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

Carolina Muñoz-González, Gilles Feron, Francis Canon

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3 **Title**

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

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8 **Author names and affiliation**

9 Carolina MUÑOZ-GONZALEZ, Gilles FERON, Francis CANON

10

11 Centre des Sciences du Goût et de l'Alimentation, UMR1324 INRA, UMR6265 CNRS

12 Université de Bourgogne, Agrosup Dijon, F-21000 Dijon, France

13

14

15 **Corresponding author:**

16 Carolina MUÑOZ-GONZALEZ

17 current phone: 0034910017900 ; current e-mail address: c.munoz@csic.es

18

19 **Abstract**

20 Malnutrition is a serious problem in the elderly while understanding flavour perception could
21 be a tool for controlling appetite or food choices. To increase our knowledge, we
22 characterised the health and oral physiology (oral volume, swallowing tongue force, number
23 of teeth, salivary flow rate, protein content and antioxidant capacity) of a cohort of 54
24 community-dwelling French elderly as well as the individual retronasal aroma release of five
25 odorants (2-pentanone, 2-nonanone, 2,3-hexanedione, octanal and linalool) by proton-
26 transfer-reaction mass spectrometry (PTR-MS). In general, large variability across
27 participants was observed in both oral physiological (>40%) and retronasal aroma release
28 (>56%) parameters. Multivariate analyses revealed a relationship between physiological
29 parameters (mostly salivary antioxidant capacity) and retronasal aroma release that explained
30 up to 46% of the variability observed. This study provides new insights to understand
31 retronasal aroma release in the elderly that could contribute to the development of
32 personalised nutrition strategies.

33

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

36

37 1. INTRODUCTION

38

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

53

54 Among the different sensory modalities involved in flavour perception, retronasal olfaction is
55 considered to be one of the major determinants together with taste and trigeminal sensations
56 for food preferences and satiation (Ruijschop, Boelrijk, de Graaf, & Westerterp-Plantenga,
57 2009). Thus, it could be a key factor for controlling food intake in the elderly (Rolls, 1999).
58 Retronasal olfaction (also known as aroma perception) is a dynamic process that occurs when
59 volatile aroma compounds are released from the food matrix within the mouth and gain access
60 to the olfactory epithelium located in the nasal cavity *via* the opening created by the velum
61 and dorsal pharyngeal wall (Hannum, Stegman, Fryer, & Simons, 2018). Once there, aroma

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

87 (e.g., fat content), it has been observed that subjects with relatively high retronasal aroma
88 release for a food product would typically show the same behaviour for another product
89 (Blee, Linforth, Yang, Brown, & Taylor, 2011; Ruijschop, Burgering, Jacobs, & Boelrijk,
90 2009). Thus, the measurement of retronasal aroma release can be considered a valid feature to
91 characterise individuals no matter what food product is measured. In food products where the
92 oral processing is to some extent simplified (such as liquid products), the number of
93 swallowing events (Repoux, Sémon, Feron, Guichard, & Labouré, 2012) and salivary related-
94 parameters (Canon, Neiers, & Guichard, 2018; Muñoz-González, Feron, & Canon, 2018) are
95 thought to be the main contributors to retronasal aroma release. In this sense, salivary
96 parameters, including salivary flow (Muñoz-González, Canon, Feron, Guichard, & Pozo-
97 Bayón, 2019) and total protein content (TPC) (Muñoz-González, Canon, Feron, Guichard, &
98 Pozo-Bayón, 2019) have been linked to differences in aroma release during wine
99 consumption (Muñoz-González, Canon, Feron, Guichard, & Pozo-Bayón, 2019). Salivary
100 proteins can impact aroma release either through noncovalent interactions (Pagès-Hélary,
101 Andriot, Guichard, & Canon, 2014) or enzymatic metabolism (Muñoz-González, Canon,
102 Feron, Guichard, & Pozo-Bayón, 2019; Muñoz-González, Feron, Brulé, & Canon, 2018).
103 Indeed, it has been recently demonstrated that these mechanisms modify aroma perception
104 (Ijichi, Wakabayashi, Sugiyama, Ihara, Nogi, Nagashima, et al., 2019), while they could be
105 modulated by other salivary parameters, including the salivary antioxidant status, that can be
106 evaluated by measuring the total antioxidant capacity (TAC) of saliva (Muñoz-González,
107 Canon, Feron, Guichard, & Pozo-Bayón, 2019; Muñoz-González, Feron, Brulé, & Canon,
108 2018). A number of these salivary parameters could vary as a consequence of the ageing
109 process, which is related to salivary disorders like hyposalivation (Affoo, Foley, Garrick,
110 Siqueira, & Martin, 2015) that might influence the total protein content and the total
111 antioxidant capacity of saliva (Muñoz-González, Brulé, Feron, & Canon, 2019).

