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A Non-invasive Measurement of Tongue Surface Temperature

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35 **Abstract**

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

51

52

53 **Keywords**

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

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61 **1. Introduction**

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

77

78 Tongue is a muscular organ responsible for food manipulation, tasting, and bolus
79 swallowing, etc. The temperature of tongue is one of the key oral physiological
80 parameters that have been shown to affect the perception of food taste, texture as well
81 as irritation (Engelen & van der Bilt, 2008). For example, it has been reported that
82 cooling tongue (or by cooling the sample solutions) affects the perceived intensity of
83 sweetness, bitterness and umami (Green, Alvarado, Andrew, & Nachtigal, 2016;
84 Green & Andrew, 2017; Green & Frankmann, 1987; Green & Nachtigal, 2012).
85 During food consumption, oral cavity including the tongue can be either heated up or
86 cooled down depending on the temperature of the consumed food, which in turn
87 physically affects the perception of food texture (Engelen et al., 2002; Engelen et al.,
88 2003; Engelen & van der Bilt, 2008). The temperature of the oral cavity (and the food)
89 will affect the perceived intensity of sensory attributes of food texture either because
90 changed enzyme activity (e.g. for texture of a starch-containing food) (Bridges,
91 Smythe & Reddrick, 2017), or tongue sensitivity (e.g. for roughness perception)
92 (Aktar, Chen, Ettelaie, et al., 2017). As for the perception of food irritation, literature
93 suggests that temperature of capsaicin solution causes a burning sensation: a weaker
94 burning sensation when capsaicin solution was served at a lower temperature (21°C),

95 but an enhanced burning sensation at a higher serving temperature (60°C) (Green,
96 1986; Lawless & Stevens, 1988; Prescott, Allen & Stephens, 1984). Therefore, it is
97 extremely important to know the surface temperature of tongue during food
98 consumption in order to optimize food design for a desirable sensory experience.

99

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

127

128 Tongue surface temperature can be affected by two very different food stimulation:

129 the temperature of the food (physical stimulation) or the chemical compounds of food
130 (chemical stimulation). Capsaicin (8-methyl-6-nonenoyl-vanillylamide) is the major
131 chemical substance in chili pepper, which gives people experience such as sweating
132 on the face and scalp, facial and neck and chest flushing, lacrimation, salivation, and
133 nasal discharge during and after consumption (Prescott & Stevenson, 1995). Inside the
134 mouth, capsaicin will activate transient receptor potential vanilloid subtype (TRPV1)
135 and produce the most intense feelings, including burning, stinging, tingling and biting
136 sensation (Prescott & Stevenson, 1995). Besides, capsaicin will induce temporary,
137 partial desensitization and affect oral and nasal sensitivity (Green, 1998; Karrer &
138 Bartoshuk, 1991; Rozin, Mark & Schiller, 1981). A very recent study showed that
139 capsaicin made no significant impact on the orthonasal aroma delivery, but it caused a
140 significant reduction in retronasal aroma release and enhanced saliva production (date
141 to be published separately). Previous physiological data suggested that capsaicin
142 produced illusory heating sensations instead of an actual temperature variation
143 (Konietzny, 1983; Petsche, Fleischer, Lembeck, & Handwerker, 1983; Green, 1986).
144 However, Boudreau et al (2009) found that the topical capsaicin application on
145 orofacial tissues increased cutaneous blood flow and elevated skin temperature. With
146 the help of IRT, therefore, it is of interest to directly observe the effect of capsaicin on
147 tongue surface temperature.

148

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

159

160 **2. Materials and Methods**

161 2.1 Material Preparation

162 Bottled non-carbonated mineral water (550 mL each, Nongfu Spring, Zhejiang, China)

163 was purchased from a local supermarket. Before sensory test, the bottled water was
164 respectively equilibrated at four different temperatures levels: cold water (0 °C), cool
165 water (20 °C), warm water (37 °C) and hot water (45 °C). The range of temperature
166 setting of bottled samples covers normal temperature range of food service, but not to
167 cause pain/damage to the tongue.

168

169 Capsaicin was used as a chemical stimulus for its effect on tongue surface
170 temperature. A stock solution (10,000 ppm) of food-grade capsaicin (Sigma-Aldrich,
171 Missouri, USA) was prepared by dissolving 1 g in 100 mL of 95% food grade alcohol.
172 The final stimulating solutions were diluted from the stock, which consisted of 5, 10
173 or 20 ppm of capsaicin. About 1 mL food-grade alcohol was dissolved in 500 mL
174 drinkable water as the control solution (0 ppm). These four solutions were kept in
175 34 °C (the normal tongue temperature at rest) water bath prior to experiments.

176

177 2.2 Apparatus Setup

178 An infrared thermal imager (Testo 875-1i, Testo Instruments International Trading Co.,
179 Shanghai, China) was used for the measurement of tongue surface temperature. The
180 emissivity value of the imager was set at 0.99 based on previous study performed by
181 Zhang et al. (1991). Infrared thermal image with the resolution of 160 × 120 pixels
182 was taken by the imager (a spectral range of 8-14 μm, noise equivalent temperature
183 distribution (NETD) ≤0.05P and a lens of 32° × 23°). Tongue surface temperature
184 measurement was performed using a dedicated software for infrared thermal images
185 elaboration (Testo IRSoft, version 4.0) and image presentations were either categories
186 of iron palettes or rainbow palettes.

