Effect of Tongue Temperature on Oral Tactile Sensitivity and Viscosity Discrimination

Cong Lv,[1] Luling Lou,[1] Ana Carolina Mosca,[1] Xinmiao Wang,[1] Ni Yang,[2] Jianshe Chen[1*]

[1]School of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou, Zhejiang, 310018, China.

[2] Division of Food Science, University of Nottingham, Sutton Bonington Campus, NG72RD, U.K.

*Correspondence

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

Email: jschen@zjgsu.edu.cn

Tele: (00)86 571 28008904

Fax: (00)86 571 28008900

Abstract

An Individual's oral capability in perceiving food texture influences greatly food appreciation and preference. While there is no doubt that one's ability of texture discrimination depends on various oral physiological characteristics of the individual, it is not yet clear how tongue surface temperature affects the sensitivity of texture discrimination. This study was designed to test the effects of tongue surface temperature on oral tactile sensitivity and viscosity discrimination. A total of twenty healthy subjects (ten females and ten males; mean age: 25 ± 1 yrs, mean body mass index: 20.5 ± 2.9 kg/m^2) participated in this study. Water at different temperatures (0, 20, 37, and 45 ^{o}C) and capsaicin solutions (5，10，and 20 ppm) were used as physical and chemical stimulations to alter tongue temperature, respectively. Semmes-Weinstein monofilaments, Bio-Thesiometer, and Touch-Test®Two-point discriminator were respectively applied to assess the tongue's sensitivity of light touch, vibratory perception and two-point discrimination before and after treatment with both physical and chemical stimuli. Maltodextrin solutions were used for oral viscosity discrimination. Tongue's vibratory perception thresholds varied significantly (P < 0.01), indicating an increase of 0.6×10^{-6}cm in vibratory perception threshold when tongue surface temperature decreased from 33^{o}C to 20 ^{o}C, while light touch and two-point discrimination thresholds remained unchanged. The application of capsaicin (5, 10, 20 ppm) produced an increase in tongue surface temperature but did not affect oral tactile sensitivity. Viscosity discrimination increased both after rinsing the mouth with water and capsaicin application (20 ppm). Capsaicin (20 ppm) increased tongue temperature by 1.3 ^{o}C and lead to a change in viscosity discrimination threshold decreased from 34.7% to 20.2%. After stimulation with water at 37 ^{o}C and 45 ^{o}C, the tongue temperature increased by 3 ^{o}C (from 34.2 ^{o}C to 37.2 ^{o}C), while threshold of viscosity discrimination decreased from 28.1% to 23.1%. When water was used to change tongue surface temperature, a positive correlation was found between vibratory perception sensitivity and viscosity discrimination ability, suggesting the capacity of discriminating viscosity might depend on vibratory perception sensitivity.

Keywords: capsaicin; tongue surface temperature; tactile sensitivity; viscosity discrimination; food oral processing

1. Introduction

Texture is a crucial sensory feature that influences consumers' appreciation and preferences toward foods (Conti-Silva, Ichiba, Silveira, Albano, & Nicoletti, 2018; van Vliet, van Aken, de Jongh, & Hamer, 2009). The definition of the term 'texture' has evolved over the decades. A currently accepted definition is that food texture is a sensory attribute derived from the food structure (at molecular, microstructure, and macroscopic levels), which involves one or many stimuli working in combination, including visual, audio, touch, and kinaesthetic (Bourne, 1975; Chen, 2009; Matz, 1962; Szczesniak, 1990). In contrast to taste, smell, vision or hearing, food texture is considerably less well understood (Kilcast, 1991). Although the physiological mechanism of texture perception is still an ongoing research, a number of scientific studies have been published and have suggested that tactile sensations may be a dominant factor in perceiving food texture during food oral processing (Booth, 2005; Bourne, 2002; Chen & Engelen, 2012; Engelen & van der Bilt, 2008; Guinard & Mazzucchelli, 1996; Lawless & Heymann, 2013; Nederkoorn, Jansen, & Havermans, 2015; Stokes, Boehm, & Baier, 2013; Strassburg, Burbidge, & Hartmann, 2009; van der Bilt, 2009).

The mouth, as Mountcastle (1974) concluded, is one of the most sensitive organs of the body because of its densely innervated nerve fibers and receptors (i.e., mechanoreceptors, thermoreceptors, nociceotors, proprioceptors, periodontal receptors, etc.). That probably is also a major reason why the mouth is exquisitely sensitive to chemical and tactile stimulations (Ringel & Ewanowski, 1965; Van Boven & Johnson, 1994). The physiology and morphology of orofacial mechanoreceptors have been characterized using the technique of microneurography and photomicrograph (Capra, 1995; Trulsson & Essick, 1997). Within the oral cavity, oral contact with foods can occur through the lips, tongue, palate, cheeks, and teeth, all of which provide textural information. It also has been demonstrated that there are several types of oral mechanoreceptors in the mouth, including SA1(slowly adapting

1), FA1(fast adapting 2), and SA2 (slowly adapting 2) (Chen et al., 2012). These oral mechanoreceptors most likely work together to relay information about touch, pressure, vibration, slip, and movement taking place in the mouth and hence play an essential role in both the sensation and perception of food texture (Trulsson & Johansson, 2002). During oral processing, textural features of the food are identified as three different modalities: mechanoreceptors in the superficial structures (hard and soft palate, tongue and gums), mechanoreceptors in the periodontal membrane (root of the teeth) and mechanoreceptors of the muscle spindles and tendons that are involved in mastication (Fujiki et al., 2001; Guinard et al., 1996). However, it remains unclear whether a specific textural feature (for example: viscosity) is produced by one mechanoreceptor or by the combined effects of several mechanoreceptors (Engelen et al., 2008). Further experiments, therefore, have been performed to explore the relationship between texture attributes and tactile sensitivity during perceiving liquid, semi-solid and solid foods. For example, Kutter, Hanesch, Rauh, and Delgado (2011) reported that the proprioception of the tongue has no influence on perceiving the thickness of semi-solid foods, but that the mechanoreceptors of the mouth contribute significantly to this sensation. Aktar, Chen, Ettelaie, and Holmes (2015a, 2015b) examined tactile sensitivity (using touch detection and two-point discrimination) and suggested that the capability to discriminate sensory attributes (i.e., viscosity, firmness, and elasticity) are seldom linked to an individual's tactile sensitivity. The extent to which tactile sensitivity is related to texture discrimination requires further investigation.