112

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

125

126 **2. MATERIAL AND METHODS**

127

128 **2.1. Aroma compounds**

129 Five aroma compounds (2-pentanone, 2-nonanone, 2,3-hexanedione, octanal, linalool)
130 belonging to three chemical classes (ketones, aldehydes, terpene-alcohol) were chosen for this
131 investigation (Table 1-Supplementary Material). Aroma compounds were of food grade and
132 purchased from Sigma-Aldrich (Steinheim, Germany). A gas chromatography–flame
133 ionization detector (GC–FID) analysis confirmed the purity of the compounds (> 98%) that
134 was taken into account for the calculations of concentration. Individual concentrated stock
135 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 (m²)). 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

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

162

163 For saliva collection, participants were asked not to consume any food or drink for at least
164 one hour before saliva was collected. Stimulated saliva samples were collected just before
165 aroma release analyses as described previously (Vandenberghe-Descamps, et al., 2016) by
166 instructing the participants to masticate a piece of pre-weighed parafilm while spitting out the
167 saliva into a pre-weighed screw-cap cup every time they felt like swallowing over a period of
168 5 min. The salivary parameters studied were stimulated flow rate (SFR), total protein content
169 (TPC) and total antioxidant capacity (TAC). To determine SFR, cups were weighed before
170 and immediately after saliva collection. Salivary flow rates were expressed in mL/min
171 assuming that 1 g of saliva corresponds to 1 mL. Saliva samples were aliquoted and
172 immediately stored at $-80\text{ }^{\circ}\text{C}$ until further biochemical analyses. TPC was obtained by
173 standard Bradford protein assay Quick Start (Bio-Rad, France) using bovine serum albumin
174 (Sigma-Aldrich, France) as standard for calibration. TAC was measured using an ORAC
175 Assay kit (CellBiolabs, San Diego, CA). This assay measures the loss of fluorescence over
176 time due to peroxy-radical formation induced by the breakdown of 2,2'-azobis-2-methyl-
177 propanimidamide dihydrochloride (AAPH). This peroxy radical oxidises fluorescein, leading
178 to a loss of fluorescence. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a
179 vitamin E analogue, served as a standard to scavenge the peroxy radical and thus inhibit
180 fluorescein fluorescence decay in a dose-dependent manner. The intensity of fluorescence was
181 measured (excitation filter: 485 nm, emission filter: 538 nm) with a microtitre plate
182 fluorometer (Victor 3-V; Perkin Elmer, Waltham, MA). The total antioxidant capacity of the
183 saliva was expressed in Trolox equivalents.

184

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 °C. 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

210 21.022 ($\text{H}_3^{18}\text{O}^+$). All the mass spectra were background-subtracted using the background
211 signal measured for 60 s before sample introduction into the mouth. After monitoring the
212 breath noise, a 12-mL disposable syringe containing the model-flavoured solution was given
213 to the subjects and they were instructed to introduce the solution completely (10 mL) into
214 their mouths at one time. Once in the oral cavity, the participants were instructed to gently
215 rinse their mouths with the solution for 30 seconds, while avoiding swallowing. During the
216 post-swallow period, the volunteers were instructed to swallow all the liquid in their mouths
217 (solution and saliva). Afterwards and every 30 seconds, subjects were instructed to continue
218 swallowing their own saliva. In total, five swallows were performed, which corresponded to
219 150 seconds of monitoring after the first swallowing. The model-flavoured solution was
220 evaluated in duplicate on two different days (once per day) by each of the participants. The
221 odorants were monitored simultaneously according to their protonated molecular ion (MH^+):
222 2-pentanone ($m/z = 87$), 2,3-hexanedione ($m/z = 115$), octanal ($m/z = 129$), and 2-nonanone
223 ($m/z = 143$) or to their protonated and dehydrated molecular ion $(\text{M}-\text{H}_2\text{O})\text{H}^+$: linalool ($m/z =$
224 137). Release curves of the monitored ions as a function of time were extracted from the mass
225 spectra. From the release curves and for each of the selected ions two main parameters were
226 extracted: the area under the curve (AUC), that corresponds to the quantity of aroma released
227 during the 150 seconds after sample swallowing and the maximum intensity (I_{max}). A
228 schema of the consumption protocol together with a typical release curve obtained is shown
229 in Figure 1. All the release data were analysed from the breath concentration (normalised
230 counts-per-second (ncps) data), using IGOR Pro (WaveMetrics, Inc., Lake Oswego, OR).

