A Novel Objective Sour Taste Evaluation Method Based on Near-infrared Spectroscopy

Ayaka Hoshi\textsuperscript{1}, Soichiro Aoki\textsuperscript{1}, Emi Kouno\textsuperscript{2}, Masashi Ogasawara\textsuperscript{2}, Takashi Onaka\textsuperscript{2}, Yutaka Miura\textsuperscript{3} and Kanji Mamiya\textsuperscript{1}

\textsuperscript{1}Central Laboratories for Key Technologies, Kirin Company Limited, 1-13-5, Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan, \textsuperscript{2}Food Research and Development Laboratory, Kirin Kyowa Foods Company Limited, 4140, Ami, Ami-machi, Inashiki-gun, Ibaraki 300-0398, Japan and \textsuperscript{3}Research Laboratories for Health Science, Kirin Company Limited, 1-13-5, Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan

Correspondence to be sent to: Ayaka Hoshi, Central Laboratories for Key Technologies, Kirin Company Limited, 1-13-5, Fukuura, Kanagawa-ku, Yokohama 236-0004, Japan. e-mail: Ayaka_Hoshi@kirin.co.jp

Accepted December 24, 2013

Abstract

One of the most important themes in the development of foods and drinks is the accurate evaluation of taste properties. In general, a sensory evaluation system is frequently used for evaluating food and drink. This method, which is dependent on human senses, is highly sensitive but is influenced by the eating experience and food palatability of individuals, leading to subjective results. Therefore, a more effective method for objectively estimating taste properties is required. Here we show that salivary hemodynamic signals, as measured by near-infrared spectroscopy, are a useful objective indicator for evaluating sour taste stimulus. In addition, the hemodynamic responses of the parotid gland are closely correlated to the salivary secretion volume of the parotid gland in response to basic taste stimuli and respond to stimuli independently of the hedonic aspect. Moreover, we examined the hemodynamic responses to complex taste stimuli in food-based solutions and demonstrated for the first time that the complicated phenomenon of the “masking effect,” which decreases taste intensity despite the additional taste components, can be successfully detected by near-infrared spectroscopy. In summary, this study is the first to demonstrate near-infrared spectroscopy as a novel tool for objectively evaluating complex sour taste properties in foods and drinks.

Key words: near-infrared spectroscopy, objective taste evaluation, sour taste stimulus

Introduction

The act of eating and drinking generates multimodal sensory stimuli, such as taste, smell, texture, and temperature, through the oral and nasal cavity. In addition, the eating experience and palatability of foods affect our overall judgment of food. Thus, humans evaluate foods from a comprehensive viewpoint.

Although sensory evaluation system is typically used for evaluating foods, it can be affected by individual differences such as personal preference, eating experience, or physical condition, and sometimes, the results fluctuate widely or are subjective. Particularly in complex samples such as foods and drinks, subjects may evaluate the same sample differently from one experiment to another. Therefore, sensory evaluation system requires the selection of suitable methods and well-trained subjects who recognize the specific features of foods and drinks.

In order to examine taste properties such as synergistic (i.e., supra-additive) taste or prolonged effects objectively, neuroimaging technologies, functional magnetic resonance imaging (fMRI) \cite{ODoherty2001, deAraujo2003, James2009}, positron emission tomography \cite{Kinomura1994}, electroencephalography \cite{Hashida2005, Ohla2010}, and magnetoencephalography \cite{Kobayakawa1994} have been used. However, these techniques are somewhat invasive, restrict the movement of subjects, and are high in cost and impractical for daily use. Therefore, a more effective method for estimating taste properties is required that is easy to use on anyone and has high sensitivity comparable to that of sensory evaluation.
Near-infrared spectroscopy (NIRS) is a noninvasive optical technique that continuously monitors changes in the concentration of hemoglobin in the cerebral vessels (Jöbsis 1977). Moreover, recording of the hemodynamic signals with NIRS enables us to test subjects in a normal, seated position with minimal restriction of movement during drinking or eating. Because of these superior features, NIRS has been recently used for measuring chemical senses, smell, and taste in the human body. For example, Sato et al. showed that multichannel NIRS can be used to measure extracranial hemodynamic signals from the parotid gland in response to a sweet stimulus (sucrose) (Sato et al. 2011). Furthermore, the effect of aroma components from dried-bonito (katsuo-bushi) broth on the salivary hemodynamic responses has been determined by using multichannel NIRS (Matsumoto et al. 2012). These studies suggest that chemical stimuli, tastes, and odors induce hemodynamic signal changes, although a correlation between the hemoglobin response and parotid gland function (i.e., salivary secretion) has not yet been demonstrated and differences in the hemodynamic responses of the parotid gland to basic tastes (sweet, umami, bitter, salt and sour) are unknown.