Capsaicin (trans-8-methyl-N-vanillyl-6-noneamide), a naturally occurring alkaloid, is the primary chemical substance extracted from the hot pepper plant that causes a strongly pungent flavor (Misery, 2016; Srinivasan, 2016). Few studies have explored the effects of capsaicin on taste, thermal perception, touch sensitivity, and desensitization as well as other sensory qualities (Drummond & Blockey, 2009; Karrer & Bartoshuk, 1995; Moon, Lee, Yoo, & Jahng, 2010; Nasrawi & Pangborn, 1989; Sizer & Harris, 1985). In particular, locally applied capsaicin on the human tongue had little effect on gustatory and tactile sensitivity but resulted in an elevated threshold of warm discrimination (Szolcsányi, 1977). With the help of an infrared thermal imager, we have demonstrated in our previous work that capsaicin can elevate tongue surface temperature by 1.3 ^{o}C (Lv, Wang, Chen, Yang, & Fisk, 2019). An interesting scientific question arising from the previous study is whether an elevated tongue surface temperature has an impact on sensory perception. In this context, this study aims to investigate the effect of tongue surface temperature on tactile sensitivity and ability to discriminate viscosity. Experiments were designed in three parts: 1) tongue temperature change as a result of different stimulation; 2) effects of tongue temperature on the tactile sensitivity; and 3) effects of tongue surface temperature on viscosity discrimination. To achieve this, both physical stimuli (hot and cold water) and chemical stimuli (capsaicin solutions) were used to vary tongue surface temperature. Tactile sensitivity of the tongue were assessed for sensation threshold of light touch detection, longitudinal vibratory perception, and two-point discrimination. A series of maltodextrin solutions with known viscosity was prepared and used for viscosity discrimination assessment. We believe that the findings from this study will improve our understanding of the mechanisms of oral texture sensation and its potential influencing factors.

2. Materials and methods

2.1 Subjects

A total of twenty subjects (ten females, ten males) volunteered to participate in each session of the sensory tests. All subjects were nonsmokers and healthy. Subjects reported no medical complications related to their oral cavity, which might have influenced the test results. They were aged between 22 and 26 years (average age: 25±1) and mean body mass index of 20.5 ± 2.9 kg/m^2. All subjects were postgraduate students recruited from the university campus. Written consents were obtained from each subject prior to the test. During the introduction session, assessors were instructed about the procedure of the test. However, they were not told of the purpose of the investigation.

2.2 Oral stimuli

Physical stimulus: Bottled water (Nongfu Spring 550 mL, Zhejiang, China) was purchased from a local supermarket and used for tongue temperature stimulation. Before sensory testing, the bottled water was equilibrated at four different temperatures: cold (about 0 ºC), cool (20 ºC), warm (37 ºC), and hot (45 ºC).

Chemical stimulus: Capsaicin was used as a chemical stimulus to change tongue surface temperature. Food grade capsaicin (Sigma-Aldrich, Missouri, USA) was dissolved in 95% food-grade alcohol to prepare a 1% stock solution (10,000 ppm). The final stimulating solutions were diluted from the stock into 5, 10, 20 ppm. Besides, 1 mL of food-grade alcohol was dissolved into 500 mL of drinkable water as the control solution (0 ppm). To keep the initial tongue surface temperature the same and to reduce the physical (temperature) effect of applied stimuli, all solutions were kept in a water bath at 34 ºC, which is the normal tongue surface temperature at rest, before the experiments.

2.3 Experimental procedure

Subjects attended two sessions of approximately 1 h each. In one session water was used as the stimulus to change tongue surface temperature and in the other capsaicin solution was used. Tests were conducted in a purposefully designated human study lab with appropriate ventilation and lighting, as well as temperature and relative humidity control. Subjects were requested to refrain from eating at least 2 hours prior to the test. During tests, subjects sat in a prearranged soft seat and rested their lower chin on the preset platform with their tongue stretched naturally out of the oral cavity for both physical and chemical stimulation and tactile assessment. Subjects were blindfold to avoid any visual cues.

2.3.1 Determination of tongue surface temperature

An infrared thermal imager (Testo 875-1i, Testo Instruments International Trading Co., Shanghai, China) was used for recording the baseline tongue surface temperature prior to stimuli application. Detailed procedures of temperature measurement can be found in our previous study (Lv, et al., 2019).

2.3.2 Stimuli application

Participants were subjected to physical stimuli and chemical stimuli on separate days. At the beginning of the test session, subjects were asked to clean the mouth with water. For the treatment with physical stimuli, subjects rinsed the mouth with bottled water at four temperatures (0, 20, 37, and 45 °C). Subjects held the water in the mouth for 10 s (a stopwatch was used for timing), then expectorated and sipped again. This process was repeated for 3 to 5 min until 550 mL of water was used. The four different temperatures were presented to the subjects randomly.

Concerning chemical stimuli, 1mL of capsaicin solution (5, 10, 20ppm) was applied evenly to the anterior tongue surface (around 2×2cm) with a cotton swab (see Figure 1). The capsaicin solutions at three different concentrations were applied in random order. A 5-min pause was taken in which subjects consume crackers and rinse the mouth with water. Then the infrared thermal imager was instantly applied to record subjects' immediate tongue surface temperature either after physical stimuli application or after chemical stimuli application. The process of measuring the tongue surface temperature took approximately (2-5 s).

2.3.3 Assessment of tongue tactile sensitivity

After stimuli application and subsequent tongue surface temperature determination, three different thresholds related to tactile sensitivity were immediately quantified using Semmes-Weinstein filaments (light touch detection threshold, taking around 15 s), Bio-Thesiometer (vibratory perception threshold, taking around 30 s), and two-point discriminator (two point discrimination thresholds, taking around 15 s).

However, the test areas for tactile threshold measurement were different. For light touch detection and two-point discrimination, the tongue tip was selected as the test area (Figure 1a). For vibratory perception measurement, a circle's area (the diameter: 1.2 cm) at the anterior tongue surface was selected as the test area since the diameter of the vibrator button was 1.2 cm (Figure 1b). The area of the circle was kept the same as that of the vibrator button because of possible effects on vibrotactile thresholds caused by contactor area and contactor configuration (Verrillo, 1963).

2.3.3.1 Light touch detection

Touch-Test® Semmes-Weinstein monofilaments (North Coast Medical, Inc., Gilroy, CA.) were selected for touch sensitivity tests (Figure 2a). This kit contained 20 monofilaments and its target force ranged from as low as 0.008 g to a maximum value of 300 g, arranged in logarithmic intervals. Light touch detection thresholds were determined using the up-down method with six calibrated monofilaments (target force of 0.008, 0.02, 0.04, 0.07, 0.16 and 0.4 g). Monofilaments of different force ratings were used in succession; if a response was elicited, then the next higher rated filament was used. The monofilament was pressed in a perpendicular direction against the tongue surface, as shown in Figure 3a, the pressing force continued to increase until it reached to a maximum when the monofilament started to bend (Aktar et al., 2015a, 2015b). Starting with 0.008 g, the monofilament was applied for approximately 1 s within the test area. If the subject failed to detect the stimulus, then the next higher force monofilament was applied. When the subject detected the presence of the stimulus, the monofilament was considered for the threshold calculation. The whole process was repeated three times. Between each test, the monofilament fiber was cleaned with an antibacterial wipe before and after measurements.

2.3.3.2 Vibratory perception tests

A Bio-Thesiometer (Bio-Thesiometer, Inc, USA) (Figure 2b) was used to determine vibratory perception thresholds, which produced a vibration of constant frequency (100 Hz). It consisted of a standard electrical vibrator and an amplitude-measuring device fastened directly to the vibrator button. The vibrator amplitude could be varied continuously over a considerable range from zero to full vibration by adjusting the control knob. A scale selector on the voltmeter provided a means of monitoring this vibration over the complete range.

