

## Effects of Fat and Sucrose in Palate Cleansers on Discrimination of Burning Sensation of Capsaicin Samples

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**Abstract** Repeated tasting of spicy foods could affect the accuracy of sensory evaluation because of the accumulating and lasting of burning sensation. Therefore, protocols are needed to overcome this problem. The objective of this study was to investigate the effects of 6 milk based palate cleansers varying in fat and sucrose level on discrimination of burning sensation of capsaicin samples prepared at different concentrations. The effects were observed at 3 different interval lengths (1, 3, and 5 min) using different subjects. Subjects rated the intensity of burning sensation of the samples using 15-point category scales and they completed 7 sessions including 1 warm up session, using a different palate cleanser in each session. At 3 min interval, fat seemed to be more effective than sucrose, and the effects of fat was still more increased with the addition of sucrose. However, consistent trends were not observed at 1 and 5 min intervals.

**Keywords:** palate cleanser, interval length, capsaicin, burning sensation

### Introduction

Capsaicin is the main cause of the burning sensation in chili peppers. Capsaicin binds to capsaicin receptors (VR-1) that are also gated by noxious heat and acidic stimuli (1). VR-1 receptors are expressed at the ends of trigeminal nociceptors that innervate the oral mucosa (2,3). Sensory responses of capsaicin include burning, irritation, and pain which are not alleviated easily. Repeated tasting of

multiple capsaicin samples produce sensitization and desensitization (4), thus, it is hard to accurately evaluate the burning sensation of each sample. Furthermore, as spicy foods gain popularity worldwide (5), development of proper protocol for the evaluation of multiple spicy foods would be very useful. Therefore, experimental procedures, such as using proper cleansers and/or adequate interval lengths, that could minimize residuals and reestablish oral environment back to baseline (6), are important to distinguish samples clearly.

There is primary reason for using palate cleanser. Palate cleanser removes residual flavor or sensation from the oral environment, which would otherwise alter the perceived intensity via adaptation (7). Lucak and Delwiche (8) mentioned that efficient palate cleanser would prevent significant differences in replicate samples. Therefore, palate cleanser is necessary to reduce confusion between samples.

In order to establish a protocol for the alleviation of burning sensation, several studies have been reported. Stevens and Lawless (9) accomplished that the burning sensation was reduced more by using sucrose solution and citric acid solution compared to using plain water, sodium chloride solution, or quinine solution. Sizer and Harris (10) evaluated the effect of rinsing with sodium chloride, sucrose, and citric acid upon the recognition threshold of capsaicin samples. They found that the sucrose solution reduced perceived burning sensation most effectively. Sucrose intake-induced suppression of pain develops quickly and lasts for minutes (11). In addition, sucrose intake-induced anti-nociceptive effects appear to be naltrexone-sensitive in rats (12), as well as in humans (13).

Baron and Penfield (14) examined the responses to capsaicin in water, cheese sauces, and starch pastes which differed in fat contents. They found that the responses decreased as the fat content increased. Additionally, they

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concluded that lipophilic substances reduced burning sensation from capsaicin which is fat-soluble phenol. Hutchinson *et al.* (15) evaluated the potential use of rice, butter, pineapple juice, and water as palate cleansers. They found that butter was slightly more effective than the others. Nasrawi and Pangborn (16) compared the effects of whole milk and skim milk as a palate cleanser. They indicated that both cleansers were effective for reducing burning sensation when they were held in the mouth, and whole milk was slightly more effective than skim milk. Although Nasrawi and Pangborn (16) studied to investigate the effects of both fat and sugar on reducing burning sensation, they did not have systematic approach to understand the effects of these 2 components. Moreover, all these reports focused on the effectiveness of fat and sugar for the reducing of burning sensation itself, but not on the discrimination of various samples.

Importance of interval length has been studied but this also was only applied to examine the effect on dissipation of perceived burning sensation (17). Dowell *et al.* (18) developed a predictive equation to estimate the time needed between spicy salsa samples in order to obtain repeatable sensory judgments. However those were concentrated on reduction of burning sensation not on the sample discrimination.

Most previous studies focused on examine the effects of palate cleansers and/or interval lengths on the reduction of burning sensation itself. However, it is also important to find out if the sample can be discriminated more clearly after the alleviation of burning sensation. The objective of this study was to demonstrate the effects of fat and sucrose in palate cleansers on the discrimination of burning sensation of capsaicin samples at several concentrations. The effects were observed at different interval lengths (1, 3, and 5 min) between samples.

## Materials and Methods

**Participants** Three groups of subjects (16 subjects/group, students of Ewha Womans University, Seoul, Korea, age

range: 20–36) were recruited by posting advertisement flyers in the campus. They were finally selected by individual interview to find out their willingness to participate. All subjects who completed the study received monetary compensation for their participation at the end of the study.

