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Simulation of retronasal aroma of white and red wine in a model mouth system. Investigating the influence of saliva on volatile compound concentrations

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ABSTRACT

The influence of saliva on aroma release from white and red wines was studied in a model mouth system. Aroma compounds were analysed in the dynamic headspace of wines by solid phase micro extraction/gas chromatography with flame ionization detection. Volatile compounds were identified by solid phase micro extraction/gas chromatography-mass spectrometry, resulting in a total of 43 compounds in white wine and 41 in red wine. The results showed a greater influence of saliva on aroma release in white wine than red wine. In white wine treated with human saliva, esters and fusel alcohols, responsible for fruity and fusel oil odours, were reduced of 32–80%; by contrast, the concentration of 2-phenylethanol and furfural, responsible for rose and toasted almond notes, increased by 27% and by 155%, respectively. In red wine, treated with human saliva, only a few esters decrease, with a reduction of 22–51% due to protein-binding ability of polyphenols that are able to inhibit the activity of the saliva. C-13 norisoprenoids, vitispirane (eucalyptol) and TDN (kerosene), decreased both in white and red wine, showing a comparable variation while, for β-damascenone, the variation was insignificant.

1. Introduction

The human olfactory system is able to distinguish between a large number of chemical compounds at very low concentrations. It is well established that, to be effective, the odorant must possess certain molecular properties, such as a partial water solubility, sufficiently high vapour pressure, low polarity, some ability to dissolve in fat (lipophilicity), and a molecular weight not greater than 300 Da.

The first impression of food *odour* takes place during inhalation when the odorants are released into the headspace and pass through the external nostrils and stimulate the olfactory receptors in the nasal cavity (orthonasal route). Whereas food *aroma* is perceived during eating when the odorants interact with receptors by travelling from the mouth to the nasal cavity via the nasopharynx (retronasal route).

The sensations of orthonasal and retronasal odours differ in the level of perception, even though they involve the same mechanisms (Burdach, Kroeze, & Koster, 1984; Kuo, Pangborn, & Noble, 1993; Linforth, Martin, Carey, Davidson, & Taylor, 2002; Voirol & Daget, 1986, 1989). In fact, tasting in the mouth can significantly increase or decrease perception of the same odour compounds via the orthonasal route. These orthonasal/retronasal differences are specific for each odorant or subject (Marie, Land, & Booth, 1987). Such differences are due to the fact that salivation, chewing and temperature are factors able to change the sensorial properties of food when it enters the mouth (Buettner & Schieberle, 2000; Taylor, 1996; van Ruth & Buhr, 2004; van Ruth & Roozen, 2000a, 2000b). Salivation plays an important role in retronasal aroma perception of foods and has been widely studied (Buettner, 2002a, 2002b; Friel & Taylor, 2001; Roberts & Acree, 1995; van Ruth & Roozen, 2000a). Saliva is a complex dilute aqueous solution containing numerous compounds, including inorganic salts, such as sodium, calcium, potassium, chloride, phosphate and bicarbonate (Drobitch & Svensson, 1992), and a great diversity of organic components, such as enzymes (amylase, lipase, esterases, and peroxidase), immunoglobulins, antibacterial proteins, proline-rich proteins and glycoprotein mucins (Beidler, 1995; Bradley, 1991; Cowman, Baron, Obenauf, & Byrnes, 1983; Humphrey & Williamson, 2001; Lindqvist & Augustinsson, 1975, 1980; Lindqvist, Nord, & Söder, 1977; Liu, Qawasmeh, & Mazbar, 1984; Tan, 1976; Weinstein, Khurana, & Mandel, 1971).

