Physiological and oral parameters contribute prediction of retronasal aroma release in an elderly cohort

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Abstract

Malnutrition is a serious problem in the elderly while understanding flavour perception could be a tool for controlling appetite or food choices. To increase our knowledge, we characterised the health and oral physiology (oral volume, swallowing tongue force, number of teeth, salivary flow rate, protein content and antioxidant capacity) of a cohort of 54 community-dwelling French elderly as well as the individual retronasal aroma release of five odorants (2-pentanone, 2-nonanone, 2,3-hexanedione, octanal and linalool) by proton-transfer-reaction mass spectrometry (PTR-MS). In general, large variability across participants was observed in both oral physiological (>40%) and retronasal aroma release (>56%) parameters. Multivariate analyses revealed a relationship between physiological parameters (mostly salivary antioxidant capacity) and retronasal aroma release that explained up to 46% of the variability observed. This study provides new insights to understand retronasal aroma release in the elderly that could contribute to the development of personalised nutrition strategies.

Keywords: interindividual differences; *in vivo* aroma release; PTR-MS; age; BMI; saliva; salivary antioxidant capacity; personalized nutrition

1. INTRODUCTION

The world's population is older than ever (UN, 2019). This global ageing is a consequence of increased life expectancy together with a decline in the global birth rate. In this regard, the population aged 65 and over is growing faster than all other age groups (UN, 2019) and represents 9 per cent of the world population that could rise further to 16 per cent (25% in Europe and Northern America) by 2050 (UN, 2019). This population group is particularly vulnerable to malnutrition (WHO, 2020), due to physiological (sensory impairment, poor oral health, loss of mobility) and socio-cultural changes (poor finances and increased isolation) associated with the ageing process that could severely compromise its health status and its immunity capacity to combat infections (Pae, Meydani, & Wu, 2012). Therefore, there is a need to develop strategies to support proper food consumption in older people. Successful dietary advice, food reformulation and intervention strategies aimed at decreasing disease risk and achieving healthier aging should consider individual determinants of food intake (Tanaka, Reed, & Ordovas, 2007). In this sense, flavour perception is considered one of the main drivers to ensure an enjoyable eating experience.

Among the different sensory modalities involved in flavour perception, retronasal olfaction is considered to be one of the major determinants together with taste and trigeminal sensations for food preferences and satiation (Ruijschop, Boelrijk, de Graaf, & Westerterp-Plantenga, 2009). Thus, it could be a key factor for controlling food intake in the elderly (Rolls, 1999). Retronasal olfaction (also known as aroma perception) is a dynamic process that occurs when volatile aroma compounds are released from the food matrix within the mouth and gain access to the olfactory epithelium located in the nasal cavity *via* the opening created by the velum and dorsal pharyngeal wall (Hannum, Stegman, Fryer, & Simons, 2018). Once there, aroma

compounds activate the olfactory epithelium generating neuronal signals that will be finally integrated by the brain to create an olfactory image of food (Hannum, Stegman, Fryer, & Simons, 2018). Thus, aroma perception will be influenced by the retronasal release of aroma compounds during consumption, which in turn, will be dependent on the physiology of individuals. Despite considerable scientific effort to help understand this problem (Blee, Linforth, Yang, Brown, & Taylor, 2011; Feron, Ayed, El Mostafa Qannari, Laboure, & Guichard, 2014; Frank, Eyres, Piyasiri, & Delahunty, 2012; Muñoz-González, Canon, Feron, Guichard, & Pozo-Bayón, 2019; Repoux, Sémon, Feron, Guichard, & Labouré, 2012), the respective impact of the different physiological parameters on retronasal aroma release is still not fully elucidated. Moreover, most of the studies dedicated to understand retronasal aroma release have been performed with young adults (Muñoz-González, Vandenberghe-Descamps, Feron, Canon, Labouré, & Sulmont-Rossé, 2018) while the older population remain underexplored. Research in this field based on young population has shown that the transfer of volatiles from the mouth to the nose can be affected by different parameters of the food oral processing (time of residence in the mouth, number of swallowings or number of chewing cycles) (Feron, Ayed, El Mostafa Qannari, Laboure, & Guichard, 2014; Labouré, Repoux, Courcoux, Feron, & Guichard, 2014; Pionnier, Chabanet, Mioche, Le Quéré, & Salles, 2004). Moreover, different oral parameters such as salivary flow and composition (Feron, Ayed, El Mostafa Qannari, Laboure, & Guichard, 2014; Muñoz-González, Canon, Feron, Guichard, & Pozo-Bayón, 2019), dental status (Duffy, Cain, & Ferris, 1999), velum opening (Labouré, Repoux, Courcoux, Feron, & Guichard, 2014; Pionnier, Chabanet, Mioche, Le Quéré, & Salles, 2004), and oral cavity volume (Mishellany-Dutour, Woda, Laboure, Bourdiol, Lachaze, Guichard, et al., 2012), have been related to the extent of retronasal aroma released. Although these factors vary depending on the food state (e.g., solid *vs* liquid state influences the mastication and the opening of the velum) and its composition

(e.g., fat content), it has been observed that subjects with relatively high retronasal aroma release for a food product would typically show the same behaviour for another product (Blee, Linforth, Yang, Brown, & Taylor, 2011; Ruijschop, Burgering, Jacobs, & Boelrijk, 2009). Thus, the measurement of retronasal aroma release can be considered a valid feature to characterise individuals no matter what food product is measured. In food products where the oral processing is to some extent simplified (such as liquid products), the number of swallowing events (Repoux, Sémon, Feron, Guichard, & Labouré, 2012) and salivary related-parameters (Canon, Neiers, & Guichard, 2018; Muñoz-González, Feron, & Canon, 2018) are thought to be the main contributors to retronasal aroma release. In this sense, salivary parameters, including salivary flow (Muñoz-González, Canon, Feron, Guichard, & Pozo-Bayón, 2019) and total protein content (TPC) (Muñoz-González, Canon, Feron, Guichard, & Pozo-Bayón, 2019) have been linked to differences in aroma release during wine consumption (Muñoz-González, Canon, Feron, Guichard, & Pozo-Bayón, 2019). Salivary proteins can impact aroma release either through noncovalent interactions (Pagès-Hélary, Andriot, Guichard, & Canon, 2014) or enzymatic metabolism (Muñoz-González, Canon, Feron, Guichard, & Pozo-Bayón, 2019; Muñoz-González, Feron, Brulé, & Canon, 2018). Indeed, it has been recently demonstrated that these mechanisms modify aroma perception (Ijichi, Wakabayashi, Sugiyama, Ihara, Nogi, Nagashima, et al., 2019), while they could be modulated by other salivary parameters, including the salivary antioxidant status, that can be evaluated by measuring the total antioxidant capacity (TAC) of saliva (Muñoz-González, Canon, Feron, Guichard, & Pozo-Bayón, 2019; Muñoz-González, Feron, Brulé, & Canon, 2018). A number of these salivary parameters could vary as a consequence of the ageing process, which is related to salivary disorders like hyposalivation (Affoo, Foley, Garrick, Siqueira, & Martin, 2015) that might influence the total protein content and the total antioxidant capacity of saliva (Muñoz-González, Brulé, Feron, & Canon, 2019).

Thus, this study aims at investigating for the first time interindividual differences on retronasal aroma release from an elderly cohort and if these differences are related to their physiology. To do that, the *in vivo* release of five aroma compounds (2-pentanone, 2-nonanone, 2,3-hexanedione, octanal and linalool), belonging to three chemical families and exhibiting different physicochemical properties was measured by *in nose*-PTR-ToF-MS in 54 French elderly during the consumption of a model-flavoured solution. The use of this instrumental approach allowed an evaluation of the effective amount of aroma compounds reaching the nasal cavity and thus, that are present at the proximity of the olfactory receptors after their oral passage. Retronasal aroma release was correlated to ten physiological variables measured in the panel (age, gender, body mass index, body fat, swallowing tongue force, number of teeth, oral volume and salivary flow rate, salivary protein content and salivary antioxidant capacity) by Spearman correlations and ANCOVA analyses.

