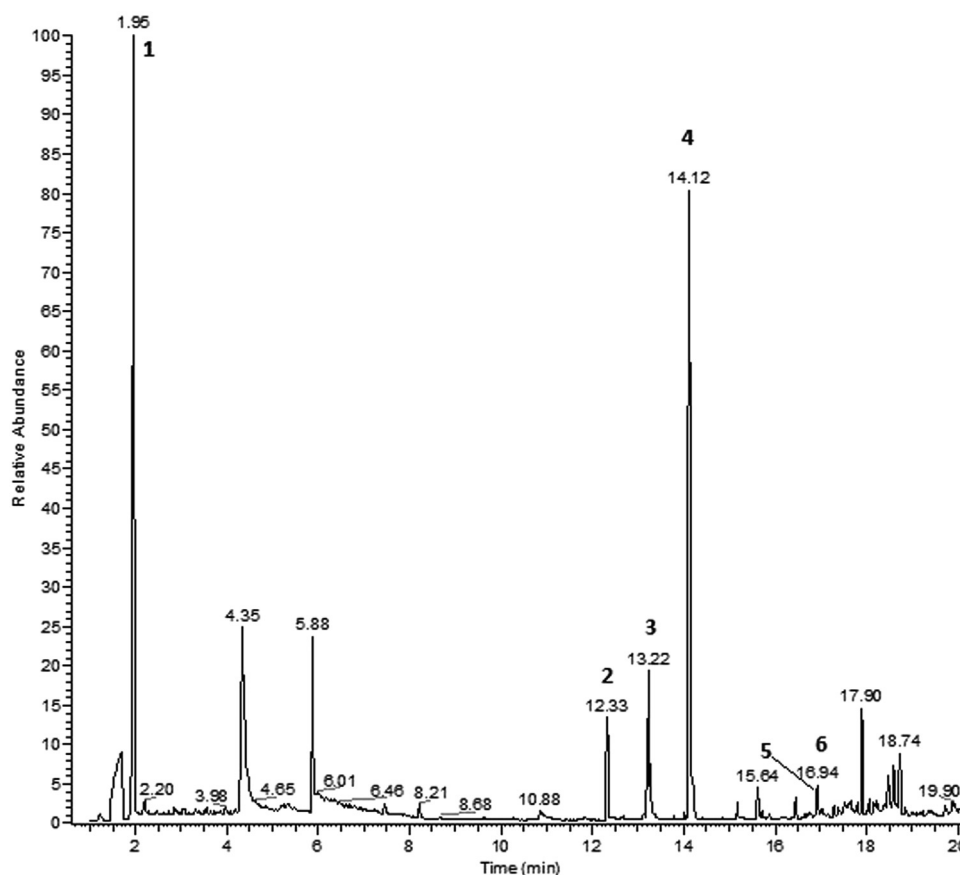


Fig. 1. Chromatogram of bioethanol (dil. 1/1000 in MeOH) determined by SPME-GC-MS/MS: 1 Ethanol; 2 α -Pinene; 3 β -Pinene; 4 *d*-Limonene; 5 Borneol; 6 α -Terpineol.

citrus peel waste undergoing yeast fermentation, chromatographic removal of limonene over a raw cotton and activated carbon column is required (Choi et al., 2015). In the present process employed in Sicily to fermentate the sugars in the aqueous residues of citrus fruit processing, *d*-limonene and four other main terpenes (α - and β -pinene, borneol and α -terpineol) do not interfere with the fermentation process and then accumulate in the distillate.

The bioproduct analyzed in this study could be used as antimicrobial and antifungal agent in medical applications, as fragrant biosolvent in spa or hotel environments (to be ventilated prior to occupancy), but also as ingredient in cosmetic formulations requiring small amounts of bioethanol and *d*-limonene, which are important ingredients in a vast array of perfume, personal care and cosmetic formulations (Naturelle Cosmetics, 2016), especially those which are labeled and certified as 'natural' and green cosmetic formulations. Following new outcomes concerning olive mill wastewater (Delisi et al., 2018) and *Opuntia ficus-indica* seed oil, (Ciriminna et al., 2017) this work further contributes to our efforts to advance the fruit, agricultural and fish waste biorefinery in Sicily, namely Italy's largest and sunniest region. New results concerning other bioproducts from biological residues will be reported shortly.

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