187 A digital thermometer (DT) with a k-type thermocouple (TES-1310, TES Electrical
188 Electronic Corp., Taiwan) was used to calibrate tongue surface temperature measured
189 by IRT. Temperature indication of the digital thermometer follows the guides set by
190 National Bureau of Standards (NBS, USA) and IEC 584 temperature/voltage
191 thermocouples.

192

193 2.3 Areas of interests at tongue surface

194 As shown in Figure 1a, tongue dorsal surface was divided into five areas: tongue tip,
195 middle, left lateral, right lateral, and tongue root (Chiu, 2000; Hsieh, Shen, & Su,
196 2016). Since dimensions of human tongue vary from one individual to another, tongue

197 areas were therefore defined in proportion to the tongue size of the individual. During
198 test, tongue was stretched out as far as possible for each individual. Tongue tip was
199 defined as the anterior one-fifth area of the tongue, tongue root was defined as the
200 posterior one-fifth of tongue, tongue laterals were defined as the lateral one-fifth of
201 the tongue on both sides, and tongue middle was defined as the area between the tip
202 and root and between the two lateral areas. On the infrared thermal image (Fig. 1b),
203 these five areas were therefore selected respectively using polygon tool in the
204 software and the temperatures of each area were automatically calculated.

205

206 2.4 Experimental setup

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

219

220 2.5 Measurements of tongue surface temperature

221

222 2.5.1 Participants preparation

223 All participants were postgraduate students recruited from the campus and consents
224 were obtained before performing tests. All subjects were non-smokers, not suffering
225 from any illness or discomfort and were not on long-term medication. Prior to
226 temperature measurements, subjects were asked to refrain from intense exercise,
227 caffeine and alcohol for at least 1 h and were asked to relax on a comfortable chair in
228 the laboratory for at least 15 min with no external disturbance (though easy listening
229 music was provided). During the tests, infrared thermal images were taken
230 immediately after subjects protruded their tongues forward and downward to the

231 desired position. Particularly, participants were told not to respire through their mouth
232 in order to minimize air movement on tongue surface.

233

234 2.5.2 Calibration of IRT

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

243

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

249

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

261

262 2.5.3 Thermal effects of water consumption

263 Ten subjects (age: 24.6 ± 1.6 yrs, F = 5, M = 5, BMI: 21.3 ± 2.2 kg/m²) were involved
264 in thermal treatments of tongue on four consecutive days. On the first day, ice-cold

265 bottled water was applied and the rest of bottled water (at different temperatures) was
266 performed one-by-one on following days in an increasing order of temperature.
267 Therefore all subjects went through all four temperatures within the four consecutive
268 days. All subjects orally took in a mouthful thermally pre-equilibrated bottled water
269 without swallowing and held the liquid in the mouth for 10 sec before expectoration.
270 With a stopwatch for timing, this process was repeated for 3 to 5 min until a total of
271 550 mL of the water was used up. Then, subjects were immediately asked to place jaw
272 to the designated position shown in Figure 2 and stretch out their tongues for 60 sec.
273 Thermal images were taken every 15 sec by the investigator.

274

275 2.5.4 Tongue surface temperature after capsaicin application

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

288

289 2.6 Data analysis

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

298

299 3. Results and discussion

300

301 3.1 Calibration of IRT

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

317

318 Integrating the information of all temperature measurements by IRT from ten
319 subjects, it was found that the mean temperature of tongue surface was 34.14°C
320 (ranging from as low as 33.2 to as high as 35.7°C). A 2.5°C variation among subjects
321 is somewhat unexpected, but not surprising. With similar controlled environment,
322 Jiang et al. (2007) reported a mean tongue surface temperature of 33.55°C (ranging
323 from 32.7 to 34.3°C) based on 20 healthy subjects with an infrared thermal imager
324 (FLIR-PM390). Zhang and Zhu (1991) reported a mean tongue surface temperature of
325 33.66°C (ranging from 32.9 to 34.4°C) based on 380 healthy subjects with a
326 self-designed infrared thermal imager. In spite of minute differences, tongue surface
327 temperatures obtained from this study are largely comparable and agreeable to
328 literature results.

329

330 When analyzing temperatures of four areas on tongue dorsum surface, it appears that
331 they are in the following order: $T_{\text{middle area}} > T_{\text{right lateral}} \approx T_{\text{left lateral}} > T_{\text{tongue tip}}$.
332 Temperature differences at different tongue surface areas could be due to the different
333 density of blood vessels at these areas. Naumova et al. (2013) found that the number
334 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.

348

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.

365

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

369 uneasy). Tongue surface temperature alteration seemed to be not long lasting, but
370 would gradually recover to its original value. The tongue surface temperature started
371 to increase shortly after being rinsed with cold and cool water, possibly due to the
372 recovered blood flow. Shortly after being rinsed with warm and hot water, on the
373 contrary, tongue surface temperature showed a continued decrease, possibly because
374 of the evaporation at tongue surface.

