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Olive oil phenolic compounds affect the release of aroma compounds

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ABSTRACT

Twelve aroma compounds were monitored and quantified by dynamic headspace analysis after their addition in refined olive oil model systems with extra virgin olive oil (EVOO) biophenols to simulate EVOO aroma. The influence of polyphenols on aroma release was studied under simulated mouth conditions by using human saliva, and SPME–GC/MS analysis. While few differences were observed in orthonasal assay (without saliva), interesting results were obtained for retronasal aroma. Biophenols caused generally the lowest headspace release of almost all volatile compounds. However, only ethyl esters and linalool concentrations were significantly lower in retronasal than orthonasal assay. Saliva also caused higher concentration of hexanal, probably due to hydroperoxide lyase (HPL) action on linoleyl hydroperoxides. Epicatechin was compared to EVOO phenolics and the behaviour was dramatically different, likely to be due to salivary protein–tannin binding interactions, which influenced aroma headspace release. These results were also confirmed using two extra virgin olive oils.

1. Introduction

Virgin olive oil (VOO) is the oil obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which has not undergone any treatment other than washing, decantation, centrifugation and filtration (EC 2568/91). It is a complex food, from a sensory and a chemical point of view. During oral processing, olive oil odorants can interact with odour receptors by moving 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 (Bojanowski & Hummel, 2012). Such differences are due to the fact that salivation, mouth size, breathing, and temperature are factors able to change the volatility of olive oil odorants and consequently VOO odour when it enters the mouth (Van Ruth, Grossmann, Geary, & Delahunty, 2001; Van Ruth & Roozen, 2000). In fact, when olive oil is put in the mouth, the odorants are affected by different factors. Initially, saliva has a hydration effect and can produce a water-in-oil emulsion in which aroma compounds are partitioned between the water and oil phase. Subsequently, they are transported from the two liquid phases to the air phase in the mouth and then they reach the olfactory receptors located in the nose. Primarily, a molecular property such as hydrophobicity (polarity), expressed as log $P_{o/w}$ (octanol–water partition coefficient), could be responsible for the mass transfer from two different liquid phases such as the organic phase of olive oil and water phase of saliva.

The activity of salivary proteins (mucins, albumin and proteins rich in proline) and enzymes (amylase, lipase, and lysozymes) are also responsible for emulsion destabilisation (Vingerhoeds, Blijdenstein, Zoet, & Van Aken, 2005). These changes in the structure of the emulsion have also an impact on the final perception (Arancibia, Jublot, Costell, & Bayarri, 2011). In addition, respiration and the dilution effect of saliva make oral processing a "dynamic process" which cause a continuous change in terms of volume, composition, and viscosity of the foods. This is the reason why VOO tasters frequently aspirate the olive oil in the mouth. In fact, this procedure favours volatilisation by increasing the surface area contact and enhances retronasal detection. Therefore, there are two major factors that control the release of volatile flavour compounds from food products: the volatility of the compounds in the product base (thermodynamic factor) and the resistance to mass transfer from product to air (kinetic factor). Only the kinetic factor is affected by the texture and this becomes apparent only under dynamic (non-equilibrium) conditions (de Roos, 2003).

Van Ruth et al. (2001) studied a model system of sunflower oil and reported a salting out effect of some volatile compounds after

Abbreviations: GC, gas-chromatography; SPME, solid-phase microextraction technique; VOO, virgin olive oil; RAS, retronasal aroma simulator; ROO, refined olive oil; ROOP, refined olive oil with added olive oil polyphenols; ROOC, refined olive oil with added catechins (epicatechin); EVOO, extra virgin olive oil.

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the addition of artificial saliva (salts, mucin, and α-amylase). The authors stated that this effect was due to the hydrophilicity of the molecules. It is also known that mucins have binding sites available to trap and decrease volatiles (Friel & Taylor, 2001). In fact, mucin can bind specific aroma compounds, principally aldehydes (Friel & Taylor, 2001; Van Ruth & Roozen, 2000), probably to form Schiff bases. Other evidence has been found for the hydrolysis of ethyl esters, according to their chemical structures, and for the oxidation of some thiols due to the enzymes in human saliva (Buettner, 2002a,b; Genovese, Piombino, Gambuti, & Moio, 2009).

Another important factor is the non-volatile matrix of a food, which could determine chemical and/or physic interactions with aroma compounds. These interactions may alter the food–air partitioning (volatility) of the aroma compounds. Therefore, they could also affect the aroma release (Van Ruth, King, & Giannouli, 2002; Van Ruth & Roozen, 2010). For other food or beverages, like red wine, a possible interaction between some phenolic and volatile compounds was reported and these interactions influenced the aroma release of wine (Pozo-Bayon & Reineccius, 2009). In addition, when saliva enters in contact with polyphenols (Bennick, 2002; De Freitas & Mateus, 2001), their interaction could affect the extent of this effect in red wine (Genovese et al., 2009; Lorrain et al., 2013; Munoz-Gonzalez, Feron et al., 2014).

Some correlations between the level of phenolic compounds and aroma compounds with sensory descriptors were also found in VOO. In particular, higher concentrations of 1-penten-3-one and phenolic compounds causes the increase of leaf odour, and an increase in the phenolics concentration causes the increase of walnut husk odour (Angerosa, Mostallino, Basti, & Vito, 2000). Although the volatile and phenolic compounds in VOO have been widely studied (for reviews see Angerosa et al., 2004; Bendini et al., 2007; Carrasco-Pancorbo et al., 2005; Kalua et al., 2007), and some studies have aimed to investigate the aroma release of volatile compounds in vegetable oil model solutions or model emulsions (Arancibia et al., 2011; Van Ruth & Roozen, 2000; Van Ruth et al., 2001; Van Ruth et al., 2002), so far, no study has aimed to verify the retronasal perception of VOO volatiles in the presence of phenolic compounds. Moreover, the majority of these *in vitro* studies employed artificial saliva. This could be a limit because the effect of numerous enzymes and proteins present in human saliva are not considered (Salles et al., 2011).

