Insights in aroma compound retention by mucosa during consumption through mathematical modelling

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ABSTRACT

A multidisciplinary approach combining physiology and physical chemistry and associating experimental measurements with *in silico* modelling was applied to explain the release of aroma compounds during food consumption. Experimental release kinetics obtained by inhaling gaseous samples through controlled protocols highlighted different release behaviours, depending on aroma compound properties. The associated mathematical model described mass transfer mechanisms between the different compartments of the naso-oro-pharyngeal cavities and included both physicochemical and physiological parameters. One of the main developments was notably to consider the possible retention of aroma compounds by wetted mucosa. Model sensitivity analysis confirmed the key role of interaction between aroma compounds and mucosa (air/mucosa partition coefficient) and of individual breath parameters (current breath volume and respiratory frequency) on the persistence of aroma compound in exhaled air. These achievements show that the association of an experimental approach and mechanistic modelling constitutes a powerful tool to improve the understanding of aroma release and persistence.

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1. Introduction

Olfactory perception is known to largely contribute to overall perception of foods and, consequently, to consumer choice and preferences. A better understanding of this specific perception is therefore of great importance and requires the identification of the main mechanisms at the origin of aroma compound release during food consumption. Several studies have notably focused on orthonasal and retronasal perceptions to highlight the origin of the main differences between these two perception pathways (Espinosa Diaz, 2004; Halpern, 2004; Heilman and Hummel, 2004; Hummel, 2008; Hummel et al., 2006; Sun and Halpern, 2005; Visschers et al., 2006; Welge-Lüssen et al., 2009). The large number and the variety of mechanisms (physical, chemical,

physiological, neurobiological, cognitive, *etc.*) that can be involved at different space and time scales largely contribute to the complexity of perception. Among them, the release dynamics of aroma compounds have long been known to be among key factors to explain aromatic perceptions (Barron et al., 2012; Biasioli et al., 2006; Déléris et al., 2011; Gierczynski et al., 2011; Heenan et al., 2009). Numerous studies in the literature have focused on the identification of the main factors that can impact release kinetics, either related to the physicochemical properties of the molecules, to product characteristics (composition, structure), to individual physiology (saliva composition and flow rate, breath flow rate) or to oral processing (chewing efficiency, product coating, etc.) (Benjamin et al., 2012; Buettner and Beauchamp, 2010; Foster et al., 2011; Frank et al., 2012; Gierczynski et al., 2011; Heath, 2002; Heenan et al., 2011). The existence of aroma compound retention by wetted mucosa has often been proposed to explain specific release behaviours, but little is known about the origin of this phenomenon. However, several mechanisms have been suggested in the literature: the interaction of aroma compounds with the constituents of the mucus layer (mucins, enzymes, antioxidants,

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ionic compounds), with saliva and/or with the mucosa tissues themselves (Buettner and Beauchamp, 2010); the role of the contact area between nasal mucus and air (Keyhani et al., 1997); the role of the physicochemical properties of aroma compounds (Ferreira et al., 2006; Tromelin et al., 2010); and the role of breath and/or salivary flow rates (Buettner and Mestres, 2005; Hodgson et al., 2004).

Performing *in vivo* experiments and developing appropriate experimental set-ups constitute the main difficulties involved in exploring this topic and in validating or not the assumptions. A previous study proposed various simple protocols to explore and quantify *in vivo* aroma release and persistence from gaseous samples, depending on the exposed physiological cavities (nose, mouth or pharynx) (Déléris et al., 2015). Results confirmed the main role of aroma compound properties and highlighted the possible occurrence of different types of mechanisms, either physical or biochemical, to explain release behaviours. The global nature of the approach and the complexity of the phenomena involved did not allow the authors to clearly identify the relative contribution of each mechanism.

The difficulty of dissociating all of the phenomena that occur during *in vivo* experiments generally prevents from determining the respective contribution of product properties or of consumer characteristics to aroma release. Due to these experimental issues, the modelling approach (*in silico*) can be a useful tool to improve the understanding. It has been largely used in the fields of pharmacokinetics and toxicology: Quantitative Structure-Activity Relationships (QSAR) (Geerts and Heyden, 2011), Physiologically-Based Pharmaco-Kinetic (PB-PK) (Corley et al., 2012; Medinsky et al., 1993; Morris, 2012) and Theoretical Passive Absorption (TPAM) (Obata et al., 2005; Takano et al., 2006) models have helped to better understand drug and toxic vapour absorption. In the field of olfaction, the QSAR approach has also been largely used to identify the main interactions between aroma compounds and olfactory receptors at the origin of perception (Anker et al., 1990; Chastrette and Rallet, 1998; Kraft et al., 2000; Rognon and Chastrette, 1994; Sanz et al., 2008). Some of these models clearly highlighted the need to consider absorption/solubilisation phenomena in tissues of the respiratory tract and/or in the mucus layer to correctly represent the availability of aroma compounds for olfactory receptors. It was demonstrated that the transport of odorant molecules in nasal mucosa clearly differs from the one within an aqueous layer (Kurtz et al., 2004). The main limitation of modelling approaches remains the lack of experimental data, notably concerning the air/mucosa partition or diffusion properties of aroma compounds within the mucus layer, or mucosa characteristics depending on its location (nasal, oral or pharyngeal cavity).