2. MATERIAL AND METHODS

2.1. Aroma compounds

Five aroma compounds (2-pentanone, 2-nonanone, 2,3-hexanedione, octanal, linalool) belonging to three chemical classes (ketones, aldehydes, terpene-alcohol) were chosen for this investigation (Table 1-Supplementary Material). Aroma compounds were of food grade and purchased from Sigma-Aldrich (Steinheim, Germany). A gas chromatography–flame ionization detector (GC–FID) analysis confirmed the purity of the compounds (> 98%) that was taken into account for the calculations of concentration. Individual concentrated stock solutions (1%) of the odorants were prepared in propylene glycol (Sigma-Aldrich, France) at

136 room temperature under magnetic stirring for 2 h. They were aliquoted and stored at 4 °C for
137 a maximum of one month.
138
139 **2.2. Panel**
140 Seventy-three individuals were selected from the AlimaSSens Project (https://anr.fr/Projet-
141 ANR-14-CE20-0003), a panel of healthy community dwelling elderly living at home in Dijon
142 (France). The recruitment criteria were the following: older than 65 years old, no acute
143 pathological episodes at the time of the experiment, and without cognitive impairment
144 measured with the mini mental state evaluation (MMSE) (Folstein, Folstein, & McHugh,
145 1975). An interview was carried out with each volunteer to ensure that they met the inclusion
146 criteria. Interested people completed a questionnaire asking them about medications.
147 Exclusionary criteria included any physiological condition or taking medications that could
148 influence salivation (for example antidepressants and antihistamines). The age and gender of
149 the panellists were reported, as well as their body mass index (BMI = weight (kg)/height
150 (m^2)). Body fat (BF) was also measured *via* impedance meter evaluation. All subjects gave
151 written informed consent to participate after receiving oral and written information. The
152 experimental protocol was approved by the French Ethics Committee for Research (CPP Est
153 I. Dijon, #14.06.03, ANSM #2014-A00071-46).
154
155 **2.3. Oral parameters**
156 The number of teeth was measured by means of a dentist evaluation that counted the number
157 of natural, restored and fixed prosthetic teeth (participants who wore dentures were asked to
158 remove them for this measurement). Swallowing tongue force was measured using the IOPO
159 ® device (Laguna, Hetherington, Chen, Artigas, & Sarkar, 2016). Oral volume was measured
160 by using an Eccovision® acoustic pharyngometer (Hood Laboratories, Pembroke, MA) as

described recently (Mishellany-Dutour, et al., 2012).

For saliva collection, participants were asked not to consume any food or drink for at least one hour before saliva was collected. Stimulated saliva samples were collected just before aroma release analyses as described previously (Vandenberghe-Descamps, et al., 2016) by instructing the participants to masticate a piece of pre-weighed parafilm while spitting out the saliva into a pre-weighed screw-cap cup every time they felt like swallowing over a period of 5 min. The salivary parameters studied were stimulated flow rate (SFR), total protein content (TPC) and total antioxidant capacity (TAC). To determine SFR, cups were weighed before and immediately after saliva collection. Salivary flow rates were expressed in mL/min assuming that 1 g of saliva corresponds to 1 mL. Saliva samples were aliquoted and immediately stored at −80 °C until further biochemical analyses. TPC was obtained by standard Bradford protein assay Quick Start (Bio-Rad, France) using bovine serum albumin (Sigma-Aldrich, France) as standard for calibration. TAC was measured using an ORAC Assay kit (CellBiolabs, San Diego, CA). This assay measures the loss of fluorescence over time due to peroxyl-radical formation induced by the breakdown of 2,2′-azobis-2-methyl-propanimidamide dihydrochloride (AAPH). This peroxyl radical oxidises fluorescein, leading to a loss of fluorescence. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a vitamin E analogue, served as a standard to scavenge the peroxyl radical and thus inhibit fluorescein fluorescence decay in a dose-dependent manner. The intensity of fluorescence was measured (excitation filter: 485 nm, emission filter: 538 nm) with a microtitre plate fluorometer (Victor 3-V; Perkin Elmer, Waltham, MA). The total antioxidant capacity of the saliva was expressed in Trolox equivalents.

185 **2.4. Retronasal aroma release measurement by *in nose*-proton-transfer reaction time-of-**
186 **flight mass spectrometry (PTR-ToF-MS)**
187
188 Immediately prior to the *in vivo* analyses, each aroma compound was diluted from the stock
189 solutions with still water (Evian, France) to obtain the following concentrations: 2-pentanone
190 (1 ppm), 2-nonanone (5 ppm), 2,3-hexanedione (20 ppm), octanal (3 ppm) and linalool (40
191 ppm). These concentrations were chosen in preliminary experiments on the basis of obtaining
192 a good sensitivity in *in nose*- (PTR-ToF-MS) analyses while avoiding instrument saturation.
193 Moreover, these concentrations allowed the complete solubility of the odorants in the sample
194 (Table 1 Supplementary Material). It was also checked that the model-flavoured solution was
195 acceptable from a sensory point of view for the subjects who participated in the study.
196
197 The retronasal aroma release measurements were conducted by monitoring the individual's
198 nosespace thanks to a Teflon nosepiece, that connected both nostrils of the subjects to a
199 proton transfer reaction-mass spectrometer (PTR-MS) equipped with a time-of-flight (ToF)
200 analyser (PTR-ToF 8000; Ionicon Analytik, Innsbruck, Austria). The connection was
201 ergonomic thanks to the use of a light helmet that enabled the participants to move their head
202 freely. The helmet was connected to the transfer line of the PTR instrument by flexible heated
203 PEEK tubing and the sampling was performed at a total flow rate of 200 mL/min with the
204 transfer line at 80 ℃. Parameters of the PTR-MS were as follows: the instrument drift tube
205 was thermally controlled (80 °C) and operated with a voltage of 490 V and a pressure of 2.3
206 mbar resulting in an E/N ratio of 112 Td. Mass spectra ranged from *m/z* 0 to 256 and were
207 acquired at a speed of 1 spectrum every 0.108 seconds. Breath volatile intensities were
208 expressed as normalised cps, taking into account corrected transmission and normalisation to
209 the protonated water monitored at their respective ^{18}O isotopic contributions found at *m/z*

21.022 ($H_3{}^{18}O^+$). All the mass spectra were background-subtracted using the background signal measured for 60 s before sample introduction into the mouth. After monitoring the breath noise, a 12-mL disposable syringe containing the model-flavoured solution was given to the subjects and they were instructed to introduce the solution completely (10 mL) into their mouths at one time. Once in the oral cavity, the participants were instructed to gently rinse their mouths with the solution for 30 seconds, while avoiding swallowing. During the post-swallow period, the volunteers were instructed to swallow all the liquid in their mouths (solution and saliva). Afterwards and every 30 seconds, subjects were instructed to continue swallowing their own saliva. In total, five swallows were performed, which corresponded to 150 seconds of monitoring after the first swallowing. The model-flavoured solution was evaluated in duplicate on two different days (once per day) by each of the participants. The odorants were monitored simultaneously according to their protonated molecular ion (MH+): 2-pentanone (*m/z* = 87), 2,3-hexanedione (*m/z* = 115), octanal (*m/z* = 129), and 2-nonanone (*m/z* = 143) or to their protonated and dehydrated molecular ion $(M\text{-}H_2O)H^+$: linalool (*m/z* = 137). Release curves of the monitored ions as a function of time were extracted from the mass spectra. From the release curves and for each of the selected ions two main parameters were extracted: the area under the curve (AUC), that corresponds to the quantity of aroma released during the 150 seconds after sample swallowing and the maximum intensity (*Imax*). A schema of the consumption protocol together with a typical release curve obtained is shown in Figure 1. All the release data were analysed from the breath concentration (normalised counts-per-second (ncps) data), using IGOR Pro (WaveMetrics, Inc., Lake Oswego, OR).

