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Variability of human saliva composition: Possible relationships with fat perception and liking

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ABSTRACT

Saliva is the medium that bathes the taste receptors in the oral cavity and in which aroma and taste compounds are released when food is eaten. Moreover saliva contains enzymes and molecules that can interact with food. To date, little research has been devoted to the intra- and inter-individual variabilities of these components and their inter-relationships. The first aim of this work was to study intra- and inter-individual variabilities over time in the composition of molecules likely to interact with food in the mouth, with particular focus on molecules that might interact with fat. The second aim was to try to relate this composition to a liking for fat and its perception. Stimulated and unstimulated saliva from 13 subjects was collected in the morning and afternoon on three occasions at 4-month intervals. Saliva characteristics such as flow, protein concentration, lipolysis, proteolysis, amylolysis, lipocalin concentration, lysozyme activity, total antioxidant status and uric acid concentrations were measured, as well as the liking for and perceived fattiness of a fat solution. The results showed that for most of the measured characteristics, intra-subject variability in saliva composition was smaller than inter-subject variability, with remarkable stability over time (8 months) in terms of flow, lypolysis, proteolysis and total antioxidant status. Relationships were found between some of these characteristics (lipolysis, lipocalin and flow) and fat-liking or perception, showing that the composition of saliva may play an important role in fat perception and liking.

1. Introduction

Whole saliva is a complex mixture of fluids from the major (parotid, submandibular, sublingual) and minor (e.g. von Ebner) salivary glands, gingival crevicular fluid, oral bacteria and food debris.[1] The regulation of secretions from these salivary glands is complex and under the control of the sympathetic and parasympathetic autonomic systems.[2]

Saliva has many roles, the main one being to protect the oral cavity against pathogens or mechanical injuries such as friction, in relation to its lubricant properties. Saliva is also involved in sensory perception. Indeed, this role has become a growing research area in recent years, and articles have stressed the fact that the salivary medium could partly explain sensory perception.[3–5]

Saliva has a strong potential to interact with all food macronutrients because of the diversity of its enzymatic composition. Its amylolytic activity is the most well known,[6,7] but proteolytic[8] and lipolytic[9,10] activities have also been reported in human saliva. The active role of these enzymatic activities at the early stages of the digestion may be limited in terms of food degradation because of the short duration of contact with food in the mouth. However, some of the molecules released or modified by these enzymes can be perceived by the taste system. This is the case of maltose for

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sweet taste, of amino acids such as glutamate or aspartate for umami and of fatty acids for the putative fat taste. Fattiness appeared recently as a possible sixth taste with discovery of the implication of three candidate receptors that are able to induce a fat taste signal transduction: CD36, delayed-rectifying potassium (DRK) channel Kv1.5 and the G protein-coupled receptor-120 (GPCR120) (see Khan and Besnard for review[11]). Some other salivary enzymes or molecules can also interact with fat or emulsions. This is the case for lysozyme, which is used to stabilize emulsions.[12] Antioxidant enzymes and compounds such as vitamins, glutathione or uric acid, which together are responsible for the antioxidant capacity of saliva,[13] may also modulate the intra-oral oxidation of lipids and/or reactive lipid species. Other proteins such as von Ebner's gland protein (VEGP)[14] belonging to the lipocalin family may also have a regulating role in fat detection as a fatty acid solubiliser.[15]

The inter-individual variability of saliva composition in the population is not well known. However, this variability could be linked to variability in food perception or liking. Perry et al.[16] showed that populations with high starch diets have more copies of the salivary amylase gene (AMY1) than those eating traditionally low-starch diets. Interestingly, the intra-individual variability of saliva appears to be quite small when compared with inter-individual variability. For instance, individual daily stability of the salivary proteome is high in a context of high inter-individual variability.[17,18]

This paper is based on the hypothesis that the composition of the salivary medium surrounding the taste receptors varies within the population (inter-individual variability) but is stable at the individual level (intra-individual variability). As a consequence, this inter-individual variability in some biochemical parameters could explain differences in the taste-active compounds released in the salivary medium and, as a result, differences in perception or liking. However, in order to prove this hypothesis, the question of the stability of salivary characteristics over time must be considered.

The first aim of this work was therefore to evaluate the intra- and inter-subject variability of food-related enzymatic activities in saliva. In view of the marked variability found in biochemical characteristics related to fat, the second objective was to try to relate saliva properties with fattiness perception and liking for fat.

2. Materials and methods

2.1. Saliva collection

Whole unstimulated and stimulated saliva was collected at 10 a.m. and 3 p.m. on three occasions at 4-month intervals from 13 subjects (8 males and 5 females), aged between 26 and 52 years. The subjects were instructed to avoid eating or drinking (although water was allowed) for at least 1 h before saliva collection.

Unstimulated saliva was collected by asking the subjects to allow their saliva to flow into pre-weighed containers for 5 min.

Stimulated saliva was collected by asking the subjects to chew a piece of Parafilm (American National Can, Chicago, IL) for 5 min and then to spit out their saliva at regular intervals. The saliva flow was calculated from the weight of saliva, assuming 1 g being equal to 1 ml.

Immediately after collection, the samples were centrifuged for 30 min at 15,000 × *g* and the supernatants were placed in 1-ml aliquots and stored at −80 °C until biochemical analyses.

The protocol of the study was approved by the appropriate French Ethics Committees: Comité de Protection des Personnes Est-1 (No. 2008/15) and Direction Générale de la Santé-France (No. DGS2008-0196).

2.2. Biochemical analyses

2.2.1. Protein concentration

The protein concentration was determined using Bradford protein assay Quick Start (Bio-Rad, France) with gamma-globulin as the standard for calibration.

2.2.2. Enzyme activities

All enzyme activities are expressed in International Enzyme Unit Activity (U) per ml of saliva. One U is defined as the amount of the enzyme that catalyses the conversion of 1 μM of substrate per minute.

2.2.3. Lipolysis

Lipolysis was determined from the method described first by Roberts[19] for the detection of lingual lipase in rats, and modified by Beisson et al.[20] The buffer contained 50 mM Tris–HCl, pH 7.5, 4 mM $CaCl_2$, 2 mM EDTA (ethylenediaminetetraacetic acid), 0.2% (w/v) NaTDC (sodium taurodeoxycholate), 1 mM PMSF (phenylmethylsulphonyl fluoride), 1 mM DTT (dithiothreitol) and 0.02% (w/v) sodium azide. The substrate solution was prepared by vortexing 19 volumes of the above buffer for 10 s with 1 volume of an ethanolic solution of 4-methylumbelliferyl 7-oleate (Sigma–Aldrich, France) to obtain a final concentration of 1 mM. The reaction was started by adding 100 μl of saliva to 400 μM of substrate solution and 4 μl ethanol. An inhibition reaction was also performed on each sample by adding 4 μl of a 125 μM ethanolic solution of THL (tetrahydrolipstatin) instead of ethanol. After 40 min incubation at 37 °C, three samples (150 μl) of the reaction medium from each single well were transferred to a microtitre plate. The intensity of fluorescence was measured (excitation filter: 355 nm; emission filter: 460 nm) using a microtitre plate fluorometer (Victor 3-V, PerkinElmer, France). Lipolysis was calculated from the difference between the average activity of each sample with and without the lipase inhibitor THL. Activity was then read against a standard curve of umbelliferone. At each set of measurements, a control of the linearity and proportionality of the reaction was performed using commercial lipase (*Aspergillus niger* Lipase, Fluka, France).

