A Non-invasive Measurement of Tongue Surface Temperature

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Abstract

Oral temperature, tongue specifically, is a key factor affecting oral sensation and perception of food flavour and texture. It is therefore very important to know how the tongue temperature is affected by food consumption. Unfortunately, traditional methods such as clinical thermometers and thermocouples for oral temperature measurement are not most applicable during food oral consumption due to its invasive nature and interference with food. In this study, infrared thermal (IRT) imager was investigated for its feasibility for the measurement of tongue surface temperature. The IRT technique was firstly calibrated using a digital thermometer (DT). The technique was then used to measure tongue surface temperature after tongue was stimulated by (1) water rinsing at different temperatures (0-45°C); and (2) treated with capsaicin solutions (5, 10, and 20 ppm). For both cases, tongue surface temperature showed significant changes as a result of the physical and chemical stimulation. Results confirm that IRT is feasible for tongue temperature measurement and could be a useful supporting tool in future for the study of food oral processing and sensory perception.

Keywords

Tongue temperature; Oral temperature; Infrared thermal (IRT) imager; Capsaicin; Food oral processing; Food sensory.
1. Introduction

Temperature is no doubt a very important factor for food oral processing and its sensory perception because of two known reasons. Firstly, temperature affects material properties of food and therefore its oral behaviour. For example, increasing temperature will normally reduce the viscosity of a fluid food or hardness/firmness of a solid or semi-solid food, an effect which will lead to a very different texture and a very different oral experience (Bozdogan, 2015; Gómez-Díaz, Navaza, & Quintáns-Riveiro, 2009). Secondly, the temperature will affect the releasing rate of flavour components and their equilibrium with food matrix and saliva. Generally speaking, a higher temperature will be beneficial for a fast release of flavour compounds, which could lead to an enhanced sensation of aroma and taste (Schoumacker et al., 2017; Seuvre, Turci, & Voilley, 2008). We further speculate that temperature effect on food oral processing and sensory perception could have its third cause, the changing of tongue surface temperature. We presume that the temperature of food can alter tongue surface temperature and then the capability and sensitivity of oral sensation and perception.

Tongue is a muscular organ responsible for food manipulation, tasting, and bolus swallowing, etc. The temperature of tongue is one of the key oral physiological parameters that have been shown to affect the perception of food taste, texture as well as irritation (Engelen & van der Bilt, 2008). For example, it has been reported that cooling tongue (or by cooling the sample solutions) affects the perceived intensity of sweetness, bitterness and umami (Green, Alvarado, Andrew, & Nachtigal, 2016; Green & Andrew, 2017; Green & Frankmann, 1987; Green & Nachtigal, 2012). During food consumption, oral cavity including the tongue can be either heated up or cooled down depending on the temperature of the consumed food, which in turn physically affects the perception of food texture (Engelen et al., 2002; Engelen et al., 2003; Engelen & van der Bilt, 2008). The temperature of the oral cavity (and the food) will affect the perceived intensity of sensory attributes of food texture either because changed enzyme activity (e.g. for texture of a starch-containing food) (Bridges, Smythe & Reddrick, 2017), or tongue sensitivity (e.g. for roughness perception) (Aktar, Chen, Ettelaie, et al., 2017). As for the perception of food irritation, literature suggests that temperature of capsaicin solution causes a burning sensation: a weaker burning sensation when capsaicin solution was served at a lower temperature (21°C),
but an enhanced burning sensation at a higher serving temperature (60°C) (Green, 1986; Lawless & Stevens, 1988; Prescott, Allen & Stephens, 1984). Therefore, it is extremely important to know the surface temperature of tongue during food consumption in order to optimize food design for a desirable sensory experience.

