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Hydrosols of *Citrus aurantium* and *Rosa sp.* support the growth of a diverse spoilage microflora impacting volatile compounds

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Citrus aurantium L. ssp. *amara* L., *Rosa centifolia* L. and *Rosa damascena* Miller hydrosols (also called hydrolats) are hydrodistillation products mainly used as food flavoring agents or ingredient in cosmetics. We analyzed 22 hydrosol samples collected from different manufacturers around the Mediterranean basin and in Eastern Europe, at storage time between 0 (sampling at processing day) and 13 months. These hydrosols contained essential oil (EO), at median concentrations ranging between 116 mg/L in the *Rosa centifolia* hydrosols mainly composed of 2-phenylethanol and citronellol, and 677 mg/L in the *Citrus aurantium* hydrosols mainly composed of linalool and alpha-terpineol. Although essential oils have been described for antimicrobial effects, with reported MIC of essential oil compounds ranging between 200 to 1000 mg/L against, there were not in sufficient concentrations to prevent microbial proliferation of hydrosol bacteria, the only micro-organisms detected in the tested hydrosols. Maximal bacterial counts in hydrosols sampled a few days to a few months storage after processing (using a Malassez counting chamber and decimal dilutions on solid medium in Petri dishes) were in the range 10⁶ - 10⁷ CFU/mL and did not comply with professional standards which recommends bacterial counts lower than 10² CFU/mL. Only hydrosols stored in a sterile packaging showed the lowest count during a three months period (<5 CFU/mL). This suggests that contaminations likely occur during operations handling hydrosols in the open air, i.e. in non-sterile conditions. The 58 hydrosol bacterial isolates were divided into 4 major branches: a *Pseudomonas sp.* branch, a *Burkholderia cepacia* complex branch, and two undefined species branches belonging to *Acetobacteraceae* and *Rhodospirillaceae*. These bacteria require low nutrients for growth and are environmental contaminants. A few of them could metabolize alpha-terpineol and geraniol, with a concomitant production of 6-methyl-5-hepten-2-one, and could also metabolize 2-phenylethyl acetate with a concomitant production of 2-phenylethanol. Challenge tests with pathogenic or reference micro-organisms (*Bacillus cereus*; *Escherichia coli*; *Staphylococcus aureus*; *Pseudomonas aeruginosa*; *Listeria monocytogenes*; *Salmonella enterica enterica* Typhimurium, *Candida albicans* and *Aspergillus brasiliensis*) showed that only *P. aeruginosa*, and *A. brasiliensis* were able to multiply at 30°C in at least one of the tested hydrosol. None of these microorganisms has been found among hydrosol isolates. In conclusion packaging in aseptic conditions could contribute to the microbiological stability of hydrosols during long-term storage. If not, a chemical stabilization with preservatives should be considered.

Keywords: Hydrosol, orange blossom water, rose water, essential oil, challenge-tests, pathogenic bacteria, spoilage bacteria.