

1
2
3
4
5
6 **A Non-invasive Measurement of Tongue Surface Temperature**
7

8
9 Cong Lv¹, Xinmiao Wang¹, Jianshe Chen^{1*}, Ni Yang², Ian Fisk²
10

11
12
13
14 ¹School of Food Science and Biotechnology, Zhejiang Gongshang University,
15 Hangzhou, Zhejiang, 310018, China.

16 ² Division of Food Science, University of Nottingham, Sutton Bonington Campus,
17 NG72RD, U.K.
18
19
20
21
22
23
24
25
26
27

28 *Correspondence

29 Jianshe Chen, School of Food Science and Biotechnology, Zhejiang Gongshang
30 University, Hangzhou, Zhejiang, 310018, China.

31 Email: jschen@zjgsu.edu.cn
32
33
34

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.

The aim of this study is to establish the feasibility of using IRT as a tool to measure tongue surface temperature, particularly in relation to food consumption and sensory perception. We hope that the methodology can be applied in future for temperature monitoring of both tongue surface and food during the process of food oral consumption. To ensure the reliability of temperature measurement, IRT was firstly calibrated against a digital thermometer. Tongue surface temperature was monitored after being stimulated by physical stimulation (water of different temperature) and chemical stimulation (capsaicin). These were achieved by rinsing mouth with water of different temperatures (0 to 45 °C) and by treatment of capsaicin solutions at different concentrations (0 to 20 ppm).

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 875-1i, 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) $\leq 0.05\text{P}$ and a lens of $32^\circ \times 23^\circ$). Tongue surface temperature measurement was performed using a dedicated software for infrared thermal images elaboration (Testo IRTSoft, 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 ($20 \pm 1^{\circ}\text{C}$) was controlled by air conditioning and the humidity ($50 \pm 10\%$) 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 $40 - 45^{\circ}$ 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

335 anterior to posterior. According to their study, tongue dorsum surface was divided into
336 the following five zones: anterior third, middle third, posterior third, lateral surface
337 and root. Blood vessels on the anterior third, middle third, posterior third and lateral
338 surface of tongue were respectively counted to be 1208, 1230, 1292 and 1048 per cm²
339 on average (Naumova, Dierkes, Sprang, & Arnold, 2013). Although the area
340 definition of Naumova's method is different from this study, it can be explained that
341 the temperature of middle area is higher than tongue tip and tongue laterals. In terms
342 of morphology, tongue dorsum surface is divided by a groove into symmetrical halves
343 by the median sulcus. The similar temperatures of right and left lateral area, therefore,
344 conform to bilateral symmetry of the tongue surface. Likewise, two deep lingual veins
345 (near the ventral surface of tongue) and associated deep lingual arteries, which
346 distribute on both sides of the tongue, may be the reason of higher lateral
347 temperatures than tongue tip but lower than the middle area.

349 3.2 Thermal effects of water consumption

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

366 Figure 5b illustrates the temperature variations of tongue surface within 60 sec after
367 water rinsing (IRT observation was not possible for longer time because of the
368 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 capasaicin 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.

References

- Aktar, T., Chen, J., Ettelaie, R., Holmes, M. & Henson, B. (2017). Human roughness perception and possible factors affecting roughness sensation. *Journal of Texture Studies*, **48**, 181-192.
- Boudreau, S. A., Wang, K., Svensson, P., Sessle, B. J., & Arendt-Nielsen, L. (2009). Vascular and psychophysical effects of topical capsaicin application to orofacial tissues. *Journal of Orofacial Pain*, **23**, 253-264.
- Bridges, J. Smythe, J. & Reddrick, R. (2017). Impact of salivary enzyme activity on the oral perception of starch containing foods. *Journal of Texture Studies*, **48**, 288-293.
- Bouzida, N., Bendada, A., & Maldague, X. P. (2009). Visualization of body thermoregulation by infrared imaging. *Journal of Thermal Biology*, **34**, 120-126.
- Bozdogan, A. (2015). Viscosity behavior of bitter orange (*Citrus aurantium*) juice as affected by temperature and concentration. *Journal of Food*, **13**, 535-540.
- Chiu, CC. (2000). A novel approach based on computerized image analysis for traditional Chinese medical diagnosis of the tongue. *Computer Methods and Programs in Biomedicine*, **61**, 77-89.
- Clark, R. P., & de Calcina-Goff, M. L. (1996). International standardisation in medical thermography-draft proposals. In: 18th Annual Conference IEEE Engineering in Medicine and Biology Society, Amsterdam, Netherlands, 2089–2090.
- Engelen, L., de Wijk, R. A., Prinz, J. F., Janssen, A. M., Weenen, H., & Bosman, F. (2003). The effect of oral and product temperature on the perception of flavor and texture attributes of semi-solids. *Appetite*, **41**, 273-281.
- Engelen, L., de Wijk, R. A., Prinz, J. F., Van der Bilt, A., Janssen, A. M., & Bosman, F. (2002). The effect of oral temperature on the temperature of liquids and semisolids in the mouth. *European Journal of Oral Sciences*, **110**, 412-416.
- Engelen, L., & Van der Bilt, A. (2008). Oral physiology and texture perception of semisolids. *Journal of Texture Studies*, **39**, 83-113.
- Erickson, R. (1980). Oral temperature differences in relation to thermometer and technique. *Nursing Research*, **29**, 157-164