Regarding retronasal aroma, saliva volume, by hydration/dilution of food, can affect the partitioning of volatile compounds over food, saliva and the air phase (van Ruth & Roozen, 2000b). Moreover, especially in foods with high fat, such as dairy products, the high polarity of saliva can change the volatility of some odour compounds (Roberts & Acree, 1996), while in food or beverages at low pH, such as wine, its neutral pH can shift the relative equilibrium between odorants (Roberts & Acree, 1995). In addition contact of food with a complex solution, such as saliva, can significantly affect volatile partitioning between the liquid and gas phases, causing an increase in headspace concentration of aroma compounds in the

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case of small-molecular-weight solutes (Chandrasekaran & King, 1972; Friel & Taylor, 2001; Taylor, 1999; van Ruth, Grossmann, Geary, & Delahunty, 2001). On the other hand, saliva is a high protein solution and the influence of macromolecules, such as proteins, on volatility of several odorous compounds has been well documented (Franzen & Kinsella, 1974; King & Solms, 1979; O'Neill, 1996).

Saliva affects odorant concentration by means of chemical and biochemical reactions between its components and food volatiles. Evidence has been found for the partial hydrolysis of several odour-active acetates (Hussein, Kachikian, & Pidel, 1983), as well as ethylic esters, according to their chemical structures, and for the oxidation of some thiols (Buettner, 2002a). It has been shown that some compounds (benzaldehyde, diacetyl, ethyl hexanoate and heptyl acetate) are affected by interaction between mucin and the type of solutes present (Friel & Taylor, 2001). Mucins are high molecular mass glycoproteins responsible for the typical viscosity and elasticity of saliva. They have binding sites, preferentially occupied by sucrose, available to trap volatiles (Friel & Taylor, 2001). In fact, mucin can bind specific aroma compounds, principally aldehydes (Friel & Taylor, 2001; van Ruth & Roozen, 2000a; van Ruth, Roozen, & Cozijnsen, 1995), probably to form Schiff bases.

An insignificant effect of salts and α-amylase on the concentrations of volatile compounds has been found (van Ruth et al., 1995). In a more recent study, aroma release was affected when α-amylase and food were in contact for an extended time (van Ruth & Roozen, 2000a) but this does not reflect the situation *in vivo*. Moreover, these enzymatic reactions, along with oxidation, may be accelerated when mastication mixes parts of the food and combines them with air. Mastication will also change the flavour profile, increasing the surface area and reducing the diffusion path from solid matrix and the vapour phase (Burdach & Doty, 1987). Further, the change of temperature that food undergoes when placed in the mouth can cause melting of some foods and other phase changes, causing modifications of the volatility and changes in the flavour perception.

Several techniques have been applied to follow the release of volatile compounds during eating, such as the trapping of exhaled volatiles on adsorptive materials (Delahunty, Piggott, Conner, & Paterson, 1996) or "real-time" measurements using mass spectrometric techniques (Soeting & Heidema, 1988; Taylor & Linforth, 1997). Unfortunately, even if this experimental approach establishes data nearest to the site of perception, the numerous oral physiological variables are difficult to control. For this reason, mouth simulators or RAS (retronasal aroma simulator), have been developed (Nassl, Kropf, & Klostermeyer, 1995; Piggott & Schaschke, 2001; Roberts & Acree, 1995; Roberts & Acree, 1996; van Ruth, Roozen, & Cozijnsen, 1994). This mouth simulator takes into account sample volume, volume of the mouth, temperature, salivation, and mastication. Deibler, Lavin, Linforth, Taylor, and Acree (2001) combined the RAS with the use of solid phase microextraction (SPME), which makes the technique more available and easy to use, and found that effluents were very similar to those monitored, breath-by-breath, by nose-space sampling.

The evidence of degradation or formation of odorous substances due to salivary activity has only been investigated in water model solutions (Friel & Taylor, 2001; Voirol & Daget, 1986; Voirol & Daget, 1989), oil model solutions (Roberts & Acree, 1995), foods such as strawberry yoghurts, pepper, beans, raspberry, emulsions and sauces (Delarue, Chanlot, Richard, & Giampaoli, 2002; Ingham, Linforth, & Taylor, 1995; Roberts & Acree, 1996; van Ruth & Roozen, 2000a; van Ruth et al., 1994; van Ruth et al., 1995), and drinks such as whisky (Margomenou, Birkmyre, Piggott, & Paterson, 2000).

