RESEARCH ARTICLE

WILEY Journal of Texture Studies

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

Carolina Muñoz-González ⬤ | Marine Brulé | Gilles Feron | Francis Canon

Centre des Sciences du Goût et de l'Alimentation, UMR1324 INRA, UMR6265 CNRS Université de Bourgogne, Agrosup Dijon, Dijon, France

Correspondence
Carolina Muñoz-González, Centre des Sciences du Goût et de l'Alimentation, UMR1324 INRA, UMR6265 CNRS Université de Bourgogne, Agrosup Dijon, F-21000 Dijon, France.
Email: carolina.munoz@inra.fr

Abstract

The aim of this work was to study the effects of interindividual variability of human elderly saliva on aroma release and metabolization by ex vivo approaches. Thirty individuals suffering or not from hyposalivation were selected from a panel formed by 110 elderly people (aged >65 years old) that were matched by age and sex. Then, their stimulated saliva samples were independently incubated in presence of three aroma compounds (ethyl hexanoate, octanal, 2-nonanone) to perform headspace-gas chromatography and liquid/liquid extraction-gas chromatography mass spectrometry analyses. These assays revealed that the extent of saliva effect on the release and metabolization of aroma compounds was highly dependent on the chemical family of the compounds (octanal>ethyl hexanoate>2-nonanone). Moreover, salivas from the hyposalivator (HPS) group exerted a significant lower release and/or higher metabolization than those of the control group for the three assayed compounds. Regarding the biochemical characterization of the saliva samples, no significant differences were found in the total protein content between the two groups. This does not preclude the involvement of specific proteins on the observed results that need to be clarified in further experiments. Saliva from the HPS group presented a significantly higher total antioxidant capacity than that of the control group, which suggests that this parameter could be related to the metabolization of aroma compounds by saliva. Such effects might alter aroma perception in individuals suffering from hyposalivation.

Practical applications

The world population is getting older so fast that most countries are not prepared to handle this demographic challenge, characterized by an increasing prevalence of noncommunicable chronic diseases (e.g., diabetes, gastrointestinal disorders) associated to inadequate eating patterns. Thus, supporting a balanced diet is one of the most cost-effective strategies to maintain a good quality of life. A suitable diet needs to take into account both, specific sensory and nutritional individual needs. However, aging is often accompanied by deterioration in oral health (e.g., low salivary secretions), which could alter the capacities to taste and smell. Results from this work contribute to a better understanding of the role of human saliva in aroma release and metabolization, a first step to comprehend retronasal aroma release and perception. This knowledge will help to propose innovative solutions for the reformulation of food products better adapted to the elderly's needs, thus allowing delayed onset of dependency.

KEYWORDS

aroma release, elderly, hyposalivation, metabolization, saliva, total antioxidant capacity

---

1 | INTRODUCTION

The interest in the role of human saliva on the food oral processing is growing in last years (Mosca & Chen, 2017; Muñoz-González et al., 2017; Muñoz-González, Feron, & Canon, 2018a; Ployon, Morzel, & Canon, 2017). In several studies, a particular attention is turned to the functions that saliva may play on aroma compounds (Buettner, 2002a, 2002b; Genovese, Piombino, Gambuti, & Moio, 2009; Munoz-Gonzalez et al., 2014; Munoz-Gonzalez, Feron, Brule, & Canon, 2018b; Pages-Helary, Andriot, Guichard, & Canon, 2014; Piombino et al., 2014). These studies, carried out ex vivo, have demonstrated that human saliva and its components can increase or decrease the release of aroma compounds via different mechanisms such as noncovalent interactions, salting-out effects or enzymatic conversion. Thus, it is believed that the variability of human saliva might be one of the factors explaining interindividual variation in retronasal aroma release and aroma perception (Feron et al., 2014; Guichard, Repoux, Qannari, Laboure, & Feron, 2017).

However, most of the studies on this topic have employed pooled salivas or individual salivas from a limited number of subjects (Buettner, 2002a, 2002b; Genovese et al., 2009; Munoz-Gonzalez et al., 2018b; Munoz-Gonzalez et al., 2014; Pages-Helary et al., 2014) that do not represent properly the well-known interindividual variability of human saliva composition in healthy individuals (Leake, Pagni, Falquet, Taroni, & Greub, 2016; Neyraud, Palicki, Schwartz, Nicklaus, & Feron, 2012). Additionally, different pathologies or functional states can provoke biochemical and microbiological changes in salivary parameters raising the differences in specific populations (Castagnola, Cabras, Vitali, Sanna, & Messana, 2011; Schipper, Silletti, & Vinyerhoeds, 2007). This fact leads to hypothesize that people experiencing salivary disorders might present an altered retronasal release and perception of aroma. Hereof, it should be mentioned an original study that showed that saliva from obese people (body mass index [BMI] > 30) suppressed aroma release from wine compared to saliva from normal-weight subjects (18.5 < BMI < 24.9; Piombino et al., 2014). Authors observed that saliva from obese individuals ($n = 28$) presented a higher total antioxidant capacity (TAC) compared to that of the controls ($n = 28$). They suggested the induction of a systemic antioxidant response in the saliva from obese subjects that might have affected the release of aroma compounds in that group.

Elderly people are another specific population affected by salivary disorders. A recent meta-analysis has shown that the aging process is associated per se with a reduced secretion of the salivary-glands (Affoo, Foley, Garrick, Siqueira, & Martin, 2015). In the elderlies, such salivary hypofunction could have a negative influence on food consumption as it has been recently reviewed (Muñoz-González et al., 2017). In that article, authors systematically reviewed the existing articles on this topic and they found that most of them showed an association between hyposalivation and a decrease in the chewing and swallowing abilities, but also in taste perception (Iwasaki et al., 2016; Samnieng et al., 2012; Solemdal, Sandvik, Willumsen, Mowe, & Hummel, 2012). A relationship between salivary hypofunction and appetite loss (Dormenval, Mojon, & Budtz-Jorgensen, 1999; Iwasaki et al., 2016) and unbalanced dietary intake was also underlined. However, in spite of the relevance of this topic, and the increased of this population group, to the authors' knowledge the effects of saliva on aroma release in elderlies have never been investigated.