2.2.4. Proteolysis

Proteolysis was determined using a Pierce Fluorescent Assay Kit (Pierce Biotechnology, Rockford, IL) as described by Wickström et al.[21] A fluorescein-labelled casein substrate liberates fluorescein fragment during proteolytic digestion (excitation at 494 nm/emission at 518 nm).

2.2.5. Lysozyme activity

Lysozyme activity was determined using an EnzCheck Lysozyme Assay Kit (Molecular Probes, The Netherlands). This kit

is based on measuring lysozyme activity on a *Micrococcus lysodeikticus* substrate labelled with fluorescein. The intensity of fluorescence proportional to lysozyme activity is read against a lysozyme standard and expressed in unit/ml/min.

2.2.6. Amylolysis

Amylolysis was determined by measuring the rate of maltose release during the incubation at 30 °C of starch with saliva extracts, using the 3,5-dinitrosalicylic acid (DNS) assay.[22] Briefly, saliva extracts were added to a solution containing 0.5% Extra Pure Starch (Merck) in phosphate buffer 0.02 M, pH 6.9. An aliquot was added to a 1% DNS solution in Na–K tartarate at 2, 4, 6, 8 and 10 min and absorbance was measured at 540 nm. The standard curve was established using a maltose solution.

2.2.7. Dot-blot immunoassay for lipocalin concentration

Dot-blot analysis was carried out using a nitrocellulose membrane (Bio-Dot Microfiltration Apparatus Instruction Manual, Richmond, CA). The membrane was first soaked with Tris-buffered saline (TBS), then it was placed in a Bio-Dot Microfiltration Apparatus (Bio-Rad). The membrane was rehydrated using 100 μl TBS per well, which was later vacuum extracted. This was followed by the fixation of 150 μl of 100 kDa ultrafiltered saliva or standard LCN1 recombinant protein (Tebu Bio, France). After complete filtration, the membrane was immersed and gently shaken for 50 min in a 0.5% Tween 20 TBS solution (TTBS) containing 10% non-fat milk. The membrane was then washed 3 times for 10 min in TTBS and immersed in a 60 ml TTBS solution added with a 5 μl LCN1 polyclonal antibody (Tebu Bio, France) and gently shaken for 50 min. After washing five times for 10 min in TTBS, the membrane was immersed and gently shaken in 30 μl of Immun Star GAM HRD conjugate and 6 μl precision strep tactin–HRP conjugate (Bio-Rad) in 60 ml TTBS. After washing four times for 10 min in TTBS and once in TBS, the membrane was gently shaken for 5 min in a 6 ml luminol enhancer and 6 ml peroxide buffer (Immun Star HRP Substrate Kit, Bio-Rad) and the luminescence was read using a microtitre plate fluorometer (Victor 3-V, PerkinElmer, France). The results are expressed as relative equivalent LCN1 per ml of saliva in reference to a calibration curve obtained on the same microplate with the standard LCN1.

2.2.8. Total antioxidant status (TAS)

The total antioxidant status was determined using an Antioxidant Assay Kit (Zen-bio, Research Triangle Park, NC). A ferryl radical is formed from metmyoglobin and hydrogen peroxide. The ferryl myoglobin radical can oxidize ABTS (2,2′-azino-bis[3-ethylbenzthiazoline-6-sulfonic acid]) to generate a radical cation, $ABTS^{\bullet+}$, measured by absorbance at 405 nm. Antioxidants inhibit the formation of the $ABTS^{\bullet+}$ radical. The antioxidant capacity of saliva is expressed in trolox equivalent, a vitamin E analogue.

2.2.9. HPLC: uric acid chromatography

The HPLC system consisting of a pump, a column oven, an auto injector, and an Integrated Amperometry detector (gold working electrode, Ag/AgCl reference electrode) came from the DIONEX ICS 3000 series. The separation of compounds was achieved using a HyperREZ Organic Acid (300 mm × 77 mm, 8 μm) column. The column oven was controlled at 40 °C. Sulphuric acid (5 mM) was used as the mobile phase. The chromatograph was operated isocratically with a flow-rate of 1 ml/min. The injection volume was 20 μl. The total run time was 15 min. The HPLC retention times were compared with those of authentic standards to enable the identification of uric acid. Results are expressed in μg of uric acid per ml of saliva.

2.3. Determinations of fat intensity and liking for fat

2.3.1. Stimulus development

The fat emulsion consisted of a mixture of half sunflower oil and half rapeseed oil in mineral water (Evian, France) at the concentration of 3.5% (w/v). To obtain a homogeneous emulsion, soy lecithin (Jerafrance, Jeufosse, France) was added at a concentration of 1% (w/v). To ensure the stability of the fat emulsion, it was carefully blended with an ultra turrax type homogenizer (Ika, Staufen, Germany) (stem KV09/04, operating speed: 9500 rpm) for 20 min. The fat emulsion was then sonicated for 16 min. It was freshly prepared for each experiment and stored at +4 °C. The stimuli were offered at room temperature in a 25-ml plastic cup containing 5 ml of solution.

The basic taste solutions were made up of mineral water (Evian, France) and food-grade or pharmacological-grade tastants (Jerafrance, Jeufosse, France). The concentrations were 0.20 M for lactose, 0.085 M for sodium chloride, 0.18 M for urea, 0.006 M for citric acid and 0.009 M for MSG.

All these solutions were also freshly prepared and stored at +4 °C.

All stimuli were offered at room temperature in a 25-ml white plastic cup containing 5 ml of solution.

2.3.2. Determinations of fat intensity and liking

The experiments were performed in a room designed for sensory evaluation. The subjects were asked to neither eat nor drink for 1 h before the session.