The vibrator handle was firmly secured on a stand (see Figure 3b). Subjects were asked to firmly attach their tongue to the surface of the vibrator button. For each subject, an ascending order and a descending order were included for the measurement of vibratory threshold (Gregg, 1951). The ascending threshold was determined by starting from 0 volts and gradually raising the voltage (vibratory amplitude) until subjects felt a vibratory sensation. The descending threshold was determined by starting from 15 volts (with apparent vibrant feeling) and gradually lowering the voltage (vibratory amplitude) until the subject could not feel the vibratory sensation. The voltage was increased or decreased 1volts every 2 s. Subjects reported the on and off of the vibration and the corresponding values of the vibratory voltage (and then vibratory amplitude) were recorded by the investigator. The vibratory threshold was then calculated as the arithmetic mean value of these two values. This procedure was repeated three times and the average of three observations was considered as the vibratory perception threshold of the subject. Between each test, the vibrator probe was cleaned with an antibacterial wipe.

2.3.3.3 Two-point discrimination

A Touch-Test® two-point discriminator (Figure 2c) (North Coast Medical, Inc., Gilroy, CA.) was selected for the determination of two-point discrimination sensitivity. The two-point discriminator was designed to measure the narrowest distance that can be sensed as two distinct pressure points. Base on a pre-test, two-point discrimination thresholds were determined using eight calibrated distance (0.25, 2, 3, 4, 5, 6, 7, and 8 mm). Static and dynamic methods are two applicable types of two-point discrimination threshold measurement. In this study, the static method was used because of its reported feasibility and reliability for the determination of the nerve integrity (Aktar et al., 2015b; Dellon, Mackinnon, & Crosby, 1987; Ferreira, Rodrigues, & Fels, 2004).

During the test, subjects were asked to stretch out their tongue, and the two-point discriminator with a preset distance between the two points was lightly pressed in a perpendicular direction against the tongue surface (see Figure 3c). Pressure applied should be just light enough so that the subjects could sense the stimulus. For each subject, the test included two ascending orders and two descending orders (Engelen, van der Bilt, & Bosman, 2004; Minato et al., 2009; Sato, Okada, Miyamoto, & Fujiyama, 1999). The ascending threshold was determined by starting from 0.25 mm and gradually increasing the distance of two points by an interval of 1 mm until the subject perceived two stimuli points. The descending threshold was determined by starting from 8 mm and gradually decreasing the distance of two points by an interval of 1 mm until the subjects identified the stimuli as one point. The threshold of each test was taken as the point where participants' sensation changed from one point to two-point detection or vice versa. Then the mean threshold of the two ascending orders and two descending orders was calculated as the tongue tip threshold. The contacting points of discriminator were cleaned with antibacterial wipes between subjects.

2.4 Viscosity discrimination

A series of maltodextrin samples (Glucidex IT6, Roquette, France) was used for viscosity discrimination assessments (Table 1). Target viscosity was chosen between 50 and 100 mPa·s (Camacho, Dop, de Graaf, & Stieger, 2015). All samples were prepared on the day of the sensory session in a lab designated as safe for food consumption.

2.4.1 Rheology measurements

The dynamic viscosities of maltodextrin samples were measured using a rheometer (TA Instruments, Ltd., USA.). Measurements were conducted at 25 ^{o}C using a cone plate geometry (HR2 geometry, 40-mm cone diameter, 2.008° cone angle and 56-μm truncation). The viscosity of maltodextrin solutions should remain steady and little changed over a wide range of shear rate due to it being close to Newtonian behavior. Viscosity values were determined three times at shear rates ranging from 1 to 1000 s^{-1} and the averages calculated for the whole set of samples (Table 1).

2.4.2 Viscosity discrimination

Sample 1 (see Table 1) was selected as the reference and all of those samples were kept at 25 ^{o}C. The just noticeable difference (JND) method was used for viscosity discrimination analysis. Participants were served with ten pairs of samples (reference versus test sample) and they were asked to report whether the viscosities of the pair were sensed as the same or different. A volume of 1 mL of maltodextrin solution was administrated in the middle of the tongue surface with the help of a syringe. Subjects were asked to apply a gentle shearing action by moving the tongue against the palate for a few seconds to sense the viscosity.

2.4.3 Viscosity discrimination affected by stimuli application

The same method of stimuli application was applied (see 2.3.2 stimuli application) before viscosity discrimination.

2.4.3.1 Viscosity discrimination affected by physical stimulus (water)

Immediately after water rinsing, tongue surface temperature was recorded using the infrared thermal imager. Following, subjects were served with a pair of test samples for viscosity discrimination. Pairs of samples were arranged in a random order.

2.4.3.2 Viscosity discrimination affected by chemical stimulus (capsaicin)

Prior to the test, participants were first requested to rinse the mouth with water at 37 ^{o}C. After that, participants sensed the reference sample and then cleaned their mouth with water at 37 ^{o}C. Following, viscosity discrimination tests were conducted with and without capsaicin application (capsaicin session and control session). In a control session, after the application of the control solution (0 ppm), subjects sensed a randomly arranged pair of samples. In a capsaicin session, 10 mL of 20 ppm capsaicin solution (37 ^{o}C) was administered to rinse the mouth for 10 s before being expectorated. We chose to use only 20 ppm capsaicin solution in the viscosity discrimination test because a pre-test indicated that this solution resulted in a reliable oral irritation with a relative strong pungent sensation without causing any discomfort. Immediately after rinsing, a pair of samples was randomly presented for viscosity discrimination judgment. In this case, a 5-min pause was included to remove residual capsaicin from the mouth by cracker consumption and water rinsing. Pairs of samples were arranged in a random order.

2.5 Data analysis

Statistical analysis was conducted using SPSS 22.0 statistical software. One-way analysis of variance (ANOVA) was used to compare physical effects of water and chemical effects of capsaicin on tongue surface temperature as well as tactile sensations.

To determine the difference in discriminating viscosity, data obtained from the viscosity perception were plotted to log-normal best-fitting lines using probit analysis including the 95% confidence intervals (Microsoft Office Excel 2019). Pearson correlations and coefficient of determination (R^2) values were calculated to establish the relationship between tactile sensitivity and viscosity discrimination.

3. **Results and discussions**

3.1 Effects of the physical stimulus (hot/cold water) on oral tactile sensitivity

By using an infrared thermal imager, tongue surface temperatures after mouth rinsing with water at different temperatures were measured. This kind of physical stimulus (0, 20, 37 and 45 ^{o}C water) could significantly vary oral temperature (see Table 2). After mouth rinsing, tactile sensitivity of the tongue tip was assessed in terms of light touch threshold, vibratory perception threshold, and two-points discrimination. Relationships between tongue surface temperature and tactile sensation are shown in Figure 3, where light touch, vibratory perception and two-point discrimination thresholds were plotted against tongue surface temperature.

