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Construction of an individualized sensory space of tastes in water using skin blood flow responses

G. Haese, P. Humeau, F. De Oliveira, P. Le Callet and P. Le Cloirec

ABSTRACT

Perception of tastes and odors in water is a major interest for water producers and distributors because off-flavors in tap water are associated with health risk by consumers. However, the taste of water is difficult to describe due to the medium itself which is supposed to have no taste. Classical sensory methodologies are difficult to adapt and only get part of the whole perception. This study suggests a new approach to qualify and quantify taste of water using multiple physiological measurements to go back up the perception chain. The four basic tastes (sweet, salty, acid and bitter) were used and diluted in Evian water as a standard, at low concentrations. Autonomic system responses were measured with skin blood flow variations. Results from skin blood flow variations are presented and indicate a high correlation between the duration and amplitude of the response with the self-reported intensity and pleasantness of the stimulus. It also shows that physiological measurements enable the significant discrimination of the different tastes even at detection threshold concentrations. The prediction of the characteristics of a stimulus might be obtained by combining multiple physiological data and sensory responses.

Key words | perception, sensory analysis, skin blood flow, taste, water

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INTRODUCTION

The perceived quality of tap water is highly dependent on its organoleptic characteristics. Off-flavors are considered by consumers as potential risks for their health and are presently a major issue for water producers and distributors. Qualifying and quantifying off-flavors of water is a serious challenge owing to the fact that water is supposed to have no taste or odor and that when it has one, off-flavors are present at very weak concentrations.

Chemical methodologies have been extensively researched to estimate the objective quality of water; they will not be developed in this paper. For example, spectroscopy (Gowen *et al.* 2012) or stir bar sorptive extraction (SBSE) coupled with gas chromatography/mass spectrometry (GC/MS) analyses (Wu & Duirk 2013) can reveal the quality, as *qualitas* (i.e. a set of inherent characteristics), but cannot necessarily reflect the consumer perception which results from complex sensory and interpretation processes (Sáenz-Navajas *et al.* 2010).

Due to weak and close to detection threshold concentrations, sensory measurement of drinking water perception and appreciation is a difficult task. Several sensory methodologies have been developed to self-report sensory perception of water taste (Teillet *et al.* 2010), the main one being the Flavor Profile Analysis (Suffet *et al.* 1988); the sensory methodologies for water taste analysis will not be described as they are not the topic of this article. These methods generally involve sensory panels responding to questionnaires about specific attributes or hedonic evaluations. Measurements obtained with sensory methodologies strive to be objective as much as possible but the response recorded is still influenced by cognitive processing of the information. The sensory response differs from the spontaneous emotional response before integration. Presently, in the particular domain of water analysis, the sensory analysis methodologies which exist all have limitations and are

difficult to link with chemical analyses. With respect to this drawback, other more objective methods might be considered using physiological measurements.

In order to go back up the perception chain, information from central and autonomic nervous systems can be recorded. In fact, following the rule ‘your body cannot lie’, physiological recordings could potentially reflect the sensation triggered by gustatory stimuli. An inventory of the different physiological methodologies which could be adapted to this context has been made by Haese *et al.* (2014). In this review, electroencephalogram, electrodermal measurements, skin blood flow (SKBF) variations and cardiac frequency changes have been described with the aim of characterizing water taste. This paper will mainly focus on SKBF experimental measurements.

There are different ways to measure SKBF variations. Many studies have used a Hematron sensor, developed by Dittmar *et al.* (1992), although the laser Doppler is a more sensitive instrument. For example, when rats were exposed to odors, the laser Doppler highlighted an increased cortical blood flow (Major & Silver 1999). It was also used on human subjects during the cephalic phase of digestion, before food had been placed into the mouth. Results showed that blood flow increased under the influence of a stimulus during this phase (Buss *et al.* 2012). Likewise, an increased blood flow was observed when subjects had to chew or eat solid food (Someya & Hayashi 2008). The laser Doppler was sensitive enough to detect changes in SKBF when small amounts of water samples were administered orally (Wipke-Tevis & Williams 2007). Using water samples, Rousmans *et al.* (2000) showed that SKBF enables each of the basic tastes to be discriminated. The amplitude of the vasoconstriction was significantly higher for bitter, acid and salty tastes than for water and significantly lower for sweet than for bitter taste. SKBF variations can be linked to hedonic responses: the more pleasant the solution, the lower the amplitude and duration. This link was confirmed by Kashima & Hayashi (2011) who demonstrated that sweet, umami, and bitter taste stimuli elicited characteristic facial SKBF responses. They showed that many facial SKBF aspects reflect the hedonic valence of the stimuli; the SKBF responses observed in the eyelid and nose represented signals of gustatory information.