The subjects were instructed to score their liking for and the intensity of the perceived fattiness. They were firstly asked to score their liking for the solution using a 9-point scale ranging from ''I do not like it at all'' scored as ''1'' to ''I like it very much'' scored as ''9''. To evaluate the intensity of perceived fattiness, the panellists were asked to use a 9-point scale from ''Really weak'' to ''Really strong''. The evaluations were performed five times because the fat emulsion was always presented in a triplet between the presentations of two water solutions with the same taste (e.g. sweet–fat emulsion–sweet). In addition to replicating the evaluations for the fat emulsion, a concomitant objective was to estimate the potential impact of tasting basic taste solutions (i.e. sweetness, saltiness, bitterness, sourness and umami) on the perception of the fat emulsion.

The data were collected with the help of FIZZ (Fizz, Biosystèmes, Couternon, France).

2.4. Data analysis

Statistical analyses were performed using STATISTICA 8 software (StatSoft, Tulsa, OK) and XL Stat software (Addinsoft,

Paris, France). Normality and variance homogeneity were checked systematically and found for all variables. For all biochemical parameters, an analysis of variance (ANOVA) and repeated-measures ANOVA were performed with a model including the main effects of the sampling time, the condition of saliva stimulation and the subject. When appropriate, differences between means were tested using Fisher's multiple range test.

Principal component analyses (PCA) were performed on median values of each biochemical parameter which did not change significantly during the three periods of sampling for each subject under stimulated and unstimulated conditions. Fat liking and fattiness perception were supplementary variables. Pearson correlation coefficients were calculated between the intensity of fat perception and liking and biochemical parameters on the mean values of the 13 subjects under stimulated and unstimulated conditions with a 5% level of statistical significance.

For all analyses, a probability of $P < 0.05$ was considered to be significant. The results are presented as mean ± SD.

3. Results

3.1. *Effects of sampling conditions on saliva characteristics*

Table 1 presents the effects of the sampling time, the type of stimulation type and the subject on saliva characteristics. Stimulated saliva flow was significantly higher (1.83 ± 0.70 ml/min) than that of unstimulated saliva flow (0.69 ± 0.43 ml/min), without any circadian effect. The effect of stimulation was significant for all variables except lipocalin and amylolysis. Values were higher under unstimulated conditions with respect to protein concentration, lipolysis, lysozyme, TAS and uric acid, and were higher under stimulated conditions for flow and proteolysis. The sampling time effect was significant with respect to protein concentration, lysozyme, lipocalin concentration, uric acid and amylolysis.

Furthermore, repeated-measures ANOVA revealed significant differences concerning the protein concentration ($F = 11.37$; $P < 0.0001$), lysozyme ($F = 13.04$; $P < 0.0001$), uric acid ($F = 72.64$; $P < 0.0001$) and amylolysis ($F = 4.22$; $P < 0.05$), indicating an effect of the month of sampling. Subject-repetition interactions were observed in particular for protein ($F = 1.68$; $P < 0.05$), uric acid ($F = 3.73$; $P < 0.0001$) and amylolysis ($F = 1.95$; $P < 0.05$). By contrast, other saliva variables displayed stability over the 8-month period, and particularly flow, lipolysis, lipocalin, proteolysis and TAS.

3.2. *Intra- and inter-subject variability of saliva characteristics*

A significant subject effect was found for all saliva characteristics (Table 1).

Fig. 1 presents the flow rates of stimulated (a) and unstimulated (b) saliva for all 13 subjects. Intra-subject variability was lower than inter-subject variability. The intra-subject coefficient of variation was 0.28 for unstimulated saliva and 0.16 for stimulated saliva, whilst inter-subject variability of the coefficient of variation was 0.62 for unstimulated saliva and 0.40 for stimulated saliva. Moreover, flow patterns differed markedly between subjects. Subjects 3, 4 and 13 had low flow rates under both conditions whereas subject 12 had a low unstimulated flow but a high stimulated flow. Subjects 7, 8 and 11 had high flows under both unstimulated and stimulated conditions.

Inter-individual variability regarding biochemical parameters can be seen in Fig. 2 for the protein concentration and

Table 1 – Effects of stimulation type (stimulated and unstimulated), of sampling time (morning and afternoon) and of subject ($n = 13$) on saliva biochemical characteristics (mean ± SD). Means with the same letter are not different ($P < 0.05$).

	Stimulated		Unstimulated		Stimulation	Sampling time	Subject
	Morning	Afternoon	Morning	Afternoon	*F/P*	*F/P*	*F/P*
Flow (ml/min)	1.84 ± 0.69^{b}	1.81 ± 0.73^{b}	0.64 ± 0.40^{a}	0.74 ± 0.45^{a}	$F = 424$ $P < 0.0001$	NS	$F = 23.4$ $P < 0.0001$
Protein (mg/ml)	1.68 ± 0.56^{a}	2.09 ± 1.12^{ab}	2.14 ± 0.94^{ab}	2.37 ± 1.33^{b}	$F = 7.02$ $P < 0.01$	$F = 5.87$ $P < 0.05$	$F = 4.55$ $P < 0.0001$
Lipolysis (U/ml)	0.27 ± 0.22^{a}	0.39 ± 0.28^{a}	0.60 ± 0.51^{b}	0.68 ± 0.62^{b}	$F = 16.9$ $P < 0.0001$	NS	$F = 3.77$ $P < 0.0001$
Lipocalin (equiv. LCN1/ml)	0.89 ± 0.51	1.03 ± 0.69	0.97 ± 0.88	1.08 ± 0.63	NS	$F = 4.53$ $P < 0.05$	$F = 2.86$ $P < 0.01$
Lysozyme (U/ml)	101 ± 75^{a}	123 ± 62^{a}	217 ± 97^{b}	267 ± 118^{c}	$F = 60.61$ $P < 0.0001$	$F = 6.86$ $P < 0.05$	$F = 2.79$ $P < 0.001$
Total antioxidant status (trolox equiv./ml/min)	47.7 ± 70.5^{a}	61.6 ± 73.5^{a}	116.4 ± 73.0^{b}	135.1 ± 79.7^{b}	$F = 100.8$ $P < 0.0001$	NS	$F = 17.5$ $P < 0.0001$
Uric acid (μg/ml)	39.4 ± 26.6^{ab}	32.6 ± 24.1^{a}	55.9 ± 28.8^{c}	45.6 ± 19.5^{b}	$F = 15.16$ $P < 0.0001$	$F = 5.04$ $P < 0.05$	$F = 4.45$ $P < 0.0001$
Proteolysis (U/ml)	0.183 ± 0.2^{ab}	0.232 ± 0.487^{b}	0.073 ± 0.075^{a}	0.085 ± 0.08^{a}	$F = 7.92$ $P < 0.01$	NS	$F = 2.07$ $P < 0.05$
Amylolysis (U/ml)	43.7 ± 18.2	47.5 ± 13.9	45.6 ± 19.8	49.6 ± 18.3	NS	$F = 4.27$ $P < 0.05$	$F = 8.52$ $P < 0.0001$