Among foods and beverages, wine is the main product in which flavour analysis is one of the most important criteria for the definition of commercial category and consumer acceptance. Although the nasal olfactory perception (and taste) of wine has been widely studied, no investigations aimed at understanding retronasal perception are known.

The aim of the present study was to investigate the influence of saliva (human and artificial) on the release of white and red wine volatile compounds by SPME/GC and SPME/GC–MS analyses, using a model mouth system that simulates the retronasal aroma of wine (RAS).

2. Materials and methods

2.1. Wine

In this experiment, Bombino white wine and Nero di Troia red wine, two Italian grape cultivars both from S. Severo–Puglia, were used.

2.2. Chemicals and reference compounds

Pure reference standards of ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, 3-methylbutyl acetate, ethyl hexanoate, hexyl acetate, ethyl lactate, methyl octanoate, ethyl nonanoate, methyl decanoate, ethyl decanoate, 3-methylbutyloctanoate, 2-methyl-1-propanol, 3-methyl-1-butanol, 1-hexanol, 3-methyl-1-pentanol, 1-octanol, benzyl alcohol, 2-phenylethanol, butanoic acid, furfural, benzaldehyde, β-damascenone and butyrolactone were supplied by Aldrich (Steinheim, Germany). 2-Phenylethyl acetate, 2-nonanol and 2-octanone were supplied by Fluka (Buchs, Switzerland). Hexanoic acid, octanoic acid, and decanoic acid were supplied by Sigma (St.Louis, USA). Ethyl acetate was supplied by Carlo Erba (Milan, Italy). Ethyl octanoate, was supplied by Lancaster (Karlsruhe, Germany). Acetic acid and ethanol were supplied by J.T.Baker (Deventer, Holland). $NaHCO_3$ was from Carlo Erba (Milan, Italy). NaCl (sodium chloride), KCl (potassium chloride), $CaCl_2 \cdot 2H_2O$ (calcium chloride dihydrate), and NaN_3 (sodium azide) were from J.T.Baker (Deventer, Holland). $K_2HPO_4 \cdot 3H_2O$ (dipotassium hydrogen phosphate trihydrate) was from Merck (Darmstadt, Germany).

2.3. Artificial saliva

Artificial saliva was composed of recommended ingredients: Ing, 5.208 $NaHCO_3$, 1.369 $K_2HPO_4 \cdot 3H_2O$, 0.877 NaCl, 0.477 KCl, 0.441 $CaCl_2 \cdot 2H_2O$, 0.5 NaN_3, 2.16 mucin (type 1-S from bovine submaxillary glands; Sigma, Milan, Italy) and 200,000 units α-amylase (DFP-treated, Type I-A from porcine pancreas; Sigma, Milan, Italy) in 1 l of distilled water (adjusted to pH 7) (van Ruth et al., 1994; van Ruth et al., 1995; van Ruth et al., 2001). The saliva was freshly prepared and brought to 37 °C prior to experimentation.

2.4. Human saliva

Mixed whole saliva (100 ml) was collected separately from six panellists 2 h after breakfast and thorough cleaning of the teeth. Panellists (three males and three females) were non-smoking volunteers from Foggia University (24–33 years of age), exhibiting no known illnesses at the time of examination and with normal olfactory and gustatory function. Before sampling, each panellist rinsed his/her mouth several times with tap water to avoid any contamination.

2.5. Release of aroma compounds in the model mouth system

Aroma compound measurements were performed under dynamic conditions using experimental RAS, as reported by several authors (Deibler et al., 2001; Nassl et al., 1995; Piggott &