The objective of the experiments described here is to build a calibrated individual sensory space based on SKBF

measurements in order to use it as a reference to characterize odor and taste problems in water samples. The aim of this paper is to display the innovative point of view of this methodology to characterize the taste of water and to show its high potential with an example of results obtained with SKBF measurement and how it correlates with sensory responses, using basic tastes at detection threshold concentrations. The subjects are used as a ‘human sensor’ and the construction of their individual sensory space is described.

MATERIALS AND METHODS

Subjects

In this study, four healthy, non-smoker volunteer subjects (two males and two females) were selected from their performances in taste recognition tasks among a dozen subjects. Their mean age was 32, ranging from 24 to 40. They attested to be free from medical treatment and from olfactory or gustative disorders and signed an informed consent explaining the procedure of the test they would have to perform. Moreover, these four subjects have been highly trained for several months on the four basic taste detection and recognition, as well as on the sensory analysis and physiological procedures.

Taste stimuli

The four basic tastes (sweet, salty, acid and bitter) were used for the tasting. The solutions were made with citric acid for acid taste, caffeine for bitter taste, sodium chloride for salty taste and sucrose (all chemicals were from Sigma Aldrich, France) for sweet taste, in Evian mineral water, used as diluents, as the control and as a blank. Each taste was presented in the range of four concentrations summarized in Table 1.

Evian mineral water was chosen for its neutral taste (Teillet *et al.* 2010) and because it has been widely used in studies on physiological reactions in response to gustative stimuli (Rousmans *et al.* 2000; Robin *et al.* 2003; Leterme *et al.* 2008). The detection threshold concentrations of the basic tastes are relatively high: the one for the citric acid is around 2 mmol.L⁻¹, for the NaCl around 10 mmol.L⁻¹, for the sucrose around 20 mmol.L⁻¹ (Purves *et al.* 2005) and the one for the caffeine between 0.26 and 1.80 mmol.L⁻¹

Table 1 | Concentrations (mmol.L⁻¹) for each gustative stimulus^a

Dilution	Citric acid (acid)	Caffeine (bitter)	NaCl (salty)	Sucrose (sweet)
1	1.6	0.72	12	8
2	2.0	0.88	17	13
3	2.5	1.13	24	21
4	3.1	1.39	34	35

^a For example, in this table, dilution 1 of citric acid (corresponding to a concentration of 1.6 mmol.L⁻¹ of citric acid) will be identified as Acid 1 in the developments of this study, and so on.

(Robinson *et al.* 2005). The concentrations of the solutions have been chosen from these threshold values and from previous tastings and a French standard (AFNOR 2012) in order to have concentrations close to the detection thresholds.

The amount of solution to be tested has been standardized to 10 mL. In order to impregnate the whole oral cavity, subjects have been asked to keep the solution in their mouth for 5 s before swallowing.

Skin blood flow system recording

The Periflux PF 5010 system (Perimed AB, Sweden) was chosen for this study and allows non-invasive monitoring of blood perfusion in capillaries, arterioles and venules. The blood perfusion unit is arbitrary, and corresponds to the product of the average speed of moving red blood cells by their concentration (Humeau *et al.* 2007). The Periflux PF 5010 system has a laser beam of 780 nm, 1 mm in diameter, with no thermal effect. A fiber optic probe PR407 is used in our work. The low-energy laser beam is transmitted by the probe to the tissue. A portion of the light is reflected on static structures, another part is reflected on the moving blood cells. When light is reflected from a moving cell, the wavelength is changed; which corresponds to the Doppler effect. The backscattered light collected by the optical fiber is used to compute the perfusion value. The measurement is expressed in perfusion units (PU). Recordings are performed on the pulp of the index of the non-dominant hand due to the strong vascularization of this area. The perfusion signal was recorded thanks to the PowerLab PL35088/35 acquisition system with eight channels, the data were collected at 1 kHz and analyzed with the Labchart[®] software (AD Instruments Ltd, UK).

