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Hydrosols of orange blossom (*Citrus aurantium*), and rose flower (*Rosa damascena* and *Rosa centifolia*) support the growth of a heterogeneous spoilage microbiota

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ABSTRACT

Hydrosols are hydrodistillation products of aromatic plants. They contain less than 1 g/L of dispersed essential oils giving organoleptic properties. Hydrosols are subjected to microbial proliferation. Reasons for spoilage have to be found in the nature of substrates supporting growth and of microbiological contaminants. The composition in essential oils and the microbiota of 22 hydrosol samples of *Citrus aurantium* L. ssp. *amara* L. (orange blossom), *Rosa damascena* Miller (rose D.), and *Rosa centifolia* L. (rose C.) flowers were analyzed to determine the factors responsible for decay. The median concentrations in essential oils were 677 mg/L for orange blossom hydrosols, 205 mg/L for rose D. hydrosols, and 116 mg/L for rose C. hydrosols. The dry matter content of these hydrosols varied between 4.0 mg/L and 702 mg/L, and the carbohydrate content varied between 0.21 mg/L and 0.38 mg/L. These non-volatile compounds were likely carried over during distillation by a priming and foaming effect, and could be used as nutrients by microorganisms. A microbial proliferation at ambient temperature and also at 5 °C has been observed in all studied hydrosols when stored in a non-sterile container. In contaminated hydrosols, maximal counts were about 7 $\log_{10}$ CFU/mL, while the French pharmacopeia recommends a maximal total bacterial count of 2 $\log_{10}$ CFU/mL. Neither yeast nor mold was detected. The isolated microbial population was composed of environmental Gram-negative bacteria, arranged in four major genera: *Pseudomonas* sp., *Burkholderia cepacia* complex, and presumably two new genera belonging to *Acetobacteraceae* and *Rhodospirillaceae*. Among those bacteria, *Burkholderia vietnamiensis* and *Novosphingobium capsulatum* were able to metabolize volatile compounds, such as geraniol to produce 6-methyl-5-hepten-2-one or geranic acid, or phenylethyl acetate to produce 2-phenylethanol. EO concentrations in hydrosols or cold storage are not sufficient to insure microbiological stability. Additional hurdles such as chemical preservatives or aseptic packaging will be necessary to insure microbial stability.

1. Introduction

Hydrosols are co-products from the steam distillation or hydrodistillation of aromatic plants for the production of the much less abundant but highly valuable essential oils (EOs). These are mainly used as food flavoring substances in a wide range of pastries and beverages of the Mediterranean basin and the Middle East. The steam distillation or hydrodistillation process of aromatic plants produces two nonmiscible phases: the EO phase containing the major part of volatile compounds, and the hydrosol phase composed of condensed water and of a low amount of dissolved EO (usually less than 1 g/L) that confers the organoleptic properties. At higher concentrations, EOs are not miscible and separate naturally from hydrosol (Fernandez, André, & Casale, 2014).

EOs in orange blossom and rose hydrosols are mostly composed of terpenoids and alcohols, such as linalool and α-terpineol in orange blossom, and 2-phenylethanol, citronellol, and geraniol in rose sp. hydrosols (Jeannot, Chahboun, Russell, & Baret, 2005; Ulusoy, Bosgelmez-Tinaz, & Secilmis-Canbay, 2009). Distillation of orange blossom (*Citrus aurantium* L. ssp. *amara* L.) results in the production of neroli EO (in a yield of about 0.1% of the distillation products) and of its co-product, the orange blossom hydrosol (also known as orange blossom water) in a yield of 99.9%. The major producers are Morocco and

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Tunisia, with annual productions of about 800 t and 300 t, respectively (Albert Vieille SAS unpublished data). Rose C. flower hydrosol (*Rosa centifolia* L.) is usually obtained by steam distillation, with quite a low yield in EO (about 0.01%). Most of the production comes from France. Rose D. flower (*Rosa damascena* Miller) hydrosol (Damascus rose water) is usually obtained by hydrodistillation, co-produced with 0.025% of EO, and most of the 1000 t annual world production comes from Bulgaria and Turkey (Fernandez et al., 2014).

Hydrosols contain EOs known for their antimicrobial effects, in particular against foodborne pathogens (Ait-Ouazzou et al., 2011; Al-Turki, 2007; Ammar et al., 2012; Burt, 2004; Chorianopoulos, Giaouris, Skandamis, Haroutounian, & Nychas, 2008; Fisher & Phillips, 2008; Sagdic, Ozturk, & Tornuk, 2013; Tornuk et al., 2011; Voon, Bhat, & Rusul, 2012). Moreover the same hydrosols must comply with professional microbiological standards that recommend a total bacterial count lower than 200 CFU/mL, a total mold and yeast count lower than 20 CFU/mL, and absence in 1 mL of the human pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa* (ANSM, 2012; Council of Europe, 2014). However, there is some concern about their microbiological stability, and about proliferation of bacteria or molds that contributes to hydrosol decay (Fernandez et al., 2014; Watt, 2015). EO concentrations may therefore not be high enough for microbiological control. The aim of this work was to characterize the microbiota of rose and orange flower hydrosols of diverse origins, its behavior in relation to chemical composition and storage conditions, and its impact on the aromatic properties of the products.