231

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

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

245

246 **2.5. Statistical analyses**

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

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

262

263 **3. RESULTS AND DISCUSSION**

264 **3.1. Panel description**

265 Seventy-three healthy elderly individuals living independently in Dijon (France) were
266 selected for this study. However, nineteen of them were not able to properly follow the
267 instructions given during the imposed consumption protocol for the retronasal aroma release
268 measurements, resulting in a final panel of fifty-four participants. Characteristics of the panel
269 are presented in Table 1. The panel was composed of 29 women and 25 men. All of them
270 were older than 67 y/o, with an average age of 74 y/o. The panel presented a mean BMI of
271 28.4 that ranged from 19.8 to 39.9. According to the WHO classification, 22 subjects were
272 obese ($\text{BMI} \geq 30 \text{ kg/m}^2$), 17 overweight ($25 \text{ kg/m}^2 \leq \text{BMI} \leq 29.9 \text{ kg/m}^2$), and 15 presented a
273 normal weight ($18.5 \text{ kg/m}^2 \leq \text{BMI} \leq 24.9 \text{ kg/m}^2$). The percentage of participants classified as
274 obese and overweight represented 69% of the panel, which is in line with the values reported
275 by European statistics for this population group (Eurostat, 2014). The average body fat (BF)
276 of the panel was 34%, which is also in line with the typical published values for elderly from
277 different regions of France (Delarue, Constans, Malvy, Pradignac, Couet, & Lamisse, 1994).

278

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

285 cm³) (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 μ M 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

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

320

321 **3.3. Interindividual differences on retronasal aroma release from elderly**

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

329

330 As can be seen, interindividual differences in the retronasal release parameters were observed
331 for all the odorants. Thus, Q3/Q1 ranged from 1.8 to 2.2. This indicates that the central 50%
332 of the 54 French elderly volunteers exhibited around 2-fold difference in the total aroma
333 released values, which is in agreement with those found in the literature for young adults. For
334 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%. and this variability was of
346 even bigger magnitude when considering the *I*_{max} 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 (*I_{max}*)). 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 *I_{max}*).
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 *I_{max}*-2-pentanone; –0.326 to *I_{max}*-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

410 swallowing instructions were not given to the individuals. Thus, interindividual variability in
411 chewing and swallowing behaviour also contributed to the interindividual variability in aroma
412 release across participants. This fact could have blurred the effect of other oral factors such as
413 saliva. It has been previously reported that differences in the composition of saliva samples of
414 groups of individuals with different BMI are related to *ex vivo* aroma release from young
415 (Piombino, Genovese, Esposito, Moio, Cutolo, Chambery, et al., 2014) and elderly people
416 (Muñoz-González, Brulé, Feron, & Canon, 2019). Interestingly, in the present study BMI was
417 significantly and positively related with salivary TAC (0.309), which in turn, was negatively
418 related to the quantity of retronasal aroma released ($p < 0.05$). Several studies have already
419 reported a higher salivary TAC in obese compared to normoweight individuals (Chielle &
420 Casarin, 2017; Piombino, et al., 2014) that could be related to the regulation of various
421 processes in the adipose tissue. Different *ex vivo* studies had reported a negative relationship
422 between salivary TAC and aroma release (Muñoz-González, Feron, Brulé, & Canon, 2018;
423 Muñoz-González, Brulé, Feron, & Canon, 2019; Piombino, et al., 2014). The effect of saliva
424 on aroma compounds depends on their structure and is thought to be a result of the activity of
425 enzymes present in saliva, which are involved in the detoxification of xenobiotics. Some of
426 these enzymes are NAD(P)H-dependent and thus depend on the oxidative state of the
427 NAD(P)H/NAD(P)⁺ couple, which is also involved in the regeneration of glutathione.
428 Glutathione is involved in the maintenance of the salivary redox balance. Thus, TAC could
429 reflect the redox status of the NAD(P)H/NAD(P)⁺ couple, which will affect the activity of
430 NAD(P)H-dependent enzymes and thus the metabolism of aroma compounds as a function of
431 their structure. The positive relation found here between salivary TAC and BMI, both of
432 which were, in turn, negatively related with retronasal aroma release, suggests that elderly
433 individuals classified as obese would present a lower retronasal aroma, and thus perception,
434 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

