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Ability of human oral microbiota to produce wine odorant aglycones from odourless grape glycosidic aroma precursors

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ABSTRACT

Grape aroma precursors are odourless glycosides that represent a natural reservoir of potential active odorant molecules in wines. Since the first step of wine consumption starts in the oral cavity, the processing of these compounds in the mouth could be an important factor in influencing aroma perception. Therefore, the objective of this work has been to evaluate the ability of human oral microbiota to produce wine odorant aglycones from odourless grape glycosidic aroma precursors previously isolated from white grapes. To do so, two methodological approaches involving the use of typical oral bacteria or the whole oral microbiota isolated from human saliva were followed. Odorant aglycones released in the culture mediums were isolated and analysed by HS–SPME–GC/MS. Results showed the ability of oral bacteria to hydrolyse grape aroma precursors, releasing different types of odorant molecules (terpenes, benzenic compounds and lipid derivatives). The hydrolytic activity seemed to be bacteria-dependent and was subject to large inter-individual variability.

1. Introduction

The aromatic profile of many wines depends on the varietal compounds of the grapes that have been employed in their production. These varietal compounds can be present in grapes as free volatile compounds and, in much higher concentrations, as aroma precursors (Baumes, 2009). Among them, non-volatile sugar-bound conjugates are odourless molecules which represent a natural reservoir of odorant compounds in wines, which can be naturally and slowly released during wine ageing, or intentionally released by using oenological enzymes during winemaking. The volatile compounds that could be released from glycosidic aroma precursors are mainly terpenes, C13 norisoprenoids, benzenic derivatives, volatile phenols and C6 compounds (Baumes, 2009). These compounds are generally potent flavour compounds characterised by low odour thresholds and interesting sensory properties (Maicas & Mateo, 2005). For example, terpenes could provide floral notes that are characteristic of some grape varieties such as Muscat (Etievant, 1991).

Although the composition of wine aroma (both, free and conjugate forms) and its impact on orthonasal aroma has been extensively studied (Escudero, Campo, Farina, Cacho, & Ferreira, 2007; Ferreira, Lopez, & Cacho, 2000; Grosch, 1997; Guth, 1997; Rapp, 1998; Sarry & Gunata, 2004), the mechanisms involved in retronasal aroma released during wine consumption and its impact on aroma perception have received very little attention. Besides the wine matrix composition or the physicochemical characteristics of the aroma compounds, other factors that can affect retronasal aroma might be dependent on human physiological parameters (oral microbiota, saliva composition, oral mucosa, temperature, in-mouth air cavity volume changes, etc.). Among these physiological factors, the influence of saliva on aroma release from wine has been recently evaluated (Genovese, Piombino, Gambuti, & Moio, 2009; Muñoz-González, Feron et al., 2014). Moreover, some *in vitro* studies with human saliva have demonstrated the role of salivary enzymes (β-glycosidases, esterases, etc.) in the degradation of free aroma volatiles (Buettner, 2002a, 2002b; Lasekan, 2013). In addition, oral microbiota could be an important parameter influencing retronasal aroma. In fact, the ability of some oral anaerobic bacteria to hydrolyse odourless cysteine-*S*-conjugates from onion, bell pepper and grapes into their corresponding odorant thiols has previously been reported (Starkenmann et al., 2008),

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which might be related to a delay in aroma perception, as was already observed by Peynaud (1996) after the consumption of Golden Sauvignon grapes. The microbiota composition of two types of saliva from obese and normal individual was characterised, although its role on aroma was only indirectly assessed (Piombino et al., 2014). More recently, the ability of human salivary enzymes to release free volatile phenols from the corresponding glycosylated phenols has been shown (Mayr et al., 2014), although exactly what role the oral microbiota play in this remains uncertain.

Oral microbiota is one of the most complex bacterial communities associated with the human body and it is formed by more than 700 bacterial species (Tian et al., 2010). These micro-organisms can be present in the saliva or they can adhere to oral surfaces and form an organised multispecies communities known as biofilms (Kuramitsu, He, Lux, Anderson, & Shi, 2007). The main sources of nutrients for oral microbiota include saliva, crevicular fluid, and host diet. Although saliva is the main nutrient source, due to its chemical composition and continuous production, food is rich in a wide variety of components, which could be used by the microbiota to generate secondary products. The relatively short residence time of wine within the oral cavity might suggest a limited effect of oral microbiota on wine aroma perception. However, results from recent research (Muñoz-González, Martín-Álvarez, Moreno-Arribas, & Pozo-Bayon, 2014), suggest a possible interaction of some wine matrix non-volatile compounds with oral and pharyngeal mucosa, which might increase the residence time of aroma precursors and free aroma compounds in the oral/pharyngeal cavities, thus, increasing their susceptibility to oral parameters (saliva, oral microbiota, etc.). Moreover, the fact that during an *in vivo* consumption situation, wine is continuously replenished in the oral cavity makes viable the idea that oral microbiota might have an influence on wine aroma perception.

