The influence of solid and liquid palate cleansers toward the neutralization effectiveness of chili’s spicy sensation

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Abstract. Capsaicin is a compound in chili which is able to bind with TRPV1 sensory nerve receptor. This sensory nerve receptor perceives pain as well as spicy sensation. Consumption of spicy foods will generally leave a residue on tongue and possibly irritate the papillae at a certain concentration. To reduce this effect, palate cleanser is required to normalize the oral cavity. Those are include, chocolate, cucumber, agar, milk and water which are normally being used as palate cleanser. The comparative study of the five palate cleanser was performed to evaluate the most effective palate cleanser in eliminating spicy sensations. This research was performed using Discrete Time Intensity (DTI) method with time interval of 30 second for 2 minutes. The data analysis was performed by GLM (General Linear Model) ANOVA and it was verified by residual saliva test on HPLC. It suggests that among the palate cleanser tested, cold fullcream milk (1.5°C-3°C) is the most effective palate cleanser for capsaicin-driven spicy sensation.

1. Introduction

Chili (Capsicum annum L.) has several types of nutrients and vitamins including calories, protein, fat, carbohydrates, fructose, calcium, vitamin A, vitamin B and vitamin C [1]. One compound that distinguishes chili with other herbs is Capsaicin (trans-8-methyl-N-vanilil-6-nonenamide). Consumption of food with excessive spicy taste can injure the tongue papillae, therefore it will cause excessive burnt sensation in the mouth. It is mainly caused by the ability of capsaicin compound to be bound by specific high heat sensory nerve receptors (TRPV1). The mechanism of TRPV1 nociceptors action can be seen in Figure 1.

Regardless its burning sensation, spicy-foods are very popular, particularly among Asian cuisines and food products. It may be attributed by the fact that capsaicin is able to stimulate the production of endorphin hormones that causes pleasant sensation [5].

Palate cleanser is any food materials that can be used to clean and neutralise oral cavity as before consumption [6]. However, it was suggested that a specific palate cleanser is required to eliminate various types of flavours and residues in the mouth [7]. The suitable palate cleanser will improve discrimination ability between products and minimize adaptation or build up [6]. It has been suggested in the literature that milk is an alternative beverage that can neutralize the spicy sensation due to its non-polar fat content [8]. In addition to the dissolution mechanism, neutralization of spicy sensation can occur due to TRPV1 local nociceptors dysfunctions caused by inhibition of mitochondrial respiration by Calcium [9-10]. Beer, sour cream, chocolate and sugar water were also assumed to
eliminate the spicy sensation as it has dominant sour and sweet taste [11]. Although in general the palate cleanser commonly applied after consuming spicy food is mineral water, it turns out that mineral water is less effective in eliminating the spicy sensation due to differences in polarity between water and capsaicin compounds. Therefore theoretically, non-polar material and Ca-containing material are more likely to be used as a palate cleanser in removing spicy residual sensations. Therefore this current study will be discussing several types of foods and beverages such as milk, water, chocolate and cucumber. Not only comparing the effectiveness of the four palate cleansers, the effectiveness and speed of time in neutralising the sensation were also observed.

Figure 1. The mechanism of action of TRPV1 [2-4]

It has been reported that palate cleanser temperature is important for cleansing roles in mouth. Regarding spicy sensation, it was suggested that warmer palate cleanser accelerates the release of spicy sensation on the tongue compared to room temperature [12-13]. Meanwhile, the influence of cold palate cleanser is relatively limited being reported in the literature. Therefore, to fill the research gap, this current study carried out a comparison between room and cold temperature palate cleanser for solid and semi-solid cleanser, while warm, room and cold temperature palate cleanser were tested for liquid cleanser.

2. Materials and methods

2.1 Materials
The palate cleansers used in this study were dark chocolate 67%, plain agar, cucumber, mineral water and pasteurized milk. The supporting material used in this study was chili powder. Materials used for
chemical analysis and analysis of capsaicin levels include ethanol pro-analysis (99%) Merck, aquaest, aqua pro injection.

2.2 Palate cleanser preparation
Agar is boiled and stirred using medium heat for 15 minutes then cooled and poured into a mold. For cucumber samples, first cut both ends and then remove the sap and wash it and the chocolate sample is only cut to size. All cut solid samples are prepared weighing 7.5 ± 8 grams and served at room temperature (25°C-27°C) and at cold temperature (1.5°C-3°C). Milk and water are heated then they are served in white cup and the temperature is kept at warm temperature (36°C -38°C), room temperature (25°C-27°C) and cold temperature (1.5°C-3°C). The chili concentration used is 0.2%; 0.4% and 0.6%. First chili powder weighed 2; 4 and 6 grams, then dissolved in 1 litre of water and heated until boiling. Then the chili solution was filtered so there are no remaining particles of chili powder which are included in the chili solution. The last, the chili solution was cooled and served in a white cup.