From the 73 individuals initially chosen, 54 were selected for their ability to follow the instructions given during the imposed consumption protocol. Their repeatability in terms of AUC and *Imax* was calculated. To do that, the replicate data for each participant was used to

produce a % coefficient of variation (%CV = 100 × standard deviation/mean). These CV were then averaged across the 54 participants to produce an average %CV for each odorant. The overall % CV of the AUC data was lower than 30% for all compounds (2-pentanone: 21% ; 2-nonanone: 28% ; 2,3-hexanedione: 25% ; linalool: 22%) except for octanal that was 38%. The overall % CV of *Imax* was lower than 35% for all compounds (2-pentanone: 32% ; 2-nonanone: 34% ; 2,3-hexanedione: 31% ; linalool: 29%) except for octanal that was 41%. Such levels of variation may be high in comparison to methods dealing with *in vitro* experiments but are comparable to those typically observed for *in vivo* aroma release measurements (Blee, Linforth, Yang, Brown, & Taylor, 2011; Frank, Eyres, Piyasiri, & Delahunty, 2012).

2.5. Statistical analyses

Gender of individuals was transformed for the statistical analyses into dichotomous variables as follows: females, 0; males, 1. Interquartile ratio (Q3/Q1), Max/Min ratio and coefficient of variation (%CV) were calculated to describe interindividual variability of the participants. The interquartile ratio (Q3/Q1) is a measure of statistical dispersion, being equal to the ratio between 75^{th} and 25^{th} percentiles (Blee, Linforth, Yang, Brown, & Taylor, 2011). Thus, it is an indication of the behaviour of 50% of the central population. The Max/Min ratio calculates the fold difference between the maximum and the minimum values observed, giving a global idea of how different the studied population is when considering the extremes. Lastly, %CV measures the dispersion of the data as the ratio of the standard deviation to the mean. The relationship between variables (AUC, *Imax* and physiological variables) was assessed by Spearman correlation analyses. Analysis of covariance (ANCOVA) was used to evaluate if differences in the total aroma released (dependent variable) might be explained by physiological parameters (explanatory variables) by controlling for confounding variables.

The significance level was $p < 0.05$ throughout the study. The XLStat program was used for data processing (StatSoft, Inc., Tulsa, OK; 2005, www.statsoft.com).

3. RESULTS AND DISCUSSION

3.1. Panel description

Seventy-three healthy elderly individuals living independently in Dijon (France) were selected for this study. However, nineteen of them were not able to properly follow the instructions given during the imposed consumption protocol for the retronasal aroma release measurements, resulting in a final panel of fifty-four participants. Characteristics of the panel are presented in Table 1. The panel was composed of 29 women and 25 men. All of them were older than 67 y/o, with an average age of 74 y/o. The panel presented a mean BMI of 28.4 that ranged from 19.8 to 39.9. According to the WHO classification, 22 subjects were obese (BMI $\geq$ 30 kg/m^2), 17 overweight (25 kg/m^2 $\geq$ BMI $\leq$29.9 kg/m^2), and 15 presented a normal weight (18.5 kg/m^2 $\geq$ BMI $\leq$ 24.9 kg/m^2). The percentage of participants classified as obese and overweight represented 69% of the panel, which is in line with the values reported by European statistics for this population group (Eurostat, 2014). The average body fat (BF) of the panel was 34%, which is also in line with the typical published values for elderly from different regions of France (Delarue, Constans, Malvy, Pradignac, Couet, & Lamisse, 1994).

Regarding the oral parameters (Table 1), six variables that can be considered as important for retronasal aroma release (swallowing tongue force, number of teeth, oral cavity volume and salivary SFR, TPC and TAC) were measured. An average swallowing tongue force of 33.9 kPa was determined for the panel. Participants presented on average 22 teeth, without counting dentures, which is in agreement with the tendency of tooth loss in the elderly. The mean oral volume (37.2 cm^3) was similar to the found for French young individuals (38.6

285 cm^3) (Feron, Ayed, El Mostafa Qannari, Laboure, & Guichard, 2014). The mean stimulated
286 salivary flow rate determined for the panel (1.5 ± 0.8 mL/min) was in the range for that
287 observed in the elderly population (Smith, Boland, Daureeawoo, Donaldson, Small, &
288 Tuomainen, 2013) but lower than that observed for French young adults (2.4 ± 1.1 mL/min
289 (Repoux, Sémon, Feron, Guichard, & Labouré, 2012); 2.6 ± 1.1 mL/min (Feron, Ayed, El
290 Mostafa Qannari, Laboure, & Guichard, 2014); 2.6 ± 1.4 mL/min (Guichard, Repoux,
291 Qannari, Labouré, & Feron, 2017)). These findings are in line with the reduced salivary flow
292 observed as a consequence of the ageing process (Affoo, Foley, Garrick, Siqueira, & Martin,
293 2015; Vandenberghe-Descamps, et al., 2016). This age-dependent salivary flow decrease
294 could be attributed to structural changes in salivary glands and/or others factors related to
295 lifestyle, such as diet or smoking habits (Vandenberghe-Descamps, et al., 2016). The mean
296 stimulated salivary TPC (0.8 ± 0.4 mg/mL) was also lower than the one observed for French
297 young adults (1.0 ± 0.3 mg/mL) (Guichard, Repoux, Qannari, Labouré, & Feron, 2017), while
298 the mean salivary TAC was 873.4 ± 350.5 μM Trolox. This value is in the same order of
299 magnitude as the values reported for normal-weight (759 ± 403) and obese (792 ± 470) young
300 adults (Besnard, Christensen, Brignot, Bernard, Passilly-Degrace, Nicklaus, et al., 2018).
301
302 **3.2. Interindividual differences on physiological parameters of elderly**
303 Table 1 shows the variability indicators (Q3/Q1 ratio, max/min ratio and % CV) calculated
304 for the physiological parameters determined in the panel of 54 French elderly volunteers. As
305 can be seen, the Q3/Q1 ratio varied from 1.1 to 1.9 for the parameters studied, which means
306 that there were no large differences in these variables for the central 50% of participants.
307 However, when looking at the max/min ratio or at the %CV, which both give an idea of the
308 global dispersion of the panel, it can be observed that these differences were of a large
309 magnitude. For instance, the oral volume showed a max/min ratio of 11.3, which means that

one participant presented an oral volume 11-fold bigger than another. Actually, the oral parameters were the physiological variables most dissimilar across participants, with percentages of variation higher than 40% for all the parameters studied. Thus, while some individuals were edentulous, others presented all their natural teeth, and while some participants were hyposalivators (SFR < 0.7 mL/min) (Närhi, Meurman, & Ainamo, 1999), others presented an elevated salivary flow rate (SFR > 4 mL/min). In addition, a relatively high dispersion was also observed for BMI (CV = 17%) and BF (CV = 21%). However, as age was an imposed criterion for the inclusion of the participants in the study, the percentage of variation of this parameter across participants was rather low (CV = 8%). The panel was balanced in terms of gender.

3.3. Interindividual differences on retronasal aroma release from elderly

In vivo release curves like the one shown in Figure 1 were obtained. From them the area under the curve (AUC) was calculated, considering the 150 seconds after sample swallowing and the maximal intensity (*Imax*). Additionally, an example of the different release curves generated by different participants can be found in Figure 1 of the Supplementary Material. The min, max, Q1, median, Q3, mean and standard deviation values for each aroma compound are shown in Table 2. Table 2 also shows the variability indicators (Q3/Q1 ratio, max/min ratio and %CV) of the retronasal aroma calculated for the panel.