For that reason, in this article, the role of interindividual differences of saliva from elderly people on aroma compounds has been studied. Thirty individuals (15 suffering from hyposalivation, 15 with normal salivary flow) were selected from a panel formed by 110 older individuals. Both groups were matched by age and sex. Saliva samples ($n = 30$) were independently incubated in presence of three aroma compounds (ethyl hexanoate, octanal and 2-nonanone). Aroma release was measured by HS-GC. Moreover, LLE-GC/MS analyses were performed to investigate the possible metabolization of aroma compounds by saliva. Biochemical analyses of the saliva samples were carried out to compare the two groups and to understand the relationship between data on saliva composition and the release and metabolization of aroma compounds.

2 | MATERIAL AND METHODS

2.1 | Aroma compounds

Three compounds were chosen on the basis of their physicochemical characteristics (similar log P and boiling point), their aroma impact, and because of their suitability for the analyses techniques regarding the detection limits and solubility (Table 1). They belong to three chemical families (ketones, aldehydes, and esters), known to be affected by saliva (Buettner, 2002a, 2002b; Munoz-Gonzalez et al., 2018b; Pages-Helary et al., 2014). The aroma compounds were of analytical grade (Aldrich, Steinheim, Germany). A gas chromatography-flame ionization detector analysis confirmed the purity of all aroma compounds (>98%), which was take into account for the calculations. Stock solutions (1%) of single aroma compounds were freshly prepared in propyleneglycol at room temperature under magnetic stirring for 2 hr. They

TABLE 1 Physicochemical properties of the aroma compounds used in this study

Aroma compounds	CAS number	Chemical family	Chemical formula	MW[a] (g/mol)	Log P[b]	BP[c] (C)	Solubility[d] (mg/L)	Aroma descriptor[e]
Ethyl hexanoate	123-66-0	Ester	$C_8H_{16}O_2$	144	2.8	168	308	Fruity
Octanal	124-13-0	Aldehyde	$C_8H_{16}O$	128	2.8	176	394	Fat, lemon, green
2-Nonanone	821-55-6	Ketone	$C_9H_{18}O$	142	2.7	185	171	Blue cheese, fatty, fruity

[a] Hydrophobic constant estimated using molecular modeling software EPI Suite (U.S. EPA 2000–2007).
[b] Boiling point estimated using molecular modeling software EPI Suite (U.S. EPA 2000–2007).
[c] Solubility in water estimated using molecular software EPI Suite (U.S. EPA 2000–2007).
[d] From Flavornet database (http://www.flavornet.org; accessed October 2009).
[e] Molecular weight.

were stored at 4C throughout the study, and their stability was periodically checked. The final concentration of the aroma compounds in the water solutions was 3 ppm.

2.2 | Saliva samples

2.2.1 | Panel of elderly individuals

A panel of 110 elderly volunteers formed the initial population (AlimaSSenS project: toward an adapted and healthy food supply for elderly people). The AlimaSSens panel was recruited from a population of elderly people living at home in Dijon (France). The recruitment criteria were the following: older than 65 years old, no acute pathological episodes at the time of the experiment, and scoring at least 24 on the mini mental state evaluation, which indicates normal cognition (Folstein, Folstein, & McHugh, 1975). An interview was carried out with each volunteer to ensure that they met the inclusion criteria. Interested people followed a dentist evaluation and completed a questionnaire asking them about medications and food habits. Exclusionary criteria included any physiological condition or taking medications that could influence salivation (e.g., antidepressants and antihistamines). Finally, their weight and their height were measured and used to calculate their body mass index (BMI = weight [kg]/height^2 [m^2]). All subjects gave written informed consent to participate in the experiment after receiving oral and written information. The experimental protocol was approved by the French Ethics Committee for Research (CPP Est I, Dijon, #14.06.03, ANSM #2014-A00071-46).

2.2.2 | Saliva collection and salivary flow rates calculation

Participants were asked not to consume any food or drink at least 1 hr before saliva was collected. Unstimulated salivary flow was measured by instructing the participants to let the saliva naturally be accumulated in the mouth and then spat it directly into a collection tube during 10 min. Stimulated salivary flow was measured 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. Cups were weighed, and salivary flow rates were expressed in ml/min assuming that 1 g of saliva corresponds to 1 ml. Only stimulated saliva samples were analyzed in this study, since this is the saliva secreted during food consumption. Moreover, it has been previously reported that this type of saliva was correlated to aroma release during food consumption (Feron et al., 2014). Saliva samples were alliquoted and stored at −80C until use.

2.3 | Saliva biochemical analysis

2.3.1 | Protein concentration

The protein concentration was determined using the Bradford protein assay with bovine serum albumin used as the standard for calibration.

2.3.2 | Protein profile

Salivary samples were separated by electrophoresis using analytical 4–20% precast polyacrylamide gels (Biorad, France). The samples (10 μg of total protein content) were diluted into a mixture (vol/vol) of 2× Laemmli buffer and Dithiothreitol (100 mM; final volume = 50 μl).

Then, they were heated at 100C during 5 min. The gels were performed on a Mini-PROTEAN Tetra Cell (BioRad). A control of saliva and a molecular weight marker Precision Plus Protein Unstained (Biorad) were loaded in each of the gels. The running buffer 1× TGS was added into the gels and a separation voltage of 200 V was applied during 40 min. After that, the gels were rinsed into the deionized water for 10 min. Gel images were acquired on a ChemiDoc MP imaging system (Biorad) for fluorescently stained gels. The time of exposure was 10 s. Molecular weight and intensity of each protein band were calculated on the normalized gels using the ImageLab software (Biorad). Spot volumes were normalized within one gel by the total volume of all valid spots for that particular gel and used for statistical analysis.

2.3.3 | Total antioxidant capacity

The total antioxidant capacity was determined using an ORAC Assay kit (Zen-bio, Research Triangle Park, NC). This assay measures the loss of fluorescein fluorescence over time due to peroxyl radical formation resulting from the breakdown of AAPH (2,2′-azobis-2-methyl-propanimidamide, dihydrochloride). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water soluble vitamin E analogue, serves as a positive control to inhibit fluorescein decay in a dose-dependent manner. The intensity of fluorescence was measured (excitation filter, 485 nm; emission filter, 538 nm) using a microtitre plate fluorometer (Victor 3-V, PerkinElmer, France). The total antioxidant capacity was expressed in micromolar Trolox equivalent.