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

511

512

513 **CRedit authorship contribution statement**

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

518

519 **Declaration of Competing Interest**

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

522

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533

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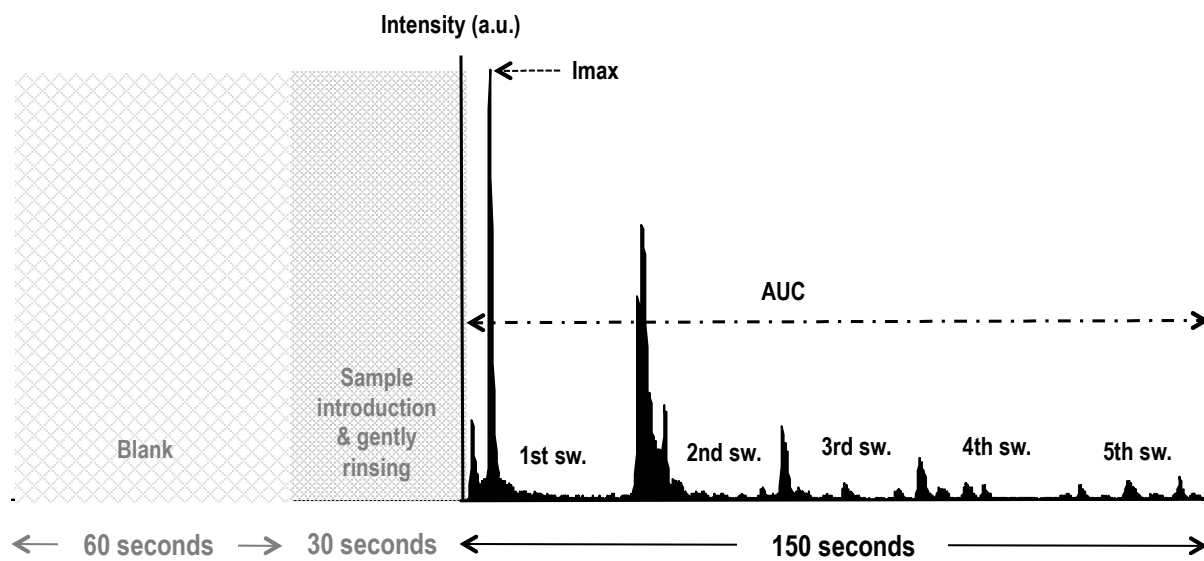


Figure 1. Consumption procedure followed during the *in vivo* retronasal aroma monitoring by PTR-ToF-MS and simulation of typical aroma release curve obtained. The quantity of aroma released (AUC) and the maximum intensity (*Imax*) were extracted from the curves (sw.= swallowing).