375

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

380

381 3.3 Tongue surface temperature after capsaicin application

382 Tongue temperature alteration after the consumption of a spicy food is of great
383 interests to food oral processing studies. Therefore, IRT technique has been tested for
384 its feasibility to monitor oral surface temperature change as influenced by the
385 chemical composition of consumed food. Capsaicin was chosen for this purpose
386 because of its well-known effect on skin stimulation and increased blood flow under
387 the skin surface. Figure 6 shows temperature variations of the entire tongue surface
388 after treated with a control solution and capsaicin solutions. Immediately after surface
389 treatment (0 sec), tongue's mean temperatures were recorded to be 33.90 (control),
390 33.87 (5 ppm), 33.80 (10 ppm), and 33.91°C (20 ppm) respectively. The application
391 temperature of capsaicin and control solutions was 34 °C, same as that of the tongue
392 at rest. After the treatment, tongue was stretched out of the oral cavity for temperature
393 measurement. Because the tongue was exposed to the open air, continuous decrease of
394 surface temperature is expected due to a lower room temperature and due to the
395 cooling effect caused by surface evaporation. As expected, temperature decreases for
396 all four cases, but at different rates. The tongue surface treated with capsaicin has a
397 higher surface temperature than that of control. Sixty seconds after capasaicin
398 treatment, tongue's mean surface temperatures became 29.87, 29.99, and 30.27 °C
399 respectively for tongue treated with 5, 10, and 20 ppm capsaicin solutions, higher than
400 that of control which was recorded at 29.51 °C ($P < 0.05$).

401

402 Even though capsaicin treatment does not cause sudden increase of tongue surface

403 temperature, the stimulation seemed to have a lasting effect on tongue surface. Figure
404 7 plots temperature change in relation to that at time zero when the tongue stretched
405 out immediately after capsaicin treatment. The negative values shown in Figure 7
406 reflect gradual temperature decrease at four different areas of the tongue surface over
407 the time. Such temperature drop is not surprising because of the lower room
408 temperature and also the surface evaporation on the tongue surface. However, we
409 would like to draw readers' attention to the different pattern of temperature decrease
410 for tongue being treated by four different solutions. Almost at all four tongue surface
411 areas (tip, middle, and two sides), the temperature remained higher after capsaicin
412 treatment than that of control. For example, one minute after the treatment, a
413 difference of 1.34°C was observed at tongue tip between the application of 20 ppm
414 capsaicin solution and the control solution (significant with $P < 0.05$). Similarly,
415 temperature differences of 0.54 and 0.73°C were found respectively on the left lateral
416 and right lateral area (significant with $P < 0.05$). A temperature difference of 1.14°C
417 was also observed at the middle area (significant with $P < 0.05$).

418

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

429

430 4. Limits and weakness of the current study

431 Research findings from this work confirm that tongue temperature could vary
432 significant after being stimulated by hot/cold water or by chemical compounds
433 (capsaicin). This work also demonstrates that tongue surface temperature can be
434 monitored reliably in a non-invasive manner by using imaging technique IRT. Despite
435 that research findings are significant, possible limits and weakness of the study should
436 be noted.

437

438 Even though IRT offers accurate and reliable temperature measurement, the technique
439 was unable to give continuous imaging. Images can only be taken at an interval of at
440 least 15 sec. Also, images were taken manually by an operator, which will inevitably
441 involve some small variations in shooting time (and then the temperature). A video
442 recording IRT will be needed for continuous monitoring of temperature change.

443

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

451

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

458

459 **5. Conclusions**

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

470

471 **Ethical statements**

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

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

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

479

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486

487

488 **References**

- 489 Aktar, T., Chen, J., Ettelaie, R., Holmes, M. & Henson, B. (2017). Human roughness
490 perception and possible factors affecting roughness sensation. *Journal of Texture*
491 *Studies*, **48**, 181-192.
- 492 Boudreau, S. A., Wang, K., Svensson, P., Sessle, B. J., & Arendt-Nielsen, L. (2009).
493 Vascular and psychophysical effects of topical capsaicin application to orofacially
494 tissues. *Journal of Orofacial Pain*, **23**, 253-264.
- 495 Bridges, J. Smythe, J. & Reddrick, R. (2017). Impact of salivary enzyme activity on
496 the oral perception of starch containing foods. *Journal of Texture Studies*, **48**,
497 288-293.
- 498 Bouzida, N., Bendada, A., & Maldague, X. P. (2009). Visualization of body
499 thermoregulation by infrared imaging. *Journal of Thermal Biology*, **34**, 120-126.
- 500 Bozdogan, A. (2015). Viscosity behavior of bitter orange (*Citrus aurantium*) juice as
501 affected by temperature and concentration. *Journal of Food*, **13**, 535-540.
- 502 Chiu, CC. (2000). A novel approach based on computerized image analysis for
503 traditional Chinese medical diagnosis of the tongue. *Computer Methods and*
504 *Programs in Biomedicine*, **61**, 77-89.
- 505 Clark, R. P., & de Calcina-Goff, M. L. (1996). International standardisation in medical
506 thermography-draft proposals. In: 18th Annual Conference IEEE Engineering in
507 Medicine and Biology Society, Amsterdam, Netherlands, 2089–2090.
- 508 Engelen, L., de Wijk, R. A., Prinz, J. F., Janssen, A. M., Weenen, H., & Bosman, F.
509 (2003). The effect of oral and product temperature on the perception of flavor and
510 texture attributes of semi-solids. *Appetite*, **41**, 273-281.
- 511 Engelen, L., de Wijk, R. A., Prinz, J. F., Van der Bilt, A., Janssen, A. M., & Bosman, F.
512 (2002). The effect of oral temperature on the temperature of liquids and semisolids in
513 the mouth. *European Journal of Oral Sciences*, **110**, 412-416.
- 514 Engelen, L., & Van der Bilt, A. (2008). Oral physiology and texture perception of
515 semisolids. *Journal of Texture Studies*, **39**, 83-113.
- 516 Erickson, R. (1980). Oral temperature differences in relation to thermometer and
517 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 using the technology of infrared
600 thermograph. *Journal of Tianjin University*, **3**, 20-24.