The resulting signal was first inverted (multiplied by -1), so that the analysis of the peak was possible in the LabChart[®] software, and its value was multiplied by 100 to convert it from volts to PU. The signal was then filtered with a low-pass filter having a cut-off frequency of 0.6 Hz to remove the characteristic frequency of the heartbeat that was not relevant in our analysis. Afterwards, the analysis of the peak was performed manually by selecting the peak in its entirety and by analyzing it with LabChart[®] software (Figure 1).

Several shape parameters were extracted from the signal (i.e. amplitude, duration, slope). They are detailed in Table 2.

Procedure

The sessions were individual and the subjects were asked not to eat or drink for at least 1 h prior to the tasting. They arrived 15 min before the beginning of the session in order to get used to the environmental conditions, particularly the temperature of the room (maintained constant at 23 °C) and to be at rest, sitting in a comfortable chair. The 16 solutions to taste and two supplementary water samples containing only Evian water, added as references, were presented in a monadic way, following a tasting order determined from a balanced incomplete block design. During one session, only nine samples out of 18 were presented to minimize sensory fatigue. Moreover, one Evian sample was always tasted as a 'warm-up' sample in order to avoid the first order effect (Lawless & Heymann 2010) and was excluded from the analysis. A light signal was used to give the order to taste the sample. When the nervous system perturbation recovered its basal level (after about

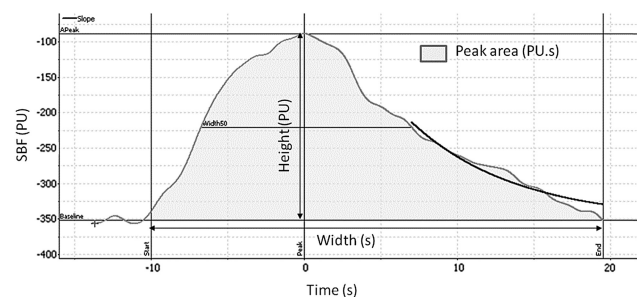


Figure 1 | Extraction of parameters from a signal of SKBF such as the height and width of the peak induced by a gustative stimulus performed with the Peak analysis tool of the Labchart[®] software. Q12

Table 2 | List of the parameters extracted with LabChart® software and their calculation

Parameters	Calculation
AMaxSlope	Sample value at MaxSlope
AMinSlope	Sample value at MinSlope
APeak	Sample value at the peak, not relative to the Baseline
Height	APeak minus the Baseline
MaxSlope	At each sample in the region from Start to End, a slope is calculated using five-point linear regression centered on the sample. The maximum of these slopes is taken as MaxSlope
MinSlope	As for MaxSlope, the minimum of these slopes are taken as MinSlope (where the minimum is the steepest downhill slope)
PeakArea	Area from Start to End
SlopeFall	Slope of the straight line through the fall points, where the fall points are the first crossings of 90 and 10% of the peak Height between the peak and End.
SlopeRise	Slope of the straight line through the rise points, where the rise points are the first crossings of 10 and 90% of the peak Height between Start and the peak.
TFall	Time between the fall points, where the fall points are the first crossings of 90 and 10% of the peak Height between the peak and End
TimeToPeak	Time from Start to APeak.
TMaxSlope	Time from Start to MaxSlope.
TMinSlope	Time from Start to MinSlope.
TRise	Time between the rise points, where the rise points are the first crossings of 10 and 90% of the peak Height between Start and the peak
Width30, Width50, Width90	WidthR (where R is one of 30, 50 and 90) is calculated as the shortest time between crossings of Baseline value plus R% of the Height either side of the event
Width	Time from Start to End

1 min), a second light signal indicated to the subjects they had to fill in a questionnaire about the stimulus they just tasted. The first part of the questionnaire was related to the identification of the taste (i.e. the determination of the taste quality); subjects had a choice between five labels: sweet, salty, acid, bitter or neutral. Then, they had to score the intensity of the taste on an 11-point scale (from 0 'not intense' (i.e. Evian taste only) to 10 'very intense') and finally to give a hedonic score on an 11-point scale (from 0 'very unpleasant' to 10 'very pleasant', 5 being 'neutral'). During this second phase, subjects could rinse their mouth with Evian water and wait for the next light signal which indicated they could taste the next stimulus. This procedure was repeated for each of the nine stimuli. One session lasted about 45 min including a debriefing at the end of the tasting in order to be sure to understand the meaning of the signal variations. The experience was repeated 12 times per subject, which means that the data is made of

six data per subject and per product and 12 data for the Evian product per subject. And thus, the database consists of 432 samples.