518 Gómez-Díaz, D., Navaza, J. M., & Quintáns-Riveiro, L. C. (2009). Effect of
 519 temperature on the viscosity of honey. *International Journal of Food Properties*, **12**,
 520 396-404.

521 Gallagher, S., Vercruyssen, M., & Deno, N. S. (1985). Hot air breathing: effects of
 522 elevated wet bulb temperatures on tissue temperatures of the mouth. *American*
 523 *Industrial Hygiene Association Journal*, **46**, 332-335.

524 Green, B. G. (1986). Sensory interactions between capsaicin and temperature in the
 525 oral cavity. *Chemical Senses*, **11**, 371-382.

526 Green, B. G. (1998). Capsaicin desensitization and stimulus-induced recovery on
 527 facial compared to lingual skin. *Physiology and Behavior*, **65**, 517-523.

528 Green, B. G., Alvarado, C., Andrew, K., & Nachtigal, D. (2016). The effect of
 529 temperature on umami taste. *Chemical Senses*, **41**, 537-545.

530 Green, B. G., & Andrew, K. (2017). Stimulus-dependent effects of temperature on
 531 bitter taste in humans. *Chemical Senses*, **42**, 153-160.

532 Green, B. G., & Frankmann, S. P. (1987). The effect of cooling the tongue on the
 533 perceived intensity of taste. *Chemical Senses*, **12**, 609-619.

534 Green, B. G., & Nachtigal, D. (2012). Somatosensory factors in taste perception:
 535 Effects of active tasting and solution temperature. *Physiology and Behavior*, **107**,
 536 488-495.

537 Helme, R. D., & McKernan, S. (1985). Neurogenic flare responses following topical
 538 application of capsaicin in humans. *Annals of Neurology*, **18**, 505-509.

539 Hofstra, W. A., & de Weerd, A. W. (2008). How to assess circadian rhythm in humans:
 540 a review of literature. *Epilepsy and Behavior*, **13**, 438-444.

541 Hsieh, S., Shen, L., & Su, S. (2016). Tongue color changes within a menstrual cycle
 542 in eumenorrheic women. *Journal of Traditional and Complementary Medicine*, **6**,
 543 269-274.

544 Jiang, Z., & Zhu, K. (2007). A comparison of infrared tongue image of healthy people
 545 in different seasons. *Traditional Chinese Medicine*, **20**, 6-7.

546 Karrer, T. & Bartoshuk, L. (1991). Capsaicin desensitization and recovery on the
 547 human tongue. *Physiology and Behavior*, **4**, 757-764.

548 Konietzny, F. (1983). The effect of capsaicin on the response characteristic of human
 549 C-polymodal nociceptors. *Journal of Thermal Biology*, **8**, 213-215.

550 Lahiri, B. B., Bagavathiappan, S., Jayakumar, T., & Philip, J. (2012). Medical
 551 applications of infrared thermography: A review. *Infrared Physics and Technology*, **55**,
 552 221-235.

553 Lawless, H. T., & Stevens., D. A. (1988). Responses by humans to oral chemical
 554 irritants as a function of locus of stimulation. *Percept Psychophys*, **43**, 72-78.

555 Moore, R. J., Watts, J. T. F., Hood, J. A. A., & Burritt, D. J. (1999). Intra-oral
 556 temperature variation over 24 hours. *European Journal of Orthodontics*, **21**, 249-261.

557 Naumova, E. A., Dierkes, T., Sprang, J., & Arnold, W. H. (2013). The oral mucosal
 558 surface and blood vessels. *Head and Face Medicine*, **9**, 1-5.

559 Nielsen, T. A., da Silva, L. B., Arendt-Nielsen, L., & Gazerani, P. (2013). The effect
 560 of topical capsaicin-induced sensitization on heat-evoked cutaneous vasomotor
 561 responses. *International Journal of Physiology, Pathophysiology and Pharmacology*,
 562 **5**, 148-160.