As can be seen, interindividual differences in the retronasal release parameters were observed for all the odorants. Thus, Q3/Q1 ranged from 1.8 to 2.2. This indicates that the central 50% of the 54 French elderly volunteers exhibited around 2-fold difference in the total aroma released values, which is in agreement with those found in the literature for young adults. For instance, Blee and co-workers (2011)(Blee, Linforth, Yang, Brown, & Taylor, 2011), found

335 an interquartile ratio (Q3/Q1) of 2.2 for the maximal intensity of menthone released by a
336 panel of 50 young individuals. Moreover, from the data published by Frank and colleagues
337 (Frank, Eyres, Piyasiri, & Delahunty, 2012) using a smaller panel (n = 8), the Q3/Q1 ratio can
338 also be calculated and this value increased to 2.9 for the release of ethyl butanoate.
339
340 To better understand the global interindividual variability of the panel, the ratio between the
341 maximum and the minimum values observed across panellists and the %CV were calculated
342 (Table 2). As can be seen, max/min ratio of AUC values ranged from 10.7 (linalool) to 43.9-
343 fold (octanal) differences. These data indicate that, for example, the amount of octanal
344 reaching the olfactory receptors in one of the participants was up to 44 times higher compared
345 to another. The %CV of the AUC values ranged from 56 to 100%. ~~a~~nd this variability was of
346 even bigger magnitude when considering the *Imax* values (Table 2). Such big differences on
347 the retronasal aroma release parameters most likely would condition differences in aroma
348 perception of the panel. Although the magnitude of this variation could be considered as
349 huge, the variability on *in vivo* aroma release across elderly people observed in this study is in
350 agreement with the great variability in retronasal aroma described for young individuals in
351 previous works (Feron, Ayed, El Mostafa Qannari, Laboure, & Guichard, 2014; Gierczynski,
352 Laboure, & Guichard, 2008; Mestres, Kieffer, & Buettner, 2006; Muñoz-González, Canon,
353 Feron, Guichard, & Pozo-Bayón, 2019). For example, in a study carried out with 8 assessors,
354 the total amount of aroma released has been found to vary on a scale from 1 to 8 (Pionnier,
355 Chabanet, Mioche, Le Quéré, & Salles, 2004), depending on the odorants studied. However,
356 this variability seems to increase as the size of the panel increases. In fact, a variability of
357 95% on the *in vivo* release of menthone in water solutions between assessors was found in a
358 panel of 50 people (Blee, Linforth, Yang, Brown, & Taylor, 2011). It should be emphasised
359 that many studies on retronasal aroma have not shown the magnitude of the interindividual

360 variation, which makes comparison across studies difficult.

361

362 **3.4. Relating retronasal aroma release to individual physiological parameters**

363 The relationship between physiology (age, gender, BMI, BF, oral parameters) and retronasal
364 aroma release was studied using Spearman correlation analyses. The results are shown in
365 Table 3. They indicate that only 4 of the 10 physiological variables studied herein were
366 correlated to retronasal aroma to some extent. These variables were: age, BMI, swallowing
367 tongue force and salivary TAC, the latter being the only variable significantly related to all
368 the aroma compounds assayed.

369

370 Table 3 shows, in spite of the small variation of age among the participants of the study (CV
371 = 8%), there was a positive correlation between this factor and the *in vivo* release of aroma
372 compounds (it was significant for 2-nonanone (AUC) and linalool (*Imax*)). Thus, the higher
373 the age of the participants was, the higher the amount of retronasal aroma released. This
374 finding seems contradictory with the retronasal olfactory impairment frequently described in
375 elderly people (Duffy, Cain, & Ferris, 1999). A possible hypothesis to explain this singularity
376 could be related to the fact that in the present study we only measured the amount of volatiles
377 that reach the olfactory receptors, but not other factors related to the integration of the sensory
378 signals in the brain and/or psychological aspects related to the experience of the consumers.
379 Thus, these results could suggest that the sensory impairment associated with increasing age
380 could be mostly due to cognitive factors than to a decrease of the amount of aroma
381 compounds that reach the olfactory receptors through the retronasal route. Nevertheless, it
382 could be also possible that the higher level of retronasal aroma released observed with
383 increasing age could lead to a mechanism of adaptation (diminished perception because of
384 overexposure to aroma) and thus to a decrease of aroma sensitivity in the elderly. However, it

385 is important to note that due to the small variation of age across participants, new studies with
386 different age groups should be performed to check this finding.
387
388 The age of the participants was also negatively correlated to their swallowing tongue force (–
389 0.351), which, in turn, was negatively related to octanal release (–0.331 AUC; –0.295 *Imax*).
390 Overall, this indicates that the higher the age of the participants was, the lower the swallowing
391 tongue force and the higher octanal release. A loss of muscle tone consequent with the ageing
392 process could be behind this phenomenon and produce less efficient swallowings (Hiramatsu,
393 Kataoka, Osaki, & Hagino, 2015), which could have affected the transfer of volatiles to the
394 nasal cavity. In addition, this fact could also indirectly indicate a loss of tone of other oral
395 muscles like the palate velum, which has the role of isolating the oral cavity from the
396 pharynx. In this scenario, the velum lock could have worked more inefficiently with
397 increasing age and aroma compounds could have been continuously transferred to the nasal
398 cavity.
399
400 As can be observed in Table 3, BMI and BF were positively (0.473) self-correlated. However,
401 only BMI was significantly related to the retronasal aroma released by the panel (–0.301 to
402 *Imax*-2-pentanone; –0.326 to *Imax*-2,3-hexanedione), which indicates that the higher the BMI
403 was, the lower the release of these compounds. In their study, Zijlstra and coworkers (Zijlstra,
404 Bukman, Mars, Stafleu, Ruijschop, & de Graaf, 2011) did not find significant differences in
405 retronasal aroma release between overweight (n = 24) and normoweight (n = 24) young
406 subjects during the consumption of a spiced rice and an apple pie yogurt. Divergences
407 between studies could be due to differences in the mode of sample consumption (free *versus*
408 imposed consumption protocol). In a free protocol, like the one in the study of Zijlstra and co-
409 workers (Zijlstra, Bukman, Mars, Stafleu, Ruijschop, & de Graaf, 2011), chewing and

swallowing instructions were not given to the individuals. Thus, interindividual variability in chewing and swallowing behaviour also contributed to the interindividual variability in aroma release across participants. This fact could have blurred the effect of other oral factors such as saliva. It has been previously reported that differences in the composition of saliva samples of groups of individuals with different BMI are related to *ex vivo* aroma release from young (Piombino, Genovese, Esposito, Moio, Cutolo, Chambery, et al., 2014) and elderly people (Muñoz-González, Brulé, Feron, & Canon, 2019). Interestingly, in the present study BMI was significantly and positively related with salivary TAC (0.309), which in turn, was negatively related to the quantity of retronasal aroma released ($p < 0.05$). Several studies have already reported a higher salivary TAC in obese compared to normoweight individuals (Chielle & Casarin, 2017; Piombino, et al., 2014) that could be related to the regulation of various processes in the adipose tissue. Different *ex vivo* studies had reported a negative relationship between salivary TAC and aroma release (Muñoz-González, Feron, Brulé, & Canon, 2018; Muñoz-González, Brulé, Feron, & Canon, 2019; Piombino, et al., 2014). The effect of saliva on aroma compounds depends on their structure and is thought to be a result of the activity of enzymes present in saliva, which are involved in the detoxification of xenobiotics. Some of these enzymes are NAD(P)H-dependent and thus depend on the oxidative state of the NAD(P)H/NAD(P)$^+$ couple, which is also involved in the regeneration of glutathione. Glutathione is involved in the maintenance of the salivary redox balance. Thus, TAC could reflect the redox status of the NAD(P)H/NAD(P)$^+$ couple, which will affect the activity of NAD(P)H-dependent enzymes and thus the metabolism of aroma compounds as a function of their structure. The positive relation found here between salivary TAC and BMI, both of which were, in turn, negatively related with retronasal aroma release, suggests that elderly individuals classified as obese would present a lower retronasal aroma, and thus perception, compared to normoweight subjects. This finding is relevant, since changes in the