2.4 | Release of aroma compounds by HS-GC

A previously validated method adapted to microvolumes of saliva was employed (Munoz-Gonzalez et al., 2018b). Briefly, 300 μl of the aroma solution (containing the three aroma compounds at 3 ppm) were placed into a 10 ml headspace vial (Agilent Technologies, Palo Alto, CA). Then, water or stimulated saliva samples (n = 30) were added to the vial until a final volume of 500 μl. Vials were immediately sealed with a PTFE/silicone septum (Supelco, Bellefonte, PA), vortexed and then incubated at 37C during 30 min. After the incubation time, 200 μl of headspace were taken automatically using a preheated (45C) 1-ml gas-tight syringe (Gerstel, manufactured by SGE, Victoria, Australia) and analyzed by gas chromatography (Agilent 7890B) in splitless mode at 240C. A capillary DW-Wax column (30 m x 0.32 mm i.d. x 0.5 μm; Agilent J&W Scientific, Folsom, CA) and a flame ionization detector set at 250C were used. The carrier gas was helium at a velocity of 30 cm^3/s. Aroma compounds were analyzed at 110C (isothermal temperature). Each salivary sample (n = 30) was analyzed independently and in triplicate (one injection per sample vial). Salivary samples were analyzed in random order.

Linearity and repeatability (calculated in three different days) of the procedure were determined in the mix of the three aroma compounds at five levels of concentration (0.6, 1.2, 2.5, 5, and 10 mg/L), with determination coefficients higher than 0.99 and relative standard deviations lower than 7% for the three assayed compounds. Absence of interference (competition, interaction…) between the aroma compounds was checked for both water and saliva (p > .05). Stability of the aroma compounds and that of the GC system was checked during

all the analysis period with no significant differences obtained across days ($p > .05$).

2.5 | Recovery of aroma compounds after incubation by LLE-GC/MS

After incubation at 37C during 30 min, 500 µl of a saturated $CaCl_2$ solution were added to the samples to inhibit possible enzymatic reactions (Buettner, 2002a, 2002b). Moreover, samples were acidified with HCl to protonate acids and increase their extraction. The solutions were extracted twice with 1 ml of dichloromethane (Carlo Erba, Val de Reuil, France) then centrifuged (5,000g, 4C, 15 min) to separate the two phases. Prior to the extraction, samples were spiked with 100 µl of the internal standard, methyl nonanoate (10 mg/L). The combined organic extracts were dried over anhydrous Na_2SO_4, then concentrated to a total volume of 100 µl. Two microliters were injected into the GC/MS in splitless mode for 1.5 min at 240C. The oven temperature was programmed to increase from 40 (5 min) to 240C at $4C/min^{-1}$ and held 5 min. Aroma compounds were separated on a DB-Wax polar capillary column (30 m × 0.32 mm i.d. × 0.50 µm film thickness) from Agilent (J&W Scientific, Folsom, CA). Helium was used as the carrier gas at a flow rate of 1.5 ml/min. For the MS system (Agilent 5973N), the temperatures of the transfer line, quadrupole, and ion source were 250, 150, and 230C, respectively. Electron impact mass spectra were recorded at 70 eV ionization voltages, and the ionization current was 10 µA. The acquisitions were performed in scan (from 35 to 350 amu) and SIM modes. The identification of compounds was based on the comparison of retention times and mass spectra from three databases: NIST 2.0 and WILEY 138. Relative peak areas (RPAs) were obtained by calculating the relative peak area in relation to that of the internal standard. The use of RPAs data to express aroma release was sufficient for this type of analysis as the aim of the work was to compare the extent of aroma recovery between saliva samples. Each salivary sample ($n = 30$) was analyzed independently and in triplicate (one injection per sample vial).

Linearity and repeatability of the procedure were determined in the aqueous solution at four levels of concentration (0.1, 1, 5, and 10 mg/L), with coefficients of determination (R^2) higher than 0.99 and relative standard deviations lower than 3% for all the assayed compounds. The same LLE procedure was carried out with the salivas and water samples without added aroma compounds to detect any possible compound coming from the salivas or any artifact formed during the extraction procedure. Stability of the aroma compounds and that of the GC/MS was checked in controls during all the analysis period and no significant differences were obtained across days ($p > .05$).

2.6 | Statistical analyses

Linear regression analyses were performed to establish the linearity of the response of each aroma compound by GC and GC/MS. Normality test on the two populations (HPS versus control) regarding the SSFR confirmed that both groups were normally distributed. ANOVA analyses and the least significant differences (LSD) test were used to determine significant differences between the groups. The significance level was $p < .05$ throughout the study. The XLstat program was used for data processing (StatSoft, Inc., 2005, www.statsoft.com).

3 | RESULTS AND DISCUSSION

3.1 | Description of the panel

As it can be seen in Table 2, the initial population was formed by 110 persons aged between 65 and 87 year olds (mean age = 72 year olds). Averaged unstimulated salivary flow rate determined for the whole group was 0.31 (±0.18) and averaged stimulated salivary flow rate was 1.63 (±0.82) ml/min.

From this population, 15 individuals presented hyposalivation (measured as the presence of a SSFR lower than 0.8 ml/min Soini, Routasalo, Lauri, & Ainamo, 2003). Therefore, these 15 individuals formed the hyposalivator group (mean SSFR for the hyposalivator group = 0.61 $\pm$ 0.18 ml/min). The control group was constituted by individuals ($n = 15$) presenting a SSFR similar to the mean SSFR of the initial population (mean SSFR for the control group = 1.69 $\pm$ 0.17 ml/min). Both groups were matched by age and sex. This resulted in a higher proportion of women than men in this study. This is because people suffering from hyposalivation in the initial population were mostly women. This gender difference on salivary flow rate has already been described in several articles, and it could be due to the smaller salivary gland size of women comparing to men (Inoue et al.,

TABLE 2 Description of the elderly panel

	Initial population	Hyposalivator group	Control group	p Value
Number of subjects	110	15	15	
Sex (male; female)	51; 59	3; 12	3; 12	
Mean age ($\pm$SD)	71.6 ($\pm$5.5)	72.3 ($\pm$5.6)	71.4 ($\pm$4.3)	
Mean BMI[1] (kg/m^2)	27.4 ($\pm$4.3)	**28.6 ($\pm$5.1)[a]**	**25.2 ($\pm$3.9)[b]**	.0500
USFR[2] (min; max) (ml/min)	0.05; 0.90	0.05; 0.41	0.16; 0.51	
Mean USFR (ml/min)	0.31 ($\pm$0.18)	**0.15 ($\pm$0.10)[b]**	**0.32 ($\pm$0.11)[a]**	.0002
SSFR (min; max) (ml/min)	0.23; 4.55	0.23; 0.80	1.35; 1.93	
Mean SSFR[3] (ml/min)	1.63 ($\pm$0.82)	**0.61 ($\pm$0.18)[b]**	**1.69 ($\pm$0.17)[a]**	$\leq$**.0001**

In bold, statistical differences between the hyposalivator and control groups. Different letters across the different groups denotes statistical differences after the application of the LSD test.
[1]Body mass index (BMI, Kg/m^2).
[2]USFR, Unstimulated salivary flow rate.
[3]SSFR, stimulated salivary flow rate.