This AAS (Atomic Absorption Spectroscopy) test was performed to determine the Ca levels of palate cleanser using AAS; AA240 Variant.

2.3 Discrete time intensity testing
This research was conducted by implementing Discrete Time Intensity method to measure the effectiveness of palate cleanser for neutralising the spicy sensation at a certain time interval. There were 26 trained panellists involved in this study. The faster palate cleanser can eliminate or reduce the spicy sensation, the more effective the palate cleanser.

Before running the main test, panellist should undergo threshold test to determine the absolute threshold of the spicy sensation by the 3-AFC method. The panellist received 5 sets of samples presented in plastic cups consisting of two blanks (mineral water) and one tastant (spicy solution) at different concentrations (0.0%; 0.10%; 0.20%; 0.40; 0.60% b/v).

The effectiveness of the palate cleanser was measured by Discrete Time Intensity method at 3 different chilli concentrations (0.2%; 0.4% and 0.6% b/v) for 30; 60; 90 and 120 seconds.

2.4 Instrumental test
AAS (Atomic Absorption Spectroscopy) test was performed to determine the Ca levels of palate cleanser using AAS (AA240 Variant).

Evacuated saliva were also tested by HPLC to measure capsaicin residue in mouth. The fourth was testing the levels of capsaicin in the results of saliva evacuation with the help of the palate cleanser using an HPLC (High Pressure Liquid Chromatography) instrument with C18 column (150 x 4.6 mm x 3 μm), mobile phase aquabides: acetonitrile (50:50) v/v, the flow rate was 0.7 ml per minute and the detection wavelength was 280 nm. Retention time for capsaicin was in the 16th minute.

2.5 Data analysis
Experimental works were conducted by Completely Randomized Block Design. The data obtained were analysed using Minitab 17 with the Analysis of Variance (ANOVA) model of the Generalized Linear Model (GLM) model and Fisher’s as the post hoc.

3. Results and Discussions

3.1 Panellist threshold
BET is a method of estimating the excitatory threshold by using a geometric average (geo-mean), the last transition from the wrong answer to the correct answer in each panellist, with a note that all stages after the transition are true [14,15,16].

In this study, BET groups of 26 panellists were sought as the basis for determining the chili concentration presented. After calculating, the score of BET groups from all panellists in this study
was 0.10%. It means that panellists will be able to sense the spicy sensation of chili at concentration of 0.10% and above.

3.2 Effectiveness of palate cleanser

A decrease in the intensity of the spicy sensation-each palate cleanser from 30 to 120 seconds is indicated by decreasing curve was observed (Figure 2). This shows that each palate cleanser worked well. The use of the palate cleanser is used to remove residual flavours and also sensations in the oral cavity [17].

As shown (Table 1), milk has the greatest ability as a palate cleanser. This is caused that milk contains non-polar compounds, which are fats can bind and dissolve capsaicin substances on the tongue [8] [11, 17-18]. Not only the fat, the great ability of milk as spicy cleanser may also be attributed by its calcium content. In this study milk contains 11.3 mg/L Ca while mineral water only contains 6.35 mg/L Ca. It was suggested that the inhibition of mitochondrial respiration occurs faster and TRPV1 nociceptors deactivation occurs in the presence of calcium [9-10].

Unlike fullcream milk which is rich of fat and calcium, water is a polar liquid which cannot bind non-polar capsaicin. But, water is still more effective palate compared to chocolate. This may be attributed by the fact that the Ca content in mineral water is still higher than that of chocolate (5.45 mg/L). The Ca content in food (extracellular Ca) which enters the mouth can cause intra-cellular calcium release activated by calcium-dependent enzymes and inhibition of mitochondrial respiration resulting in deactivation or impaired local nociceptive function (TRPV1) [9-10].

In addition, the mechanism of water in neutralising spicy sensation water is also related to hedonic factors, as it was suggested that water is the most preferred beverage as a palate cleanser for spicy sensation [19].

The decrease intensity of spicy sensation contributed by solid food including chocolate, agar and cucumber were lower than those of liquid cleansers. This may be related to salivary cleansing. Salivary secretion can be stimulated in several ways such as mechanical stimulation by chewing food and chemical stimuli with food taste, neuronal stimulation, psychic stimulation and pain stimulation [20]. Saliva has many functions such as lubricants, protectors, cleansers, solvents, agent of flavour-forming or sensations compounds [21]. It was reported that the saliva volume reached 1.7 ml/minute while chewing and reached 0.33-0.65 ml/minute without chewing. Thus chewing enhances the cleansing effect in mouth [21]. However, considering the palate cleansers tested in this current study, liquid milk was observed as the most superior cleanser for spicy sensation of chili.