435 concentration of retronasal aroma have been related to affect food intake (Ruijschop,
436 Burgering, Jacobs, & Boelrijk, 2009). Thus, this knowledge could be useful to develop new
437 nutrition strategies for targeted population groups.
438
439 The remaining physiological parameters studied (gender, oral volume, number of teeth,
440 salivary flow and TPC) were not significantly correlated with retronasal aroma by means of
441 Spearman correlation analyses. Gender is a parameter not traditionally studied in the literature
442 in terms of retronasal aroma release, but the present results would suggest that it is not
443 directly related. Meanwhile, the contribution of dentition to aroma release has been more
444 explored in relation to chewing solid food than for liquid foods. However, the oral volume,
445 salivary flow and TPC had already been related to aroma release in young individuals (Duffy,
446 Cain, & Ferris, 1999; Mishellany-Dutour, et al., 2012; Muñoz-González, Canon, Feron,
447 Guichard, & Pozo-Bayón, 2019), which leads us to think that either the effect of these
448 physiological parameters on retronasal aroma is different between young and older people, or
449 that differences across studies (different methodology, different odorants, population from
450 different countries) could have influenced these relationships. Thus, more studies are needed
451 to confirm these points.
452
453 In order to assess further the strength of the relationships while controlling for confounding
454 factors, ANCOVA analyses were performed. Table 4 shows the ANCOVA models that were
455 significant ($p < 0.05$). As can be seen, four out of five compounds assayed presented
456 significant models for AUC values that explained between 39 to 41% of variation observed.
457 In the case of *Imax*, the five compounds generated significant ANCOVA models that
458 explained 37 to 46% of variation observed. Figure 2 shows the β-standardised coefficients of
459 the physiological parameters that significantly contributed to the models. For AUC (Figure

460 2a), these parameters were number of teeth, salivary TPC and TAC and *Imax* (Figure 2b), in
461 addition to the previous three, BMI also contributed in a significant way to the models.
462
463 Regarding the number of teeth, negative β-standardised coefficients were obtained (Figure 2),
464 which indicates that having more teeth was related to lower quantities of retronasal release.
465 However, it is important to keep in mind that the number of teeth reported in this study did
466 not take into account the use of removable dentures, while their use was allowed during the *in*
467 *vivo* analyses. Therefore, a person with no teeth reported here could have worn a complete
468 removable denture during the *in vivo* analyses, which could have been related with a more
469 difficult control of the velum during swallowing than subjects with all their natural teeth or
470 with fixed teeth. Regarding salivary parameters, two of them significantly contributed to the
471 ANCOVA models. Positive β-standardised coefficients were obtained for salivary TPC and
472 negative for TAC (Figure 2). This means that the higher the TPC of saliva and the lower the
473 TAC, the higher the retronasal aroma release of the participants. The contribution of TPC to
474 retronasal aroma release was already shown in a previous study (Muñoz-González, Canon,
475 Feron, Guichard, & Pozo-Bayón, 2019), and was attributed to different phenomena, such as a
476 salting out effect of odorants in the mouth or to a higher retention of odorants by salivary
477 proteins of the mucosal pellicle (mostly mucins) in the mouth that could have affected their
478 transfer to the nose over the 150 seconds of monitoring time. Salivary TAC was the parameter
479 more highly associated to AUC and *Imax* and it was negatively related to them. To the
480 authors' knowledge this is the first time that an association between salivary TAC and *in vivo*
481 retronasal aroma release has been observed. The hypothesis about the mechanism of action of
482 TAC on aroma release was already pointed out above. Lastly, BMI significantly contributed
483 to the models of *Imax* for the three ketones. This indicates that a higher BMI was related to a
484 lower intensity of ketones released. Spearman correlations (Table 3) revealed that BMI was

485 correlated to the number of teeth and salivary TAC. Herein we can observe that these two
486 factors contributed to the models that explained retronasal aroma release. This finding is
487 interesting since it suggests that changes in the physiological status of individuals (i.e. ageing
488 and obesity) would contribute to modifying the extent of retronasal aroma release. Thus, this
489 work supposes a step forward to a better understanding of flavour perception.
490

491 **4. CONCLUSIONS**

492 A panel formed by 54 elderly volunteers older than 67 y/o and balanced for gender was
493 characterised in terms of physiology (BMI, BF, oral parameters) and retronasal aroma release.
494 A high interindividual variation (40–49%) of the panel was found for their oral (swallowing
495 tongue force, oral volume, number of teeth, salivary flow rate, salivary TPC, salivary TAC) as
496 well as for retronasal aroma release parameters (56–132%). Spearman analyses revealed that
497 retronasal aroma release was correlated to age, BMI, swallowing tongue force and salivary
498 TAC of the participants. Multivariate analyses revealed that physiological parameters
499 explained up to 46% of the retronasal aroma variation although only four parameters (salivary
500 TAC, salivary TPC, number of teeth and BMI) significantly contributed to the models. Thus,
501 variables such as the gender of the participants appear not be related to retronasal aroma
502 release in the elderly, while others such as age, BMI and mostly salivary TAC were important
503 factors to explain interindividual variability in retronasal aroma release from an elderly
504 population. This highlights the need to deeply characterise the mechanisms of action of these
505 factors, and especially of salivary TAC, involved in retronasal aroma release and perception.
506 In addition, a substantial amount of the variability was not explained by the parameters
507 measured in the current study, suggesting that other factors may be considered in future
508 investigations. Overall, this ambitious study presents new and relevant information to

understand retronasal aroma release in elderly people that can be potentially useful when redesigning food products according to their needs.

CRediT authorship contribution statement

CMG designed the study, contributed to data acquisition, performed data analysis, interpretation of results and wrote the manuscript. GF and FC contributed to design the study and interpretation of results and revised the manuscript for important intellectual content. All authors gave final approval for manuscript revision and submission.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Affoo, R. H., Foley, N., Garrick, R., Siqueira, W. L., & Martin, R. E. (2015). Meta-analysis of salivary flow rates in young and older adults. *Journal of the American Geriatrics Society, 63*(10), 2142-2151.

Besnard, P., Christensen, J. E., Brignot, H., Bernard, A., Passilly-Degrace, P., Nicklaus, S., de Barros, J.-P. P., Collet, X., Lelouvier, B., & Servant, F. (2018). Obese subjects with specific gustatory papillae microbiota and salivary cues display an impairment to sense lipids. *Scientific reports, 8*(1), 1-15.

Blee, N., Linforth, R., Yang, N., Brown, K., & Taylor, A. (2011). Variation in aroma release between panellists consuming different types of confectionary. *Flavour and fragrance journal, 26*(3), 186-191.

Canon, F., Neiers, F., & Guichard, E. (2018). Saliva and flavor perception: perspectives. *Journal of agricultural and food chemistry, 66*(30), 7873-7879.

Chielle, E. O., & Casarin, J. N. (2017). Evaluation of salivary oxidative parameters in overweight and obese young adults. *Archives of endocrinology and metabolism, 61*(2), 152-159.

Delarue, J., Constans, T., Malvy, D., Pradignac, A., Couet, C., & Lamisse, F. (1994). Anthropometric values in an elderly French population. *British journal of nutrition, 71*(2), 295-302.

Duffy, V. B., Cain, W. S., & Ferris, A. M. (1999). Measurement of sensitivity to olfactory flavor: application in a study of aging and dentures. *Chemical Senses, 24*(6), 671-671.

Eurostat. (2014). https://ec.europa.eu/eurostat/statistics-explained/index.php?title=File:Share_of_overweight_population_by_sex_and_age, _2014.png. (Access date: 23/04/2020).

Feron, G., Ayed, C., El Mostafa Qannari, P. C., Laboure, H., & Guichard, E. (2014). Understanding aroma release from model cheeses by a statistical multiblock approach on oral processing. *PloS one, 9*(4).

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. *Journal of psychiatric research, 12*(3), 189-198.