3.1.1 Light touch threshold

Using the Semmes-Weinstein monofilaments, there was no evidence that the tongue's light touch thresholds differed between 0, 20, 37 and 45 ^{o}C oral water application (Table 2). As shown in Figure 4, we observed a floor effect that all subjects could sense the weakest monofilament with the smallest point stress (0.008 g). This means that all subjects likely required a smaller point stress to determine their thresholds. By using Von Frey monofilaments, Santagiuliana et al. (2019) observed a similar experimental result with more than 90% of the participants detecting the slimmest monofilaments with the smallest stress applied (16.08 mN/mm^2). Because of the tongue's high light touch sensitivity, currently available monofilaments might not be suitable to estimate the light touch threshold of healthy young adults. Although no apparent evidence supported tongue's light touch thresholds change, we cannot conclude that tongue surface temperature had little impact on light touch thresholds of the tongue. Devices with higher sensitivity are recommended for exploring the relationship between light touch threshold and tongue surface temperature.

3.1.2 Vibratory perception threshold

To quantify the effect of tongue surface temperature on tongue's vibratory sensation, subjects' vibratory perception thresholds after rinsing water at 0, 20, 37, and 45 ^{o}C were compared. Figure 4 shows individual vibratory perception threshold after water rinsing at different temperatures. As expected, a decrease in tongue surface temperature increased vibratory perception thresholds. However, the effects of tongue surface warming on vibratory perception thresholds were small. Figure 4 also shows that vibratory perception thresholds possibly reached the lowest point when tongue surface temperature was near body temperature. By inspecting Table 2, statistical analysis (ANOVA) suggested that there were significant differences among vibratory perception thresholds after rinse with water of 0, 20, 37, and 45 ^{o}C ($P < 0.01$).

In hairy skin, it has been demonstrated that a decreased surface temperature is likely to reduce the sensitivity of vibratory perception and some sensory perceptions (i.e., roughness) (A. Gescheider, Thorpe, Goodarz, & Bolanowski, 1997; Green, Lederman, & Stevens, 1979; Harazin & Harazin-Lechowska, 2007). For example, Green (1977) investigated that vibratory perception thresholds exhibited a U-shaped function relative to skin surface temperature during the period of cooling or warming the skin. One possible reason why cooling decreased skin's vibratory sensitivity derives from the thermosensitive properties of the Pacinian corpuscles (PCs), which are a kind of sensory receptor that perceive pressure and high-frequency vibration (> 80 Hz). PCs belong to a type of thermosensitive receptor, whose sensitivity to vibration is affected by surface temperature (Inman & Peruzzi, 1961; Merzenich & Harrington, 1969). Similar conclusions can also be drawn when paying attention to the human's tongue surface. Fucci et al. (1976) found an increased vibrotactile threshold when cooling and warming the tongue and suggested that the point of average body temperature (37^{o}C) was concomitant with minimal lingual vibrotactile thresholds. Green (1987) also reported a significant loss of vibrotactile sensitivity when the tongue was exposed to cold stimulation. However, previous researchers generally agreed with the absence of the Pacinian corpuscles in the dorsal surface of the tongue (Gairns, 1953; Verrillo, 1966). Why is there a similar relation between surface temperature and vibratory perception thresholds either on the skin or on the tongue surface? One interesting feature of vibratory perception is that the threshold is not solely determined by the presence or absence of Pacinian corpuscles s in the dorsal surface of the tongue but also contributed to other mechanoreceptor systems (Green, 1987). Although few studies have documented the histological structure of the tongue, Green (1987) suggested as that other mechanoreceptors sensitive to lower vibratory frequency, deep-lying Pacinian corpuscles, are located at tongue tissues (nerve bundles and blood vessels) and underside of the tongue work together to mediate detection of vibration. Apart from surface temperature, two aspects are influencing vibratory perception thresholds of a particular body site. One point is concerned with intrinsic features of vibratory stimuli, including vibratory frequency, duration, pressure, and contactor surface area as well as contactor configuration (Bikah, Hallbeck, & Flowers, 2006; Fucci & Petrosino, 1982; Gilmer, 1937; Gregg, 1951; Harris, Fucci, & Petrosino, 1988; Verrillo, 1963, 1992). Another aspect associated with vibratory perception depends on factors like age, gender, and BMI (Calhoun, Gibson, Hartley, Minton, & Hokanson, 1992; Verrillo, 1977; R. T. Verrillo, 1979; Ronald T. Verrillo, 1979; Verrillo, 1980; Wiles, Pearce, Rice, & Mitchell, 1991). Vibratory stimuli and test areas in this study have been strictly specified and subjects were homogeneous concerning age and BMI and balanced for gender. Thus, we conclude that a decrease in tongue surface temperature may impair vibratory perception sensitivity.

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3.1.3 Two-point discrimination threshold

To estimate how tongue surface temperature affected two-point discrimination, subjects' two-point discrimination thresholds after rinsing with water at 0, 20, 37 and 45 ^{o}C were compared. By inspecting Table 2, we observed that the average two-point discrimination threshold of tongue tip (37 ^{o}C) across 20 subjects was 1.3 ± 0.1 mm. This result is comparable with that of previous investigators: Maeyama and Plattig (1989) reported 1.6 ± 0.4 mm, Toshihide Sato et al. (1999), 1.7 ± 0.1 mm, Minato et al. (2009), 1.1 ± 0.4 mm, and Aktar et al. (2015b), 0.6 ± 0.9 mm, in spite of using other stimulation devices. However, there was no evidence to support that cooling or warming the tongue would change the tongue's two-point discrimination sensitivity. Each subject's two-point discrimination threshold is shown in Figure 4. As a matter of fact, all the subjects could tell the difference between one- and two-point stimulation using their tongue tip. Therefore, it is not possible to conclude that tongue surface temperature can have an impact on sensing two-point discrimination.

When it comes to skin, it is quite clear that a lowering temperature can cause a decrease in two-point discrimination sensitivity at sensory spots like fingers, hand, upper limbs, and lower limbs (Mills, 1956; Provins & Morton, 1960; Stevens, 1989). Regarding the tongue, however, we did not observe a decrease in two-point discrimination sensitivity when cooling the tongue surface. One conceivable fact is that the tongue feels more sensitive than other parts of the body according to experimental data (Ringel et al., 1965). The two-point discriminator may not be accurate enough to determine the tongue's two-point discrimination threshold, although this device is widely used for estimating the sensitivity of various body loci in a lot of medical practices. A more sensitive alternative thus should be found to measure the tongue's two-point discrimination threshold.

3.2 Effects of the chemical stimulus (capsaicin) on oral tactile sensitivity

By using the infrared thermal imager, tongue surface temperatures after applying 0 (control), 5, 10, and 20 capsaicin solutions were measured. Unlike physical stimulus, this kind of chemical stimulus (0, 20, 37 and 45 ºC water) could produce a slight increase in tongue surface temperature (see Table 3).