In the present study, we first evaluated the hemodynamic responses to basic tastes using salivary-dedicated NIRS, which is the original model for measuring the specific hemodynamics of the parotid gland. Next, we examined hemodynamic responses to complex taste stimuli in food-based solutions with or without rice vinegar (RV), which has the effect of masking the acid taste of high acidity vinegar (HAV). We demonstrate that salivary hemodynamic signals measured by NIRS are a useful objective indicator for evaluating food properties.

Materials and methods

NIRS system

We used an optical topography system that was modified from the model used for prefrontal cortex (HOT-121B, Hitachi, Tokyo, Japan) to enable the measurement of extracranial hemoglobin (Hb) signals from the parotid gland. This system has 1 head probe, which consists of a source–detector pair (Figure 1). A light emitting diode with a wavelength of 810 nm was used as the light emitter. Reflected light was detected every 0.1 s by a detector located 30 mm from the emitter. The optode was carefully positioned on the right or left temple area. The suitability of the temple-area position was evaluated in a pretest (drinking water) before experiment 1 or experiment 2.

Experiment 1 (5 basic taste stimuli)

Subjects

Eleven healthy adults (mean age, 32.4 years; 8 males, 3 females) participated in experiment 1. All subjects were free from medication and did not report any olfactory or gustatory disorders. The subjects reviewed and gave written informed consent before the experiment. Study approval was obtained from the Ethics Committee of Central Laboratories for Key Technologies, Kirin Company, Limited.

Taste stimuli

The following 5 taste solutions (sweet, umami, bitter, salt, and sour) were prepared: sucrose (Wako Pure Chemical Industries) for sweet, NaCl (Wako Pure Chemical Industries) for salt, citric acid (Wako Pure Chemical Industries) for sour, caffeine (Shiratori Yakuhin) for bitter, and sodium glutamate (Kirin Food Tech) for umami. Each of the taste stimuli was prepared at 3 different concentrations (Table 1, experiment 1). Volvic water (Kirin MC Danone Water) was used as the diluent and served as the control. All solutions were dispensed in a 20-mL volume as a test solution using a disposable cup and presented at a constant temperature (23–25 °C). Each taste concentration was determined as described in previous studies (Chee et al. 2005; Leow et al. 2007).

Table 1 Experiment 1: the tested concentrations for each basic taste substance

<table>
<thead>
<tr>
<th>Basic taste</th>
<th>Stimuli</th>
<th>Concentrations (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td>Sweet</td>
<td>Sucrose</td>
<td>58.1</td>
</tr>
<tr>
<td>Umami</td>
<td>MSG</td>
<td>27.4</td>
</tr>
<tr>
<td>Bitter</td>
<td>Caffeine</td>
<td>2.99</td>
</tr>
<tr>
<td>Salt</td>
<td>NaCl</td>
<td>61.3</td>
</tr>
<tr>
<td>Sour</td>
<td>Citric acid</td>
<td>6.56</td>
</tr>
</tbody>
</table>

MSG: monosodium glutamate.
Each taste concentration was determined so as to the equivalent taste intensities as described in previous studies (Chee et al. 2005; Leow et al. 2007).

Figure 1 The device for measuring the hemodynamic responses of the parotid gland. Left image, face-on view; right image, side view.
NIRS test procedure

The subjects were each requested to abstain from eating or drinking for 1 h before the test. They were seated in a comfortable chair in a room at a constant temperature (23–25 °C). After a subject was verbally informed about the procedure, the optode device was placed on his or her temple area. The task and rest procedure consisted of sequential periods of a 60-s rest before the task, the 90-s task, and a 60-s rest after task. The tasting sequence consisted of 5 steps: (i) holding and raising the cup to the mouth for 4 s, (ii) taking the contents of the cup into the mouth for 4 s, (iii) holding and tasting the contents in the mouth for 4 s, (iv) swallowing the contents and returning the cup to the desk for 1 s, and (v) recording for 80 s (Figure 2A). The subjects individually participated in the test session, which lasted about 60 min. Water and 3 taste solutions of different concentrations (low, middle, and high) were numbered (1)–(4) and dispensed to the subjects in the order of Figure 2A. Subjects were instructed to rinse their mouth with Volvic water ad libitum after each test solution. After NIRS measurement, each subject completed a taste visual analog scale (VAS) for pleasantness/unpleasant and intensity. The 5 different tastes were tested on 5 separate days.