Thermometers and thermocouples are probably the most commonly used conventional devices to measure oral temperature (Erickson, 1980; Gallagher, Vercruyssen, & Deno, 1985; Moore, Watts, Hood, & Burritt, 1999). Despite that thermometers can quickly detect the temperature, it is only for a single point measurement, i.e., it is not capable of mapping temperature distribution at tongue surface. Conventional methods are also not applicable when the temperature of materials is not in equilibrium (such as during food oral processing). Moreover, these probes will interfere with food movement during oral processing and cause uncomfortable feelings. The infrared thermal (IRT) imager has been developed as a fast, non-contact and non-invasive technology for surface temperature measurement, including body surface (Ring, 2007). By using IRT, temperature can be easily visualized on site and recorded continuously with high resolution images. Generally, IRT maps the temperature distribution of regions of interest. Modern image processing software can be applied for data analysis and numerical modeling; a temperature profile can be obtained almost instantly as a function of either the location or the time. To achieve an accurate and reliable infrared thermal image, several fundamental experimental requirements have been established, including the setup of examination room (ambient temperature, humidity, air circulation, lighting, etc.), subject control (physically relaxed or in exercise, definition of regions of interest, etc.), subject information screening (gender, age, body mass index, etc.), imaging system and image conditions (lens focus, distance to subjects, lens angle, etc.), image processing and results analysis (minimum, maximum and mean values) and so on (Clark & de Calcina-Goff, 1996; Ring, 1995; Ring & Ammer, 2012; Ring et al., 2004; Ring, Mcevoy, Jung, Zuber & Machin, 2010). Despite few literature on IRT application for tongue surface temperature measurement, no such measurement has been performed for the purpose of food oral processing and sensory perception research.

Tongue surface temperature can be affected by two very different food stimulation:
the temperature of the food (physical stimulation) or the chemical compounds of food (chemical stimulation). Capsaicin (8-methyl-6-nonenoyl-vanillylamide) is the major chemical substance in chili pepper, which gives people experience such as sweating on the face and scalp, facial and neck and chest flushing, lacrimation, salivation, and nasal discharge during and after consumption (Prescott & Stevenson, 1995). Inside the mouth, capsaicin will activate transient receptor potential vanilloid subtype (TRPV1) and produce the most intense feelings, including burning, stinging, tingling and biting sensation (Prescott & Stevenson, 1995). Besides, capsaicin will induce temporary, partial desensitization and affect oral and nasal sensitivity (Green, 1998; Karrer & Bartoshuk, 1991; Rozin, Mark & Schiller, 1981). A very recent study showed that capsaicin made no significant impact on the orthonasal aroma delivery, but it caused a significant reduction in retronasal aroma release and enhanced saliva production (date to be published separately). Previous physiological data suggested that capsaicin produced illusory heating sensations instead of an actual temperature variation (Konietzny, 1983; Petsche, Fleischer, Lembeck, & Handwerker, 1983; Green, 1986).

However, Boudreau et al (2009) found that the topical capsaicin application on orofacial tissues increased cutaneous blood flow and elevated skin temperature. With the help of IRT, therefore, it is of interest to directly observe the effect of capsaicin on tongue surface temperature.

2. Materials and Methods

2.1 Material Preparation

Bottled non-carbonated mineral water (550 mL each, Nongfu Spring, Zhejiang, China)
was purchased from a local supermarket. Before sensory test, the bottled water was respectively equilibrated at four different temperatures levels: cold water (0 °C), cool water (20 °C), warm water (37 °C) and hot water (45 °C). The range of temperature setting of bottled samples covers normal temperature range of food service, but not to cause pain/damage to the tongue.

Capsaicin was used as a chemical stimulus for its effect on tongue surface temperature. A stock solution (10,000 ppm) of food-grade capsaicin (Sigma-Aldrich, Missouri, USA) was prepared by dissolving 1 g in 100 mL of 95% food grade alcohol. The final stimulating solutions were diluted from the stock, which consisted of 5, 10 or 20 ppm of capsaicin. About 1 mL food-grade alcohol was dissolved in 500 mL drinkable water as the control solution (0 ppm). These four solutions were kept in 34 °C (the normal tongue temperature at rest) water bath prior to experiments.

2.2 Apparatus Setup
An infrared thermal imager (Testo 875-li, Testo Instruments International Trading Co., Shanghai, China) was used for the measurement of tongue surface temperature. The emissivity value of the imager was set at 0.99 based on previous study performed by Zhang et al. (1991). Infrared thermal image with the resolution of 160 × 120 pixels was taken by the imager (a spectral range of 8-14 μm, noise equivalent temperature distribution (NETD) \( \leq 0.05P \)) and a lens of 32° × 23°). Tongue surface temperature measurement was performed using a dedicated software for infrared thermal images elaboration (Testo IRSoft, version 4.0) and image presentations were either categories of iron palettes or rainbow palettes. A digital thermometer (DT) with a k-type thermocouple (TES-1310, TES Electrical Electronic Corp., Taiwan) was used to calibrate tongue surface temperature measured by IRT. Temperature indication of the digital thermometer follows the guides set by National Bureau of Standards (NBS, USA) and IEC 584 temperature/voltage thermocouples.