2006). Table 2 shows that, as expected, the hyposalivator group presented significantly lower unstimulated and stimulated salivary flow rates than the control group. Moreover, the body mass index (BMI) of people forming the hyposalivator group was significantly higher than that of those from the control group (Table 2). An association between high BMI and hyposalivation has already been found by Flink, Bergdahl, Tegelberg, Rosenblad, and Lagerlof (2008). However, the explanations for this association are so far inconclusive. It has been suggested that both parameters could be related to malnutrition, by the appearance of diabetes, cardiovascular diseases, cancer, osteoporosis and caries, or other chronic diseases that might affect both the weight and/or the salivary gland function of the people (Flink et al., 2008).

3.2 | Aroma release after the interaction of the selected aroma compounds with water or with salivas from the hyposalivator and control groups
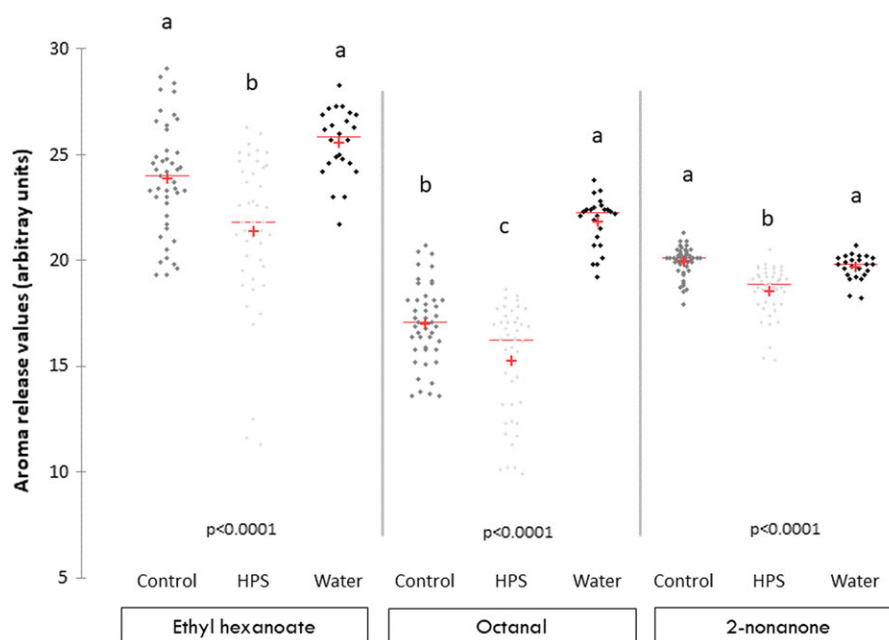
Figure 1 shows the aroma release values obtained for each of the three aroma compounds in the water and saliva samples (hyposalivator and control groups) together with the results of the LSD test. As it can be seen, all the assayed compounds were significantly less released in the hyposalivator group compared to the control group and the water samples, although the extent of the effect varied among compounds. Interestingly, octanal was the compound most affected by saliva and the only significantly different between the control group and the water samples. The differences observed among compounds cannot be only explained on the basis of some physicochemical properties of the aroma compounds, such as hydrophobicity or boiling point, as these characteristics were similar for the tested molecules (see Table 1). The effect of saliva on aroma compounds varied as a function of their chemical family. The higher effect of human saliva on aldehydes and esters than on

monoketones has been previously reported in several publications performed under ex vivo conditions (Buettner, 2002a, 2002b; Munoz-Gonzalez et al., 2018b; Pages-Helary et al., 2014).

The decrease in the release could be mostly due to two phenomena. On the one hand, aroma compounds might be retained by salivary components (such as proteins), as it has been previously suggested (Pages-Helary et al., 2014). On the other hand, these results could be explained by a transformation of the aroma compounds (Buettner, 2002a, 2002b; Munoz-Gonzalez et al., 2018b; Pages-Helary et al., 2014) through enzymatic or nonenzymatic reactions by saliva. In any case, the fact that in average, salivas from elderly people suffering from hyposalivation decreased more the release of aroma compounds than saliva from individuals presenting a normal salivary flow could be at the origin of aroma perception anomalies in this population. To the best of the authors' knowledge, this is the first time that this finding has been shown. However, in our study, people from the hyposalivator group presented also a higher BMI compared to those of the control group. In this regard, Piombino et al. (2014) have already shown that saliva from young obese individuals (high BMI) supresses aroma release compared to saliva from normal-weight subjects. Therefore, in vivo studies controlling for confounding factors (such as BMI) will be needed to elucidate the role of hyposalivation on retronasal aroma release and perception.

3.3 | Metabolization of aroma compounds by salivas from the hyposalivator and control groups

The possible metabolization of the assayed aroma compounds by saliva was investigated by LLE-GC/MS. After incubating the aroma compounds in the presence of each of the 30 saliva samples or water during 30 min at 37C, aroma compounds were extracted with dichloromethane and analyzed by GC–MS. Blanks of saliva without the

FIGURE 1 Aroma release values obtained after the incubation of the three aroma compounds at 37C during 30 min with water or with salivas (n = 30) from the HPS or control groups and analyzed by HS-GC. Different letters across the different groups denote statistical differences after application of the LSD test

aroma compounds or the aroma compounds without saliva were also analyzed in the same conditions.