601 **Captions**

602

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

606

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

612

613 Figure 3. Infrared thermal image analysis and histogram generation.

614

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

621

622

623 Figure 5. Temperature variations of subjects ($n = 10$) within 60 sec after rinsing with 0°C cold water
624 (\blacksquare), 20°C cool water (\bullet), 37°C warm water (\blacktriangle) and 45°C hot water (\times).

625

626 Figure 6. Temperature variations of tongue surface after treatment of control (\blacksquare), 5 ppm (\bullet), 10
627 ppm (\blacktriangle) and 20 ppm (\times) capsaicin solution ($n = 20$).

628

629 Figure 7. Variations of temperature on different areas of tongue surface ($n = 20$): (a) tip, (b) left
630 lateral, (c) right lateral and (d) middle area within 60 sec after treatment of control (\blacksquare), 5 ppm
631 (\bullet), 10 ppm (\blacktriangle) and 20 ppm (\times) capsaicin solution. Temperature variation was given in
632 negative values, which was the measured temperature in relation to that measured immediately
633 after capsaicin treatment.

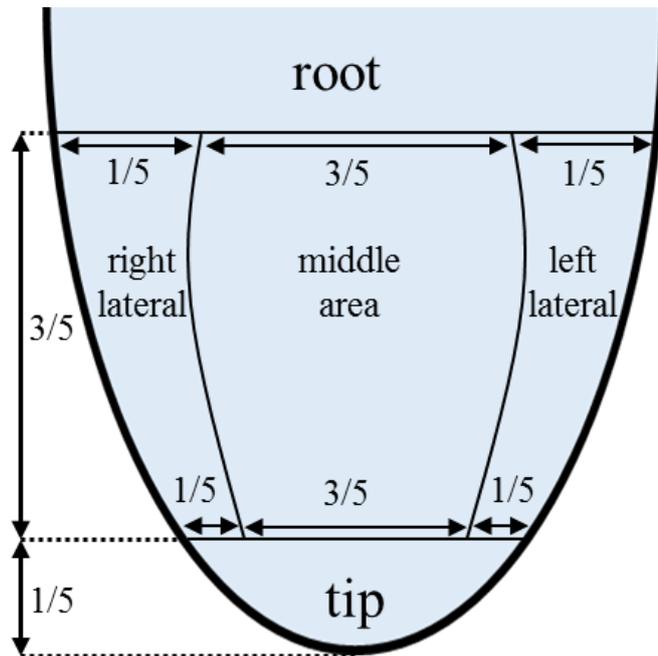
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(a)



(b)

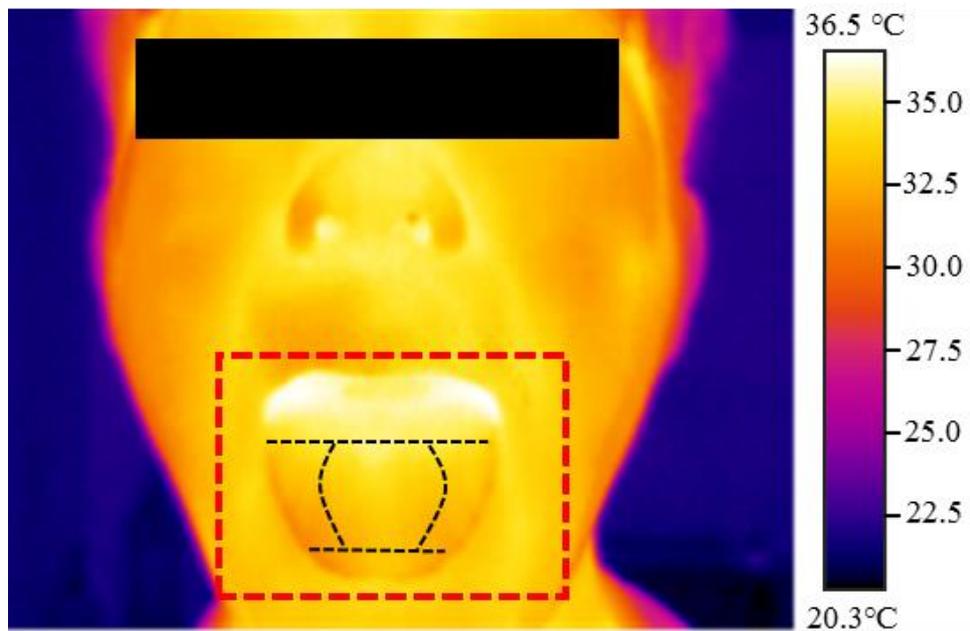


Figure 1. Division of tongue surface: (a) sketch of four areas of the tongue surface for temperature measurement, and (b) tongue surface highlighted on infrared thermal image (160×120 pixels) with a temperature scale.