563 Petsche, U., Fleischer, E., Lembeck, F., & Handwerker, H. O. (1983). The effect of
 564 capsaicin application to a peripheral nerve on impulse conduction in functionally
 565 identified afferent nerve fibres. *Brain Research*, **265**, 233-240.

566 Prescott, J., Allen, S., & Stephens, L. (1984). Interactions between oral chemical
 567 irritation, taste and temperature. *Chemical Senses*, **18**, 389-404.

568 Prescott, J., & Stevenson, R. J. (1995). Pungency in food perception and preference.
 569 *Food Reviews International*, **11**, 665-698.

570 Ring, E. F. J. (1995). Criteria for thermal imaging in medicine. In: Proc. 17th Annual
 571 Conference of IEEE Engineering in Medicine and Biology Society, Montreal, Canada,
 572 1697-1698.

573 Ring, E. F. J. (2007). The historical development of temperature measurement in
 574 medicine. *Infrared Physics and Technology*, **49**, 297-301.

575 Ring, E. F. J., & Ammer, K. (2012). Infrared thermal imaging in medicine.
 576 *Physiological Measurement*, **33**, R33-R46.

577 Ring, E. F. J., Ammer, K., Jung, A., Murawski, P., Wiecek, B., Zuber, J., Zwolenik, S.,

578 Plassmann, P., Jones, C., & Jones, B.F. (2004). Standardization of infrared imaging. In:
579 Proc. 26th Annual International Conference of the IEEE Engineering in Medicine and
580 Biology Society, San Francisco, USA, 1183-1185.

581 Ring, E. F. J., Mcevoy, H., Jung, A., Zuber, J., & Machin, G. (2010). New standards
582 for devices used for the measurement of human body temperature. *Journal of Medical*
583 *Engineering and Technology*, **34**, 249-253.

584 Rozin, P., Mark, M., & Schiller, D. (1981). The role of desensitization to capsaicin in
585 chili pepper ingestion and preference. *Chemical Senses*, **1**, 23-31.

586 Schoumacker, R., Martin, C., Thomas-Danguin, T., Guichard, E., Quéré, J. L. L., &
587 Labouré, H. (2017). Fat perception in cottage cheese: The contribution of aroma and
588 tasting temperature. *Food Quality and Preference*, **56**, 241-246.

589 Seuvre, A. M., Turci, C., & Voilley, A. (2008). Effect of the temperature on the release
590 of aroma compounds and on the rheological behaviour of model dairy custard. *Food*
591 *Chemistry*, **108**, 1176-1182.

592 Standring, S. (2008). *Gray's Anatomy: the Anatomical Basis of Clinical Practice*. (40th
593 ed.). London: Bailliere, (Chapter 26).

594 Waterhouse, J., Drust, B., Weinert, D., Edwards, B. J., Gregson, W., Atkinson, G., Kao,
595 S.Y., Aizawa, S. & Reilly, T. (2005). The circadian rhythm of core temperature: Origin
596 and some implications for exercise performance. *Chronobiology International*, **22**,
597 207-225.

598 Zhang, X., Zhu, K., Li, W., Zhang, Q., Zhang, B., Zhang, J., & Liu, H. (1991). A
599 study of the surface temperature of human suing the technology of infrared
600 thermograph. *Journal of Tianjin University*, **3**, 20-24.

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 as 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 produce to illustration purpose and was not to the proportion.

Figure 3. Infrared thermal image analysis and histogram generation.

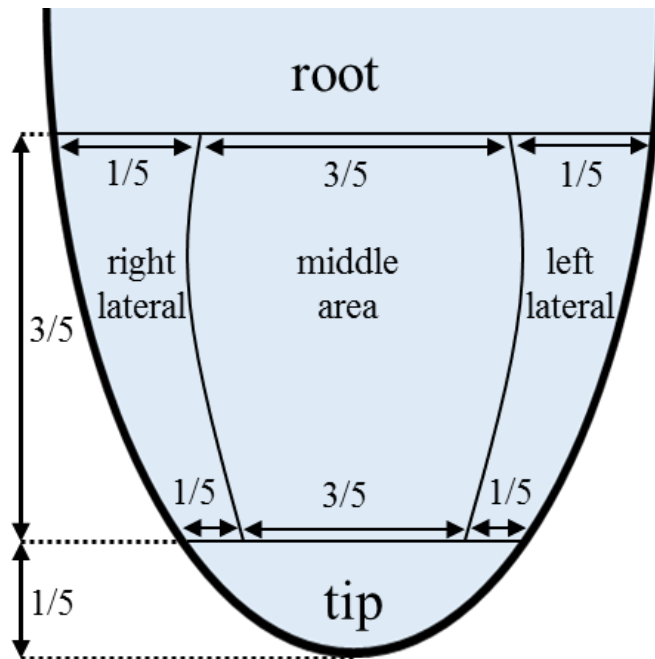
Figure 4. Images (160×120 pixels) of one representative subject and corresponding histogram of temperature distribution after his/her mouth rinsed with water of different temperatures: (a) cold water (0°C), (b) cool water (20°C), (c) warm water (37°C) and (d) hot water (45°C). The horizontal axis of 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.