**Stimuli** Capsaicin was incorporated into samples using an aqueous stock solution which was prepared according to the modified ASTM method E 1083-00 (19). Capsaicin (0.02 g) (95%; Sigma-Aldrich, St. Louis, MO, USA) was mixed with 0.7 g of food grade polysorbate-80 (Sigma-Aldrich) and heated on a hot plate ( $30 \pm 2^\circ\text{C}$ , HMS-10; Yong-Ji Hana Tech., Seoul, Korea) until the capsaicin was completely dissolved (about 5 min). After transferring the capsaicin-polysorbate 80 mixture with  $70^\circ\text{C}$  water into a 100-mL volumetric flask, the capsaicin concentration in the resulting stock solution was 200 ppm. Experimental capsaicin samples (0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 ppm) were prepared by diluting the stock solution with water ( $22 \pm 2^\circ\text{C}$ ) and stored in refrigerator ( $4 \pm 1^\circ\text{C}$ ). Capsaicin samples were served at room temperature ( $22 \pm 2^\circ\text{C}$ ).

**Palate cleansers** Six palate cleansing strategies with different levels of fat and sucrose were used in this study (Table 1). Palate cleansers included skim milk (SKM), mixture of skim milk and 10% sucrose (SKS), mixture of skim milk and heavy whipping cream (SKW), mixture of skim milk, heavy whipping cream, and 10% sucrose (SWS), heavy whipping cream (WCR), and mixture of heavy whipping cream and 10% sucrose (WCS). Palate cleansers were stored in the refrigerator ( $4 \pm 1^\circ\text{C}$ ) and served in thermos (250-mL, AP-250; Apollo Co., Ltd., Yangju, Korea) to maintain the serving temperature ( $5 \pm 1^\circ\text{C}$ ).

**Evaluation procedure** Subjects participated in series of 7 sessions (1 for each palate cleanser) which include 1 extra warm up session for a period of 4 weeks. The sessions were 2 to 4 days apart to reduce the learning effect. Prior to evaluating the capsaicin samples, the participants were instructed not to swallow any capsaicin samples, palate cleansers, and water during the evaluation

**Table 1. Information of palate cleansers used in the study**

| Sample identification | Major ingredients <sup>1)</sup>          | Fat and sucrose contents     |
|-----------------------|--|------------------------------|
| SKM                   | Skim milk                                | 0.35% milk fat, 0% sucrose   |
| SKS                   | Skim milk, sucrose                       | 0.35% milk fat, 10% sucrose  |
| SKW                   | Skim milk, heavy whipping cream          | 19.68% milk fat, 0% sucrose  |
| SWS                   | Skim milk, heavy whipping cream, sucrose | 19.68% milk fat, 10% sucrose |
| WCR                   | Heavy whipping cream                     | 39.00% milk fat, 0% sucrose  |
| WCS                   | Heavy whipping cream, sucrose            | 39.00% milk fat, 10% sucrose |

<sup>1)</sup>Skim milk (Seoul Milk Co., Ltd., Geochang, Korea); Sucrose (Beksul, CJ Cheiljedang Corp., Nonsan, Korea); Heavy whipping cream (Seoul Milk Co., Ltd., Yangju, Korea)

session. Five mL of the capsaicin sample were served in a small size paper cup (2.5 oz; Easepack Co., Namyangju, Korea) coded with 3-digit random numbers. Participants received 6 capsaicin samples at a time and the 6 samples were served in a random order except that the lowest concentration of capsaicin sample was never followed by the highest concentration and *vice versa*. Additionally, the palate cleanser was served in thermos (250 mL, AP-250; Apollo Co., Ltd.) to maintain the temperature ( $5\pm1^{\circ}\text{C}$ ), with a small size paper cup containing a line marked at 15 mL above the bottom of the cup. Each participant received 1 palate cleanser in 1 session and the order of 6 palate cleansers was randomized. Warm water ( $50\pm2^{\circ}\text{C}$ ) was provided to remove the palate cleanser from the mouth.

The warm up session was held 2 or 3 days before the evaluation started. In the warm up session, participants received detailed written and verbal instructions of the tasting procedure, rinsing procedure, and how to use the scale. Additionally, they rated the intensity of the burning sensation of 6 different capsaicin concentrations using warm water and 3 min interval length between each sample in order to recognize the range of capsaicin samples.