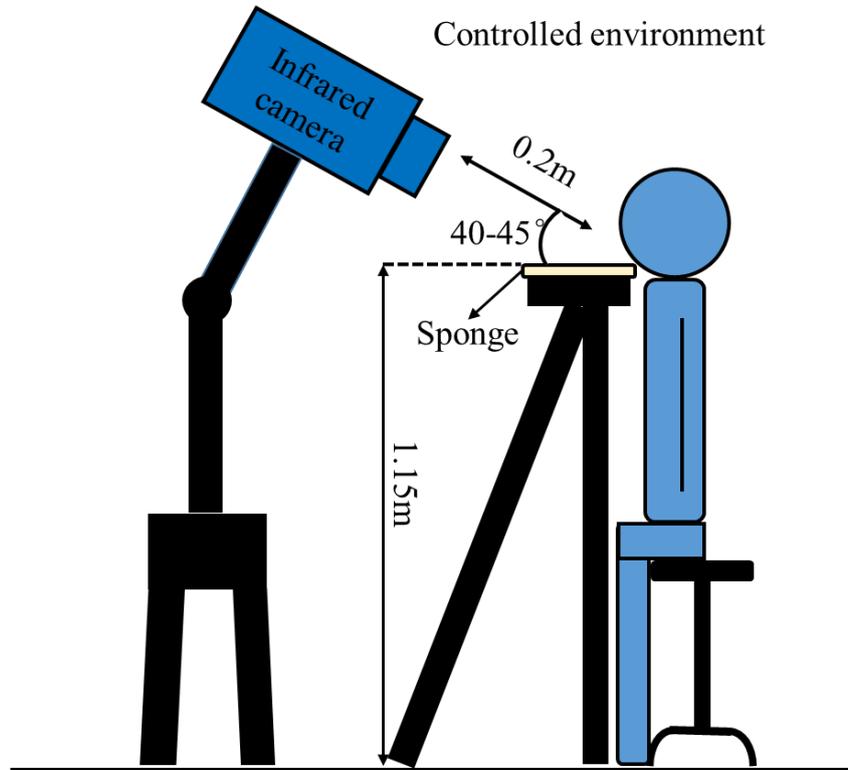


Figure 2. Sketch of experimental set-up for thermography experiment. The temperature and humidity of the laboratory is kept within a comfortable limit. The lens of IRT is positioned as perpendicularly to tongue surface to minimize geometrical errors. The distance and angle of lens is fixed so that approximately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

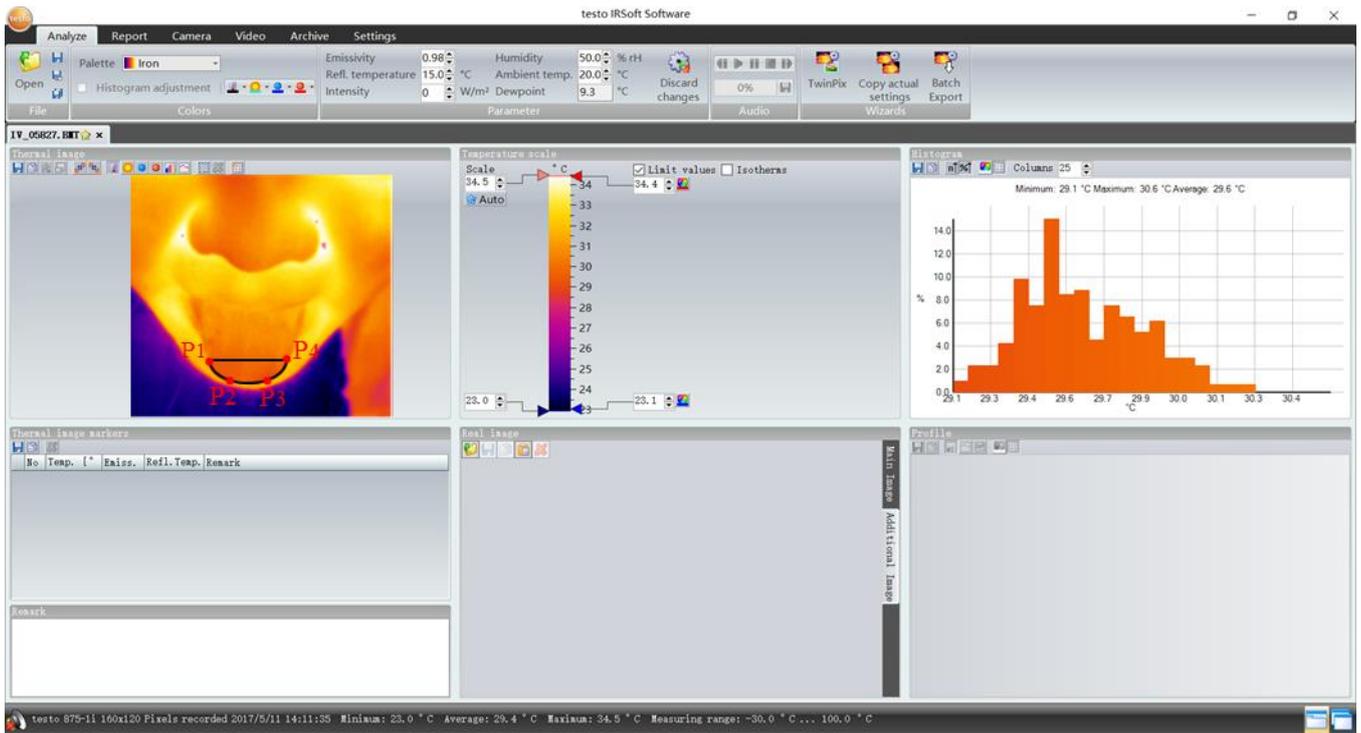


Fig 3. Infrared thermal image analysis and histogram generation.