Statistical analysis

All analyses were performed using SensoMineR (Husson & Lê 2009) and FactoMineR (Lê *et al.* 2008) implemented in R Q1 (version 2.14.1) and XLSTAT-Pro 2010.3.01 software.

Sensory responses

The effect of the taste on intensity and hedonic scores has been analyzed by an ANalysis Of VAriance (ANOVA) including the product effect, the subject effect and the interaction product-subject. The subject effect was fixed because there were only four subjects and the results can only be

applied for this particular design. Differences were considered significant at a level of 0.05.

Skin blood flow variations

Principal Component Analysis (PCA) has been used:

- to study the individuals (i.e. the taste stimuli): two stimuli are close if they share similar results, the interest being the variability between individuals,
- to study the variables (i.e. the physiological parameters): it enables the visualization of the correlations between the physiological variables in order to find synthetic variables,
- to link the two studies by characterizing groups of individuals with variables (Escofier & Pagès 2008).

Then, the SKBF responses were analyzed individually with one-way ANOVA (product effect), subjects having their own 'preferential channel' (Lacey *et al.* 1953). Indeed, some subjects responded with SKBF variations, others with

EDR variations, and physiological measures are known to show large individual differences, which leads to the necessity to individualize the analysis (Johannes & Gaillard 2014).

Finally, correlations between intensity or hedonic mean scores by product and nervous system responses averaged by product were calculated using Pearson coefficient. Differences were considered significant at a level of 0.05.

RESULTS

Sensory evaluation

Recognition rates

Salty and sweet tastes were well identified by the panel for all the concentrations with a recognition rate of 100% for the three higher ones (Table 3). Highest acid concentrations

Table 3 | Individual recognition counts for each gustative stimulus and recognition rates (%) on the four subjects

		Citric acid				Caffeine				Evian	NaCl				Sucrose				
		1	2	3	4	1	2	3	4		1	2	3	4	1	2	3	4	
Subject 1	Acid	1	1	6	6	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Bitter	1	1	0	0	5	6	6	6	4	0	0	0	0	0	0	0	0	0
	Neutral	3	4	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0
	Salty	0	0	0	0	0	0	0	0	1	6	6	6	6	0	0	0	0	0
	Sweet	1	0	0	0	0	0	0	0	4	0	0	0	0	6	6	6	6	6
Subject 2	Acid	0	2	6	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Bitter	0	0	0	0	6	4	6	6	3	0	0	0	0	0	0	0	0	0
	Neutral	5	3	0	0	0	1	0	0	7	0	0	0	0	0	0	0	0	0
	Salty	0	0	0	0	0	0	0	0	0	6	6	6	6	0	0	0	0	0
	Sweet	1	1	0	0	0	1	0	0	2	0	0	0	0	6	6	6	6	6
Subject 3	Acid	1	1	6	6	0	1	0	0	1	0	0	0	0	0	0	0	0	0
	Bitter	2	4	0	0	6	5	6	6	3	0	0	0	0	0	0	0	0	0
	Neutral	3	1	0	0	0	0	0	0	8	0	0	0	0	3	0	0	0	0
	Salty	0	0	0	0	0	0	0	0	0	6	6	6	6	0	0	0	0	0
	Sweet	0	0	0	0	0	0	0	0	0	0	0	0	0	3	6	6	6	6
Subject 4	Acid	1	4	6	6	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Bitter	3	1	0	0	3	5	4	5	8	1	0	0	0	0	0	0	0	0
	Neutral	2	1	0	0	3	1	2	0	4	0	0	0	0	0	0	0	0	0
	Salty	0	0	0	0	0	0	0	0	0	5	6	6	6	0	0	0	0	0
	Sweet	0	0	0	0	0	0	0	0	0	0	0	0	0	6	6	6	6	6
Recognition rates	Acid	13	33	100	100	0	4	0	4	4	0	0	0	0	0	0	0	0	0
	Bitter	25	25	0	0	83	84	92	96	38	4	0	0	0	0	0	0	0	0
	Neutral	54	38	0	0	17	8	8	0	44	0	0	0	0	12	0	0	0	0
	Salty	0	0	0	0	0	0	0	0	2	96	100	100	100	0	0	0	0	0
	Sweet	8	4	0	0	0	4	0	0	12	0	0	0	0	88	100	100	100	100