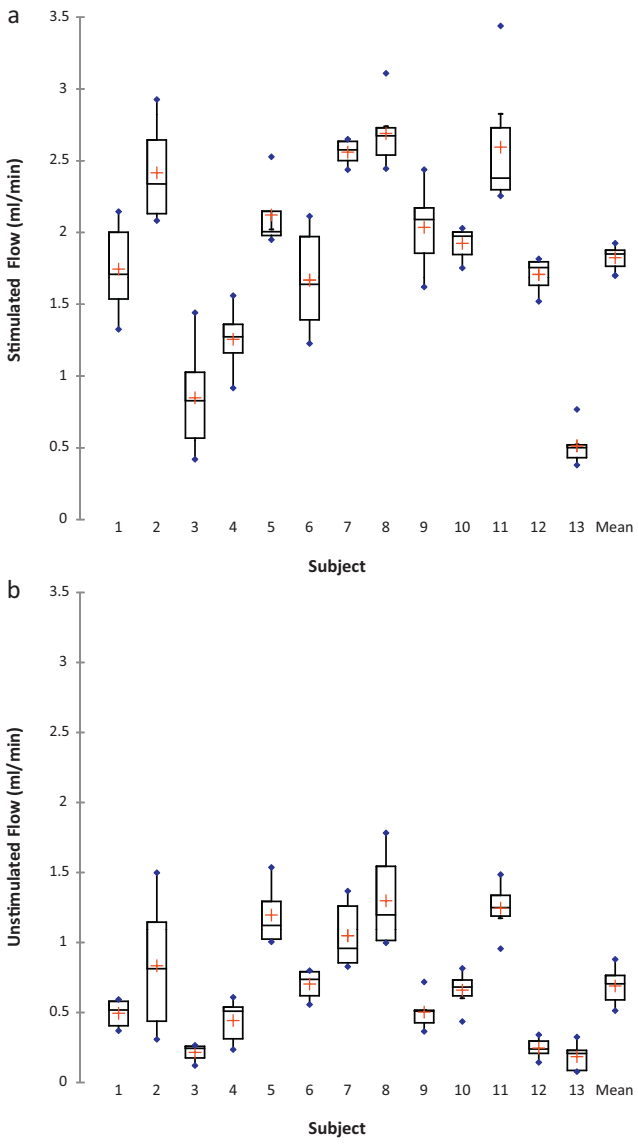

Fig. 1 – Box plot representation of stimulated (a) and unstimulated (b) salivary flow (n = 6) collected from the 13 subjects. Box outline represents lower quartile and upper quartile, outermost points correspond to the smallest and largest observation, the horizontal line is the median value, and the cross in the box is the mean value.

main enzyme activities (lipolysis, amylolysis and proteolysis) and in Fig. 3 for other characteristics. For all parameters and under both types of stimulation, inter-individual variability was systematically higher than intra-individual variability (results not shown).

3.3. *Relationships between saliva characteristics and fat-liking and fattiness perception*

The mean score for fattiness perception of the fat emulsion was 6.1 (median = 6.0), with subject means ranging from 2.6 to 9.0 (Fig. 4a), whilst the mean score for liking was 2.5 (median = 2.6), with subject means ranging from 1.0 to 4.8 (Fig. 4b). No significant effect of the tastant tasted before the fat stimulus was observed, showing that fat intensity (F = 0.29) and liking (F = 0.98) were not affected by a previous stimulation with another tastant. No effects of fat stimulation were observed on the intensity scores for each tastant before and after stimulation. However, significant effects were observed on the liking scores for saltiness ($P < 0.001$) – with liking scores of 6 before and 2.5 after fat stimulation – and umami ($P < 0.05$), with liking scores of 2 before and 6 after fat stimulation.

Fig. 5 presents the PCA of the different biochemical parameters in stimulated saliva (Fig. 5a) and unstimulated saliva (Fig. 5b), with fattiness intensity and liking for fat as supplementary variables. To prevent any bias in interpretation, only variables that did not show any significant differences by repeated measure ANOVA were considered, i.e. flow, lipolysis, lipocalin, proteolysis and TAS.

For stimulated saliva, the first two axes explain 64.34% of variance. The first axis (42.19%) mainly represents information