Visual analog scales

A VAS was presented to the subjects after NIRS measurement. Subjects were asked to rate its pleasantness/unpleasantness and its intensity. Participants rated pleasantness on a scale ranging from 0 (neutral) to 5 (extremely pleasant) or unpleasantness ranging from 0 (neutral) to −5 (extremely unpleasant). Intensity was rated on a scale of 0 (neutral) to 10 (extremely intense).

Data analysis

The waveform of Hb changes during the tasks was calculated for each of the subjects. We used “peak height” and “rate of increase” indices to evaluate the waveform for the 90-s recording—that is, 80 s recording after swallowing and 10 s before swallowing. Peak height was determined as the highest point of Hb changes, and rate of increase was calculated as the Hb change rate (Δy) (10–90%) divided by time change rate (Δx) (10–90%) (Figure 3). To compare the responses induced by basic taste stimuli, the 2 evaluation points, peak height and rate of increase, were normalized by the average indices calculated from data obtained for the control, water stimulus. The mean relative values of peak height and increasing rate during the 90-s task were compared using 2-way repeated measure analysis of variance (ANOVA) with the “strength of taste stimuli” (low, middle, and high) and “repeated time” (first, second, and third). Statistical analyses were performed using Predictive Analytics SoftWare (PASW) statistics (version 18.0.0. IBM).

Measurement of salivary secretion volume

The subjects were each requested to abstain from eating or drinking for 1 h before the test. The test conditions were almost the same as those for NIRS measurement. To collect parotid gland saliva, a KUBOKI saliva collector (SANTO) was carefully inserted in the oral cavity and mounted at the stensen’s duct of the parotid gland saliva. A subject remained seated in a chair until the instrument inside the mouth had no effect on salivation. The task and rest procedure were followed as described above for NIRS (Figure 2A). The amount of collected saliva was measured by a microbalance (Sartorius Japan), and data from the 5 basic taste stimuli were normalized by average data obtained for the control, water stimulus.

Figure 2  Schematic diagrams of the data measurement sequence for taste stimuli (A) Experiment 1, basic taste stimuli; (B) Experiment 2, acid taste stimuli. Each test solution was tasted 3 times in experiment 1 and 4 times in experiment 2. For each test solution, the tasting sequence consisted of 5 steps: raising glass to mouth (4 s), taking contents into mouth (4 s), holding contents (4 s), swallowing contents (1 s), and recording after swallowing contents (80 s). The content of each solution is indicated in the table on the right.
**Experiment 2 (acid taste stimuli)**

**Subjects**

Eight healthy adults (mean age 37.2 years, 3 males, 5 females) participated in experiment 2. All subjects were free from medication and did not report any olfactory or gustatory disorders. The subjects reviewed and gave written informed consent before the experiment. Study approval was obtained from the Ethics Committee of Central Laboratories for Key Technologies, Kirin Company, Limited.

**Taste stimuli**

Three kinds of sweetened vinegar sauce, designated HAV, mixed vinegar (MV), and RV, were used in experiment 2. The vinegar content of HAV, MV, and RV was 14.7% HAV (Kewpie Jyozo), 10% HAV and 10% RV (Kirin Kyowa Foods), and 31.3% RV, respectively (Table 2, experiment 2). The sweetened vinegar sauce base consisted of 12% white superior soft sugar (Dai-Nippon Meiji Sugar), 5% dried-bonito stock (Kirin Kyowa Foods), 3% light soy sauce (Yamasa), and 1% table salt (Naruto Salt Mfg). The test solutions were adjusted for pH (3.5) and acidity (0.74%) (Table 2: experiment 2). All solutions were dispensed in a 3-mL volume as a test sample using a disposable cup and were presented at room temperature (23–25 ºC). The stimuli and concentrations were chosen on the basis of psychophysical tests performed on a panel of subjects.

**Procedure**

The task sequence was almost the same as that in experiment 1. The 3 taste solutions (HAV, MV, and RV sweetened vinegar sauce) were numbered (1)–(3) and presented to the subjects in the order (1), (2), and (3) (Figure 2B). Subjects were instructed to rinse their mouth with Volvic water ad libitum after each solution. At the end of measuring NIRS, the subject completed VAS for the intensity of acid taste.