were also perfectly recognized and bitter taste was relatively well identified (respectively 92% and 96% of right answers for concentrations 3 and 4). However, low acid concentrations were not recognized and were classified as 'neutral' most of the time (respectively 54% and 38% for concentrations 1 and 2). Evian mineral water was often perceived bitter (in 38% of cases), but as it was used for all the dilutions, results are comparable.

Intensity scores

Intensity scores were highly correlated with recognition rates ($r=0.75$, $P < 0.001$). They confirmed that low acid concentrations 1 and 2 were scored as little intense as Evian mineral water (respectively 1.2 ± 1.5 , 1.0 ± 1.0 and 0.8 ± 1.2); they were just below detection thresholds. The effect of the product was highly significant on mean intensity scores ($P < 0.001$). Concentrations 4 of acid, salty and sweet tastes got the highest scores with, respectively, 8.4 ± 1.2 , 8.6 ± 1.0 and 8.3 ± 0.9 . Bitter solutions were less differentiated from one another and got the highest standard deviations; concentrations 3 and 4 were not significantly different. Concentrations 1 and 2 of salty taste were not significantly different either. The only taste for which every concentration was significantly rated different than the previous one was sweet (Figure 2).

The interaction product-subject was significant ($P < 0.001$); the effect of the product was not the same depending on the subject. In this study this interaction was mainly due to one subject who had difficulties in discriminating bitter tastes. Training tends to reduce this effect in the context of a description of the characteristics of the products (Lawless & Heymann 2010).

Hedonic scores

Mean hedonic scores were significantly different among the products ($P < 0.001$). Evian mineral water obtained a neutral hedonic score (5.2 ± 1.5), as well as the weakest concentrations of the different tastes close to the detection thresholds. As concentration rose, the hedonic score of acid, bitter and salty solutions decreased, whereas the hedonic scores of sweet solutions rose in the same direction as their concentrations.

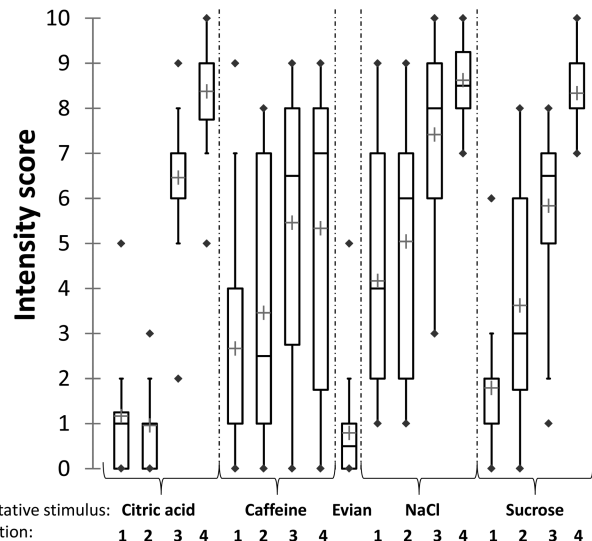


Figure 2 | Boxplots of the intensity scores obtained for each gustative stimulus and for each dilution (1, 2, 3, 4, cf. Table 1), averaged on the four subjects with six replicates each (i.e. a total of 24 intensity marks by product), 12 replicates for the Evian sample (i.e. a total of 48 intensity marks).

The concentration 4 of acid, concentrations 3 and 4 of caffeine and each concentration of NaCl obtained significantly lower hedonic scores than the other solutions (respectively 3.3 ± 2.4 , 3.5 ± 1.8 , 3.5 ± 2.3 and 1.3 ± 0.9), whereas all sweet solutions obtained significantly higher hedonic scores (7.0 ± 2.6 for the highest sucrose concentration).