3.2.1 Light touch threshold

Given the data summarized in Table 3, the subjects' average light touch thresholds are 0.008 g. Figure 5 shows individual light touch thresholds with the application of control solution and 5, 10, 20 ppm capsaicin solution. Similarly, the method used to determine the tongue's light touch sensitivity affected by capsaicin was also clearly limited by a floor effect (also see section 3.1.1). Actual tongue's light touch thresholds of subjects in this study were below the smallest point stress that can be applied with monofilaments. Thus, we cannot examine the hypothesis that light touch sensitivity of the tongue is affected by capsaicin application.

Studies have reported an increased touch threshold with the topically applied capsaicin in the skin (Drummond et al., 2009; Kauppila, Mohammadian, Nielsen, Andersen, & Arendt-Nielsen, 1998). By performing experiments on animals, it was indicated that capsaicin application depletes neuropeptides like substance P (putative neurotransmitters of nociceptive processing) in small primary afferent neurons and results in a long-lasting but reversible decrease of sensibility induced by capsaicin (Amann & Lembeck, 1990). This capsaicin-induced decrease of sensibility can also occur on the tongue with the application of a low concentration (3-30 ppm) of capsaicin (Green, 1993; Karrer & Bartoshuk, 1991). Although our current results do not support previous findings, we believe that the threshold of light touch might be affected by capsaicin. Further studies focused on the relation between light touch sensitivity and capsaicin can be continued.

3.2.2 Vibratory perception threshold

Each subject's vibratory perception threshold is shown in Figure 5. Across all participants, vibratory perception thresholds did not differ significantly after the application control solution and 5, 10, 20 ppm capsaicin solution (Table 3), suggesting that vibratory perception sensitivity was less susceptible to capsaicin. Similar results were also obtained by Tandan, Lewis, Badger, and Fries (1992), Kauppila et al. (1998), and Privitera et al. (2017), with no significant change in vibratory perception thresholds being observed when applying capsaicin.

Capsaicin produces typically a significant increase in blood flow and temperature accompanied by burning pain (Boudreau, Wang, Svensson, Sessle, & Arendt-Nielsen, 2009). Based on our observation, the treatment with 20 ppm capsaicin solution could lead to a temperature increase of 1.3 ^{o}C at the tongue tip (Lv et al., 2019). In spite of Pacinian corpuscles' thermosensitive property, an increase ranged from 1 to 2 ^{o}C in the tongue surface temperature might be unable to alter the tongue's vibratory perception sensitivity.

3.2.3 Two-point discrimination threshold

Subjects' two-point discrimination thresholds are compared in Table 3. The present results show that two-point discrimination thresholds did not differ significantly between applying control solution and applying 5, 10, 20 ppm capsaicin solution. Each subject’s two-point discrimination threshold is shown in Figure 5. There might also exist a floor effect on two-point discrimination as previously discussed (also see section 3.1.3).

Interestingly, it was reported that capsaicin led to impairment of two-point discrimination ability when testing on the hand, suggesting this capsaicin-induced impairment of two-point discrimination ability was most likely relative to the primary afferents of hyperalgesia (Kauppila et al., 1998). Also, there are a lot of responsive and unresponsive C nociceptors in human skin, including units responding only to mechanical stimuli (CM), units responding only to heat (CH), mechano-heat-responsive units (CMH) and mechano-heat-insensitive units (CM_iH_i). Schmidt et al. (1995) mentioned the responsive alternation of these C nociceptors to mechanical stimuli after the application of capsaicin: for instance, 2 of 15 tested CM_iH_i units became sensitive to heating but not to mechanical stimuli while a unit became sensitized to mechanical stimulation but not to heat. Since we used a psychophysical method that determined the summed effects (simultaneously produced by several various tactile sensing systems) of the capsaicin-induced alternations on two-point discrimination sensitivity, rather than a single effect in one single part of these sensing systems aimed for two-point discrimination. We cannot rule out the possibility that some direct mechanisms of oral capsaicin could have participated in the perception of tongue’s tactile sensation.

3.3 Viscosity discrimination

3.3.1 Viscosity discrimination affected by physical stimulus (water)

The population threshold was calculated as the cumulative median (50%) of the cumulative population distribution (Lawless et al., 2013). Figure 6 summarized subjects’ capability of viscosity discrimination after mouth rinsing with: (a) 0 ^{o}C water; (b) 20 ^{o}C water; (c) 37 ^{o}C water; and (d) 45 ^{o}C water. Cumulative JND response (population percentage) was plotted against the logarithmic percentage viscosity difference of the reference sample (see Eq. 1):

$$\%\ \text{Viscosity difference} = \frac{\eta_1 - \eta_0}{\eta_0} \times 100 \qquad (1)$$

where η_0 and η_1 are the viscosity of the reference sample and testing sample, respectively.

In Figure 6a (rinsing 0 °C water), tongue surface temperature of the subjects was cooled down to only 21.3 °C ± 1.1 °C. The population threshold (cumulative median) of viscosity discrimination was 41.3% (CI = 40.2% - 42.5%), suggesting a 41.3% change in viscosity from the reference sample would be the minimum detectable change by the tongue. In Figure 6b (rinsing 20 °C water), tongue surface temperature across the subjects reached 27.2 ± 0.8 °C, and the population threshold of viscosity discrimination was found to be 34.7% (CI = 33.3% - 36.1%). After mouth rinsing with 37 °C water (Figure 6c), tongue surface temperature became 34.2 ± 0.4 °C and the population threshold of viscosity discrimination was 28.1% (CI = 26.5% - 29.6%). When rinsing with 45 water (Figure 6d), tongue surface temperature was lightly elevated to just above body temperature at 37.2 ± 0.5 °C. In this case, the population threshold of viscosity discrimination reduced to 23.1% (CI = 21.4% - 24.9%). These findings show that decreasing tongue surface temperature leads to a decrease in sensitivity to discriminate viscosity, while an increase in temperature increases tongue´s sensitivity.

3.3.2 Viscosity discrimination affected by chemical stimulus (capsaicin)

Figure 7 summarized the tongue’s capability of viscosity discrimination with the control solution and 20 ppm capsaicin solution application. In the control session, the population threshold of viscosity detection across all subjects was 34.7% (CI = 33.3% - 36.1%). With a similar approach, however, the population threshold of viscosity discrimination across all subjects decreased to only 20.5% (CI = 18.2% - 22.1%) in the capsaicin session. This suggests that capsaicin application can enhance tongue’s ability of viscosity discrimination, although underlying reasons are still unclear.

3.4 Relation between tactile sensitivity and viscosity discrimination ability

3.4.1 Effects of tongue surface temperature

No relationship was found between the individual viscosity discrimination ability and either light touch or two-point discrimination sensitivity. Conversely, individual viscosity discrimination thresholds were positively correlated with vibratory perception thresholds (viscosity difference %) (see Figure 8) when water was used to change tongue surface temperature. This correlation suggests that an individual who possesses a lower vibratory perception threshold has a better ability to discriminate viscosity. Additionally, this correlation seems to remain at different oral temperatures, despite both viscosity discrimination thresholds and vibratory perception thresholds being significantly influenced by the tongue surface temperature. This result suggests that the ability to discriminate viscosity might depend on vibratory perception sensitivity.