To our knowledge, the transformation of wine odourless glycosidic aroma precursors into odorant aglycones by human microbiota is unstudied. To check this hypothesis, a glycosidic precursor extract isolated from white grapes was incubated with representative oral bacteria species and with human oral microbiota isolated from human saliva, thereby considering the complexity of the whole oral microbiota. Odorant aglycones were isolated from the cultures and analysed by HS–SPME–GC/MS, and chemometric tools were applied, in order to gain insight into the effect of different experimental factors (bacteria type, growing requirements, incubation time, saliva treatment, intra/inter-individual differences) on the ability of oral bacteria to hydrolyse wine glycosidic aroma precursors.

2. Material and methods

2.1. Reagents and solvents

Solvents (ethanol, dicloromethane, pentane, ethyl acetate and methanol) were obtained from Merck (Darmstadt, Germany) and LabScan (Gliwice, Poland). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). Sodium chloride, sodium phosphate dibasic, sodium phosphate monobasic monohydrate and Octyl-β-D-glucopyranoside were provided by Panreac (Barcelona, Spain) and Sigma–Aldrich (Steinheim, Germany).

2.2. Preparation of white grape precursor extract

Methodologies based on the protocol already published by Rodríguez-Bencomo, Selli, Muñoz-González, Martín-Álvarez, and Pozo-Bayón (2013) were followed to obtain the white grape aroma precursor extract. Briefly, white grapes were de-stemmed, crushed and filtered and glycosidic aroma precursors were isolated by retention on Amberlite XAD-2 resins from Supelco (Bellefonte, PA, USA) and eluted with a mixture of ethyl acetate/methanol (9:1 v:v). The eluted sample was then evaporated to dryness, dissolved in Milli-Q water and stored at −20 °C. The absence of free volatiles in the aroma precursor extract was tested before the experiments.

2.3. Experiment 1: in vitro oral microbiota experiments

2.3.1. Bacterial strains and growth conditions

The oral bacteria assayed in this study and the growth conditions are described in Table 1. These bacterial species are naturally present in the oral cavity (*Streptococcus sanguinis*, *Streptococcus oralis*, *Actinomyces naeslundii*) and commonly encountered in the supragingival plaque (*Streptococcus mutans*, *Veillonella dispar*, *Fusobacterium nucleatum*) (Bik et al., 2010). Under certain circumstances, some of them (*Staphylococcus aureus*, *Enterococcus faecalis*) may be found in the mouth of healthy individuals. The anaerobe facultative and anaerobe micro-organisms were incubated under 5% CO_2 atmosphere and in an anaerobic chamber (nitrogen 90%, carbon oxide 5%, hydrogen 5%), respectively. All strains were cryo-preserved at −80 °C in a sterilised mixture of culture medium and glycerol (50:50, v/v).

2.3.2. Wine odourless glycosidic aroma precursor biotransformation by isolated oral bacteria

In a preliminary experiment the antimicrobial effect of the grape extract on the growth of selected oral bacteria was tested. For this purpose, a microdilution method described by Cueva et al. (2010) was used with some modifications. Briefly, in sterile 96-well microtitre plates, 100 μL of the grape extract (equivalent to 40 g of grapes) were diluted with broth medium and placed into a well containing 100 μL of bacterial suspension (1×10^6 cfu/mL). A parallel series of mixtures with uninoculated broth was prepared (blank samples). Finally, a growth control with 100 μL of broth and 100 μL of bacterial suspension was included. After incubation at 37 °C at different times (Table 1), the bacterial growth was determined by reading the absorbance at 550 nm. The inhibition percentage of the tested micro-organisms was calculated by comparing the control growth with those obtained from cultures with grape extract. For the HS–SPME analysis, the contents of the 10-well (2 mL) microtitre plates were placed in a 20-mL headspace vial to analyse the release of volatile compounds. All experiments were performed in duplicate.

Table 1
Oral bacteria assayed in this study.

Micro-organisms	Growth conditions
Aerobe	
Staphylococcus aureus ATCC 25923 *Enterococcus faecalis* V583 (clinical isolate)	TSB, 24 h, 37 °C
Anaerobe facultative	
Streptococcus sanguinis DSMZ 20,567	Modified TSB, 24 h, 37 °C
Streptococcus oralis CECT 907 *Streptococcus mutans* CECT 479	BHI, 24 h, 37 °C
Actinomyces naeslundii CECT 806	Modified BHI, 24–48 h, 37 °C
Anaerobe	
Granulicatella adiascens DSMZ 9848 *Veillonella dispar* DSMZ 20735 *Fusobacterium nucleatum* DSMZ 15643	WC, 24–48 h, 37 °C

TSB: tryptic soy broth (Difco); modified TSB (TSB containing 0.3% yeast extract); BHI: brain heart infusion (Difco); modified BHI (BHI containing 1% casein, 0.5% glucose and 0.5% yeast extract); WC: Wilkins Chalgren anaerobe broth (Difco).