In food science, some mechanistic models describing volatile release have been proposed and sometimes compared to experimental *in vivo* data (Buffo et al., 2005; Harrison, 2000; Harrison and Hills, 1997; Hodgson et al., 2005; Normand et al., 2004; Wright and Hills, 2003). These models, based on physical, chemical and physiological parameters, led to more or less good predictions of the release kinetics of aroma compounds, but only for liquid food products. Only the models of (Wright and Hills, 2003) and (Normand et al., 2004) included a term representing possible interactions between aroma compounds and mucosa and/or salivary constituents. Even though many publications exist on molecular mechanisms that explain interactions between aroma compounds and proteins in the mucus of the nasal cavity of rats (Odorant Binding Proteins, OBPs), results cannot be directly used to explain *in vivo* release kinetics in humans (Borysik et al., 2010; Yabuki et al., 2011). All of these studies constitute a first step in describing the phenomena involved but do not yet provide a clear understanding. In a previous publication, a mathematical model was proposed to predict *in vivo* aroma release from masticated food products that considered food properties and the physiological characteristics of the individuals (Doyennette et al., 2014). Comparison between experimental and predicted kinetics highlighted the possible specific retention of one hydrophobic aroma compound by wetted mucosa and mucus in the naso-oro-pharyngeal cavities. This model thus needs to be further developed to propose a satisfactory quantitative description of the retention phenomenon at the origin of aroma persistence.

In this context, the main goal of the present study is to better understand the mechanisms underlying aroma release and persistence. The originality of the proposed approach is to combine: (i) *in vivo* aroma release measurements (using controlled protocols to ensure aroma supply by flavoured air inhalation, without the interference of any food product); with (ii) the detailed mechanistic modelling of mass transfer to investigate the key mechanisms responsible for the release profiles and/or retention of aroma compounds.

2. Material and methods

Even if this study was not performed in the field of medical research, a detailed research protocol containing the relevant information in agreement with the World Medical Association Declaration of Helsinki was done. Only single-use materials were used with panellists. Aroma compounds were all food grade and their liquid concentrations were adjusted to limit gaseous concentration and ensure panellist comfort and avoid sensory saturation. Only one session (45 min) per week was planned for each panellist and the number of samples during one session was limited to five. Samples were coded to protect the privacy of panellist and the confidentiality of their personal information. Subjects were clearly informed of the observational nature of this study, gave their free and informed consent and received compensation for their participation.

2.1. Aroma compounds

Food grade quality aroma compounds (ethyl propanoate, 2-nonanone and (*Z*)-3-hexen-1-ol) were purchased from Sigma Aldrich (France) (Table 1).

They were selected since they belong to several chemical classes and present different physicochemical properties and different release behaviours in terms of persistence (Déléris et al., 2015). Concentrated stock solutions were prepared in polypropylene glycol (Sigma Aldrich, France) and used throughout the study. Diluted solutions were prepared extemporaneously.

2.2. Gaseous sample preparation

An aroma compound mixture was used to reduce the number of experimental sessions. Gaseous samples were prepared as previously described (Déléris et al., 2015). The concentrations of aroma compounds in the liquid phase were high enough to be detected during PTR-MS measurements, while being acceptable from a sensory point of view for the panellists: 1000 mg/kg for (*Z*)-3-hexen-1-ol, 150 mg/kg for ethyl propanoate and 100 mg/kg for 2-nonanone.

Twenty-five mL of flavoured aqueous solution were stored at ambient temperature for 4 h before measurements in 250-mL flasks (Schott, France), closed by caps equipped with valves (equilibrium establishment). To control the inhaled volume of gaseous sample (and, therefore, the amount of inhaled aroma compounds) between the different assays, a specific set-up was developed to prepare gaseous samples (Fig. 1): a manual pump was connected to