2.3 Areas of interests at tongue surface
As shown in Figure 1a, tongue dorsal surface was divided into five areas: tongue tip, middle, left lateral, right lateral, and tongue root (Chiu, 2000; Hsieh, Shen, & Su, 2016). Since dimensions of human tongue vary from one individual to another, tongue
areas were therefore defined in proportion to the tongue size of the individual. During test, tongue was stretched out as far as possible for each individual. Tongue tip was defined as the anterior one-fifth area of the tongue, tongue root was defined as the posterior one-fifth of tongue, tongue laterals were defined as the lateral one-fifth of the tongue on both sides, and tongue middle was defined as the area between the tip and root and between the two lateral areas. On the infrared thermal image (Fig. 1b), these five areas were therefore selected respectively using polygon tool in the software and the temperatures of each area were automatically calculated.

2.4 Experimental setup
All tests were performed in a laboratory designated specifically for human studies. The laboratory entry was so designed to prevent any external disturbance and interference. Room temperature (20 ± 1°C) was controlled by air conditioning and the humidity (50 ± 10 %) was controlled by a humidifier. Proper and stable lighting was maintained by fluorescent lighting and no direct ventilation was allowed in the lab. During tests, a jaw-shaped metal support was placed in front of the subject so that the subject could sit in a relaxed position with jaw comfortably resting on a sponge padding. A visible mark was made on the sponge so that subjects can place the jaw precisely on the spot. IRT was placed ahead of the support and the lens was set with a distance of 0.20 m in the direction perpendicular to tongue surface. Therefore, an angle of 40 - 45° between lens and sponge padding was maintained (Fig. 2). The focus was manually adjusted to ensure high quality imaging.

2.5 Measurements of tongue surface temperature
2.5.1 Participants preparation
All participants were postgraduate students recruited from the campus and consents were obtained before performing tests. All subjects were non-smokers, not suffering from any illness or discomfort and were not on long-term medication. Prior to temperature measurements, subjects were asked to refrain from intense exercise, caffeine and alcohol for at least 1 h and were asked to relax on a comfortable chair in the laboratory for at least 15 min with no external disturbance (though easy listening music was provided). During the tests, infrared thermal images were taken immediately after subjects protruded their tongues forward and downward to the
desired position. Particularly, participants were told not to respire through their mouth in order to minimize air movement on tongue surface.

2.5.2 Calibration of IRT

Although plenty of practical applications have demonstrated that IRT is accurate and reliable for surface temperature measurement, calibration has also been conducted to ensure the reliability. DT was used to calibrate tongue surface temperature. Ten subjects (age: 24.7 ± 1.3 yrs, F = 5, M = 5, BMI: 20.4 ± 2.5 kg/m²) participated in the test and their tongue surface temperatures were recorded once a day over three consecutive days. During the test, IRT was firstly applied and then the thermocouple of DT was used to measure tongue surface temperature with a minimum interval of approximately 1 sec between courses (that is to press camera shutter).

For measurement of surface temperature with the aid of DT, the thermocouple was consecutively placed at tongue tip, left lateral, right lateral and middle area. On each area, temperature was taken at three randomly selected points and the mean temperature was defined as the arithmetic mean for the area. Thermocouple was cleaned with an antibacterial wipe each time before and after measurement.

For measurement with the IRT, infrared thermal images were taken immediately when subjects protruded their tongues. Mean temperatures of tongue surface were calculated using the polygon tool (curve selection) for each tongue surface area (see Fig. 1(b)). Take one of the subjects for example, polygon tool was applied on the thermal image and tongue tip area was selected by linking up the four points (P1, P2, P3 and P4) (see Fig. 3). Similarly, other three areas (middle and two sides) can also be selected by same means. After selecting a tongue area, histograms can be generated for its temperature analysis. Practically, the color histogram can be used for various purposes including such as image retrieval, segmentation, temperature and intensity-based clustering, individual identification and authentication using biometric approach.