**Visual analog scale**

To evaluate each taste solution (HAV, MV or RV sweetened vinegar sauce) subjectively, a VAS was presented to the subject after NIRS measurement. Subjects rated the intensity of acid taste on a scale of 0 (neutral) to 7 (extremely intense).

**Data analysis**

NIRS data were analyzed in the same manner as in experiment 1. The mean relative values of peak height and rate of increase during the 90-s task were compared using a 2-way repeated measure ANOVA with “taste stimuli” (HAV, MV, and RV) and “repeated time” (first, second, third, and fourth). Statistical analyses were performed using PASW statistics (version 18.0.0. IBM).

**Threshold analysis of vinegar**

The analysis was performed as described previously (Sakamoto et al. 2009). In brief, sample solutions for the threshold analysis of vinegar comprised a dilution series with an acetic acid content ranging from $3.08 \times 10^{-2}$% to $9.38 \times 10^{-7}$%, which is prepared by a series of 2-fold dilutions of HAV. Subjects were given 2 cups containing either a sample solution or Volvic water and asked if they could recognize the taste. Sample solutions were offered, starting with the lowest acidity solution ($9.38 \times 10^{-7}$%); the solution for which acidity was first detected was defined as the “detection threshold value” and that for which sour taste was detected was defined as the “recognition threshold value.”

**Chemical analysis**

The acidity of HAV and RV was defined as the volume of sodium hydrate (Junsei Chemical) needed for neutralization titration analysis. To measure the free amino acid (FAA) concentration in HAV and RV, sample solutions were diluted 2-fold with 2% sulfoalicylic acid, sonicated, and filtered on 0.45-µm filters and then applied to ion exchange chromatography with ninhydrin detection (JLC-500V; Jeol). To measure the total amino acid (TAA) concentration in HAV

---

**Table 2** Experiment 2: composition of sweetened vinegar sauce

<table>
<thead>
<tr>
<th>Composition (%)</th>
<th>HAV</th>
<th>MV</th>
<th>RV</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAV</td>
<td>14.7</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>RV</td>
<td>0</td>
<td>10</td>
<td>31.3</td>
</tr>
<tr>
<td>White superior soft sugar</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Dried-bonito stock</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Light soy sauce</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Table salt</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

HAV, MV, and RV were adjusted for same pH (3.5) and acidity (0.74%).
and RV, 1-mL sample solutions were added to 7mL of 6N hydrochloric acid, incubated for 1h in −20 °C, and deaerated. The samples were then heated for 21h at 110 °C, and the degradation products were evaporated and dissolved in 3mL of 2% sulfosalicylic acid. Samples were filtered on 0.45-μm filters and then applied to ion exchange chromatography with ninhydrin detection (JLC-500V; Jeol).

Results

Pleasantness/unpleasantness intensity rating

The VAS intensity ratings for each basal taste are shown in Figure 4A. The intensity scores of all taste stimuli increased as the concentration of the solution increased. The water control evoked a mean intensity rating of 0. The 5 basal taste stimuli successfully provoked various hedonic scores (Figure 4B). Only the sweet taste stimulus induced a pleasant emotion, and the pleasant score increased with the sucrose concentration of the solution. Whereas the sour taste stimulus provoked various emotions among subjects, the other taste stimuli (umami, bitter and salt) did not evoke increases in hemoglobin even for high intensity stimuli. The 2 indices used to evaluate hemoglobin changes in this study, that is, peak height and rate of increase, were calculated independently and the values were normalized to the respective mean values for the control, water stimulus (Figures 5B and C, respectively).

For statistical analysis, the relative values of both the peak height and the rate of increase were examined using a 3 (strength of taste stimuli: low, middle, and high) × 3 (repeated time: first, second, and third) 2-way repeated measure ANOVA. On the basis of peak height, the 2-way repeated measure ANOVA indicated no significant effect of “repeated time” for any of the basic taste stimuli, whereas a significant major effect of “strength of taste stimuli” was observed for bitter and sour taste (bitter: \(F = 4.442, P = 0.025\); sour: \(F = 22.7, P = 4.77 \times 10^{-7}\)). Sheffe’s test showed that the peak height of the high concentration bitter stimulus was significantly lower than that of the low concentration bitter stimulus (\(P = 0.015\)) and that of the high concentration sour stimulus was significantly higher than those of the low- and middle-concentration sour stimuli (\(P = 4.39 \times 10^{-7}\) and \(P = 3.35 \times 10^{-4}\), respectively) (Figure 5B).