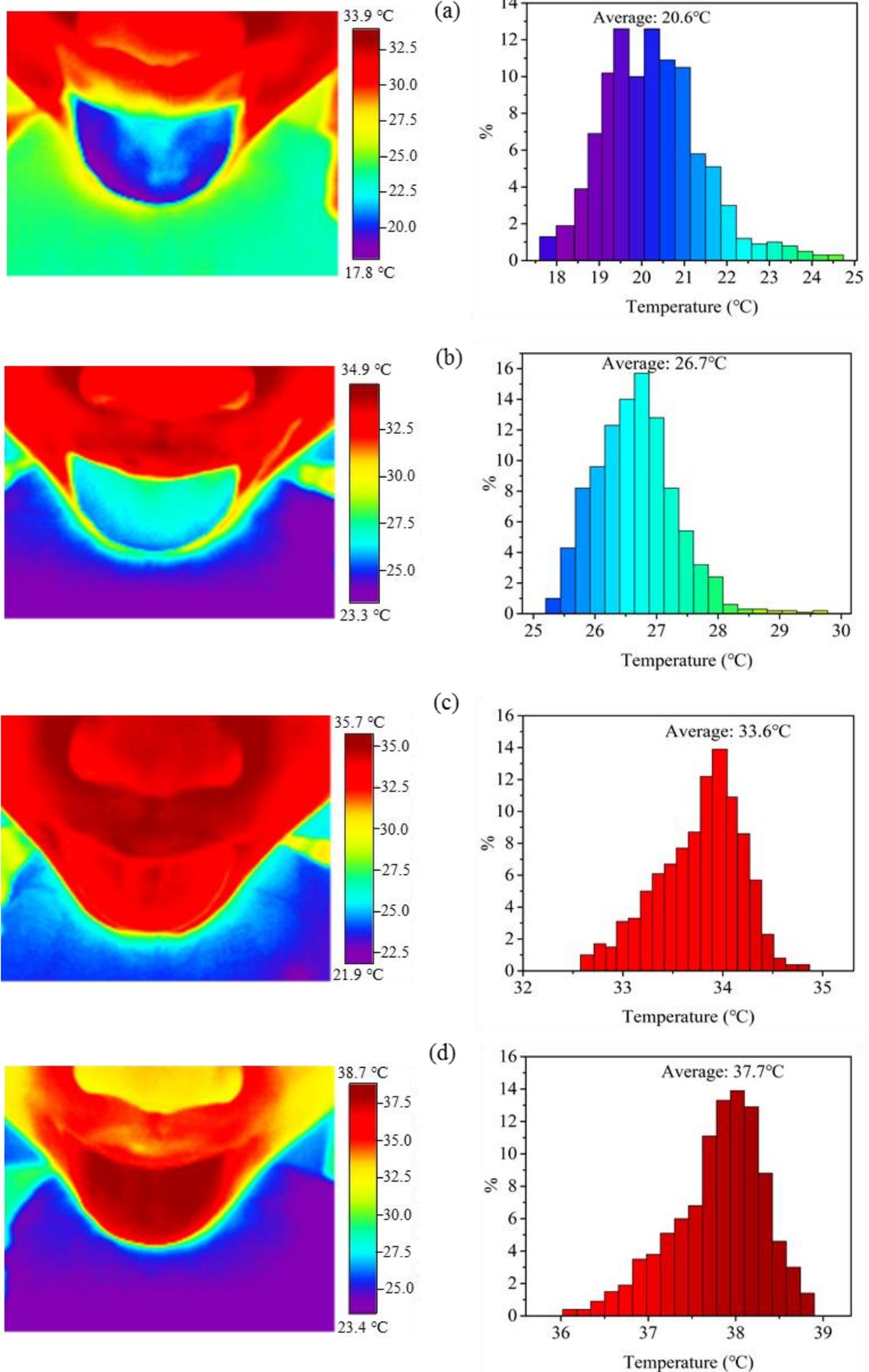


Figure 4. Images (160×120 pixels) of one representative subject and corresponding histogram

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.