The interaction product-subject was significant ($P < 0.001$); the effect of the product was not the same depending on the subject. In the case of hedonic ratings, this interaction is not problematic since some subjects may, for example, appreciate the acid taste whereas other subjects find it very unpleasant.

These sensory results confirmed the choice of the basic tastes and the choice of the different concentrations. Indeed, the 17 stimuli are staggered on the intensity scale, from the detection thresholds to higher intensities, and enable understanding covering the entire extent of the hedonic scale, even if the hedonic valence is taken as individual information in this study.

Physiological evaluation will now be focused on subject 3's results only.

Physiological evaluation: skin blood flow variations analysis

PCA was performed in order to visualize data obtained with physiological parameters extracted from individual SKBF

variations from subject 3 (cf. Figure 1 and Table 2). This analysis enables the representation of all the variables on a circle of correlations (Figure 3(a)) and all the products on a two-dimension space (Figure 3(b)). The first map represented 70.54% of the initial variability.

The first PCA axis was characterized by a group of variables of duration and amplitude of the physiological response (Figure 3(a)). This way, the Acid 3 and 4, Caffeine 3 and 4, NaCl 4 products, located on the right part of the map (Figure 3(b)) tended to have high values for the variables ‘width’, ‘height’ and ‘peak area’.

The second axis principally separated the variables of ‘slope’. Products located on the top of the graph tended to cause a faster fall of the signal after the stimulation, whereas the products on the bottom induced a slower return to the baseline level before stimulation.

The intensity and hedonic variables were processed as illustrative variables; they did not participate in the construction of the axes. However, they seemed to be highly correlated with physiological variables. The perceived intensity was significantly positively correlated to the first dimension ($r = 0.72$, $P = 0.001$), while the hedonic variable was significantly negatively correlated with the first dimension ($r = -0.65$, $P = 0.005$). Products perceived intense and unpleasant seemed to be characterized by stronger physiological reactions unlike pleasant and less intense stimuli.

According to the ANOVA, the most discriminant variables for the products were the width of the signal, i.e. its duration ($P < 0.001$), and the area of the peak ($P < 0.001$). Acid 4, NaCl 4 and Caffeine 3 induced significantly stronger perturbation than the others. Parameters being mathematically linked, the height of the peak, i.e. its amplitude, was also a discriminant parameter ($P = 0.004$) for the stimuli Acid 4 and Caffeine 3.

Correlational analysis confirmed these results. The width of the signal was significantly correlated with the intensity ($r = 0.68$, $P = 0.003$) and with the pleasantness ($r = -0.63$, $P = 0.007$), as well as the peak area ($r = 0.65$, $P = 0.004$ and $r = -0.65$, $P = 0.005$ respectively) and the height of the peak ($r = 0.68$, $P = 0.003$ and $r = -0.68$, $P = 0.003$ for intensity and pleasantness, respectively).

DISCUSSION

The aim of the study is to highlight the potential of SKBF to reflect the intensity of a stimulus or its hedonic valence at detection threshold concentrations in water and to evaluate the repeatability and the reproducibility of these kinds of measurements. This paper described the results obtained with SKBF measurements, which were linked with perception reports of one subject considered as a ‘human sensor’.