On the basis of rate of increase, the 2-way repeated measure ANOVA indicated no significant effect of “repeated time” for any of the 5 basic taste stimuli, whereas it indicated a significant major effect of “strength of taste stimuli” for sour taste stimuli (\(F = 17.27; P = 6.07 \times 10^{-5}\)). Sheffe’s test showed that the rate of increase of the high concentration sour stimulus was significantly higher than that of the low- and middle-concentration sour stimuli (\(P = 6.12 \times 10^{-5}\) and \(P = 2.47 \times 10^{-2}\), respectively) (Figure 5C).

With the aim of confirming a direct correspondence between hemoglobin response and parotid gland function, we measured the salivary secretion volume of the parotid gland. Figure 5D shows the mean salivary secretion volume of the parotid gland in response to basic taste stimuli. Each value was normalized to the mean volume obtained for the control water stimulus. The 2-way repeated measure ANOVA indicated no significant effect of “repeated time” for any of the basic taste stimuli but a significant major effect of strength of taste stimuli for salt and sour taste stimuli (salt: \(F = 5.164; P = 0.036\); sour: \(F = 11.297; P = 0.005\)). Sheffe’s test showed that the salivary secretion volume of the high concentration salt stimulus was significantly higher than that of the low concentration salt stimulus (\(P = 0.036\)) and that
of the high concentration sour stimulus was significantly higher than that of the low- and middle-concentration sour stimuli (\( P = 0.007 \) and \( P = 0.019 \), respectively) (Figure 5D). Collectively, these results show that the hemodynamic responses and salivary secretion of the parotid gland correlate well with the subjects’ sensory reaction to sour taste stimulus.

**RV decreased “acid taste” in a sensory evaluation**

RV is vinegar seasoning made from rice and contains high levels of FAA and TAA (FAA: 37.2mg/100mg, TAA: 282mg/100mg) compared with HAV (FAA: 4.2mg/100mg, TAA: 37.2mg/100mg) (Table 3). In addition, the acidity of RV is lower (4.7 w/v%) than that of HAV (10.1 w/v%) (Table 4). To evaluate the characteristics of a complex sour
stimulus, we compared the intensity score for acid taste between \( RV \) and \( HAV \) stimuli by sensory evaluation. The test solutions, comprising sweetened vinegar sauce containing \( RV \) and \( HAV \) (\( RV: HAV = 2:1 \)), or \( HAV \), were adjusted for pH (3.5) and acidity (0.74\%) (Table 2, experiment 2).

The intensity scores for acid taste are shown in Figure 6A. The 2-way repeated measure ANOVA indicated no significant effect of “repeated time” but a significant major effect of acid taste stimulus \( (F = 20.708, P = 7.4 \times 10^{-7}) \). Sheffe’s test showed that the intensity score for \( HAV \) was significantly higher than that for \( MV \) and \( RV \) \( (P = 8.5 \times 10^{-4} \) and \( P = 4.8 \times 10^{-7} \), respectively), whereas there was no significance in the intensity scores between \( MV \) and \( RV \) \( (P = 0.057) \) (Figure 6A).

**Salivary hemodynamic responses to RV**

We assessed the characteristics of the hemodynamic responses of the parotid gland to the \( HAV \), \( RV \), and \( MV \) acid taste stimuli. Figure 6B shows the mean hemodynamic responses. The largest and long-lasting increase in hemoglobin was observed for the \( HAV \) stimulus, and there was no difference in the hemodynamic response between \( MV \) and \( RV \). The 2 indices used to evaluate hemoglobin changes, that is, peak height and rate of increase, were calculated (Figures 6C and D, respectively).