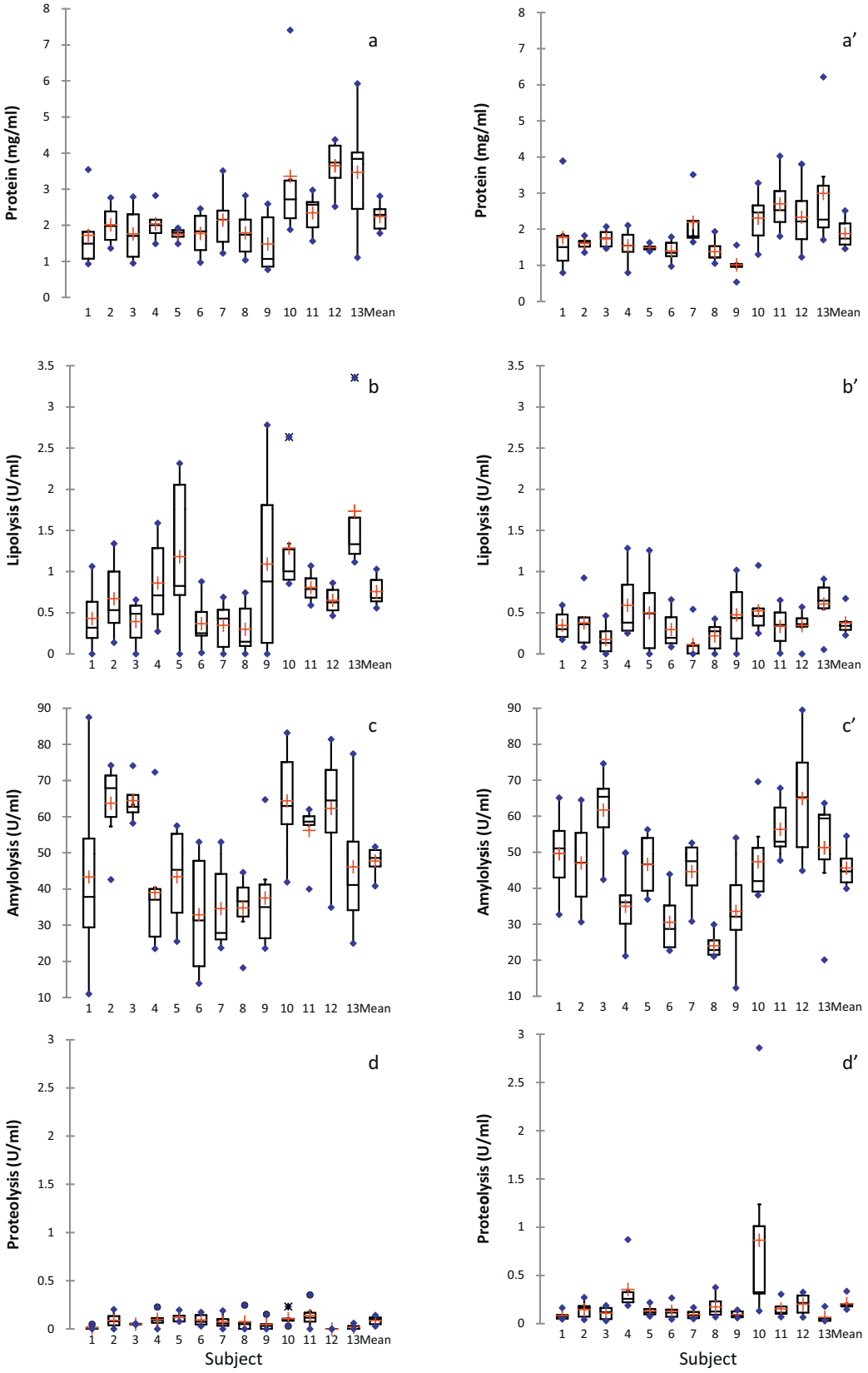

Fig. 2 – Box plot representations (n = 6) of protein concentration (a, a′), lipolysis (b, b′), amylolysis (c, c′) and proteolysis (d, d′) of saliva from the 13 subjects collected without (left figures, x) or with stimulation (right figures, x′). Box outline represents lower quartile and upper quartile, outermost points correspond to the smallest and largest observation, the horizontal line is the median value, and the cross in the box is the mean value.

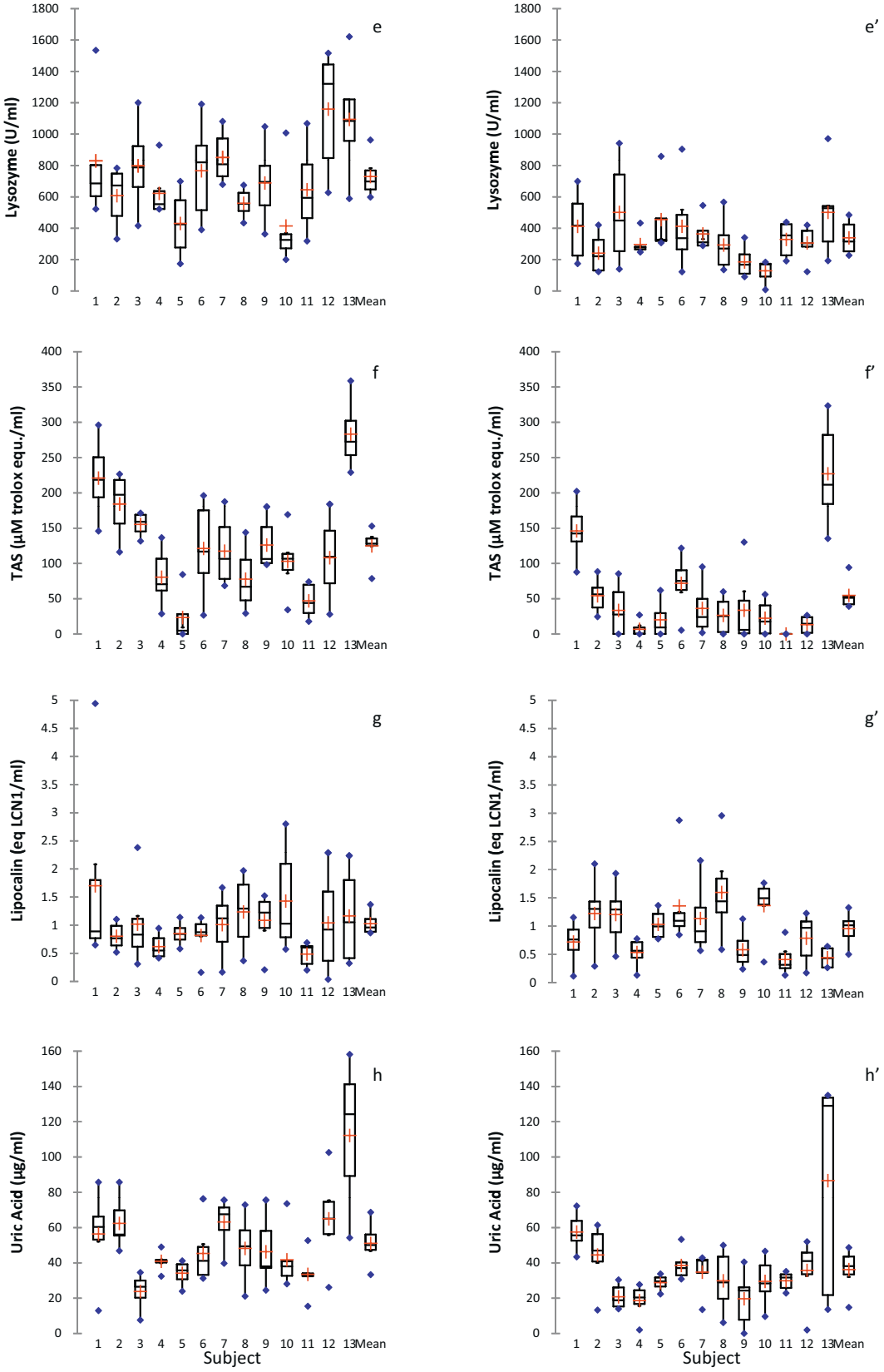

Fig. 3 – Box plot representations ($n = 6$) of lysozyme activity (e, e′), total antioxidant status (f, f′), lipocalin level (g, g′) and uric acid concentration (h, h′) of saliva from the 13 subjects collected without (left figures, x) or with stimulation (right figures, x′). Box outline represents lower quartile and upper quartile, outermost points correspond to the smallest and largest observation, the horizontal line is the median value, and the cross in the box is the mean value.