A one-way ANOVA analysis was performed on the extracted amounts of octanal, ethyl hexanoate, 2-nonanone after their incubation in the presence of the 30 saliva samples or water. These results are shown in Table 3. As it can be observed, the three assayed compounds presented a significantly lower recovered quantity of the three aroma compounds in the saliva samples from the hyposalivator group compared to the water samples and to the salivas from the control group. In agreement with the aroma release analyses, octanal was the only compound significantly different between the control group and the water samples. Table 3 also shows that the recovered quantity of the three aroma compounds was significantly lower in the salivas from the hyposalivator group compared to those of the control group. Assuming the fact that the addition of the solvent to the samples would loss the integrity of salivary proteins, noncovalent interactions between protein and aroma compounds are unlikely to survive to the solvent changes. Therefore, they are unlikely to explain the different recovery between the two groups. Therefore, a stronger metabolization of the three compounds in the hyposalivator group compared to the control group is the most plausible mechanism to explain our results, despite that the involvement of noncovalent interactions cannot be excluded.
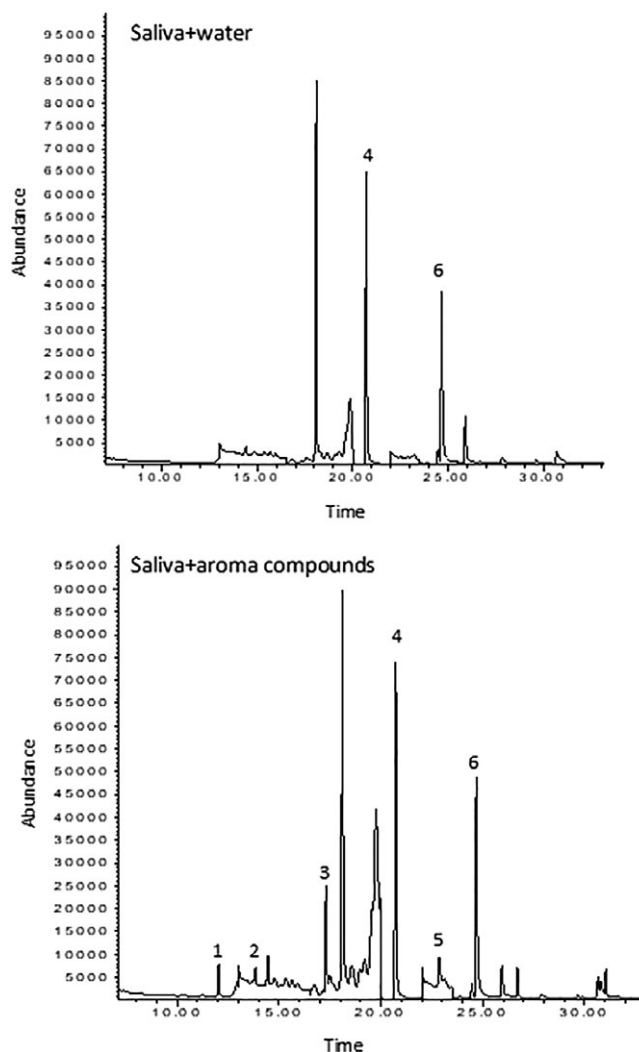
Additionally, the presence of metabolites was checked to investigate which type of conversion suffered the aroma compounds by saliva. The type of reaction was clearly confirmed for octanal that was reduced to 1-octanol (Figure 2). In agreement, no octanol was found in the water samples and a significant lower production of 1-octanol was obtained in the control group compared to the hyposalivator group (Table 3). In the case of ethyl hexanoate, its expected metabolite (the hexanoic acid) was also detected in the blanks of saliva without aroma compounds added (Figure 2). Therefore, its formation by the effect of salivary components could not be definitely proven in the present study. Finally, no metabolite was detected for the 2-nonanone (Figure 2), which was possibly due to a limitation on the sensibility of our method.

Overall, these findings confirm a different metabolization rate of aroma compounds in the two salivary groups. Therefore, these results suggest that elderly people suffering from hyposalivation could present an altered aroma perception (e.g., due to a lower availability of aroma compounds to reach the olfactory receptors retronasally and/or to a higher formation of new aroma compounds [i.e., metabolites] which might present different odor thresholds and qualities). This relevant finding needs to be verified in vivo.

FIGURE 2 Chromatograms corresponding to one saliva sample incubated with water or the same saliva incubated with the aroma compounds selected for this study. Numbers in the chromatogram correspond to the compounds: 1. Ethyl hexanoate, 2. Octanal, 3. 2-nonanone, 4. Methyl nonanoate (IS), 5. Octanol, and 6. Hexanoic acid

3.4 | Researching the origin of the differences observed on aroma release and metabolism between the hyposalivator and the control groups

In a previous study performed with artificial or pooled human saliva, it has been suggested that salivary proteins such as mucin and alpha-amylase could interact with aroma compounds, affecting their release to the headspace (Pages-Helary et al., 2014). In another work, Piombino et al. (2014) showed that saliva from obese individuals presented a significantly higher total antioxidant capacity and lower ex vivo aroma release than saliva from normal-weight subjects. In agreement with those papers, Munoz-Gonzalez et al. (2018b) observed that the highest the total protein content and the total antioxidant capacity of the saliva samples, the lowest the ex vivo aroma release. However, the latter study was performed with a limited number of saliva samples ($n = 3$). Consequently, the protein content and the total antioxidant seem to be two factors affecting the behavior of aroma compounds in presence of saliva. To validate this hypothesis,

TABLE 3 Relative peak areas (related to the internal standard) of the aroma compounds extracted after the incubation at 37°C during 30 min with water or the salivas from the hyposalivator and control groups and determined by LLE-GC/MS

	Ethyl hexanoate	Octanal	2-Nonanone	Octanol
Water	0.41[a]	0.27[a]	1.66[a]	0.00[c]
Control	0.37[a]	0.19[b]	1.54[a]	0.24[b]
Hyposalivator	0.25[b]	0.15[c]	1.39[b]	0.33[a]
p Value	<.0001	<.0001	.0003	<.0001

Different letters across the different groups denote statistical differences after application of the LSD test.
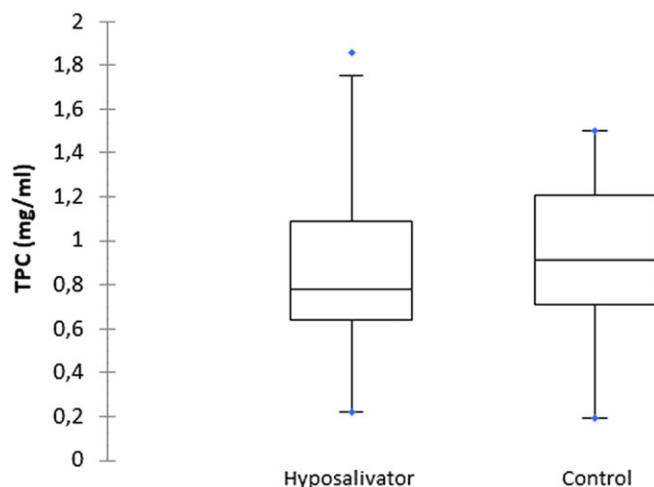
these parameters were analyzed in the 30 saliva samples selected for this study and results are shown below.