Generally speaking, viscosity is considered as a sensation that arises from the combination of both touch and pressure mechanical receptors (Smith, Logemann, Burghardt, Carrell, & Zecker, 1997). That is, sensing viscosity is a human's intrinsic ability and independent of temperature. The correlation between vibratory perception sensitivity and viscosity discrimination ability also proves this point. However, viscosity itself is greatly influenced by temperature. When consuming foods, the heat interactions between food and oral mucous are inevitable. Considering this, the heat exchange between maltodextrin samples (served at 25 ^{o}C) and oral mucous (normally 37 ^{o}C) must occur, which may in turn change the viscosity of maltodextrin samples.

3.4.2 Effects of capsaicin

No significant correlations were found between individual viscosity discrimination ability and tactile sensations measure by the three methods either after application of a control solution (0 ppm capsaicin) and 20 ppm capsaicin solution. Although chemical stimulation with capsaicin increased the tongue surface temperature by 1.3 ^{o}C, no direct evidence suggests why viscosity discrimination shows a significant difference when comparing the control session or capsaicin session.

A comparison between the results observed for water and capsaicin should be noted. Capsaicin increased tongue temperature by 1.3 ^{o}C and the change in viscosity discrimination threshold decreased from 34.7% to 20.2%. After stimulation with water at 37 ^{o}C and 45 ^{o}C, the tongue temperature increased by 3 ^{o}C (from 34.2 ^{o}C to 37.2 ^{o}C), while threshold of viscosity discrimination decreased from 28.1% to 23.1%. That means that capsaicin had a higher impact on viscosity discrimination threshold than water, suggesting that other factors besides temperature might play a role on sensitivity. It should be noted that all the subjects reported an increased saliva secretion after oral capsaicin application (saliva flow rate was not measured). This is in agreement with previous studies that reported that capsaicin promoted saliva secretion in animal and human salivary glands (Ding et al., 2010; Zhang et al., 2006). Generally, salivary secretion can affect food texture perception due to either its dilution effect or lubrication properties during food oral processing (Engelen et al., 2005; Ranc et al., 2006). Considering this, an increase in saliva secretion might be an explanation for the enhanced ability of viscosity discrimination after stimulation of the tongue with a 20 ppm capsaicin solution.

3.5 Limitations

Notes should be made about the limitations of this study. Firstly, only tongue surface temperature was assessed, but we referred to it in some cases to oral temperature, believing that the tongue played a core role in oral sensation even though other parts of oral surfaces are also involved in viscosity sensation. Secondly, the Touch-Test® Semmes-Weinstein monofilaments and the two-point discriminator were shown not to be sensitive enough to assess the tactile sensitivity of the human tongue. Our results suggest that the tactile sensitivity threshold for both touching and two-point discrimination fell below the testing limit of the technique. This may explain why we observed a temperature increase and an enhanced capability of viscosity discrimination for capsaicin treatment without a clear evidence of associated tactile sensitivity increase. Therefore, one should remain cautious in concluding that touching sensitivity and two-point discrimination did not influence the capability of viscosity discrimination. Thirdly, this study did not include a vibratory perception test with a higher frequency. Fucci et al. (1982) obtained different suprathreshold magnitude functions at the three frequencies of 100, 250, and 400 Hz. Thus, it remained unclear how capsaicin would affect the vibratory perception threshold at higher frequencies. Fourthly, it also should be made clear that the tongue surface temperature will eventually return to its normal value after the removal of both physical and chemical stimulation. Despite conducting experimental tests as soon as the stimulation was applied (within 15 to 30 s), time variation is inevitable. This could be a source of experimental error in this study. Finally, this study might ignore the effects of other physiological factors on viscosity discrimination. For example, Steele (2018) found that tongue strength will influence oral viscosity discrimination.

4. Conclusion

This study aimed to examine how oral temperature impacted oral tactile sensitivity and capability of viscosity discrimination. Our results show that only vibratory perception thresholds differed significantly after rinsing the mouth with water at different temperatures. Tactile sensitivity did not change with the application of capsaicin to the tongue surface. Both stimuli, water and capsaicin, changed significantly the ability of discriminating viscosity. A positive correlation was found between vibratory perception and viscosity discrimination when water was used as physical stimuli, suggesting that the ability of viscosity discrimination might depend on vibratory perception sensitivity. In the case of capsaicin however, correlations between tactile sensitivity and viscosity discrimination were not observed probably due to effects of capsaicin-induced saliva secretion.

5 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.

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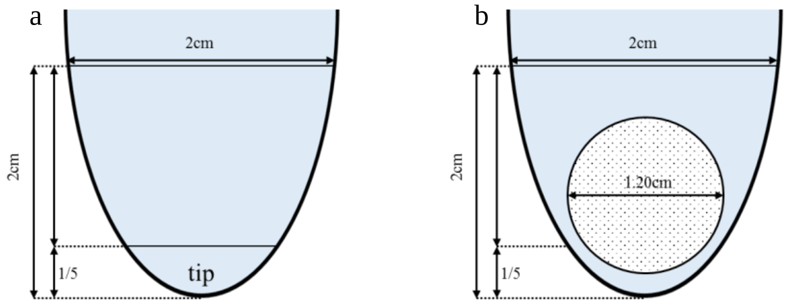

Fig. 1. Testing area on tongue surface: (a) for light touch and two-point discrimination and (b) for vibratory perception.

Fig. 2. Equipments applied in the threshold measurement of tactile sensitivity：(a) Semmes-Weinstein monofilaments; (b) Bio-Thesiometer; and (c) Touch-Test ®Two-point discriminator.