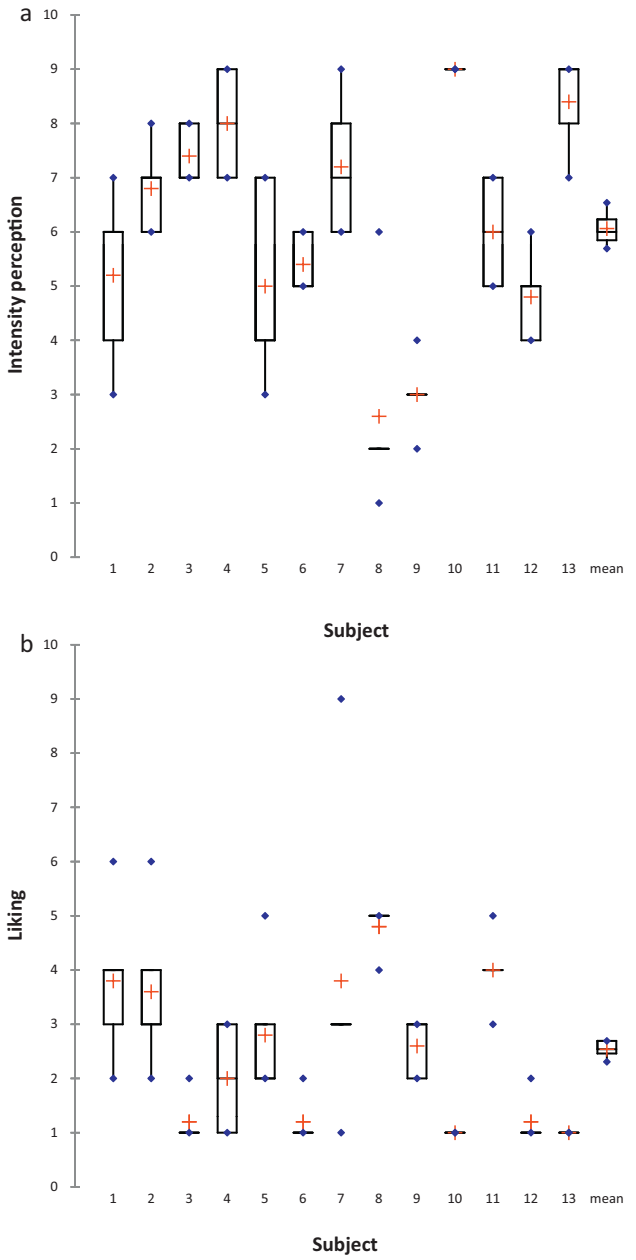

Fig. 4 – Box plot representations ($n = 5$) of fat model emulsion intensity perception (a) and liking (b) measured for the 13 subjects. Box outline represents lower quartile and upper quartile, outermost points correspond to the smallest and largest observation, the horizontal line is the median value, and the cross in the box is the mean value.

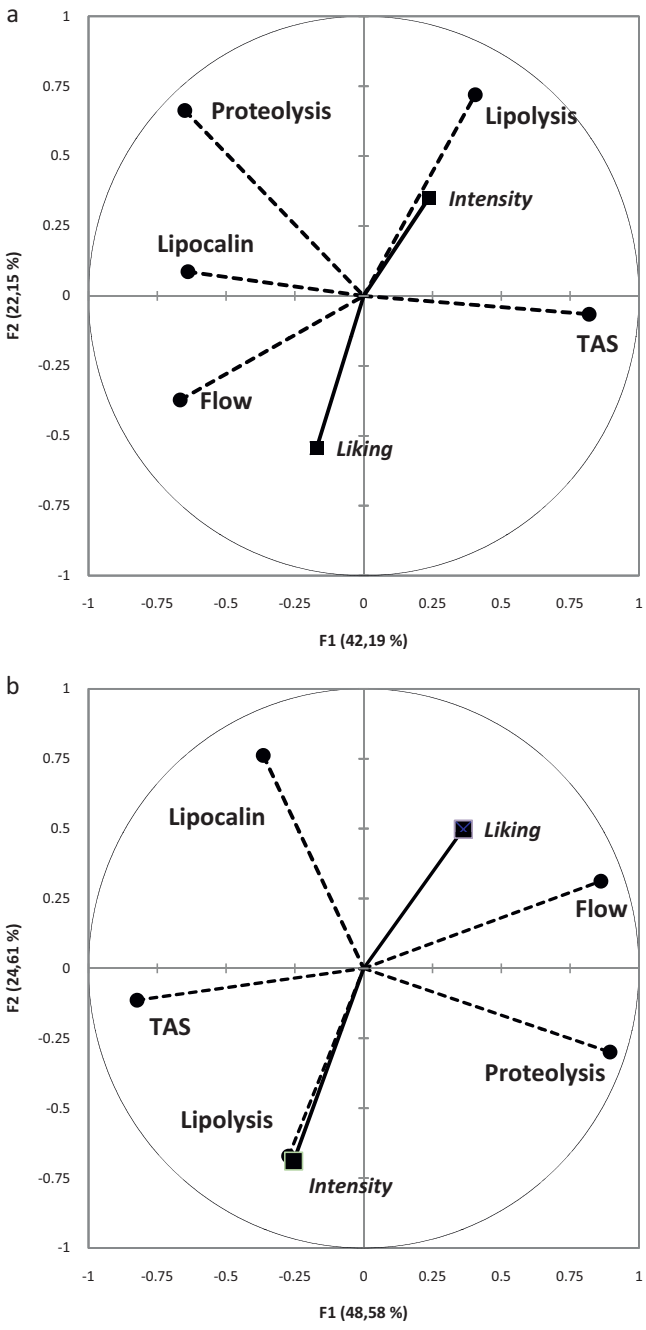

Fig. 5 – Principal component analyses of stimulated (a) and unstimulated (b) saliva characteristics. Data were obtained from the median values of the 13 subjects. Liking and intensity were supplementary variables.

linked to TAS on the one hand and flow and lipocalin on the other. Information regarding proteolysis and lipolysis, as well as supplementary variables, fat intensity and fat liking were mainly borne by the second axis (22.15%). In particular, liking for fat is negatively correlated to the second axis, unlike lipolysis which is positively correlated.

For unstimulated saliva, the first two axes explain 73.19% of the information on saliva composition. Information on the first dimension (48.58%) is carried mainly by TAS on the one hand and flow and proteolysis on the other. Lipocalin and lipolysis are correlated (+0.761 and −0.672, respectively) with the second dimension (24.61%). Interestingly, liking and fat intensity perception are also mainly projected in the second dimension (+0.498 and −0.689, respectively), suggesting that these sensory variables were most closely related to the characteristics of unstimulated saliva.

Moreover, significant correlations were found between flow and fat liking in stimulated ($R = 0.78$; $P < 0.01$) and unstimulated saliva ($R = 0.75$; $P < 0.01$).