2.5.3 Thermal effects of water consumption

Ten subjects (age: 24.6 ± 1.6 yrs, F = 5, M = 5, BMI: 21.3 ± 2.2 kg/m²) were involved in thermal treatments of tongue on four consecutive days. On the first day, ice-cold
bottled water was applied and the rest of bottled water (at different temperatures) was performed one-by-one on following days in an increasing order of temperature. Therefore all subjects went through all four temperatures within the four consecutive days. All subjects orally took in a mouthful thermally pre-equilibrated bottled water without swallowing and held the liquid in the mouth for 10 sec before expectoration. With a stopwatch for timing, this process was repeated for 3 to 5 min until a total of 550 mL of the water was used up. Then, subjects were immediately asked to place jaw to the designated position shown in Figure 2 and stretch out their tongues for 60 sec. Thermal images were taken every 15 sec by the investigator.

2.5.4 Tongue surface temperature after capsaicin application
Twenty subjects (age: 25.6 ± 1.4 yrs, F = 10, M = 10, BMI: 20.1 ± 2.2 kg/m²) participated in this test on four consecutive days. One reference solution and three capsaicin solutions (5, 10 and 20 ppm) were used for the test. For each day, one of the four solutions was randomly provided to the subjects; and then the tongue surface was monitored for temperature changes. Therefore all subjects went through all four solutions within the four consecutive days. To avoid the effect of saliva mixing, all four solutions were respectively rubbed or rolled onto the whole anterior area of tongue with cotton swabs instead of subjects sipping the capsaicin solution. Subjects were then asked to stretch out their tongues for around 60 sec and tongue surface temperatures were measured every 15 sec with IRT. The tongue surface temperature was taken immediately after tongue stretching and used as the baseline, and the temperature variation (ΔT) was also determined over 1 minute time.

2.6 Data analysis
Statistical analyses were performed using SPSS 23.0 statistical software package. The differences of tongue surface temperature measured by IRT and DT were compared using a paired t-test. The difference in standard deviation of temperature measurement was performed using F-test. Analysis of Variance (ANOVA) was performed to evaluate significant differences in the mean temperature among treatments of capsaicin solutions and Tukey Means Comparison was used to determinate significant differences on temperature. For all statistical analyses, P < 0.05 was considered to be significantly different.

3. Results and discussion
3.1 Calibration of IRT

The temperatures of tongue tip, left lateral, right lateral and middle areas determined by IRT and DT are shown in Table 1. One can see that, over three consecutive days of observations, no significant difference was observed between IRT and DT on all four tongue areas ($P > 0.05$). Further $F$-test also showed that there was no significant difference in measurement accuracy between IRT and DT ($P > 0.05$). According to the experimental data in Table 1, the temperature measurements of IRT were fairly reproducible over the three days measurements. No statistical significance was found between this technique and the conventional DT technique. For the four different areas over the tongue surface, the temperature appears to be somewhat fluctuated, but with no clear pattern. Experimental error is probably the cause of the observed fluctuation. Over the three days measurement, tongue surface temperature remains steady, also reflecting the reliability of the IRT technique. Temperatures of the entire tongue surface from ten subjects measured by IRT are shown in Table 2. Again no significant difference was found during the three consecutive days ($P > 0.05$) for all subjects.

Integrating the information of all temperature measurements by IRT from ten subjects, it was found that the mean temperature of tongue surface was 34.14°C (ranging from as low as 33.2 to as high as 35.7°C). A 2.5°C variation among subjects is somewhat unexpected, but not surprising. With similar controlled environment, Jiang et al. (2007) reported a mean tongue surface temperature of 33.55°C (ranging from 32.7 to 34.3°C) based on 20 healthy subjects with an infrared thermal imager (FLIR-PM390). Zhang and Zhu (1991) reported a mean tongue surface temperature of 33.66°C (ranging from 32.9 to 34.4°C) based on 380 healthy subjects with a self-designed infrared thermal imager. In spite of minute differences, tongue surface temperatures obtained from this study are largely comparable and agreeable to literature results.

When analyzing temperatures of four areas on tongue dorsum surface, it appears that they are in the following order: $T_{\text{middle area}} > T_{\text{right lateral}} \approx T_{\text{left lateral}} > T_{\text{tongue tip}}$. Temperature differences at different tongue surface areas could be due to the different density of blood vessels at these areas. Naumova et al. (2013) found that the number of blood vessels increased in accordance with the increasing tongue surface from
anterior to posterior. According to their study, tongue dorsum surface was divided into the following five zones: anterior third, middle third, posterior third, lateral surface and root. Blood vessels on the anterior third, middle third, posterior third and lateral surface of tongue were respectively counted to be 1208, 1230, 1292 and 1048 per cm² on average (Naumova, Dierkes, Sprang, & Arnold, 2013). Although the area definition of Naumova’s method is different from this study, it can be explained that the temperature of middle area is higher than tongue tip and tongue laterals. In terms of morphology, tongue dorsum surface is divided by a groove into symmetrical halves by the median sulcus. The similar temperatures of right and left lateral area, therefore, conform to bilateral symmetry of the tongue surface. Likewise, two deep lingual veins (near the ventral surface of tongue) and associated deep lingual arteries, which distribute on both sides of the tongue, may be the reason of higher lateral temperatures than tongue tip but lower than the middle area.