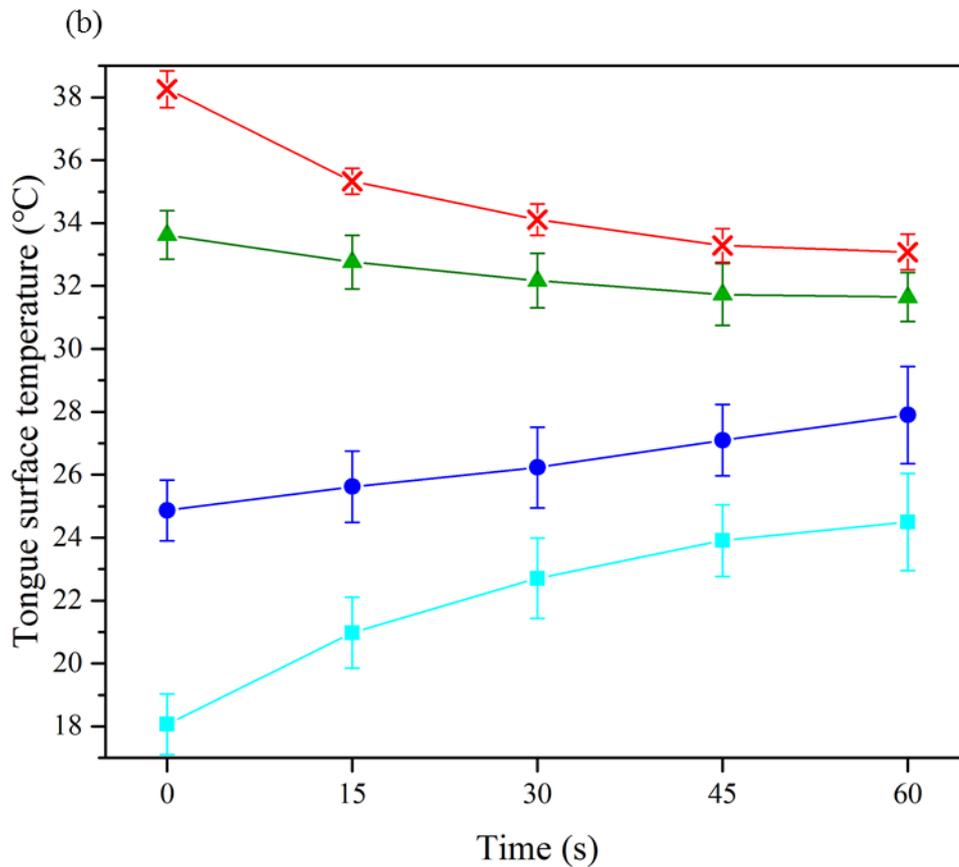
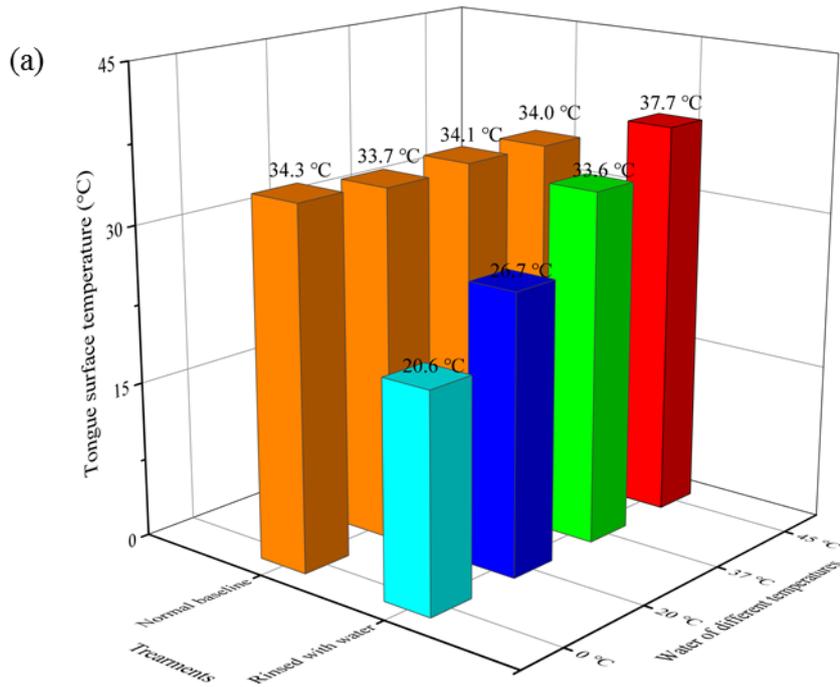


Figure 5. (a) Instant temperatures of the subject's tongue surface before (orange column) and 1 min after rinsed with water with corresponding temperatures (teal: cold; blue: cool; green: warm; red: hot); and (b) Temperature variations of subjects (n=10) within 60 sec after rinsing with 0°C cold water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