4. Discussion

4.1. *Variability of saliva composition*

Inter-individual variability in sensory perception was rather large. To explain such variability, genetic or cognitive factors have been studied extensively. However, during consumption, food mixes with saliva so that it is not the food itself which is perceived but the products of its interactions with saliva. For this reason, the role of saliva in sensory perception appears to be essential, even though it has been insufficiently investigated. When subjects display marked differences in their saliva composition, their oral potential of interaction with food may differ, as may the subsequent release and perception of taste compounds. For instance, sensory scores for saltiness and thickness obtained for savoury liquids thickened with starches or non-starch hydrocolloid hydroxypropylmethyl cellulose (HPMC) were shown to be correlated with amylase activity in 14 panellists.[23] To date, the variability of saliva composition has been poorly described, with the exception of common characteristics such as flow. During the present study, the intra-individual variability of flow observed during the 8-month period was remarkably low.

However, when focusing on enzymatic activities related to macronutrients, the literature is quite scarce. During this study, proteolysis was more elevated in stimulated than in unstimulated saliva. Proteolysis already occurs in salivary glands as a posttranslational proteolytic process, but once in the oral cavity, proteolysis also arises from enzymes deriving from bacteria, epithelial cells and other host cells.[8] Any increase in proteolysis in stimulated saliva may thus be due to a marked rise in the content of both bacterial and epithelial cells immediately after stimulation starts.[24] This activity contrasts with that of lipolysis, lysozyme activity or TAS, which we found to be more elevated in unstimulated saliva. These characteristics are more likely to interact with fatty products, and interestingly a strong stimulation effect was found for lipolysis, with a higher activity in unstimulated saliva. The existence of a human lingual lipase is still a controversial subject,[25] because the enzyme has not been clearly identified in humans. However, we and many authors found a low level of lipolysis in humans when compared to rodents.[10,26,27] In humans, this activity seems to be definitely too low to be actively involved in the digestion of triglycerides, but may be implicated in ''fat taste'' perception, as suggested previously.[26] For this reason, an attempt was made to relate lipolysis to the lipocalin concentration. This was based on the hypothesis that the taste buds/von Ebner salivary gland complex present in rat foliate and circumvallate papillae[28] might also exist in humans and be dedicated to fat perception.[11] In rodents, saliva secreted by von Ebner's glands contains a lipase and a lipocalin; the von Ebner's gland protein (VEGP).[14] Lipase hydrolyses the triglycerides in glycerol and fatty acids and lipocalin may act as a solubiliser of these fatty acids and enable their transport to the taste buds. Then, in the cleft of papillae, saliva secreted by von Ebner glands would contain higher concentrations of lipase and lipocalin than the rest of the mouth, explaining why lipolysis in the rest of the mouth remains at a low level. The lower lipolysis found in unstimulated saliva could be explained by dilution. When stimulated, saliva from minor glands (e.g. von Ebner) is diluted by saliva from the parotid glands. Molecules secreted by the von Ebner glands then have a lower concentration in stimulated saliva than in unstimulated saliva. We observed this phenomenon for lipolysis, but contrastingly, the level of lipocalin was found to be identical under both conditions of saliva collection, indicating an increase in secretion under stimulation. Results similar to those for lipolysis were found for TAS and uric acid. These findings were in accordance with those of Nagler et al.[29] who found similar results with a higher TAS in unstimulated *versus* stimulated saliva, and a higher concentration of uric acid in unstimulated saliva (28.7 mg/l) than in stimulated saliva (11.5 mg/l). This type of relationship is not surprising, since the most important antioxidant molecule in saliva is uric acid.[29] Lysozyme activity appears to follow a pattern similar to that of TAS, with higher levels in unstimulated saliva. Lysozyme also acts as an antibacterial substance, so together with TAS, they appear to play a protective role for the oral cavity throughout the day during unstimulated periods, and their direct involvement during stimulation (sensory or mechanical) does not seem to be important, as their levels of expression decrease during stimulated periods. However, their basal concentrations in unstimulated saliva could affect the perception of certain substances; for example, those which are sensitive to oxidation.

4.2. *Relationships between saliva composition, fat liking and fat perception*

In view of the important inter-subject variability in the composition of saliva and the relationships existing between markers, our last goal was to assess whether a liking for fat or fattiness perception were linked to certain saliva characteristics. To do this, only biochemical variables displaying good stability over time were included: flow, lipolysis, lipocalin, proteolysis and TAS. In particular, our main hypothesis concerned a potential relationship between fat-related markers such as TAS, lipolysis and the lipocalin concentration fat liking or fattiness perception, indicating that the enzymatic potential of saliva against fat might drive or reflect the sensory perception of fat. The PCA on unstimulated saliva characteristics provided the strongest evidence of a possible relationship between lipolysis and lipocalin levels and fat intensity when compared to other saliva markers. We found a positive relationship between lipolysis and fat intensity. This seems logical under the hypothesis that more triglycerides are hydrolysed; more fatty acids are released and then perceived. However, we found a negative relationship between lipocalin concentrations and fat intensity. The role of lipocalin in this mechanism remains an enigma. Interestingly, a significant correlation was observed between flow rate and fat liking. According to Vingerhoeds et al.,[30] after flocculation, emulsions are described as rough, dry or astringent, which are considered to be unpleasant attributes. The positive

correlation between flow rate and fat liking could then be explained by the fact that the emulsion we used was quite unacceptable for the panellists because of its oxidation off-flavours. At a high flow rate, larger amounts of saliva might contribute to an easier washing of the emulsion when tasted, leading to a less unpleasant scoring. This could explain the contrasting relationship observed between fattiness perception and a liking for fat.

Because the solution used was sensitive to oxidation, a relationship between antioxidant status and sensory attributes was anticipated but was not in fact observed. The oxidation of fatty acids may be modulated by the antioxidant capacity of saliva. However, perception phenomena are more complex for oxidized fatty acids or their oxidation products which are also perceived by the olfactory system, such as metallic-smelling compounds from linoleic acid or odorant precursors[31,32] or rancidity.[33] The perception of oxidation products may thus be perceived by different sensory modalities that are not solely linked to fat perception.

5. Conclusion

The aim of the present work was to study the biochemical composition of saliva in 13 subjects, with special focus on characteristics known to interact with lipids. The results gave rise to some interesting and novel observations. The main one was the light shed on the low intra-subject variability and important inter-subject variability of the characteristics measured. In addition, remarkable stability over time was observed for some saliva characteristics throughout the 8-month experiment. This was particularly relevant in a context of identifying salivary markers of sensory perception because only stable variables can be considered. Generally speaking, a similar inter-individual variability *versus* intra-individual stability could be observed in terms of sensory perception. We advanced the hypothesis that the salivary medium could, to some extent, drive sensory perception, and that the over- or under-expression of some salivary markers might be specific to some sensory phenotypes. We were able to determine some relationships between stable salivary characteristics (lipolysis, lipocalin and flow) and fat liking or perception. These results indicate that the oral medium differs markedly between subjects, and our approach suggests that the composition of saliva may affect fat perception and liking.