For the statistical analysis, the relative values of peak height and rate of increase were compared using a 3 (taste stimuli: \( HAV \), \( MV \), and \( RV \)) × 4 (repeated time: first, second, third, and fourth), 2-way repeated measure ANOVA. On the basis of peak height, the 2-way repeated measure ANOVA indicated no major effect of “repeated time” but a significant major effect of “taste stimulus” \( (F = 6.007, P = 0.004) \). Sheffe’s test showed that the peak height of \( HAV \) was significantly higher than that of \( MV \) and \( RV \) \( (P = 0.044 \) and \( P = 0.006 \), respectively) (Figure 6C). On the basis of the rate of increase, the 2-way repeated measure ANOVA indicated no major effect of “repeated time” but a significant major

### Table 3 Amino acid composition of studied vinegars

<table>
<thead>
<tr>
<th>FAA (mg/100g)</th>
<th>TAA (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( HAV )</td>
</tr>
<tr>
<td>( HAV )</td>
<td>( RV )</td>
</tr>
<tr>
<td>Asp</td>
<td>0.215</td>
</tr>
<tr>
<td>Thr</td>
<td>0.191</td>
</tr>
<tr>
<td>Ser</td>
<td>0.170</td>
</tr>
<tr>
<td>Glu</td>
<td>0.131</td>
</tr>
<tr>
<td>Gly</td>
<td>0.239</td>
</tr>
<tr>
<td>Ala</td>
<td>0.326</td>
</tr>
<tr>
<td>Val</td>
<td>0.208</td>
</tr>
<tr>
<td>Cystine</td>
<td>n.d.</td>
</tr>
<tr>
<td>Met</td>
<td>n.d.</td>
</tr>
<tr>
<td>Ile</td>
<td>0.166</td>
</tr>
<tr>
<td>Leu</td>
<td>0.449</td>
</tr>
<tr>
<td>Tyr</td>
<td>n.d.</td>
</tr>
<tr>
<td>Phe</td>
<td>n.d.</td>
</tr>
<tr>
<td>His</td>
<td>n.d.</td>
</tr>
<tr>
<td>Lys</td>
<td>0.399</td>
</tr>
<tr>
<td>Arg</td>
<td>2.03</td>
</tr>
<tr>
<td>Pro</td>
<td>n.d.</td>
</tr>
<tr>
<td>total</td>
<td>4.53</td>
</tr>
</tbody>
</table>

n.d.: not detected (under detection threshold).

### Table 4 Chemical analysis of studied vinegars

<table>
<thead>
<tr>
<th></th>
<th>( HAV )</th>
<th>( RV )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidity (w/v%)</td>
<td>10.1</td>
<td>4.70</td>
</tr>
<tr>
<td>pH</td>
<td>2.30</td>
<td>3.00</td>
</tr>
</tbody>
</table>

**Figure 6** Intensity comparison of acid taste by sensory evaluation and NIRs. (A) Bar chart showing differences in the mean scores of acid taste intensity during sensory evaluation. (B) Mean changes in hemoglobin signal in response to taste stimuli. Black, blue, and red lines show \( HAV \), \( MV \), and \( RV \), respectively. (C) and (D): Bar chart showing differences in the mean peak height (C) and rate of increase (D). (A), (C), and (D): White, gray, and black squares show \( HAV \), \( MV \), and \( RV \), respectively. Asterisks indicate significant differences \( (**P < 0.01, ^*P < 0.05) \) between taste stimuli. Error bars indicate the SE \( (n = 8 \) subjects who tasted each stimulus 4 times).
the analysis of nerve responses to basic taste stimuli, it was shown that sour taste stimuli (e.g., citric acid and hydrochloric acid) elicited a strong response in the glossopharyngeal nerve (GL), which innervates posterior tongue taste buds of the foliate papillae, circumvallate papillae, and pharynx (Hanamori et al. 1988; Hanamori and Ishiko 1993). Previous studies demonstrated that the foliate papillae were much more sensitive to sour taste than the tongue tip (fungiform papillae) via a filter paper disk method, which was used to measure taste thresholds in humans (Collings 1974; Nilsson 1979). In addition, anatomical studies indicate that GL innervates the parotid gland (Holsinger and Bui 2007). Therefore, sour taste stimulus received from taste cells on the foliate papillae are directly transmitted to the parotid gland through GL, and these physiological and anatomical properties rationally support our finding that sour taste stimulus elicited specific hemodynamic responses of the parotid gland (Figures 5A–D). By contrast, studies showed that the foliate papillae and circumvallate papillae were much more sensitive to bitter taste than the tongue tip (Nilsson 1979, Acta Odontol Scand). Besides, bitter taste stimulus elicited more response in the GL than other taste stimuli, sweet and salt (Hanamori et al. 1988; Hanamori and Ishiko 1993). In the present study, however, the bitter taste stimulus of caffeine did not elicit hemodynamic responses (Figures 5A–C) and salivary secretion from the parotid gland (Figure 5D). Previous studies (Hodson and Linden 2006) also support our results in that bitter stimulus showed the lowest salivary flow rate of parotid gland in 5 basic tastes. These data suggest that the neural pathways from the bitter taste cells to the parotid glands, which induce salivary secretion, may be different from those from the bitter taste cells to the brain, which induce bitter taste recognition. Further studies are necessary to clarify the physiological mechanism of the differences between hemodynamic responses of the parotid gland and GL responses to taste stimuli.