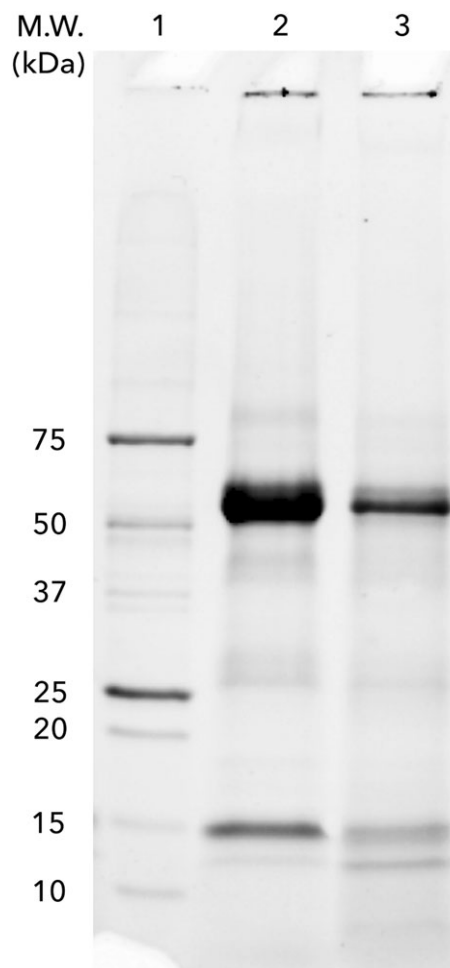### 3.4.1 | Total protein content and protein profile of saliva from hyposalivators or control subjects

As it can be seen in Figure 3, the total protein content (TPC) values between the hyposalivator and control groups were not significantly different ($p = .710$). Therefore, this parameter did not seem to be enough to explain the differences in the aroma release and metabolization observed in the two salivary groups of the present study. These differences could be more likely due to specific noncovalent interactions or enzymatic reactions. In this regard, Pages-Helary et al. (2014) suggested that specific salivary proteins such as mucin and alpha-amylase affect the release of esters and monoketones. Consequently, we further explored the protein profile of the 30 saliva samples through electrophoresis to know if the relative amount of specific proteins could better explain the aroma release and metabolization data. One example of the protein profiles obtained is shown in Figure 4. From that analysis, seven spots were identified in more than the 80% of the saliva samples and their relative abundances determined. However, the determination of mucins could not be possible due to the high molecular weight of these proteins and no significant differences were observed between groups in the band corresponding to the protein alpha-amylase between groups (55 kDa). This could be due to the fact that different proteins could have comigrated in the same band of the gel, which could have masked the differences of specific proteins between groups. Interestingly, three bands, in the range of 10–15 kDa, were significantly different between the two salivary groups ($p < .1$). Consequently, these preliminary results open the door to new proteomics studies to identify the protein(s) involved in the observed results.

3.4.2 | Total antioxidant capacity of saliva from hyposalivators or control subjects

To check a possible role of TAC on the differences on aroma release and metabolism observed in the two groups, TAC was analyzed in the

FIGURE 4 Protein profiles corresponding to the protein standard (1) and the stimulated salivary samples from two subjects: one hyposalivator (2) and one control (3)
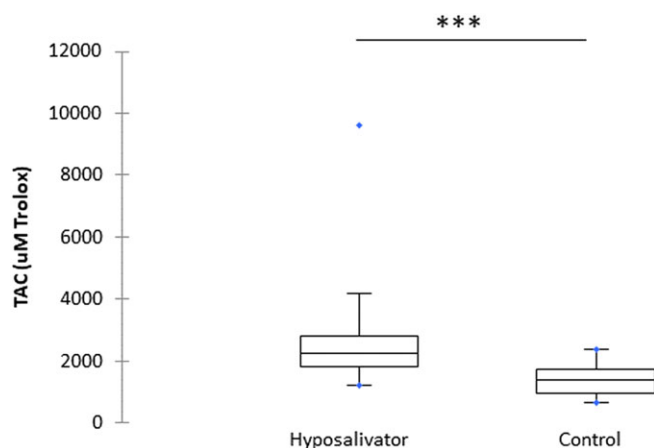

FIGURE 3 Box plot representation of the TPC determined in the salivas from the hyposalivator and control groups. Box outline represents lower and upper quartiles, the line inside the box is the median value and the circles correspond to the smallest and largest observations

30 saliva samples and results showed significant differences in the TAC values between both groups (Figure 5). In this regard, the hyposalivator group presented significantly higher TAC values than those of the control group, which might explain at least partially the higher metabolization rate and thus, the lower aroma release observed for this group. In previous papers, it has been suggested a negative association between the salivary TAC and the ex vivo release of aroma compounds (Munoz-Gonzalez et al., 2018b; Piombino et al., 2014). Although the mechanisms on how TAC impact aroma release are to date unclear, these effects could be related to the oxidative status of the saliva samples, which could affect the aroma compounds prone to redox reactions (Piombino et al., 2014). Recently, it was ascertained that the presence of NADH helped to take place at a higher rate the enzymatic conversion of aroma compounds (octanal) by saliva (Munoz-Gonzalez et al., 2018b). NADH is a coenzyme, which is used as a reducing agent. Moreover, it has been suggested a relation between the antioxidant status and the total amount of reducing agents in saliva (Kohen, Tirosh, & Kopolovich, 1992). Therefore, it is possible that people with high TAC have also high concentration of NADH in their saliva. However, further research is required to validate this hypothesis.