(a)



(b)

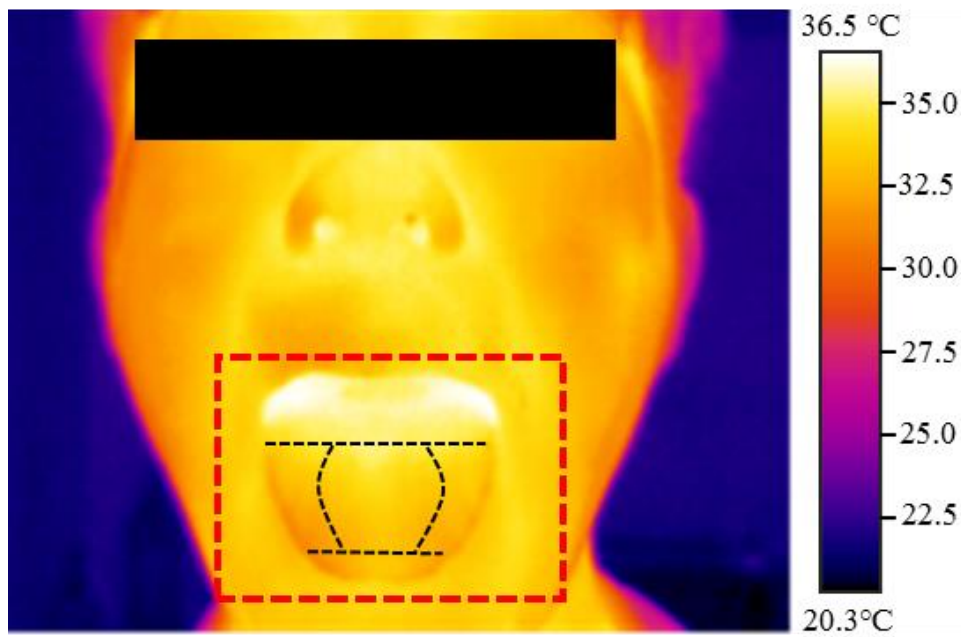


Figure 1. Division of tongue surface: (a) sketch of four areas of the tongue surface for temperature measurement, and (b) tongue surface highlighted on infrared thermal image (160×120 pixels) with a temperature scale.

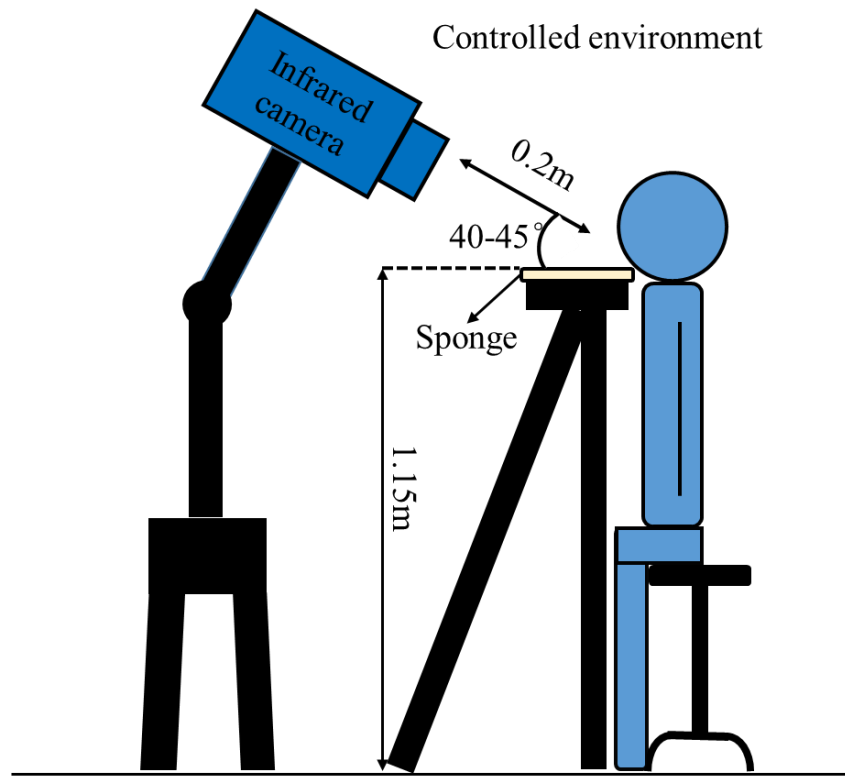


Figure 2. Sketch of experimental set-up for thermography experiment. The temperature and humidity of the laboratory is kept within a comfortable limit. The lens of IRT is positioned as perpendicularly to tongue surface to minimize geometrical errors. The distance and angle of lens is fixed so that approximately the same number of pixels is covered each time during experiments.