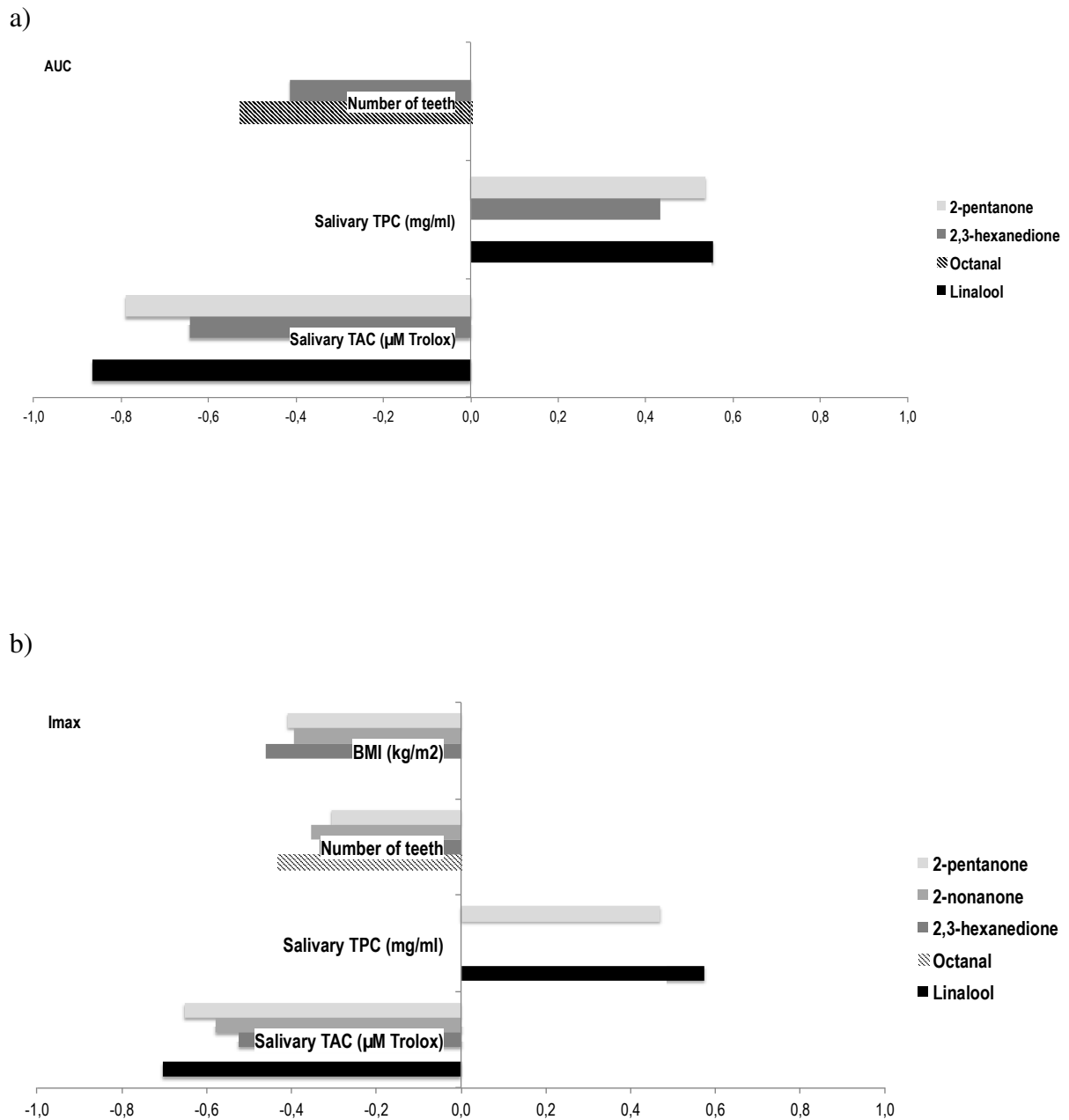


Figure 2. β -coefficients of the physiological parameters that significantly ($p < 0.05$) contributed to the ANCOVA models performed with the retronasal aroma release parameters (AUC (a) and I_{max} (b)) obtained from a panel of 54 French seniors during the consumption of a model flavoured solution.

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

	<i>n</i>	Min	Max	1st Quartile	Median	3rd Quartile	Mean	Standard deviation (<i>n</i> -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											
<i>Women</i>	29										
<i>Men</i>	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/m ² (<i>Obesity</i>)	22	30.0	39.9	31.0	33.1	33.7	33.1	2.7	1.1	1.3	8
25–29.9 kg/m ² (<i>Overweight</i>)	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/m ² (<i>Normoweight</i>)	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 (cm ³)	54	6.7	75.6	28.3	37.8	45.2	37.2	15.1	1.6	11.3	41
Stimulated salivary parameters :											
<i>Flow Rate (SFR) (mL/min)</i>	54	0.0	4.2	1.1	1.4	1.9	1.5	0.8	1.8	-	49
<i>Total Protein Content (TPC) (mg/mL)</i>	53	0.3	2.2	0.6	0.7	1.0	0.8	0.4	1.6	7.9	48
<i>Total Antioxidant Capacity (TAC) (μM Trolox)</i>	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, *I*_{max}) 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 (<i>n</i> -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
<i>I</i>_{max}										
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				<i>l</i> _{max}					
	2-Pentanone	2,3-Hexanedi one	Octanal	Linalool	2-Pentanone	2-Nonanone	2,3-Hexanedi one	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/m²)	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 (cm³)	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 ($p < 0.05$))