2. Materials and methods

2.1. Origin and preparation of the analyzed hydrosols

Samples of commercial rose (*R. damascena* Miller and *R. centifolia* L.) and orange blossom (*C. aurantium* L. ssp. *amara* L.) hydrosols were collected by Albert Vieille SAS (Vallauris, France) from different manufacturers at different locations in Europe and around the Mediterranean basin. Selected and examined hydrosols were in conformity with market practices. These hydrosols were produced by steam distillation or hydrodistillation of fresh flowers (Fig. 1). A second distillation of hydrosols may occasionally be performed, in case of non-compliance to microbiological or aromatic standards. For all hydrosols analyzed in the present work, volumes of 5 L to 10 L were sampled at different times of processing and storage (Table 1). Then, 50 mL to 100 mL aliquots of each hydrosol sample were aseptically separated for chemical and microbiological analysis. Among these, four hydrosol samples have been followed during a three-month storage period. Two rose C. samples (#18 and #22), and two orange blossom samples (#7 and #8) were collected in the industrial storage and collection tanks (therefore not maintained in a sterile environment and/or packaging during a few days after distillation) and poured into 10 L sterile containers stored at 5 °C (samples #18.1, #22.1, #7.1, and #8.1) or at ambient temperature (samples #18.2, #22.2, #7.2, and #8.2). Changes in bacterial populations were followed over a three-month storage period by sampling 50 mL volumes at regular time intervals. Two hundred grams of fresh rose C. flowers were collected before distillation (#17) for determination of microbiological counts.

2.2. Chemical analysis

2.2.1. Extraction, identification and quantification of volatile compounds

Volatile compounds were extracted from 4 mL volumes of hydrosol by three successive liquid–liquid extractions with 1.5 mL of n-hexane (VWR International, Fontenay-sous-Bois, France). Water traces remaining in the organic phase were absorbed on Na_2SO_4 (VWR). Volatile compounds were analyzed and quantified with a gas chromatograph mass spectrometer (GCMS-QP2010; Shimadzu, Kyoto, Japan). Samples were

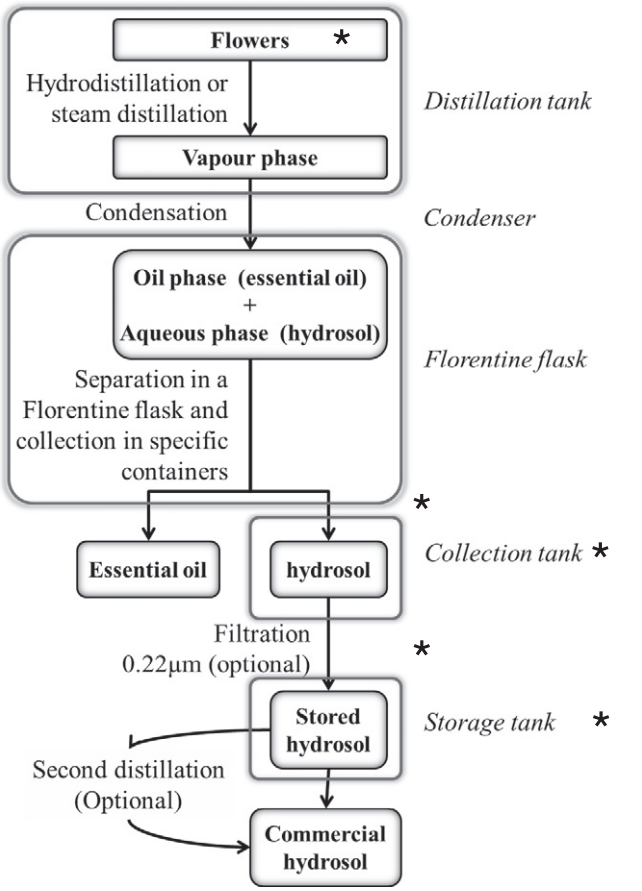

Fig. 1. Diagram of hydrosol production showing the product at different stages of processing, and physical and chemical processes applied to the product. Containers and process equipment are indicated in italics. Products and fractions are in bold characters. * indicates sampling locations.

injected with an auto-sampler (AOC-5000; Shimadzu) in splitless mode at 250 °C (purge opened after 0.5 min), then separated with a UBWAX column (30 m × 0.25 mm, 0.5 µm) (Interchim, Montluçon, France). The carrier gas was helium at a constant velocity of 35.5 cm/s. The oven program temperature was as follows: 50 °C, 4 °C/min to 230 °C and 5 min hold time. The mass spectrometer was operated in the electron impact mode at 70 eV in the *m/z* range 29–450 at a speed of 1.7 scans/s. The temperatures of the ion source and of the transfer line were respectively 200 °C and 250 °C. Mass spectral matches were performed by comparison of experimental mass spectra with the ones of the Wiley Mass Spectral library (8th edition) and NIST/EPA/NIH Mass Spectral Library (NIST 08). Experimental retention indices (RI) were determined for 31 constituents by injecting a series of n-alkanes (C7–C30) (Supelco, Bellefonte, USA) and were then compared to the values given in the literature (www.pherobase.com and www.flavornet.org) to confirm identification. Quantifications was done by area comparisons, using n-hexadecane (Sigma-Aldrich, Saint-Quentin-Fallavier, France) as internal standard, and measurement of the response factors of pure linalool, α-terpineol, citronellol, nerol, geraniol (all Sigma-Aldrich), and 2-phenylethanol (Merck, Darmstadt, Germany), representing respectively 85% and 75% of volatile compounds of *Rosa* sp. and orange blossom hydrosols. Volatile compound concentrations expressed in mg/L or % surface area were the mean of two replicate extractions. The reproducibility of extractions and analyses was estimated by % mean deviations. These were lower than 5.3% for EO concentrations and lower than 3.4% for peak areas of volatile compounds.