The sketch was produce to illustration purpose and was not to the proportion.

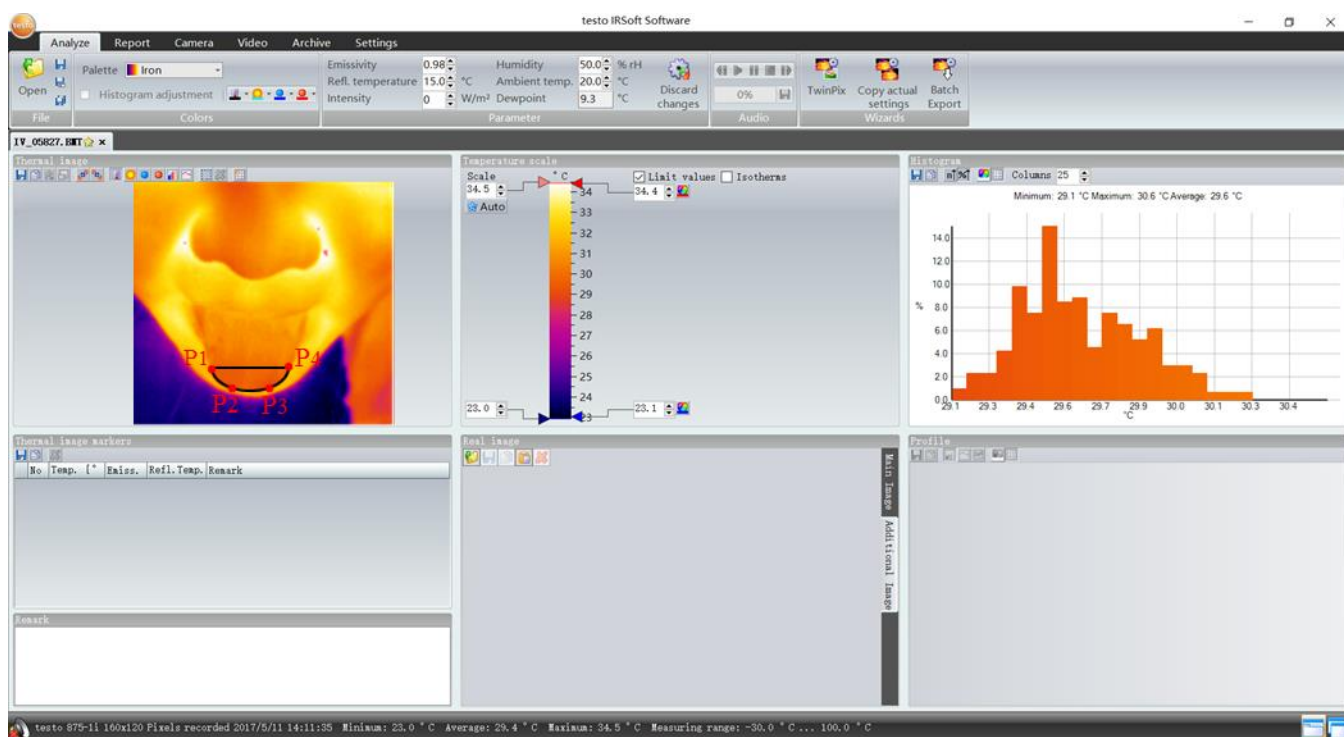


Fig 3. Infrared thermal image analysis and histogram generation.