3.2 Thermal effects of water consumption

Altogether ten subjects participated in this test. Color-coded infrared thermal images from one representative subject as a function of temperature are shown in Figure 4. Histograms of temperature distribution are correspondingly presented alongside each image. From the infrared thermal images, mouth rinses with water of different temperatures were found to alter the temperature of the entire tongue surface. After subjects repeatedly rinsed their mouth with water, tongue surfaces were cooled down by cold and cool water and heated up by warm and hot water. Further data analysis gives the mean temperature of the subject’s entire tongue surface of 20.6, 26.7, 33.6 and 37.7°C, respectively. Temperature differences of the subject’s tongue surface before and after water rinsing are shown in Figure 5a. Rinsed with a cold (0°C) water and a cool (20°C) water, tongue surface temperature decreased by 13.7°C and 7.0°C, respectively, from its normal baseline. After rinsed with a warm (37°C) water, the tongue surface temperature became 33.6 °C, only 0.2 °C difference from its normal figure. For hot (45°C) water, tongue surface was heated up and its temperature reached to as high as 37.7°C.

Figure 5b illustrates the temperature variations of tongue surface within 60 sec after water rinsing (IRT observation was not possible for longer time because of the buildup of mouth water which dripped from the open mouth and made subjects very
uneasy). Tongue surface temperature alteration seemed to be not long lasting, but
would gradually recover to its original value. The tongue surface temperature started
to increase shortly after being rinsed with cold and cool water, possibly due to the
recovered blood flow. Shortly after being rinsed with warm and hot water, on the
contrary, tongue surface temperature showed a continued decrease, possibly because
of the evaporation at tongue surface.

Above results show that water rinsing can indeed alter the temperature at tongue
surface. We tend to believe that the range of temperature change was so large and
significant that its effects on tongue’s sensory functionality (e.g. the perception of
tastes, discrimination of texture, and etc) cannot be ignored.

3.3 Tongue surface temperature after capsaicin application

Tongue temperature alteration after the consumption of a spicy food is of great
interests to food oral processing studies. Therefore, IRT technique has been tested for
its feasibility to monitor oral surface temperature change as influenced by the
chemical composition of consumed food. Capsaicin was chosen for this purpose
because of its well-known effect on skin stimulation and increased blood flow under
the skin surface. Figure 6 shows temperature variations of the entire tongue surface
after treated with a control solution and capsaicin solutions. Immediately after surface
treatment (0 sec), tongue’s mean temperatures were recorded to be 33.90 (control),
33.87 (5 ppm), 33.80 (10 ppm), and 33.91°C (20 ppm) respectively. The application
temperature of capsaicin and control solutions was 34 °C, same as that of the tongue
at rest. After the treatment, tongue was stretched out of the oral cavity for temperature
measurement. Because the tongue was exposed to the open air, continuous decrease of
surface temperature is expected due to a lower room temperature and due to the
cooling effect caused by surface evaporation. As expected, temperature decreases for
all four cases, but at different rates. The tongue surface treated with capsaicin has a
higher surface temperature than that of control. Sixty seconds after capsaicin
treatment, tongue’s mean surface temperatures became 29.87, 29.99, and 30.27 °C
respectively for tongue treated with 5, 10, and 20 ppm capsaicin solutions, higher than
that of control which was recorded at 29.51 °C (P < 0.05).