In the main test session, the subjects were instructed to rinse their mouth with 15 mL of palate cleanser, then, warm water twice to remove the palate cleanser, which would have remained in the mouth, followed by 1, 3, or 5 min break. Subjects placed the 5 mL of capsaicin sample in their mouths, swirled it around their mouths and expectorated it, and rated the perceived intensity of the burning sensation on a 15-point category scale. After they marked the intensity of the burning sensation, they used the same rinsing and resting procedure for the next 5 samples. The scale was labeled with 'weak' on the left end and 'strong' on the right end. All sessions were evaluated in individual booths to promote privacy during the session and subjects were not permitted to eat or drink anything other than water 1 h prior to the session.

**Statistical analysis** To determine which palate cleansers allowed subjects to discriminate the samples better, the *F*-values of palate cleanser were compared (20,21). In addition, analysis of variance (ANOVA) using the procedure general lineal model (GLM) was conducted to examine the effects of each palate cleanser on capsaicin samples ( $p<0.05$ ). Duncan's multiple range test was conducted as a follow-up *post hoc* comparison between the samples ( $p<0.05$ ). In the models, the ratings of burning sensation were the dependent variable and palate cleanser and samples were the independent variables. Separated ANOVA were conducted on each group. All statistical analyses were performed using SPSS for Windows software (version 18.0; SPSS Inc., Chicago, IL, USA).

**Table 2.** *F*-value of each palate cleanser at different interval lengths

| Palate cleanser | Interval length (min) |        |        |
|-----------------|-----------------------|--------|--------|
|                 | 1                     | 3      | 5      |
| SKM             | 24.611                | 35.205 | 31.353 |
| SKS             | 24.722                | 35.965 | 32.254 |
| SKW             | 23.532                | 37.130 | 61.191 |
| SWS             | 16.262                | 41.993 | 40.841 |
| WCR             | 30.108                | 43.063 | 34.607 |
| WCS             | 18.437                | 43.751 | 73.691 |

## Results and Discussion

**Effect of fat and sucrose** There were significant differences among the 6 palate cleansers at all interval conditions ( $p<0.001$ ). When comparing the *F*-values of each palate cleansers (Table 2), in 1 min interval group, panelist discrimination was the highest when the heavy whipping cream (WCR) was used and it was the lowest when mixture of skim milk, heavy whipping cream, and sucrose (SWS) was used. On the other hand, in 3 min interval group, the panelist discrimination was the highest when mixture of heavy whipping cream and sucrose (WCS) was used and it was the lowest when skim milk (SKM) was used. In addition, the panelist discrimination increased as the fat and sucrose level increased but the effects of fat was more obvious than sucrose. This effectiveness of fat was confirmed by Nasrawi and Pangborn (16), Baron and Penfield (14), Hutchison *et al.* (15), and Carden *et al.* (22) who found that fat reduced the burning sensation. In the 5 min interval group, panelist discrimination was the highest when WCS was used and mixture of skim milk and heavy whipping cream (SKW) was also very effective. Moreover, in 3 and 5 min interval groups, panelist discrimination tended to increase as the fat content increased when sucrose was in the palate cleanser. It seemed that the effects of fat were markedly increased by the addition of sucrose. This result was supported by the previous research which showed the effects of sucrose. Sweet taste is known to produce pleasant emotions in adults and even in infants within hours of birth (23) and this allows people and animals to tolerate pain better while they taste sweetness (24,25).

**Effect of interval time** There were significant differences among the capsaicin samples at all interval lengths tested ( $p<0.05$ ), and the burning sensation tended to increase as the capsaicin concentration increased (Table 3). In 3 min interval, the intensity of burning sensation consistently increased as the capsaicin concentration increased in all palate cleansers. However, in 1 and 5 min interval, burning