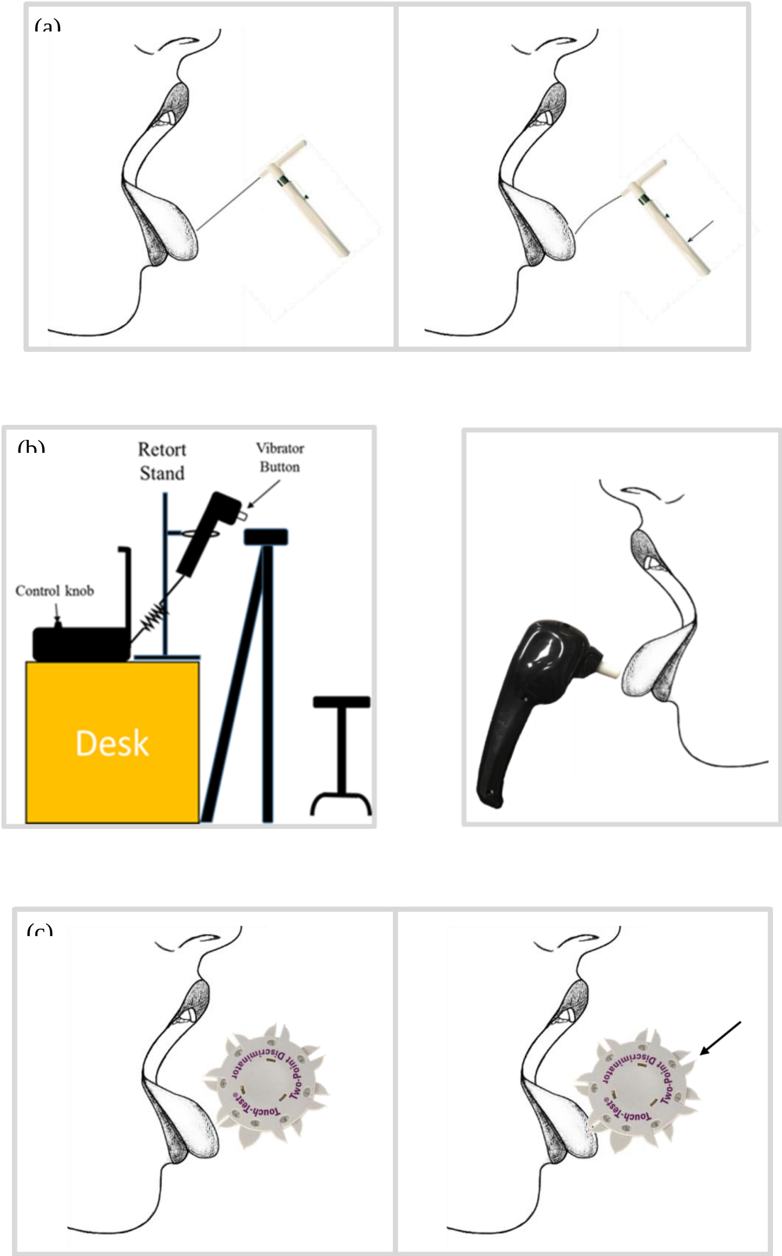

Fig. 3. Sketch illustration of experimental set up for tactile sensation tests: (a) light touch detection, (b) vibratory perception test, and (c) two-point discrimination.

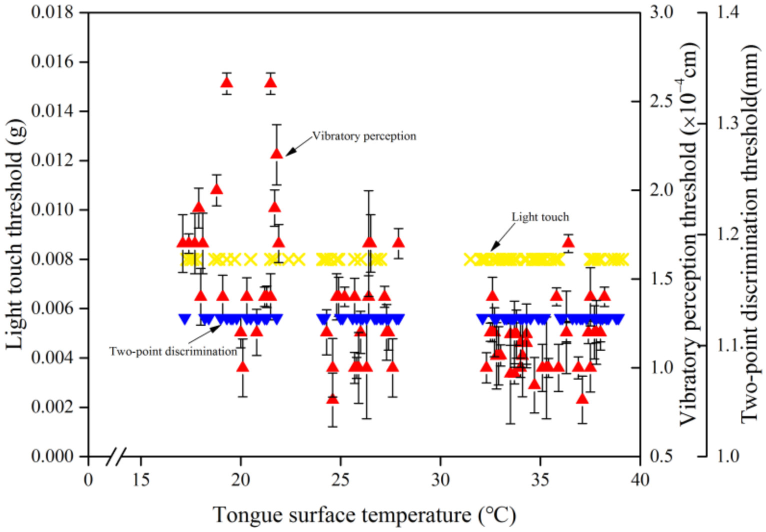

Fig 4 Effects of tongue surface temperature after mouth rinsing with hot and cold water on light touch, vibratory perception and two-point discrimination thresholds (n=20).

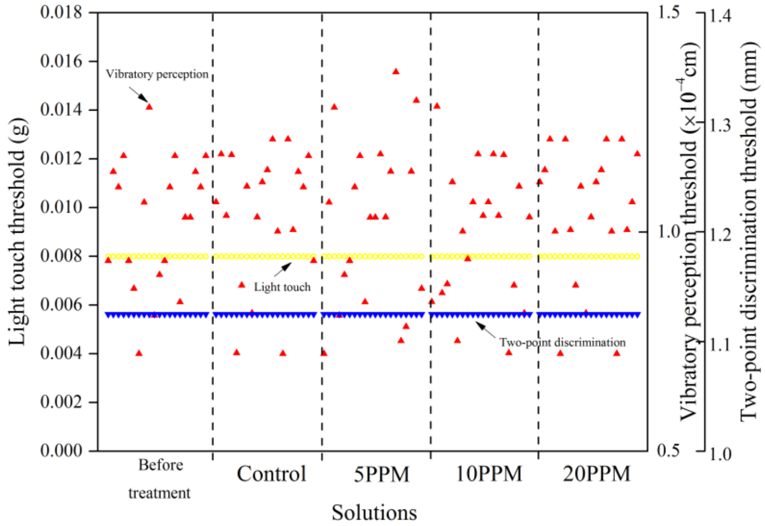

Fig. 5. Effects of capsaicin treatment on light touch, vibratory perception and two-point discrimination thresholds (n=20).

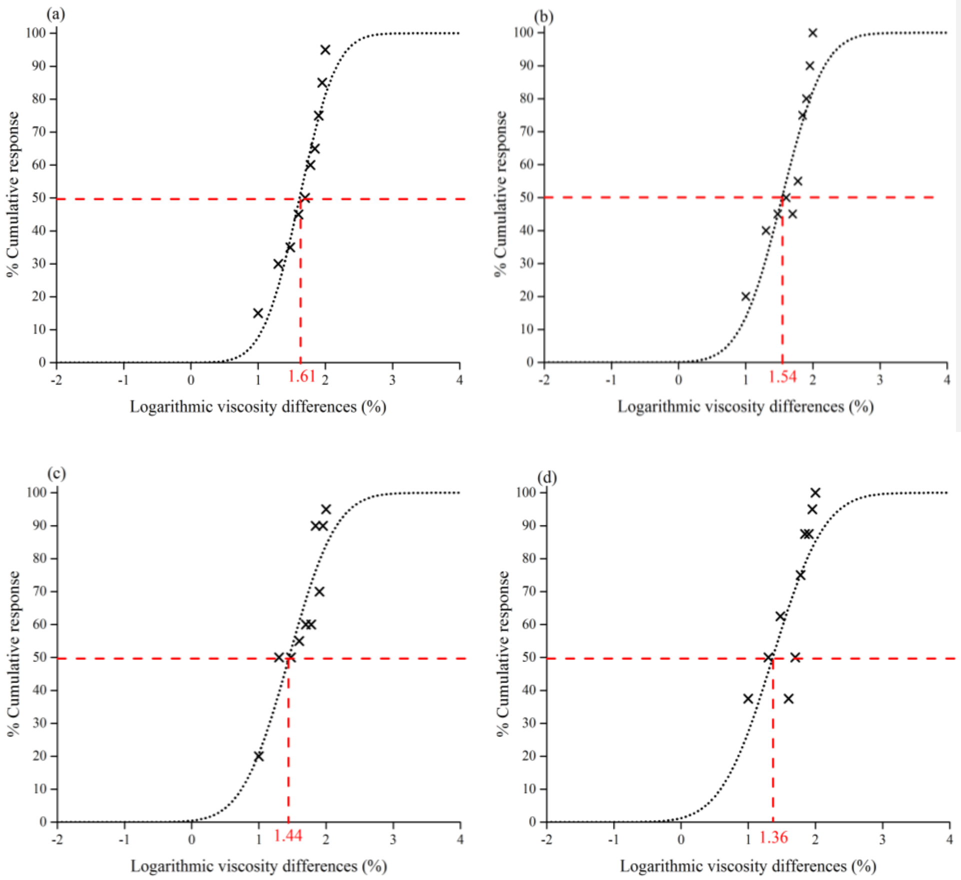

Fig. 7. Log-normal best fitted (Probit analysis) cumulative responses of subjects shown as population against the logarithmic viscosity difference (%) after mouth rinsing with water of different temperatures (n=20): (a) 0℃; (b) 20℃; (c) 37; and (d) 45℃.