Interestingly, for the salt taste stimulus, the peak height and rate of increase tended to increase according to the taste intensity (Figures 5B, C). Furthermore, the salivary secretion volume of the parotid gland in response to the high salt stimulus was significantly higher than that of the low salt stimulus (Figure 5D). Recently, Oka et al. (2013) showed that high concentration salt recruits the primary aversive taste

### Table 5 The acid threshold value

<table>
<thead>
<tr>
<th>Detection-threshold value</th>
<th>Recognition-threshold value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Recognition</td>
<td>0.0016</td>
</tr>
<tr>
<td>No recognition</td>
<td>0.0126</td>
</tr>
<tr>
<td>Total</td>
<td>0.0057</td>
</tr>
</tbody>
</table>

SE: standard error.
Recognition: $n = 5$; no recognition: $n = 3$. 

### Relationship between an acid threshold value and NIRS

Finally, to assess the relationship between sensitivity to acid taste and the hemodynamic responses of the parotid gland, the threshold values for diluted vinegar were studied by a sensory evaluation. The 8 subjects were classified into 2 groups according to the peak height of the hemodynamic response of the parotid gland. The “recognition” group included subjects who showed the largest increase in hemoglobin in response to HAV (1 male, 4 females), whereas the “no recognition” group included subjects who showed the largest increase in hemoglobin in response to MV or RV (2 males, 1 female). Comparison of the mean detection- or recognition-threshold value between the 2 groups showed that both values were significantly lower for the recognition group than for the no recognition group (detection-threshold value, $P = 0.0066$; and recognition-threshold value, $P = 0.0066$; paired $t$-test) (Table 5).

### Discussion

The present study demonstrated that a sour taste stimulus elicited specific hemodynamic responses in the parotid gland whereas other basic taste stimuli (sweet, umami, bitter, and salt) did not (Figure 5A). The 2 indices used to evaluate hemoglobin changes, namely peak height and rate of increase, increased with the intensity of sour taste stimulus (Figures 5B, C). Moreover, the hemodynamic response of the parotid gland was closely correlated to the salivary secretion volume of the parotid gland in response to sour taste stimulus (Figure 5D).

The findings of the present study indicate that the specific hemodynamic responses of the parotid gland to sour taste stimulus are closely related to taste nerve responses. From the analysis of nerve responses to basic taste stimuli, it was shown that sour taste stimuli (e.g., citric acid and hydrochloric acid) elicited a strong response in the glossopharyngeal nerve (GL), which innervates posterior tongue taste buds of the foliate papillae, circumvallate papillae, and pharynx (Hanamori et al. 1988; Hanamori and Ishiko 1993). Previous studies demonstrated that the foliate papillae were much more sensitive to sour taste than the tongue tip (fungiform papillae). By contrast, studies showed that the foliate papillae and circumvallate papillae were much more sensitive to bitter taste than the tongue tip (Nilsson 1979, Acta Odontol Scand). Besides, bitter taste stimulus elicited more response in the GL than other taste stimuli, sweet and salt (Hanamori et al. 1988; Hanamori and Ishiko 1993). In the present study, however, the bitter taste stimulus of caffeine did not elicit hemodynamic responses (Figures 5A–C) and salivary secretion from the parotid gland (Figure 5D). Previous studies (Hodson and Linden 2006) also support our results in that bitter stimulus showed the lowest salivary flow rate of parotid gland in 5 basic tastes. These data suggest that the neural pathways from the bitter taste cells to the parotid glands, which induce salivary secretion, may be different from those from the bitter taste cells to the brain, which induce bitter taste recognition. Further studies are necessary to clarify the physiological mechanism of the differences between hemodynamic responses of the parotid gland and GL responses to taste stimuli.