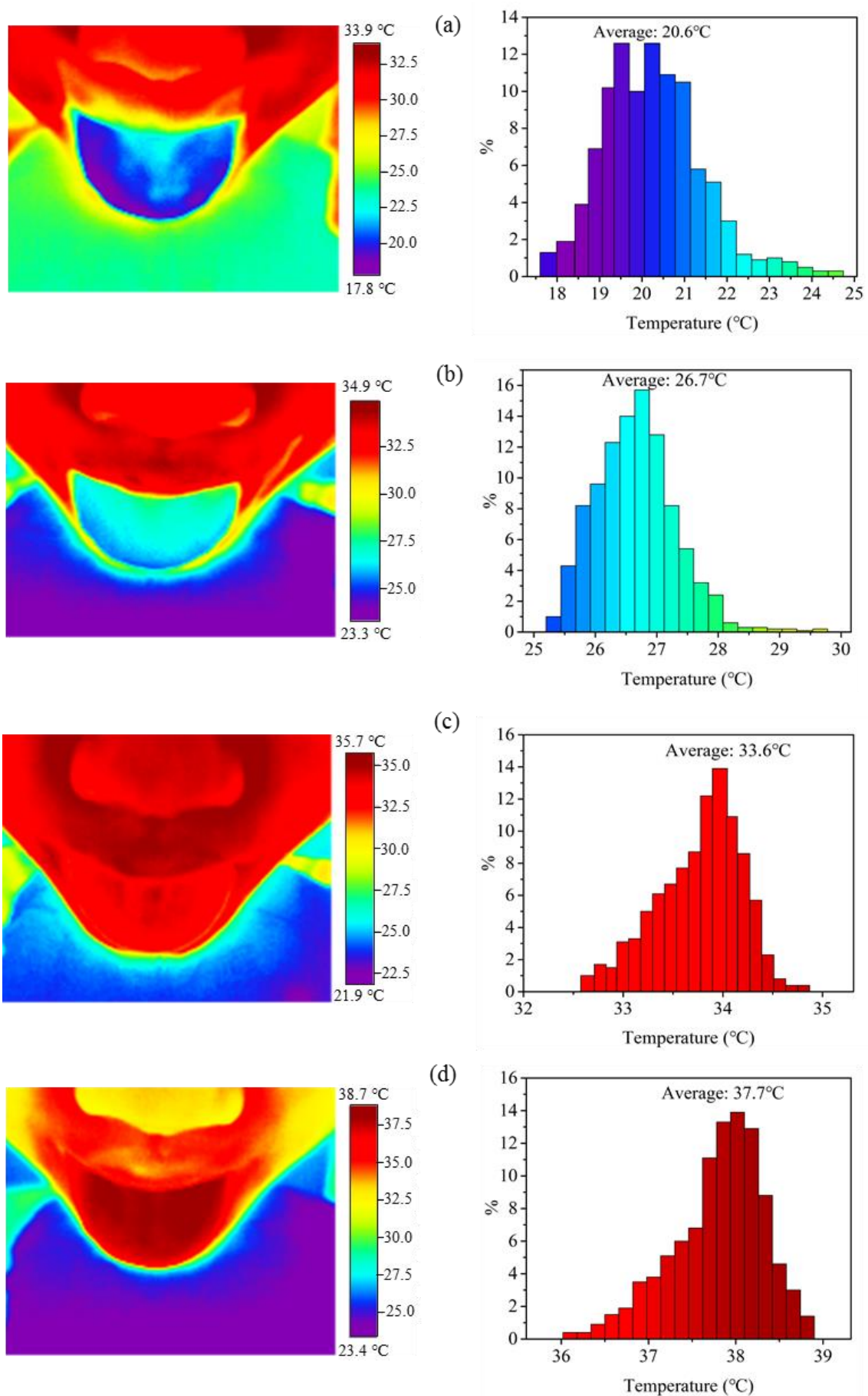


Figure 4. Images (160×120 pixels) of one representative subject and corresponding histogram

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.