Finally, it should be mentioned that the effects of hyposalivation on aroma compounds need to be validated in vivo, taking into account

FIGURE 5 Box plot representation of the TAC determined in the salivas from the hyposalivator and control groups. Box outline represents lower and upper quartiles, the line inside the box is the median value and the arms of the box correspond to the smallest and largest observations (***Significant differences $p < .0001$)

the control of confounding factors (age, gender, BMI, drug intake, diseases, mental status, or dental status). As stated above, salivary parameters might be modified by the health status of individuals. For example, it has been seen that obese people presented higher TAC values than normal-weight subjects (Piombino et al., 2014) and that patients with dementia presented lower TAC values than control subjects (Choromanska et al., 2017). Moreover, hyposalivation is frequently present in diverse systemic diseases and immunological disorders, such as obesity (Flink et al., 2008), diabetes mellitus (Conner, Iranpour, & Mills, 1970), or dementia (e.g., Alzheimer's dementia; Choromanska et al., 2017; Flink et al., 2008), and it can be also an adverse effect of the treatments (such as radiotherapy) used to treat them (Narhi et al., 1992; Schubert & Izutsu, 1987; Sreebny & Valdini, 1988; Valdez, Atkinson, Ship, & Fox, 1993). Therefore, as hyposalivation might have different origins, the dispersion of saliva composition of the subjects suffering it (and therefore its effect on aroma perception) could also be very high. Moreover, some of these conditions (e.g., dementia) could affect directly the sensory integration in the brain, masking the contribution of saliva to aroma perception.

4 | CONCLUSIONS

In conclusion, from a panel formed by 110 elderly people, 15 of them suffered from hyposalivation (measured as presenting a salivary flow rate lower than 0.8 ml/min), which represented the 13.6% of the population. Most of them were women.

Moreover, this work has demonstrated for the first time that interindividual differences of saliva from elderly people affect aroma release and ex vivo metabolization of aroma compounds. In particular, saliva from elderly people suffering from hyposalivation presented a lower release and/or a higher metabolization of aroma compounds (octanal, ethyl hexanoate, and 2-nonanone) than saliva from the control group. Although no differences on the total protein content between groups were found, the involvement of specific proteins on the present results cannot be discarded. Moreover, saliva from the

hyposalivator group presented a significantly higher TAC mean than that of the control group.

These findings could indicate that people suffering from hyposalivation could present an altered aroma perception due to a different amount of aroma compounds reaching the olfactory receptors by the retronasal pathway, concomitantly with the dissimilar formation of new metabolites with different sensory properties. However, this finding found under ex vivo conditions will need to be confirmed in an in vivo and more realistic situation, in which the influence of other orophysiological parameters can be taken into account and in times comparable with the eating process. Moreover, the control of confounding factors in future research is mandatory to assess if these differences among salivary groups observed ex vivo can have a sensory meaning.

ETHICAL STATEMENTS

Conflict of Interest: The authors declare no conflict of interest in publishing this work.
Ethical Review: This study was approved by the French Ethics Committee for Research (CPP Est I, Dijon, #14.06.03, ANSM #2014-A00071-46).
Informed Consent: Written informed consent was obtained from all study participants.

ORCID

Carolina Muñoz-González https://orcid.org/0000-0001-6185-7049

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. https://doi.org/10.1111/jgs.13652

Buettner, A. (2002a). Influence of human saliva on odorant concentrations. 2. Aldehydes, alcohols, 3-alkyl-2-methoxypyrazines, methoxyphenols, and 3-hydroxy-4,5-dimethyl-2(5H)-furanone. *Journal of Agricultural and Food Chemistry*, *50*(24), 7105–7110. https://doi.org/10.1021/jf020714o

Buettner, A. (2002b). Influence of human salivary enzymes on odorant concentration changes occurring in vivo. 1. Esters and thiols. *Journal of Agricultural and Food Chemistry*, *50*(11), 3283–3289. https://doi.org/10.1021/jf011586r

Castagnola, M., Cabras, T., Vitali, A., Sanna, M. T., & Messana, I. (2011). Biotechnological implications of the salivary proteome. *Trends in Biotechnology*, *29*(8), 409–418. https://doi.org/10.1016/j.tibtech.2011.04.002

Choromanska, M., Klimiuk, A., Kostecka-Sochon, P., Wilczynska, K., Kwiatkowski, M., Okuniewska, N., ... Maciejczyk, M. (2017).

Antioxidant defence, oxidative stress and oxidative damage in saliva, plasma and erythrocytes of dementia patients. Can salivary AGE be a marker of dementia? *International Journal of Molecular Sciences*, 18(10). pii: E2205. https://doi.org/10.3390/ijms18102205

Conner, S., Iranpour, B., & Mills, J. (1970). Alteration in parotid salivary flow in diabetes mellitus. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics*, 30(1), 55. https://doi.org/10.1016/0030-4220(70)90011-3

Dormenval, V., Mojon, P., & Budtz-Jorgensen, E. (1999). Associations between self-assessed masticatory ability, nutritional status, prosthetic status and salivary flow rate in hospitalized elders. *Oral Diseases*, 5(1), 32–38.

Feron, G., Ayed, C., Qannari, E. M., Courcoux, P., Laboure, H., & Guichard, E. (2014). Understanding aroma release from model cheeses by a statistical multiblock approach on oral processing. *PLoS One*, 9(4), e93113. https://doi.org/10.1371/journal.pone.0093113

Flink, H., Bergdahl, M., Tegelberg, A., Rosenblad, A., & Lagerlof, F. (2008). Prevalence of hyposalivation in relation to general health, body mass index and remaining teeth in different age groups of adults. *Community Dentistry and Oral Epidemiology*, 36(6), 523–531. https://doi.org/10.1111/j.1600-0528.2008.00440.x

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). Mini-mental state - practical method for grading cognitive state of patients for clinician. *Journal of Psychiatric Research*, 12(3), 189–198. https://doi.org/10.1016/0022-3956(75)90026-6

Genovese, A., Piombino, P., Gambuti, A., & Moio, L. (2009). Simulation of retronasal aroma of white and red wine in a model mouth system. Investigating the influence of saliva on volatile compound concentrations. *Food Chemistry*, 114(1), 100–107. https://doi.org/10.1016/j.foodchem.2008.09.022