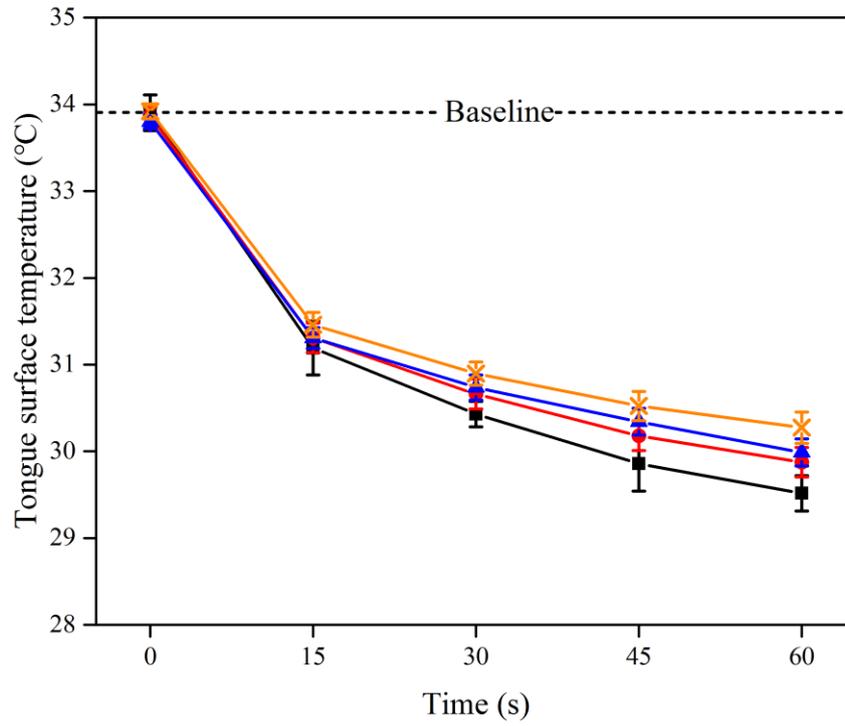


Figure 6. Temperature variations of tongue surface after treatment of control (■), 5 ppm (●), 10 ppm (▲) and 20 ppm (×) capsaicin solution (n=20).

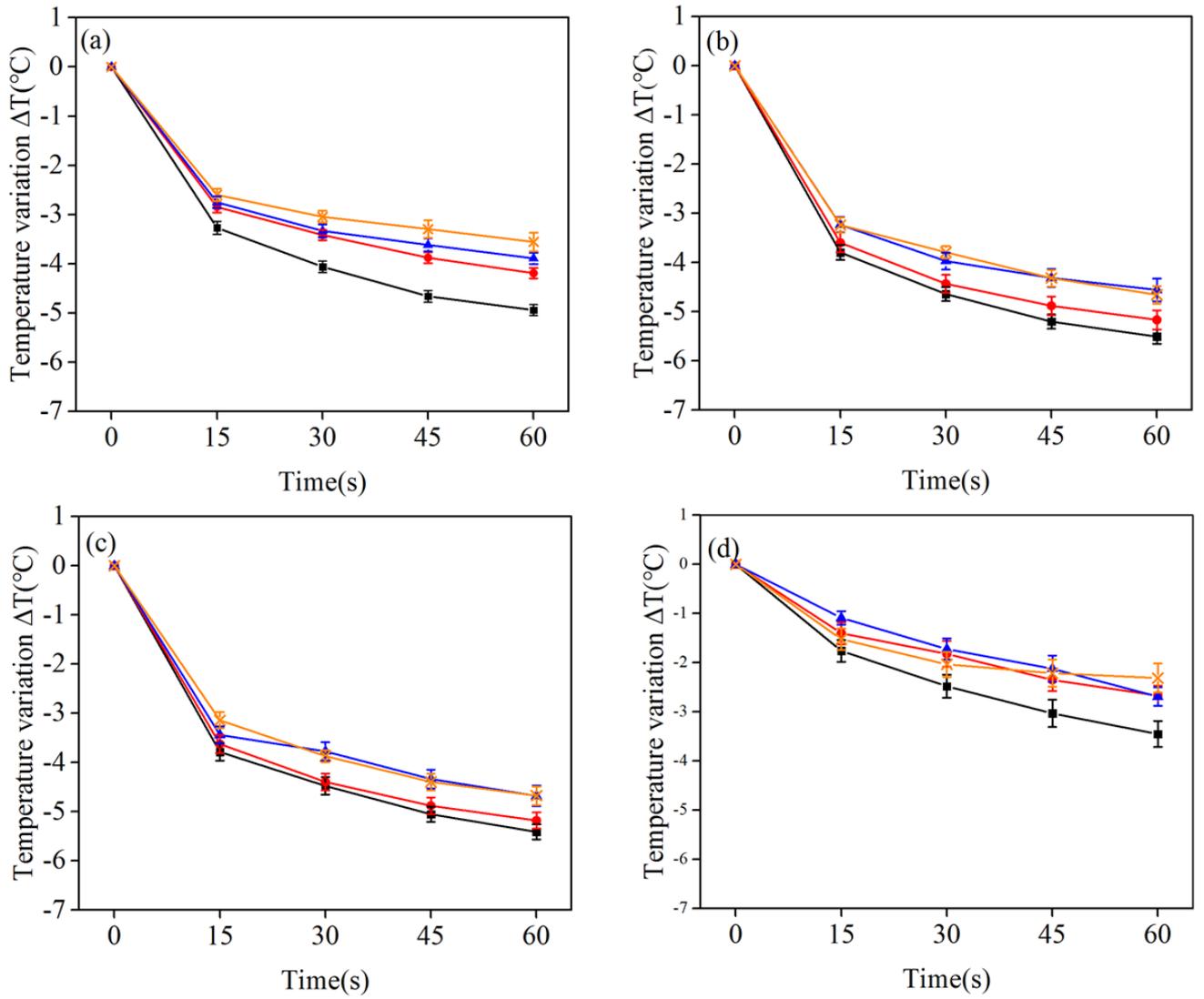


Figure 7. Variations of temperature on different areas of tongue surface (n=20): (a) tip, (b) left lateral, (c) right lateral and (d) middle area within 60 sec after treatment of control (■), 5 ppm (●), 10 ppm (▲) and 20 ppm (×) capsaicin solution. Temperature variation was given in negative values,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

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 ($^{\circ}$ 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