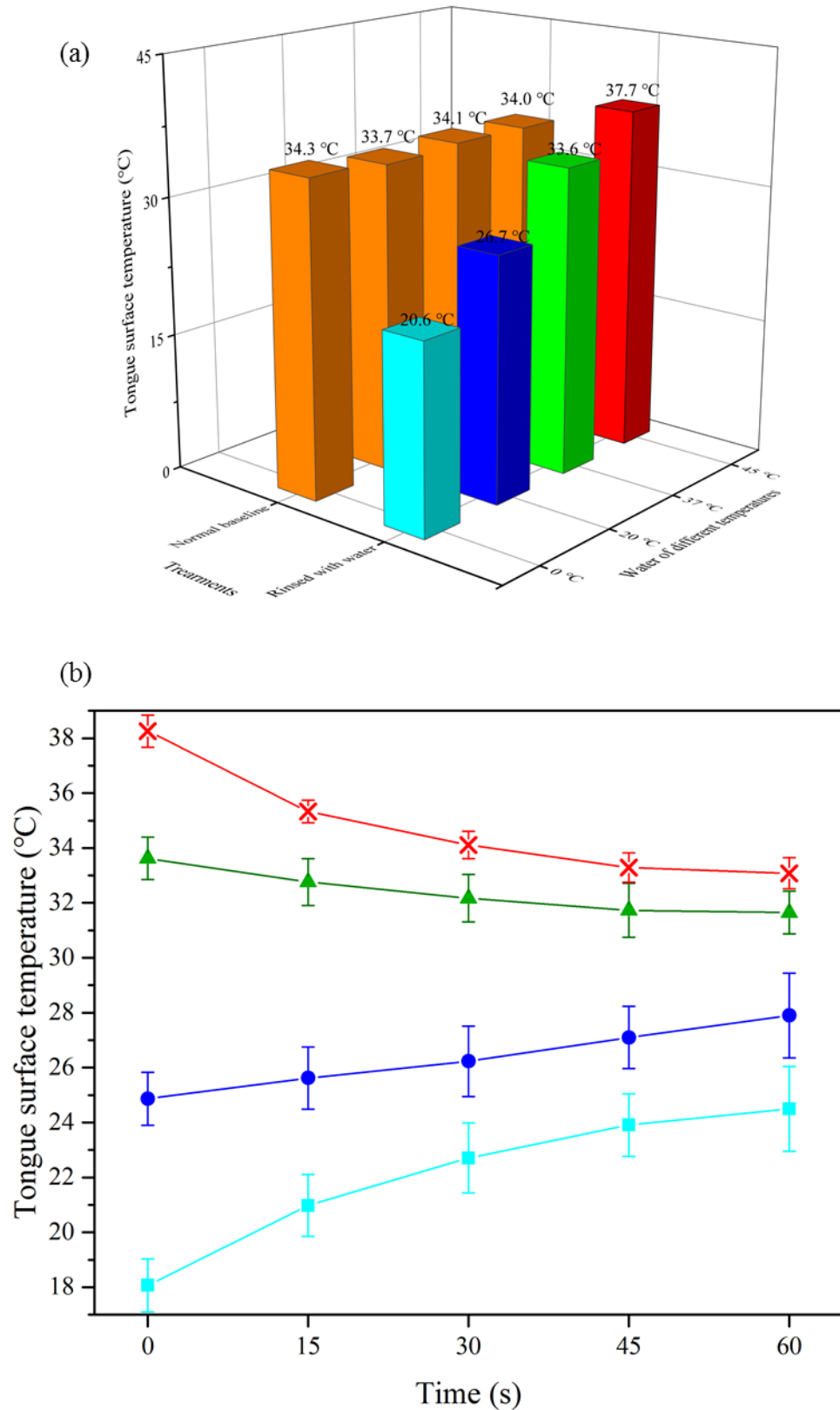


Figure 5. (a) Instant temperatures of the subject's tongue surface before (orange column) and 1 min after rinsed with water with corresponding temperatures (teal: cold; blue: cool; green: warm; red: hot); and (b) Temperature variations of subjects (n=10) within 60 sec after rinsing with 0°C cold water (■), 20°C cool water (●), 37°C warm water (▲) and 45°C hot water (×).

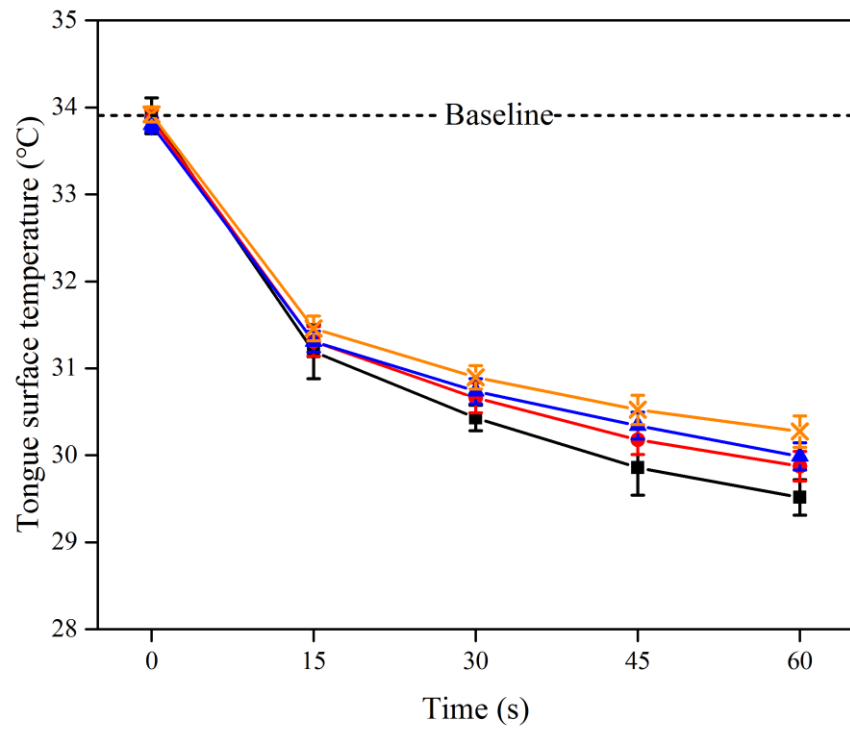


Figure 6. Temperature variations of tongue surface after treatment of control (■), 5 ppm (●), 10 ppm (▲) and 20 ppm (×) capsaicin solution (n=20).