Frank, Eyres, G., Piyasiri, U., & Delahunty, C. (2012). Effect of food matrix structure and composition on aroma release during oral processing using in vivo monitoring. *Flavour and fragrance journal, 27*(6), 433-444.

Gierczynski, I., Laboure, H., & Guichard, E. (2008). In vivo aroma release of milk gels of different hardnesses: Inter-individual differences and their consequences on aroma perception. *Journal of agricultural and food chemistry, 56*(5), 1697-1703.

Guichard, E., Repoux, M., Qannari, E., Labouré, H., & Feron, G. (2017). Model cheese aroma perception is explained not only by in vivo aroma release but also by salivary composition and oral processing parameters. *Food & function, 8*(2), 615-628.

Hannum, M., Stegman, M. A., Fryer, J. A., & Simons, C. T. (2018). Different olfactory percepts evoked by orthonasal and retronasal odorant delivery. *Chemical Senses, 43*(7), 515-521.

Hiramatsu, T., Kataoka, H., Osaki, M., & Hagino, H. (2015). Effect of aging on oral and swallowing function after meal consumption. *Clinical interventions in aging, 10*, 229.

Ijichi, C., Wakabayashi, H., Sugiyama, S., Ihara, Y., Nogi, Y., Nagashima, A., Ihara, S., Niimura, Y., Shimizu, Y., Kondo, K., & Touhara, K. (2019). Metabolism of Odorant Molecules in Human Nasal/Oral Cavity Affects the Odorant Perception. *Chemical Senses*.

Labouré, H., Repoux, M., Courcoux, P., Feron, G., & Guichard, E. (2014). Inter-individual retronasal aroma release variability during cheese consumption: Role of food oral processing. *Food research international, 64*, 692-700.

Laguna, L., Hetherington, M. M., Chen, J., Artigas, G., & Sarkar, A. (2016). Measuring eating capability, liking and difficulty perception of older adults: A textural consideration. *Food Quality and Preference, 53*, 47-56.

Mestres, M., Kieffer, R., & Buettner, A. (2006). Release and perception of ethyl butanoate during and after consumption of whey protein gels: Relation between textural and physiological parameters. *Journal of agricultural and food chemistry, 54*(5), 1814-1821.

Mishellany-Dutour, A., Woda, A., Laboure, H., Bourdiol, P., Lachaze, P., Guichard, E., & Feron, G. (2012). Retro-nasal aroma release is correlated with variations in the in-mouth air cavity volume after empty deglutition. *PloS one, 7*(7).

Muñoz-González, C., Canon, F., Feron, G., Guichard, E., & Pozo-Bayón, M. A. (2019). Assessment Wine Aroma Persistence by Using an in Vivo PTR-ToF-MS Approach and Its Relationship with Salivary Parameters. *Molecules, 24*(7), 1277.

Muñoz-González, C., Feron, G., Brulé, M., & Canon, F. (2018). Understanding the release and metabolism of aroma compounds using micro-volume saliva samples by ex vivo approaches. *Food chemistry, 240*, 275-285.

Muñoz-González, C., Feron, G., & Canon, F. (2018). Main effects of human saliva on flavour perception and the potential contribution to food consumption. *Proceedings of the Nutrition Society, 77*(4), 423-431.

Muñoz-González, C., Vandenberghe-Descamps, M., Feron, G., Canon, F., Labouré, H., & Sulmont-Rossé, C. (2018). Association between salivary hypofunction and food consumption in the elderlies. A systematic literature review. *The journal of nutrition, health & aging, 22*(3), 407-419.

Muñoz-González, C., Brulé, M., Feron, G., & Canon, F. (2019). Does interindividual variability of saliva affect the release and metabolization of aroma compounds ex vivo? The particular case of elderly suffering or not from hyposalivation. *Journal of texture studies, 50*(1), 36-44.

Närhi, T. O., Meurman, J. H., & Ainamo, A. (1999). Xerostomia and hyposalivation. *Drugs & aging, 15*(2), 103-116.

Pae, M., Meydani, S. N., & Wu, D. (2012). The role of nutrition in enhancing immunity in aging. *Aging and disease, 3*(1), 91.

Pagès-Hélary, S., Andriot, I., Guichard, E., & Canon, F. (2014). Retention effect of human saliva on aroma release and respective contribution of salivary mucin and α-amylase. *Food Research International, 64*, 424-431.

Piombino, P., Genovese, A., Esposito, S., Moio, L., Cutolo, P. P., Chambery, A., Severino, V., Moneta, E., Smith, D. P., & Owens, S. M. (2014). Saliva from obese individuals suppresses the release of aroma compounds from wine. *PloS one, 9*(1).

Pionnier, E., Chabanet, C., Mioche, L., Le Quéré, J.-L., & Salles, C. (2004). 1. In vivo aroma release during eating of a model cheese: Relationships with oral parameters. *Journal of agricultural and food chemistry, 52*(3), 557-564.

628 Repoux, M., Sémon, E., Feron, G., Guichard, E., & Labouré, H. (2012). Inter-individual
629 variability in aroma release during sweet mint consumption. *Flavour and*
630 *fragrance journal, 27*(1), 40-46.
631 Rolls, B. J. (1999). Do chemosensory changes influence food intake in the elderly?
632 *Physiology & behavior, 66*(2), 193-197.
633 Ruijschop, R. M., Boelrijk, A. E., de Graaf, C., & Westerterp-Plantenga, M. S. (2009).
634 Retronasal aroma release and satiation: a review. *Journal of agricultural and food*
635 *chemistry, 57*(21), 9888-9894.
636 Ruijschop, R. M., Burgering, M. J., Jacobs, M. A., & Boelrijk, A. E. (2009). Retro-nasal
637 aroma release depends on both subject and product differences: a link to food
638 intake regulation? *Chemical Senses, 34*(5), 395-403.
639 Smith, C. H., Boland, B., Daureeawoo, Y., Donaldson, E., Small, K., & Tuomainen, J. (2013).
640 Effect of aging on stimulated salivary flow in adults. *Journal of the American*
641 *Geriatrics Society, 61*(5), 805-808.
642 Tanaka, T., Reed, D. R., & Ordovas, J. M. (2007). 10 Taste as the Gatekeeper of
643 Personalized Nutrition. *Personalized Nutrition: Principles and Applications*, 115.
644 UN. (2019). https://www.un.org/en/sections/issues-depth/ageing/. *United*
645 *Nations*(Access date: 23/04/2020).
646 Vandenberghe-Descamps, M., Labouré, H., Prot, A., Septier, C., Tournier, C., Feron, G., &
647 Sulmont-Rossé, C. (2016). Salivary flow decreases in healthy elderly people
648 independently of dental status and drug intake. *Journal of texture studies, 47*(4),
649 353-360.
650 WHO. (2020). https://www.who.int/nutrition/topics/ageing/en/index1.html. *World*
651 *Health Organization*(Access date: 24/06/2020).
652 Zijlstra, N., Bukman, A. J., Mars, M., Stafleu, A., Ruijschop, R. M., & de Graaf, C. (2011).
653 Eating behaviour and retro-nasal aroma release in normal-weight and
654 overweight adults: a pilot study. *British journal of nutrition, 106*(2), 297-306.
655