Figure 2. The changes of spicy sensation over 2 minutes observation
Table 1. Changes of spicy sensation intensity every 30s by 5 different palate cleansers

| Palate Cleanser | Intensity at 30s | 60s | 90s | 120 s | AUC (Area Under Curve) |
|----------------|-----------------|-----|-----|-------|------------------------|
| Water          | 2.3<sup>c</sup> | 1.6<sup>c</sup> | 1.0<sup>b</sup> | 0.5<sup>b</sup> | 122.2<sup>c</sup> |
| Milk           | 1.5<sup>d</sup> | 0.9<sup>d</sup> | 0.5<sup>c</sup> | 0.2<sup>c</sup> | 70.3<sup>d</sup> |
| Chocolate      | 2.5<sup>bc</sup> | 1.7<sup>bc</sup> | 1.1<sup>ab</sup> | 0.6<sup>ab</sup> | 136.7<sup>bc</sup> |
| Agar           | 3.0<sup>a</sup> | 2.1<sup>a</sup> | 1.4<sup>a</sup> | 0.8<sup>a</sup> | 178.9<sup>a</sup> |
| Cucumber       | 2.8<sup>ab</sup> | 2.0<sup>ab</sup> | 1.3<sup>ab</sup> | 0.7<sup>ab</sup> | 153.1<sup>ab</sup> |

*Different superscript at the same column indicates significant different at 95% confidence interval

As shown in Table 2, cold palate cleansers were more effective than those of higher temperature cleansers. TRPV1 receptors will respond when the temperature in the mouth reached 42°C or more [22]. Increased temperature in the mouth can be affected by the temperature of the food consumed [23]. Thus when holding cold food in mouth, the increasing temperature in the mouth will be slow. It has been suggested that cold temperatures can slow down the process of consumer to feel the spicy sensation [24]. This may also be attributed to psychological influences, as in general people prefer to consume cold beverages to experience fresh perception [25].

Table 2. The influences of palate cleanser serving temperature to the changes of spicy intensity

| Palate Cleanser Temperature | Intensity at |
|-----------------------------|--------------|
|                             | 30s | 60s | 90s | 120 s |
| Warm (36-38°C)              | 2.4<sup>b</sup> | 1.7<sup>b</sup> | 1.0<sup>b</sup> | 0.5<sup>b</sup> |
| Room (25-27°C)              | 3.0<sup>a</sup> | 2.1<sup>a</sup> | 1.4<sup>a</sup> | 0.8<sup>a</sup> |
| Cold (1.5-3°C)              | 1.8<sup>c</sup> | 1.3<sup>c</sup> | 0.8<sup>b</sup> | 0.4<sup>b</sup> |

*Different superscript at the same column indicates significant different at 95% confidence interval

3.3 Correlation of area under curve (AUC) spicy intensity changes and capsaicin concentration in saliva by different palate cleansers

![Graph](image)

Figure 3. (a) AUC value of palate cleanser samples and (b) Capsaicin concentration on saliva for various palate cleansers

In this study Area under Curve (AUC) was calculated as this parameter showed the total response of the spicy sensation felt by panellists within 2 minutes records. The greater the AUC value indicates the slower reduction of spicy sensation felt by panellist (Figure 3a). The observation shows that milk provided the smallest AUC among all cleansers and it is correlated to the highest capsaicin
concentration in the panellist’s saliva. This suggests that milk are able to neutralize the spicy sensation faster compared to other palate cleanser (Figure 3b, Figure 4).

![Graph of Pure Capsaicin from Chili Powder and Evacuated Capsaicin on Saliva as Effected by Various Palate Cleansers](image)

Note: a = Pure Capsaicin; b = Chili Powder; c = Milk; d = water; e = Chocolate; f = Agar; g = Cucumber)

**Figure 4.** HPLC Graph of Pure Capsaicin from Chili Powder and Evacuated Capsaicin on Saliva as Effected by Various Palate Cleansers

### 4. Conclusion

This current study suggests that different palate cleanser and temperature influence chili’s spicy sensation neutralisation (p<0.05). Among tested palate cleansers, milk is proven to be the most effective in eliminating the spicy sensation. It may be attributed to milk fat as it may bind capsaicin and thus induces capsaicin dissolution and therefore reduce spicy sensation. Milk also contains high calcium, in which contributing to deactivate TRPV1 nociceptors. As milk was tested in its liquid form, milk can also reach and clean the inside of the mouth more thoroughly. In terms of temperature, the more effective way to eliminate the spicy sensation is cold cleansers as it slowdowns activation of the high heat sensor in TRPV1.

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