Interestingly, for the salt taste stimulus, the peak height and rate of increase tended to increase according to the taste intensity (Figures 5B, C). Furthermore, the salivary secretion volume of the parotid gland in response to the high salt stimulus was significantly higher than that of the low salt stimulus (Figure 5D). Recently, Oka et al. (2013) showed that high concentration salt recruits the primary aversive taste
pathways by activating sour- and bitter-taste-sensing cells. According to the results of the present study, a very high concentration salt stimulus might significantly induce hemodynamic responses and salivary secretion from the parotid gland through the sour taste-activated pathway. Therefore, in addition to sour taste stimulus, NIRS analysis might be suitable for evaluating high concentration salt taste stimulus.

A recent study demonstrated a significant correlation between the subjective hedonic scores (pleasantness/unpleasantness score) accompanying taste stimuli and changes in facial skin blood flow (Kashima and Hayashi 2011). That study showed that skin blood flow in the eyelid increased in response to sweet and umami taste stimuli whereas that in the nose decreased in response to a bitter stimulus (Kashima and Hayashi 2011). In contrast, in our study, the hedonic scores did not have any relationship with the changes in hemodynamic responses of the parotid gland (Figures 4B and Figure 5A–C). Salivary secretion is a result of the reflex response. Thus, the hemodynamic changes observed above may be influenced not by taste palatability but by chemical characteristics of the taste solutions. Therefore, our results suggest that NIRS is a useful tool for taste evaluation, which reacts to taste stimuli independently of the hedonic aspect and the food consumption experience.

Our results demonstrated that RV decreased “acid taste” both in the sensory evaluation (Figure 6A) and in the hemodynamic responses detected by NIRS (Figures 6B–D). These findings suggest that a complex sour taste, namely acid taste in food solutions, can be objectively evaluated by the hemodynamic responses of the parotid gland. Although a previous study showed that the synergistic effect of adding aroma compounds in food-based taste solutions can be evaluated by NIRS (Matsumoto et al. 2012), the present study is the first to demonstrate that a complicated phenomenon such as the “masking effect,” which decreases taste intensity despite additional taste components (RV), can be successfully detected by NIRS.

The RV contained more FAA and TAA than the HAV (Table 3); therefore, one of the reasons for the masking effect of RV might be the high amino acid content. Previous studies showed that basic amino acids decreased the intensity of sour taste in a sensory evaluation (Nishimura and Kato 1988). The mechanism underlying the masking of sour taste by amino acids is unknown, but the present study demonstrated that the masking effect of RV that may be induced in taste receptors or neural activity influenced the hemodynamic responses of the parotid gland. This finding provides an interesting issue for future investigations.

The threshold values for diluted vinegar among subjects indicated an important correlation between the subjective sensory evaluation of acid taste and the objective evaluation made by measuring changes in the hemodynamic response of the parotid gland (Table 5). Thus, subjects who have high sensitivity to sour taste also induce high hemodynamic responses to sour taste stimulus. These results raise the possibility that recording the salivary hemodynamic signals in each subject by NIRS will provide objective measurement data useful for detecting sour taste sensitivity among subjects.

In summary, our findings demonstrated that NIRS can detect sour taste strength by measuring activation of the salivary gland response, which presumably correlates with personal sensitivity toward sour taste. Moreover, the hemodynamic responses measured by NIRS exhibited close similarity to the salivary secretion of the parotid gland. Our study is the first to demonstrate a direct correspondence between hemodynamic response and parotid gland function. The hemodynamic responses of the parotid gland are induced due to an influx mechanism independent of hedonic information; as a result, this novel taste evaluation system based on NIRS would be very objective, especially in sour taste stimulus, and applicable to all subjects without training. Used alongside a traditional method, sensory evaluation, this novel method of recording salivary hemodynamic responses by NIRS would help to improve our understanding of the complex sour taste properties experienced when people intake foods and drinks.

Acknowledgements
We thank Masachika Okamura, Yuji Morita, and Tadayoshi Katsumata for valuable discussions.

Conflict of interest
We have the following interests: Dr Hoshi, Dr Aoki, Dr Kouno, Dr Ogasawara, Dr Onaka, Dr Miura, and Dr Mamiya are employed by Kirin Kyowa Foods Co, Ltd, and Kirin Co, Ltd. There are no patents, products in development or marketed products to declare.

References
Hodson NA, Linden RW. 2006. The effect of monosodium glutamate on parotid salivary flow in comparison to the response to representatives of the other four basic tastes. Physiol Behav. 89(5):711–717.