Even though capsaicin treatment does not cause sudden increase of tongue surface
temperature, the stimulation seemed to have a lasting effect on tongue surface. Figure 7 plots temperature change in relation to that at time zero when the tongue stretched out immediately after capsaicin treatment. The negative values shown in Figure 7 reflect gradual temperature decrease at four different areas of the tongue surface over the time. Such temperature drop is not surprising because of the lower room temperature and also the surface evaporation on the tongue surface. However, we would like to draw readers’ attention to the different pattern of temperature decrease for tongue being treated by four different solutions. Almost at all four tongue surface areas (tip, middle, and two sides), the temperature remained higher after capsaicin treatment than that of control. For example, one minute after the treatment, a difference of 1.34°C was observed at tongue tip between the application of 20 ppm capsaicin solution and the control solution (significant with $P < 0.05$). Similarly, temperature differences of 0.54 and 0.73°C were found respectively on the left lateral and right lateral area (significant with $P < 0.05$). A temperature difference of 1.14°C was also observed at the middle area (significant with $P < 0.05$).

Above results suggest that capsaicin causes a long lasting increase tongue surface temperature. The temperature variation is detectable by the IRT technique and is statistically significant. The cause behind temperature increase is possibly related to the increased cutaneous blood flow of tongue after capsaicin stimulation. Previous study has already reported neurogenic mediated responses of vasodilation or even red flare followed topical application of capsaicin on skin (Helme & McKernan, 1985). Nielsen et al. (2013) investigated the effects of capsaicin on skin of forearm and demonstrated increases in cutaneous blood flow and elevated skin temperature. Bouzida et al (2009) also found that increases in blood flow and temperature were paralleled with intense burning pain when capsaicin was applied on orofacial tissues.

4. Limits and weakness of the current study

Research findings from this work confirm that tongue temperature could vary significant after being stimulated by hot/cold water or by chemical compounds (capsaicin). This work also demonstrates that tongue surface temperature can be monitored reliably in a non-invasive manner by using imaging technique IRT. Despite that research findings are significant, possible limits and weakness of the study should be noted.
Even though IRT offers accurate and reliable temperature measurement, the technique was unable to give continuous imaging. Images can only be taken at an interval of at least 15 sec. Also, images were taken manually by an operator, which will inevitably involve some small variations in shooting time (and then the temperature). A video recording IRT will be needed for continuous monitoring of temperature change.

Another limitation of the experimental design is the number of subjects. This study included 20 subjects for tongue temperature observation after capsaicin treatment, but only 10 volunteers for the test of IRT calibration and thermal impact of water. It is well known that intra-oral temperature variation could be highly dependent of subjects’ gender, pre-test physiological and psychological conditions as well as other factors. Despite results obtained from those subjects are conclusive, more subjects will be needed to further confirm the observed effects.

It should also be mentioned that this study did not perform a screening to volunteers for their sensitivity to capsaicin. Participated subjects were students coming from different provinces or regions in China, with possibly very different food culture and very different previous food exposure. It could be reasonable to assume that regional and diet differences of subjects might give difference responses to capsaicin, but this has not been taken into consideration in the analysis.

5. Conclusions
This study investigated the feasibility of using IRT technique as a non-invasive method for the measurement of tongue surface temperature after being treated with hot/cold water and capsaicin solutions. Our findings demonstrated that tongue surface could have a large temperature variation after physical and chemical stimulation and IRT is an effective technique for an instant measurement of tongue temperature. This non-contact and non-invasive technique ensures subjects comfort-ability of temperature measurement during food oral processing. Our long term aim is to reveal the thermal impact of food (and its components) on tongue surface and more importantly on tongue’s sensory capability. The technique established in this work will be used for the following study on tongue’s sensory sensitivity.
Ethical statements

Ethical Review: Ethic permission was obtained from the school of Food Science and Biotechnology at Zhejiang Gongshang University and all test procedures followed the ethical rules and regulations set by the University.

Informed Consent: All tests were conducted in a purposely designated human study laboratory. Consent forms were obtained from each subject before the test.

Conflict of interests: Authors declare that authors have no conflict of interests in conducting this project.

Acknowledgements

The authors would like to thank Dr. Carol Mosca for her assistance in sensory analysis. This study was financially supported by the National Key Research and Development of China (Grant number 2017YFD0400101) awarded by the Ministry of Science and Technology and by the scheme of Zhejiang Provincial Top Key Discipline of Food Science and Biotechnology.
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Captions

Figure 1. Division of tongue surface: (a) sketch of four areas of the tongue surface for temperature measurement, and (b) tongue surface highlighted on infrared thermal image (160 × 120 pixels) with a temperature scale.