Since VOO odour plays an important role in the quality, as well as in the classification of the virgin olive oil commercial categories, it could be very important to understand the retention and/or the release of volatile compounds from olive oil-in-water emulsion produced in mouth with human saliva in relation to VOO phenolic compounds, which could affect the aroma and the sensory perception.

Therefore, the aim of the present study was to investigate the effect of phenolic compounds on aroma release of VOO under simulated mouth conditions by using a RAS (retronasal aroma simulator) device. For this purpose, we set up a "two-phase" model system without saliva, composed of a liquid phase (olive oil) and air phase (headspace), with the aim of simulating the orthonasal conditions, *i.e.* olive oil odour. The second system simulating retronasal condition, i.e. olive oil aroma, consisted of a "three-phase" system after saliva addition: the lipid phase (olive oil), water phase (human saliva), and air phase (headspace).

2. Material and methods

2.1. Samples, standards and reagents

The refined olive oil (ROO) and extra virgin olive oil (EVOO) from *Coratina* cultivar were supplied by IOBM (Industria Olearia Biagio Mataluni, Montesarchio, Benevento). Two EVOO from *Ravece* cultivar were provided by APOOAT Soc. Coop arl (Avellino, Italy) in 250 mL green glass bottles. The olive oil samples were stored under suitable conditions avoiding light exposure and high temperatures in order to prevent oxidation and were used within eight months from their production (November 2013).

Ethyl isobutyrate (99%), ethyl butyrate (99%), ethyl 2-methylbutyrate (99%), hexyl acetate (99%), *cis*-3-hexenyl acetate (98%), hexanal (98%), *trans*-2-pentenal (95%), *trans*-2-hexenal (98%), 1-hexanol (99%), *cis*-3-hexen-1-ol (99%), linalool (97%), and 1-penten-3-one (97%) were supplied by Sigma–Aldrich (St. Louis, MO). The following reagents were used for the analysis: hexane (95%), methanol (99.9%), glacial acetic acid, trifluoroacetic acid, acetonitrile, diethyl ether, distilled water, supplied by Romil (Cambridge, England). Potassium iodide and sodium carbonate were provided by AppliChem (Darmstadt, Germany). Ammonium acetate, Folin–Ciocalteau solution and sodium hydroxide were purchased from Sigma–Aldrich. Sodium hydroxide, phenolphthalein and starch were provided by Titolchimica S.p.A. (Rovigo, Italy). Sodium thiosulfate was supplied by Fluka (Buchs, Switzerland), and chloroform was supplied by LabScan (Dublin, Ireland). The (−)-epicatechin (90%) was supplied by Sigma–Aldrich.

2.2. Sample preparation

To study the effect of phenolic compounds on the release of olive oil aroma, the experimental plan reported in Fig. 1 was applied. Six model systems were set up in order to use known amounts of aroma compounds in refined olive oil with added phenolic extract (Fig. 1A). In order to verify our results, obtained by using model systems, two EVOO were also analysed (Fig. 1B).

Human saliva was added to the model systems and the samples were subsequently analysed by using a retronasal aroma simulator, a dynamic headspace device simulating mouth conditions (RAS). In the systems without saliva, 20 glass balls were added to obtain the same headspace volume in all the samples.

2.2.1. Preparation of the refined olive oil sample with added virgin olive oil phenolic compounds (ROOP)

The phenolic extract was obtained from extra virgin olive cultivar Coratina, typically known for its high content of phenolic compounds. An aliquot of the oil sample (200 g) was dissolved in hexane (200 mL) and vigorously shaken for 10 s. A subsequent extraction was carried out by using a water/methanol mixture (40/60 v/v) in a separating funnel. This step was repeated three times by using a total of 420 mL solvent. Subsequently, the obtained hydro-alcoholic extract was washed with hexane to remove any oil contamination and was centrifuged for 10 min at 3500 rpm (PK-120; ALC International s.r.l., Milan, Italy). The organic phase was removed from the sample, and the hydro-alcoholic phase was collected in the flask and evaporated under vacuum in a rotary evaporator at 40 °C (VV 2000; Heidolph, Schwabach, Germany). The phenolic compounds were suspended using 40 mL methanol. An aliquot of the extract was used for the HPLC and Folin–Ciocalteau analyses. An amount of 2 mL phenolic extract was adjusted to volume using refined olive oil in a 100-mL volumetric flask and the oil mixture was treated in an ultrasonic bath for 15 min. Then, methanol was evaporated using a vacuum evaporator (Heidolph VV 2000) at 38 °C for 15 min (Garcia-Mesa, Pereira-Caro, Fernàndez-Hernàndez, Garcìa-Ortìz Civantos, & Mateos, 2008). The amount of phenolic compounds added to refined olive oil (334 mg kg^{-1}) was chosen on the basis of the average levels reported in the literature for extra virgin olive oil, equivalent to about 260 mg kg^{-1} total phenolic compounds according to Bayram et al. (2012), and 220–340 mg kg^{-1} (slight bitterness taste of VOO) as indicated by Beltrán, Ruano, Jiménez, Uceda, and Aguilera (2007). The