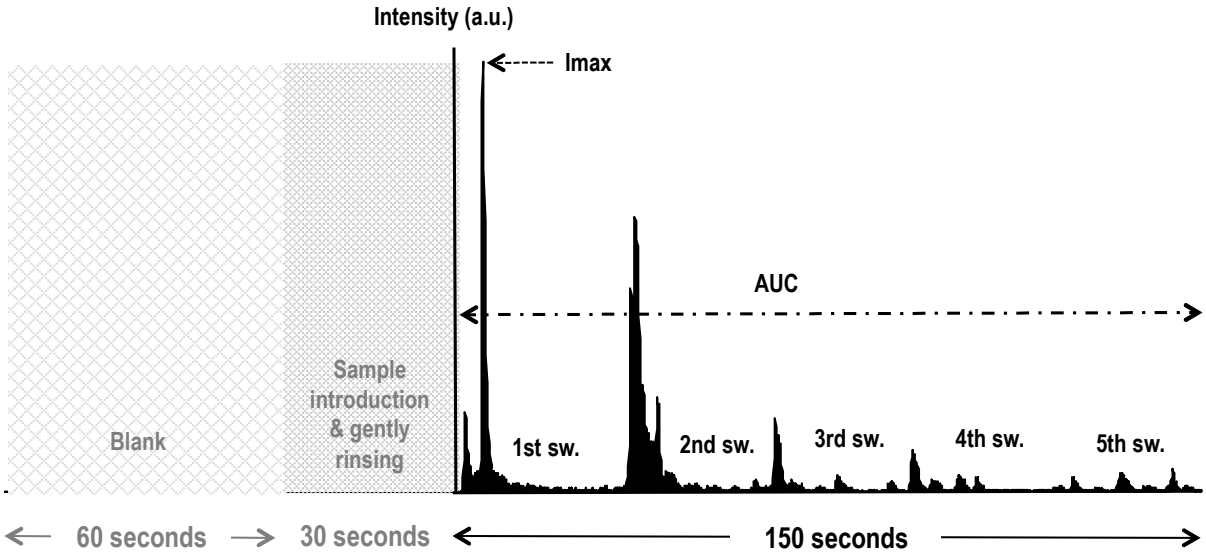
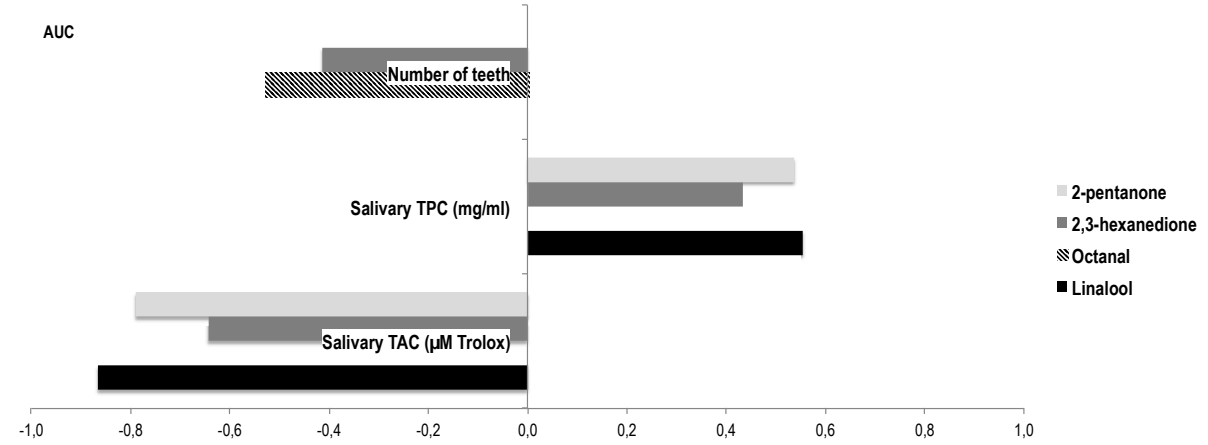


Figure 1. Consumption procedure followed during the *in vivo* retronasal aroma monitoring by PTR-ToF-MS and simulation of typical aroma release curve obtained. The quantity of aroma released (AUC) and the maximum intensity (*Imax*) were extracted from the curves (sw.= swallowing).

a)



b)

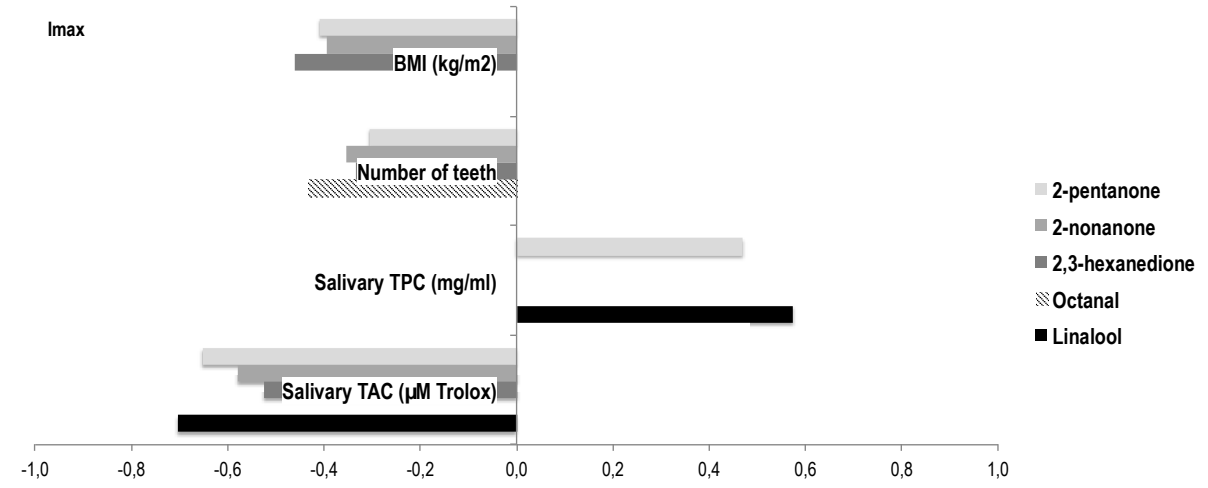


Figure 2. β-coefficients of the physiological parameters that significantly ($p < 0.05$) contributed to the ANCOVA models performed with the retronasal aroma release parameters (AUC (a) and *Imax* (b)) obtained from a panel of 54 French seniors during the consumption of a model flavoured solution.

Table 1. Characteristics of the 54 subjects included in the study.

	n	Min	Max	1st Quartile	Median	3rd Quartile	Mean	Standard deviation (*n*–1)	Q3/Q1	Max/Min	% CV
AGE (y/o)	54	67.0	87.0	69.0	73.5	78.7	74.0	5.6	1.1	1.3	8
GENDER											
Women	29										
Men	25										
BMI (kg/m²)	54	19.8	39.9	24.5	28.5	31.4	28.4	4.8	1.3	2.0	17
≥30 kg/m² (Obesity)	22	30.0	39.9	31.0	33.1	33.7	33.1	2.7	1.1	1.3	8
25–29.9 kg/m² (Overweight)	17	25.1	29.2	26.5	27.3	28.6	27.3	1.3	1.1	1.2	5
18.5–24.9 kg/m² (Normoweight)	15	19.8	24.9	22.0	22.9	23.8	22.7	1.6	1.1	1.3	7
BF (%)	54	14.7	48.8	29.0	34.1	39.9	34.2	7.3	1.4	3.3	21
ORAL PARAMETERS											
Swallowing tongue force (kPa)	53	8.7	73.3	23.3	32.3	44.7	33.9	14.2	1.9	8.5	42
Number of teeth	54	0.0	32.0	16.5	26.0	28.0	22.0	9.6	1.7	-	44
Oral volume (cm³)	54	6.7	75.6	28.3	37.8	45.2	37.2	15.1	1.6	11.3	41
Stimulated salivary parameters :											
Flow Rate (SFR) (mL/min)	54	0.0	4.2	1.1	1.4	1.9	1.5	0.8	1.8	-	49
Total Protein Content (TPC) (mg/mL)	53	0.3	2.2	0.6	0.7	1.0	0.8	0.4	1.6	7.9	48
Total Antioxidant Capacity (TAC) (µM Trolox)	53	308.2	1858.9	614.0	850.4	1095.7	873.4	350.5	1.8	6.0	40

Q3/Q1 : interquartile ratio
Max/Min : ratio between the maximum and the minimum value observed
% CV= 100 × (standard deviation (*n*–1)/mean)

Table 2. Description of the retronasal aroma release parameters (AUC, *Imax*) obtained from a panel of 54 French seniors during the consumption of a model-flavoured solution.