Figure 2. Sketch of experimental set-up for thermography experiment. The temperature and humidity of the laboratory is kept within a comfortable limit. The lens of IRT is positioned perpendicularly to tongue surface to minimize geometrical errors. The distance and angle of lens is fixed so that approximately the same number of pixels is covered each time during experiments. The sketch was produced to illustration purpose and was not to the proportion.

Figure 3. Infrared thermal image analysis and histogram generation.

Figure 4. Images (160 × 120 pixels) of one representative subject and corresponding histogram of temperature distribution after his/her mouth rinsed with water of different temperatures: (a) cold water (0°C), (b) cool water (20°C), (c) warm water (37°C) and (d) hot water (45°C). The horizontal axis of histogram represents the range of temperature variations and the vertical axis presents the percentage of pixels in the corresponding temperature variation. The colors of columns in histograms corresponded to the color of temperature scale in the infrared thermal images.

Figure 5. Temperature variations of subjects (n = 10) within 60 sec after rinsing with 0°C cold water (■), 20°C cool water (●), 37°C warm water (▲) and 45°C hot water (×).

Figure 6. Temperature variations of tongue surface after treatment of control (■), 5 ppm (●), 10 ppm (▲) and 20 ppm (×) capsaicin solution (n = 20).

Figure 7. Variations of temperature on different areas of tongue surface (n = 20): (a) tip, (b) left lateral, (c) right lateral and (d) middle area within 60 sec after treatment of control (■), 5 ppm (●), 10 ppm (▲) and 20 ppm (×) capsaicin solution. Temperature variation was given in negative values, which was the measured temperature in relation to that measured immediately after capsaicin treatment.
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Figure 5. (a) Instant temperatures of the subject's tongue surface before (orange column) and 1 min after rinsed with water with corresponding temperatures (teal: cold; blue: cool; green: warm; red: hot); and (b) Temperature variations of subjects (n=10) within 60 sec after rinsing with 0°C cold water (■), 20°C cool water (●), 37°C warm water (▲) and 45°C hot water (×).
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Table 1. Results of temperature distributions on four tongue surface areas measured with IRT and DT (n=10).

Table 2. Temperature variations during three consecutive days of monitoring with IRT (n=10).
Table 1. Results of temperature distributions on four tongue surface areas measured with IRT and DT (n=10)

| Area | Method | Day 1 | Day 2 | Day 3 | P Value | Day 1 | Day 2 | Day 3 | P Value | Day 1 | Day 2 | Day 3 | P Value |
|------|--------|-------|-------|-------|---------|-------|-------|-------|---------|-------|-------|-------|---------|
|      | IRT    | DT    |       |       |         | IRT   | DT    |       |         | IRT   | DT    |       |         |
| Tip  | 32.72  | 32.14 | 0.301 | 32.50 | 32.84  | 0.128 | 33.20 | 33.26 | 0.095   |       |       |       |         |
|      | (0.56) | (0.61) |       | (0.57) | (0.50) |       | (0.49) | (0.26) |         |       |       |       |         |
| Left | 33.66  | 33.84 | 0.304 | 33.84 | 33.82  | 0.468 | 34.20 | 34.24 | 0.230   |       |       |       |         |
|      | (0.32) | (0.59) |       | (0.68) | (0.29) |       | (0.60) | (0.28) |         |       |       |       |         |
| Right| 33.68  | 33.76 | 0.156 | 33.78 | 33.96  | 0.097 | 33.98 | 34.18 | 0.158   |       |       |       |         |
|      | (0.36) | (0.54) |       | (0.58) | (0.18) |       | (0.66) | (0.52) |         |       |       |       |         |
| Middle| 34.06 | 33.92 | 0.224 | 33.20 | 34.14  | 0.287 | 34.24 | 34.42 | 0.467   |       |       |       |         |
|      | (0.78) | (0.33) |       | (0.49) | (0.57) |       | (0.67) | (0.25) |         |       |       |       |         |

Data were presented as mean (± standard deviation). IRT stands for Infrared Thermal imager; DT stands for Digital Thermometer.

Table 2. Temperature variations during three consecutive days of monitoring with IRT (n=10)

| Test day | Infrared thermal imager (°C) | Min | Max | Mean | SD |
|----------|-----------------------------|-----|-----|------|----|
| Day 1    |                             | 33.2| 35.1| 34.10| 0.59|
| Day 2    |                             | 33.0| 35.3| 34.17| 0.64|
| Day 3    |                             | 33.2| 35.7| 34.17| 0.79|