Guichard, E., Repoux, M., Qannari, E. M., Laboure, 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. https://doi.org/10.1039/c6fo01472k

Inoue, H., Ono, K., Masuda, W., Morimoto, Y., Tanaka, T., Yokota, M., & Inenaga, K. (2006). Gender difference in unstimulated whole saliva flow rate and salivary gland sizes. *Archives of Oral Biology*, 51(12), 1055–1060. https://doi.org/10.1016/j.archoralbio.2006.06.010

Iwasaki, M., Yoshihara, A., Ito, K., Sato, M., Minagawa, K., Muramatsu, K., ... Miyazaki, H. (2016). Hyposalivation and dietary nutrient intake among community-based older Japanese. *Geriatrics & Gerontology International*, 16(4), 500–507. https://doi.org/10.1111/ggi.12500

Kohen, R., Tirosh, O., & Kopolovich, K. (1992). The reductive capacity index of saliva obtained from donors of various ages. *Experimental Gerontology*, 27(2), 161–168. https://doi.org/10.1016/0531-5565(92)90040-7

Leake, S. L., Pagni, M., Falquet, L., Taroni, F., & Greub, G. (2016). The salivary microbiome for differentiating individuals: Proof of principle. *Microbes and Infection*, 18(6), 399–405. https://doi.org/10.1016/j.micinf.2016.03.011

Mosca, A. C., & Chen, J. S. (2017). Food-saliva interactions: Mechanisms and implications. *Trends in Food Science & Technology*, 66, 125–134. https://doi.org/10.1016/j.tifs.2017.06.005

Muñoz-González, C., Feron, G., & Canon, F. (2018a). Main effects of human saliva on flavour perception and the potential contribution to food consumption. *Proceedings of the Nutrition Society*, 77, 423–431. https://doi.org/10.1017/S0029665118000113

Munoz-Gonzalez, C., Feron, G., Brule, M., & Canon, F. (2018b). Understanding the release and metabolism of aroma compounds using micro-volume saliva samples by ex vivo approaches. *Food Chemistry*, 240, 275–285. https://doi.org/10.1016/j.foodchem.2017.07.060

Munoz-Gonzalez, C., Feron, G., Guichard, E., Rodriguez-Bencomo, J. J., Martin-Alvarez, P. J., Moreno-Arribas, M. V., Pozo-Bayon, M. A. (2014). Understanding the role of saliva in aroma release from wine by using

static and dynamic headspace conditions. *Journal of Agricultural and Food Chemistry*, 62(33), 8274–8288. https://doi.org/10.1021/jf503503b

Muñoz-González, C., Vandenberghe-Descamps, M., Feron, G., Canon, F., Labouré, H., & Sulmont-Rossé, C. (2017). Association between salivary hypofunction and food consumption in the elderlies. A systematic literature review. *The Journal of Nutrition, Health & Aging*, 22, 407–419. https://doi.org/10.1007/s12603-017-0960-x

Narhi, T. O., Meurman, J. H., Ainamo, A., Nevalainen, J. M., Schmidtkaunisaho, K. G., Siukosaari, P., ... Mäkilä, E. (1992). Association between salivary flow-rate and the use of systemic medication among 76-year-old, 81-year-old, and 86-year-old inhabitants in Helsinki, Finland. *Journal of Dental Research*, 71(12), 1875–1880. https://doi.org/10.1177/00220345920710120401

Neyraud, E., Palicki, O., Schwartz, C., Nicklaus, S., & Feron, G. (2012). Variability of human saliva composition: Possible relationships with fat perception and liking. *Archives of Oral Biology*, 57(5), 556–566. https://doi.org/10.1016/j.archoralbio.2011.09.016

Pages-Helary, S., Andriot, I., Guichard, E., & Canon, F. (2014). Retention effect of human saliva on aroma release and respective contribution of salivary mucin and alpha-amylase. *Food Research International*, 64, 424–431. https://doi.org/10.1016/j.foodres.2014.07.013

Piombino, P., Genovese, A., Esposito, S., Moio, L., Cutolo, P. P., Chambery, A., ... Ercolini, D. (2014). Saliva from obese individuals suppresses the release of aroma compounds from wine. *PLoS One*, 9(1), e85611. https://doi.org/10.1371/journal.pone.0085611

Ployon, S., Morzel, M., & Canon, F. (2017). The role of saliva in aroma release and perception. *Food Chemistry*, 226, 212–220.

Samnieng, P., Ueno, M., Shinada, K., Zaitsu, T., Wright, F. A. C., & Kawaguchi, Y. (2012). Association of hyposalivation with oral function, nutrition and oral health in community-dwelling elderly Thai. *Community Dental Health*, 29(1), 117–123. https://doi.org/10.1922/CDH_2690Ueno07

Schipper, R. G., Silletti, E., & Vinyerhoeds, M. H. (2007). Saliva as research material: Biochemical, physicochemlical and practical aspects. *Archives of Oral Biology*, 52(12), 1114–1135. https://doi.org/10.1016/j.archoralbio.2007.06.009

Schubert, M. M., & Izutsu, K. T. (1987). Iatrogenic causes of salivary-gland dysfunction. *Journal of Dental Research*, 66, 680–688. https://doi.org/10.1177/00220345870660s213

Soini, H., Routasalo, P., Lauri, S., & Ainamo, A. (2003). Oral and nutritional status in frail elderly. *Special Care in Dentistry*, 23(6), 209–215.

Solemdal, K., Sandvik, L., Willumsen, T., Mowe, M., & Hummel, T. (2012). The impact of oral health on taste ability in acutely hospitalized elderly. *PLoS One*, 7(5), e36557. https://doi.org/10.1371/journal.pone.0036557

Sreebny, L. M., & Valdini, A. (1988). Xerostomia. 1. Relationship to other oral symptoms and salivary-gland hypofunction. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics*, 66(4), 451–458. https://doi.org/10.1016/0030-4220(88)90268-x

Valdez, I. H., Atkinson, J. C., Ship, J. A., & Fox, P. C. (1993). Major salivary-gland function in patients with radiation-induced xerostomia—Flow-rates and sialochemistry. *International Journal of Radiation Oncology Biology Physics*, 25(1), 41–47. https://doi.org/10.1016/0360-3016(93)90143-j