	Min	Max	1st Quartile	Median	3rd Quartile	Mean	Standard deviation (*n*–1)	Q3/Q1	Max/Min	% CV
AUC										
2-Pentanone	2324	41570	6187	9370	12405	10990	7167	2.0	17.9	65
2-Nonanone	2040	46057	10250	15281	22404	17559	9773	2.2	22.6	56
2,3-Hexanedione	4028	62176	11470	16424	22239	18731	10632	1.9	15.4	57
Octanal	97	4271	377	535	791	758	756	2.1	43.9	100
Linalool	4764	51027	13159	15740	24024	19900	11074	1.8	10.7	56
Imax										
2-Pentanone	36	776	84	129	183	155	122	2.2	21.8	79
2-Nonanone	20.0	915	76	122	165	146	134	2.2	45.9	91
2,3-Hexanedione	106	2129	269	406	505	433	297	1.9	20.0	69
Octanal	3	221	11	16	24	25	34	2.1	68.6	132
Linalool	15	270	36	58	74	68	52	2.0	18.4	76

Q3/Q1 : interquartile ratio
Max/Min : ratio between the maximum and the minimum value observed
% CV= 100 × (standard deviation (*n*–1)/mean)

Table 3. Spearman correlation coefficients among age, gender, BMI, BF, oral-related parameters and retronasal aroma release parameters of five compounds determined in 54 French seniors.

Variables	Age	Gender	BMI (kg/m^2)	BF (%)	Sw. force (kPa)	Number of teeth	Oral volume (cm^3)	Salivary flow (mL/min)	Salivary TPC (mg/mL)	Salivary TAC (µM Trolox)
Age	**1**	–0.067	0.075	0.089	**–0.351**	–0.159	0.085	–0.200	0.244	0.019
Gender	–0.067	**1**	–0.020	**–0.720**	0.045	0.206	**–0.343**	0.280	–0.113	**–0.312**
BMI (kg/m^2)	0.075	–0.020	**1**	**0.473**	–0.040	**–0.338**	–0.129	–0.004	0.050	**0.309**
BF (%)	0.089	**–0.720**	**0.473**	**1**	0.016	**–0.343**	0.212	–0.269	0.181	**0.474**
Sw. force (kPa)	**–0.351**	0.045	–0.040	0.016	**1**	–0.099	–0.087	–0.017	–0.015	0.060
Number of teeth	–0.159	0.206	**–0.338**	**–0.343**	–0.099	**1**	–0.112	0.134	–0.007	–0.188
Oral volume (cm^3)	0.085	**–0.343**	–0.129	0.212	–0.087	–0.112	**1**	–0.192	–0.074	0.040
Salivary flow (mL/min)	–0.200	0.280	–0.004	–0.269	–0.017	0.134	–0.192	**1**	**–0.318**	**–0.604**
Salivary TPC (mg/mL)	0.244	–0.113	0.050	0.181	–0.015	–0.007	–0.074	**–0.318**	**1**	**0.541**
Salivary TAC (µM Trolox)	0.019	**–0.312**	**0.309**	**0.474**	0.060	–0.188	0.040	**–0.604**	**0.541**	**1**
AUC_2-Pentanone	0.256	–0.035	–0.281	–0.019	–0.215	–0.003	0.151	0.056	0.022	**–0.323**
AUC_2-Nonanone	**0.300**	–0.090	–0.205	0.016	–0.143	0.082	0.135	0.073	–0.017	**–0.311**
AUC_2,3-Hexanedione	0.212	–0.052	–0.221	–0.033	–0.113	–0.110	0.154	0.141	–0.016	**–0.345**
AUC_Octanal	0.279	–0.040	–0.138	–0.073	**–0.331**	–0.125	–0.031	0.194	0.037	**–0.339**
AUC_Linalool	0.201	0.072	–0.182	–0.030	–0.096	0.111	0.172	0.044	0.083	**–0.303**
Imax_2-Pentanone	0.280	–0.179	**–0.301**	0.138	–0.193	–0.030	0.130	–0.001	0.083	–0.209
Imax_2-Nonanone	0.280	–0.243	–0.264	0.150	–0.213	0.061	0.128	–0.020	0.156	–0.213
Imax_2,3-Hexanedione	0.217	–0.147	**–0.326**	0.042	–0.106	0.051	0.132	0.111	0.028	–0.279
Imax_Octanal	0.166	–0.121	–0.205	–0.044	**–0.295**	–0.077	0.033	0.115	0.017	–0.253
Imax_Linalool	**0.318**	–0.055	–0.241	0.055	–0.211	0.120	0.206	0.063	0.091	**–0.292**

Values in bold denote statistically significant differences between correlations ($p < 0.05$)

Table 4. ANCOVA results (R^2, F, Pr > F) obtained to check the strength of the relationship between physiological and retronasal aroma release parameters obtained from a panel of 54 French seniors during the consumption of a model flavoured solution.

		AUC				*Imax*				
		2-Pentanone	2,3-Hexanedione	Octanal	Linalool	2-Pentanone	2-Nonanone	2,3-Hexanedione	Octanal	Linalool
	R^2	0.387	0.395	0.413	0.395	0.398	0.464	0.428	0.373	0.403
	F	2.394	2.485	2.678	2.482	2.514	3.294	2.839	2.263	2.561
	Pr > F	**0.026**	**0.021**	**0.014**	**0.021**	**0.020**	**0.004**	**0.010**	**0.034**	**0.018**
Age	F	0.357	0.092	1.735	0.330	0.609	3.910	1.822	2.669	0.664
	Pr > F	0.554	0.763	0.196	0.569	0.440	0.055	0.185	0.111	0.420
Gender	F	1.133	0.270	0.383	2.548	0.700	0.344	0.120	0.140	2.266
	Pr > F	0.294	0.606	0.540	0.119	0.408	0.561	0.731	0.710	0.141
BMI (kg/m^2)	F	3.362	3.448	2.715	1.419	4.676	4.897	6.236	2.659	2.644
	Pr > F	0.075	0.071	0.108	0.241	**0.037**	**0.033**	**0.017**	0.111	0.112
BF (%)	F	1.662	0.842	1.017	2.534	2.032	2.601	1.589	0.511	2.836
	Pr > F	0.205	0.365	0.320	0.120	0.162	0.115	0.215	0.479	0.100
Sw. force (kPa)	F	0.997	0.341	0.293	0.161	0.191	0.103	0.002	0.059	0.609
	Pr > F	0.324	0.563	0.592	0.691	0.664	0.750	0.962	0.809	0.440
Number of teeth	F	3.206	7.879	12.247	0.121	4.315	6.531	5.433	8.285	1.453
	Pr > F	0.081	**0.008**	**0.001**	0.730	**0.045**	**0.015**	**0.025**	**0.007**	0.236
Oral volume (cm^3)	F	0.101	0.240	0.100	1.518	0.417	0.449	0.593	0.090	2.107
	Pr > F	0.753	0.627	0.754	0.225	0.522	0.507	0.446	0.766	0.155
Salivary flow (mL/min)	F	0.425	0.004	0.019	1.945	0.234	0.114	0.032	0.019	0.080
	Pr > F	0.518	0.947	0.890	0.171	0.631	0.737	0.858	0.890	0.778
Salivary TPC (mg/ml)	F	7.095	4.696	2.394	7.712	5.555	3.459	3.235	1.602	8.374
	Pr > F	**0.011**	**0.037**	0.130	**0.008**	**0.024**	0.071	0.080	0.213	**0.006**
Salivary TAC (µM Trolox)	F	10.669	7.188	3.790	12.996	7.405	6.559	5.060	3.122	8.684
	Pr > F	**0.002**	**0.011**	0.059	**0.001**	**0.010**	**0.015**	**0.030**	0.085	**0.005**

R^2 : Coefficient of determination; F: F statistic; Pr > F: Probability values (values in bold are statistically significant ($p < 0.05$))