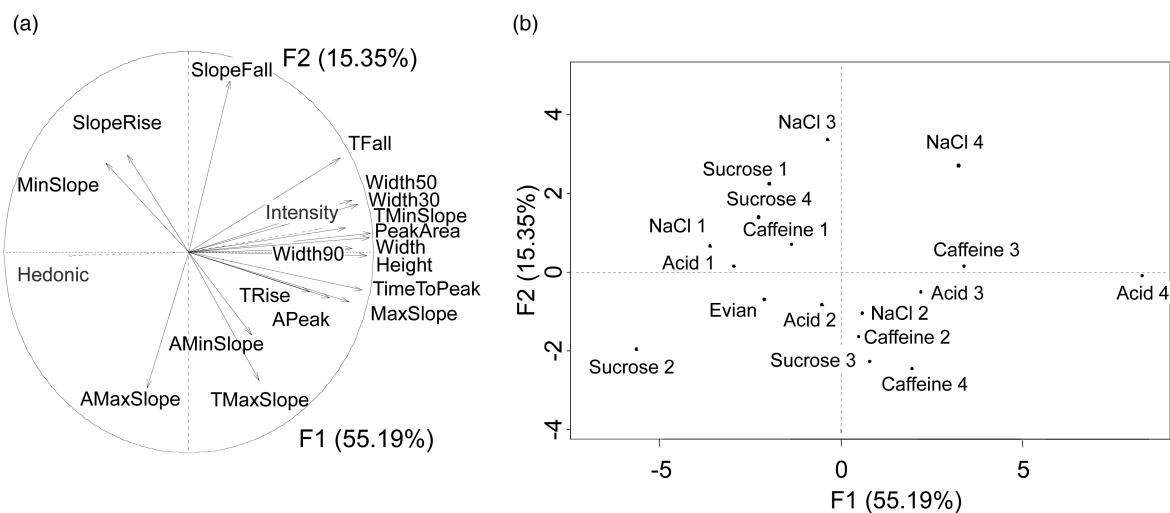


Figure 3 | (a) Representation of the variables (continuous line for active variables, dotted line for illustrative ones), (b) Representation of the gustative stimuli from the PCA (F1-F2) on blood flow data obtained with one subject.

The analysis had to be individualized because each subject responded on their own ‘preferential channel’ (Lacey *et al.* 1953) and because of the chosen concentrations which were much lower than in the literature. The state of the art showed that with obvious concentrations in water, the results taken on a global panel vary in the same direction. However, small variations induced by detection threshold concentrations have to be handled more carefully and the human subject has to be calibrated as a unique sensor in order to build an individualized sensory space.

The results obtained here for one subject were consistent with the literature since intense and unpleasant stimuli induced higher SKBF variations in duration and in amplitude than non-intense and pleasant ones. In fact, Rousmans *et al.* (2000) showed that basic tastes had a significant effect on skin blood flow amplitude (vasoconstriction). The different tastes were associated with significantly different autonomic nervous system responses: sweet taste, which has a positive hedonic valence (pleasant) and is innate-accepted, caused the weakest ANS response, while salty, acid and bitter tastes, which are rather unpleasant, elicited stronger ANS responses, the innate-rejected bitter taste inducing the strongest ones. Robin *et al.* (1998) demonstrated the same tendency with odors and showed that a pleasant smell induced weaker autonomic responses than an unpleasant one. A high correlation was found between hedonic evaluation and the autonomic estimation of the basic emotions (Ekman *et al.* 1983; Alaoui-Ismaili *et al.* 1997; Robin *et al.* 1998).

Subject 3 has the most common way to appreciate tastes but each subject has his own specific liking. Indeed, some subjects like bitterness and this taste may even be expected in some food, such as coffee for example (Drewnowski & Gomez-Carneros 2000). This is why ANS responses have to be interpreted with the knowledge of the subject’s individual liking.

CONCLUSION

This study highlighted high correlations between the intensity and the hedonic dimensions of a stimulus in water and the duration and amplitude of the SKBF variations of the subject considered as a ‘human sensor’. As far as we know, low

concentration tastes have never been used for this kind of experiment; studies have always employed obvious stimuli in water. Nevertheless, our results highlighted a high sensitivity of the physiological measurements even with stimuli close to detection thresholds. We also proposed in this work an innovative way of analyzing and correlating individualized physiological and sensory data, using factorial and univariate analyses.

Given the small number of participants in this study, it would be interesting to repeat the same protocol on a larger number of subjects to ensure that the same tendencies can be observed and that the construction of an individual sensory space built from physiological measurements is possible. Since subjects respond individually, and not always on the same physiological channel, the same pattern analysis will be performed for electrodermal and heart rate variability data and presented in another paper. The limits of biological methods in terms of detection could be identified, an intensity or hedonic law might be obtained for each taste, the different tastes could be classified based on the physiological reactions and a methodology might be suggested for each taste.

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- Q1** Lê (2008) has been changed to Lê *et al.* (2008) as per the reference list.
- Q2** In supplied Figure 1 is not sufficient print quality. Please resupply as a high resolution file (300 dpi or above) with sharp lines and text.
- Q3** Please check the suggested running title is ok.