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REFERENCES

1. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. *J Prosthet Dent* 2001;**85**(2):162–9.
2. Proctor GB, Carpenter GH. Regulation of salivary gland function by autonomic nerves. *Auton Neurosci Basic Clin* 2007;**133**(1):3–18.
3. Spielman AI. Interaction of saliva and taste. *J Dent Res* 1990;**69**(3):838–43.
4. Matsuo R. Role of saliva in the maintenance of taste sensitivity. *Crit Rev Oral Biol Med* 2000;**11**(2): 216–29.
5. Chen JS. Food oral processing—a review. *Food Hydrocolloids* 2009;**23**(1):1–25.
6. Engelen L, van den Keybus PAM, de Wijk RA, Veerman ECI, Amerongen AVN, Bosman F, et al. The effect of saliva composition on texture perception of semi-solids. *Arch Oral Biol* 2007;**52**(6):518–25.
7. de Wijk RA, Prinz JF, Engelen L, Weenen H. The role of alpha-amylase in the perception of oral texture and flavour in custards. *Physiol Behav* 2004;**83**(1):81–91.
8. Helmerhorst EJ. Whole saliva proteolysis—wealth of information for diagnostic exploitation. In: Malamud D, Niedbala RS, editors. *Oral-based diagnostics*. Oxford: Blackwell Publishing; 2007. p. 454–60.
9. Drago SR, Panouille M, Saint-Eve A, Neyraud E, Feron G, Souchon I. Relationships between saliva and food bolus properties from model dairy products. *Food Hydrocolloids* 2011;**25**(4):659–67.
10. Stewart JE, Feinle-Bisset C, Golding M, Delahunty C, Clifton PM, Keast RSJ. Oral sensitivity to fatty acids, food consumption and BMI in human subjects. *Br J Nutr* 2010;**104**(1):145–52.
11. Khan NA, Besnard P. Oro-sensory perception of dietary lipids: new insights into the fat taste transduction. *Biochim Biophys Acta Mol Cell Biol Lipids* 2009;**1791**(3):149–55.
12. Silletti E, Vingerhoeds MH, Norde W, van Aken GA. Complex formation in mixtures of lysozyme-stabilized emulsions and human saliva. *J Colloid Interface Sci* 2007;**313**(2):485–93.
13. Battino M, Ferreiro MS, Gallardo I, Newman HN, Bullon P. The antioxidant capacity of saliva. *J Clin Periodontol* 2002;**29**(3):189–94.
14. Kock K, Ahlers C, Schmale H. Structural organization of the genes for rat von Ebners gland protein-1 and protein-2 reveals their close relationship to lipocalins. *Eur J Biochem* 1994;**221**(3):905–16.
15. Gilbertson TA. Gustatory mechanisms for the detection of fat. *Curr Opin Neurobiol* 1998;**8**(4):447–52.
16. Perry GH, Dominy NJ, Claw KG, Lee AS, Fiegler H, Redon R, et al. Diet and the evolution of human amylase gene copy number variation. *Nat Genet* 2007;**39**(10):1256–60.
17. Neyraud E, Sayd T, Morzel M, Dransfield E. Proteomic analysis of human whole and parotid salivas following stimulation by different tastes. *J Proteome Res* 2006; **5**(9):2474–80.
18. Quintana M, Palicki O, Lucchi G, Ducoroy P, Chambon C, Salles C, et al. Inter-individual variability of protein patterns in saliva of healthy adults. *J Proteomics* 2009;**72**(5):822–30.
19. Roberts I. Hydrolysis of 4-methylumbelliferyl butyrate: a convenient and sensitive fluorescent assay for lipase activity. *Lipids* 1985;**20**:243–7.
20. Beisson F, Aoubala M, Marull S, Moustacas-Gardies AM, Voultoury R, Verger R, et al. Use of the tape stripping

technique for directly quantifying esterase activities in human stratum corneum. *Anal Biochem* 2001;**290**(2):179–85.
21. Wickström C, Herzberg MC, Beighton D, Svensater G. Proteolytic degradation of human salivary MUC5B by dental biofilms. *Microbiology (UK)* 2009;**155**:2866–72.
22. Ghose TK. Measurement of cellulase activities. *Pure Appl Chem* 1987;**59**(2):257–68.
23. Ferry ALS, Mitchell JR, Hort J, Hill SE, Taylor AJ, Lagarrigue S, et al. In-mouth amylase activity can reduce perception of saltiness in starch-thickened foods. *J Agric Food Chem* 2006;**54**(23):8869–73.
24. Dawes C, Tsang RWL, Suelzle T. The effects of gum chewing, four oral hygiene procedures, and two saliva collection techniques, on the output of bacteria into human whole saliva. *Arch Oral Biol* 2001;**46**(7):625–32.
25. Mattes RD. Is there a fatty acid taste? *Annu Rev Nutr* 2009;**29**:305–27.
26. Kawai T, Fushiki T. Importance of lipolysis in oral cavity for orosensory detection of fat. *Am J Physiol Regul Integr Comp Physiol* 2003;**285**(2):R447–54.
27. Denigris SJ, Hamosh M, Kasbekar DK, Lee TC, Hamosh P. Lingual and gastric lipases species differences in the origin of prepancreatic digestive lipases and in the localization of gastric lipase. *Biochim Biophys Acta* 1988;**959**(1):38–45.
28. Spielman AI, Dabundo S, Field RB, Schmale H. Protein analysis of human von Ebner saliva and a method for its collection from foliate papillae. *J Dent Res* 1993; **72**(9):1331–5.
29. Nagler RM, Klein I, Zarzhevsky N, Drigues N, Reznick AZ. Characterization of the differentiated antioxidant profile of human saliva. *Free Radic Biol Med* 2002;**32**(3): 268–77.
30. Vingerhoeds MH, Silletti E, de Groot J, Schipper RG, van Aken GA. Relating the effect of saliva-induced emulsion flocculation on rheological properties and retention on the tongue surface with sensory perception. *Food Hydrocolloids* 2009;**23**(3):773–85.
31. Lawless HT, Schlake S, Smythe J, Lim JY, Yang HD, Chapman K, et al. Metallic taste and retronasal smell. *Chem Senses* 2004;**29**(1):25–33.
32. Glindemann D, Dietrich A, Staerk HJ, Kuschk P. The two odors of iron when touched or pickled: (skin) carbonyl compounds and organophosphines. *Angew Chem Int Ed* 2006;**45**(42):7006–9.
33. Dransfield E. The taste of fat. *Meat Sci* 2008;**80**(1):37–42.