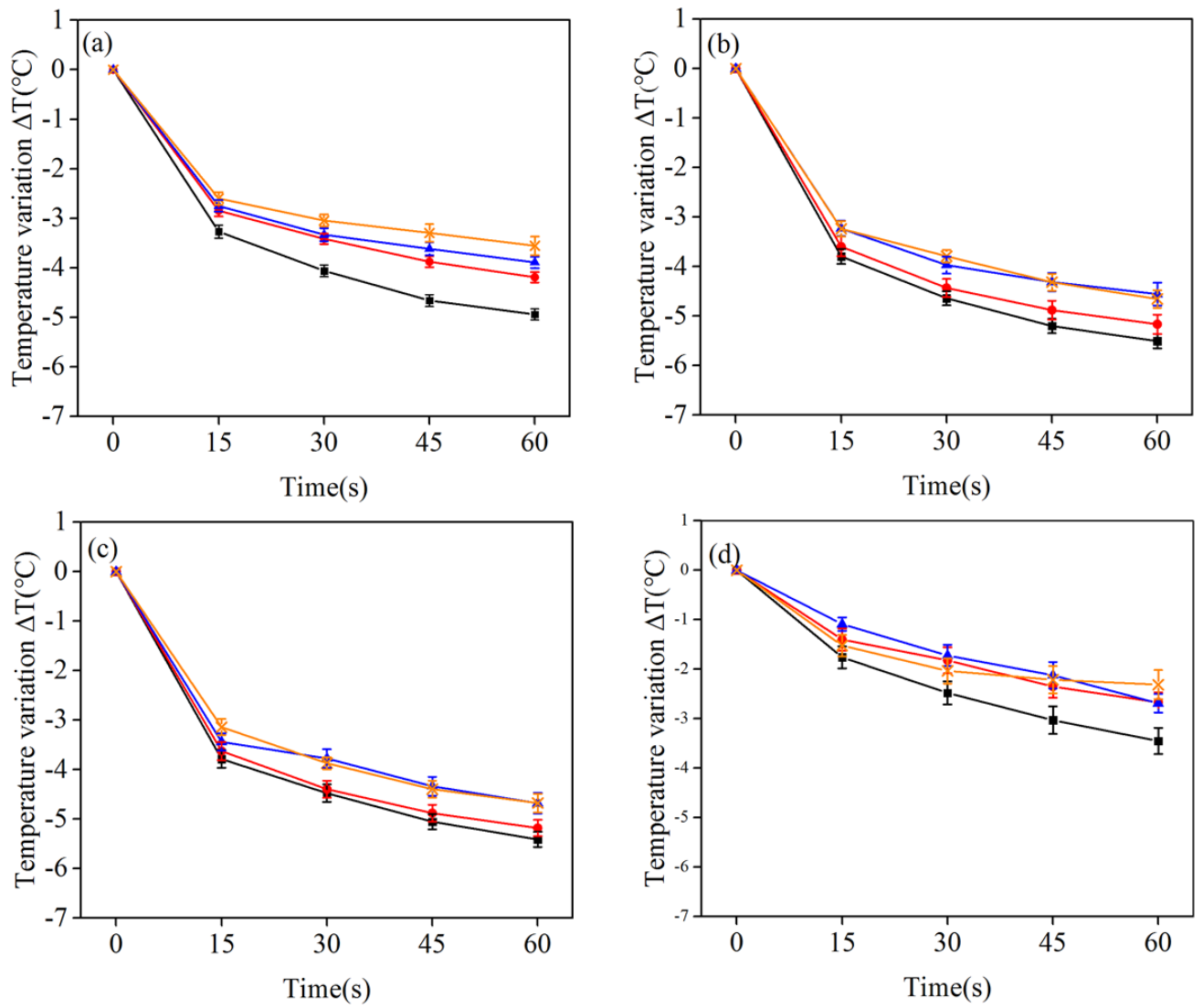


Figure 7. Variations of temperature on different areas of tongue surface (n=20): (a) tip, (b) left lateral, (c) right lateral and (d) middle area within 60 sec after treatment of control (■), 5 ppm (●), 10 ppm (▲) and 20 ppm (×) capsaicin solution. Temperature variation was given in negative values, which was the measured temperature compared to that measured immediately after capsaicin treatment.

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)

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

Data were presented as mean (\pm 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 ($^{\circ}\text{C}$)			
	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