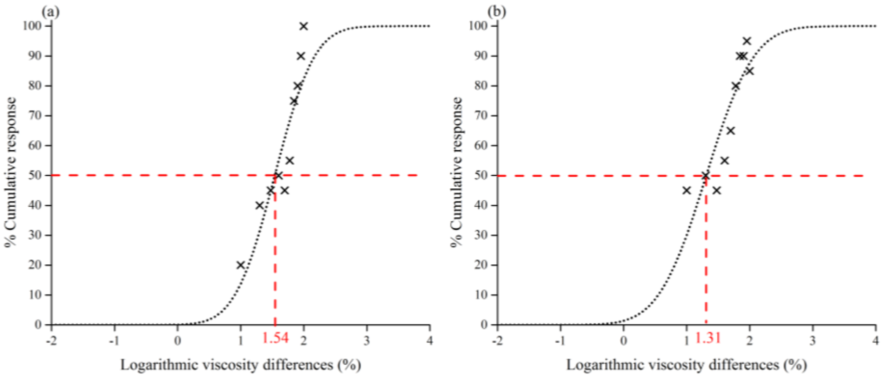

Fig. 7. Log-normal best fitted (Probit analysis) cumulative responses of subjects shown as population against the logarithmic viscosity difference (%) (n=20): (a) control group and (b) capsaicin treatment.

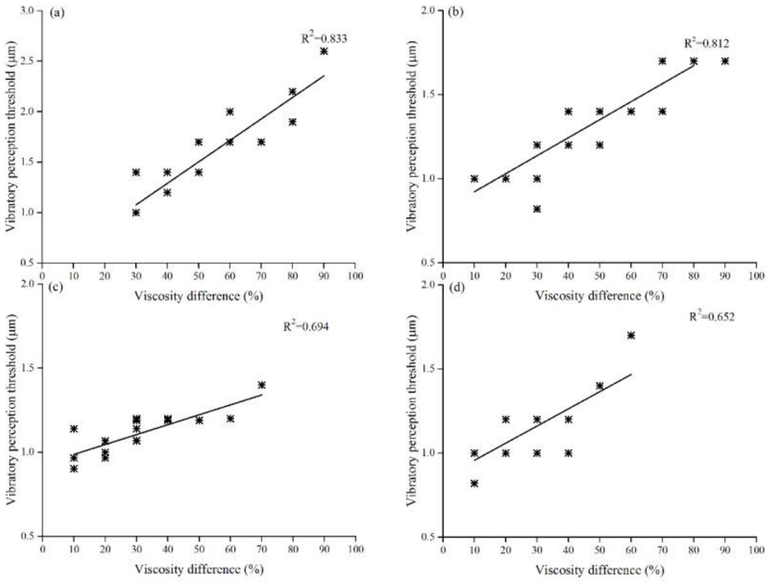

Fig. 8. Correlation between the capability of shear viscosity discrimination and the vibratory perception sensitivity for subjects after mouth rinsing with water of different temperatures: (a) 0°C; (b) 20°C; (c) 37°C; and (d) 45°C.

Table 1. Properties of maltodextrin test samples (25℃)

Sample number	Target Viscosity (mPa • s)	Actual viscosity (mPa • s)	Actual concentration (%)	Viscosity difference from the reference (△*I*)
*1	50	49.9 ± 0.48	30.50	0%
2	55	54.6 ± 0.48	31.50	10%
3	60	60.2 ± 0.39	32.10	20%
4	65	65.1 ± 0.19	32.48	30%
5	70	69.4 ± 0.79	32.91	40%
6	75	74.9 ± 0.80	33.34	50%
7	80	80.1 ± 0.23	33.78	60%
8	85	84.8 ± 0.53	34.40	70%
9	90	89.7 ± 1.01	34.64	80%
10	95	95.1 ± 0.92	34.75	90%
11	100	100.6 ± 1.07	35.76	100%

*Reference sample

Table 2. Summary of tactile sensation thresholds with physical stimuli across all participants (n=20)

Tactile test	Test results	0 ºC (n=20)	20 ºC (n=20)	37 ºC (n=20)	45 ºC (n=20)	*p*-value
Light touch	Tongue surface temperature (ºC)	18.1 ± 0.9	24.9 ± 1.0	33.6 ± 0.8	38.3 ± 0.6	< 0.01
	Thresholds (g)	0.008	0.008	0.008	0.008	-
Vibratory perception	Tongue surface temperature (ºC)	19.7 ± 1.1	25.6 ± 1.2	33.4 ± 0.7	36.8 ± 0.5	< 0.01
	Thresholds (10^{-6}cm)	1.7 ± 0.4[a]	1.3 ± 0.3[b]	1.1 ± 0.1[c]	1.2 ± 0.2[b]	<0.01
Two-point discrimination	Tongue surface temperature (ºC)	19.5 ± 1.0	24.5 ± 0.8	34.0 ± 0.8	37.7 ± 0.4	< 0.01
	Thresholds (mm)	1.4 ± 0.2	1.3 ± 0.2	1.3± 0.1	1.3± 0.1	0.14

Table 3. Summary of tactile sensation thresholds with chemical stimuli across all participants (n=20)

Tactile test	Test results	Control solution application (n=20)	Capsaicin solution application (n=20)			*p*-value
			5 ppm	10 ppm	20 ppm	
Light touch	Tongue surface temperature (°C)	33.6 ± 0.7	35.1 ± 0.4	35.3 ± 0.5	35.9 ± 0.7	<0.05
	Thresholds (g)	0.008	0.008	0.008	0.008	-
Vibratory perception	Tongue surface temperature (°C)	33.3 ± 0.5	34.7 ± 0.6	34.6 ± 0.6	35.1 ± 0.6	<0.05
	Thresholds (10^{-6}cm)	1.1 ± 0.3	1.0 ± 0.3	1.1 ± 0.2	1.0 ± 0.3	0.88
Two-point discrimination	Tongue surface temperature (°C)	33.4 ± 0.5	33.9 ± 0.6	34.0 ± 0.7	34.3 ± 0.7	<0.05
	Thresholds (mm)	1.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.1	1.3 ± 0.1	0.89