**Table 3. Mean intensity scores for capsaicin samples with different palate cleansers in 1, 3, and 5 min interval**

| Interval length | Palate cleanser | Capsaicin sample (ppm)                       |                              |                             |                              |                              |                              |
|-----------------|-----------------|--|------------------------------|-----------------------------|------------------------------|------------------------------|------------------------------|
|                 |                 | 0.5  | 1.0                          | 2.0                         | 4.0                          | 6.0                          | 8.0                          |
| 1 min           | SKM             | 2.50 <sup>d 1)</sup><br>(1.46) <sup>2)</sup> | 2.25 <sup>d</sup><br>(1.18)  | 5.00 <sup>c</sup><br>(3.56) | 7.63 <sup>b</sup><br>(4.21)  | 9.13 <sup>ab</sup><br>(3.98) | 9.69 <sup>a</sup><br>(4.56)  |
|                 | SKS             | 2.25 <sup>d</sup><br>(1.84)                  | 3.25 <sup>cd</sup><br>(2.35) | 4.38 <sup>c</sup><br>(3.22) | 7.62 <sup>b</sup><br>(4.43)  | 9.50 <sup>ab</sup><br>(4.10) | 10.00 <sup>a</sup><br>(2.94) |
|                 | SKW             | 1.81 <sup>d</sup><br>(1.56)                  | 2.94 <sup>cd</sup><br>(2.84) | 4.00 <sup>c</sup><br>(3.06) | 5.88 <sup>b</sup><br>(3.07)  | 8.62 <sup>a</sup><br>(4.00)  | 9.94 <sup>a</sup><br>(3.07)  |
|                 | SWS             | 2.63 <sup>d</sup><br>(2.28)                  | 2.88 <sup>d</sup><br>(2.63)  | 5.44 <sup>c</sup><br>(3.16) | 5.75 <sup>c</sup><br>(3.84)  | 7.75 <sup>b</sup><br>(4.33)  | 10.06 <sup>a</sup><br>(3.80) |
|                 | WCR             | 1.81 <sup>e</sup><br>(1.11)                  | 2.75 <sup>de</sup><br>(2.57) | 3.87 <sup>d</sup><br>(2.90) | 6.81 <sup>c</sup><br>(3.39)  | 9.06 <sup>b</sup><br>(4.64)  | 11.00 <sup>a</sup><br>(3.85) |
|                 | WCS             | 2.88 <sup>d</sup><br>(2.31)                  | 2.69 <sup>d</sup><br>(1.62)  | 4.94 <sup>c</sup><br>(3.26) | 7.44 <sup>b</sup><br>(3.90)  | 8.00 <sup>ab</sup><br>(3.14) | 9.50 <sup>a</sup><br>(3.80)  |
| 3 min           | SKM             | 2.37 <sup>c</sup><br>(1.86)                  | 3.13 <sup>c</sup><br>(2.00)  | 5.06 <sup>b</sup><br>(3.42) | 8.94 <sup>a</sup><br>(3.70)  | 9.75 <sup>a</sup><br>(3.34)  | 10.44 <sup>a</sup><br>(3.79) |
|                 | SKS             | 2.25 <sup>d</sup><br>(1.18)                  | 2.94 <sup>d</sup><br>(1.98)  | 5.56 <sup>c</sup><br>(2.71) | 7.44 <sup>b</sup><br>(3.37)  | 9.88 <sup>a</sup><br>(3.58)  | 10.62 <sup>a</sup><br>(3.34) |
|                 | SKW             | 2.06 <sup>d</sup><br>(1.65)                  | 4.63 <sup>c</sup><br>(3.12)  | 5.50 <sup>c</sup><br>(2.94) | 7.50 <sup>b</sup><br>(3.20)  | 10.50 <sup>a</sup><br>(3.41) | 12.19 <sup>a</sup><br>(3.10) |
|                 | SWS             | 2.38 <sup>d</sup><br>(1.20)                  | 3.44 <sup>cd</sup><br>(2.25) | 4.38 <sup>c</sup><br>(3.05) | 6.56 <sup>b</sup><br>(3.54)  | 9.75 <sup>a</sup><br>(3.45)  | 11.13 <sup>a</sup><br>(2.68) |
|                 | WCR             | 2.63 <sup>d</sup><br>(1.96)                  | 3.06 <sup>d</sup><br>(1.88)  | 5.00 <sup>c</sup><br>(2.73) | 7.69 <sup>b</sup><br>(3.95)  | 10.25 <sup>a</sup><br>(2.91) | 11.75 <sup>a</sup><br>(2.86) |
|                 | WCS             | 2.06 <sup>d</sup><br>(1.44)                  | 3.00 <sup>d</sup><br>(1.51)  | 5.31 <sup>c</sup><br>(2.60) | 7.87 <sup>b</sup><br>(3.30)  | 9.75 <sup>a</sup><br>(2.67)  | 10.88 <sup>a</sup><br>(2.50) |
| 5 min           | SKM             | 2.00 <sup>c</sup><br>(1.36)                  | 3.06 <sup>c</sup><br>(2.29)  | 6.00 <sup>b</sup><br>(3.88) | 10.06 <sup>a</sup><br>(3.31) | 10.25 <sup>a</sup><br>(3.83) | 10.94 <sup>a</sup><br>(2.95) |
|                 | SKS             | 2.38 <sup>c</sup><br>(2.36)                  | 3.19 <sup>c</sup><br>(1.83)  | 7.31 <sup>b</sup><br>(3.49) | 7.25 <sup>b</sup><br>(3.80)  | 11.13 <sup>a</sup><br>(3.20) | 12.00 <sup>a</sup><br>(3.54) |
|                 | SKW             | 1.88 <sup>d</sup><br>(1.08)                  | 3.19 <sup>d</sup><br>(2.13)  | 5.00 <sup>c</sup><br>(2.30) | 8.69 <sup>b</sup><br>(3.57)  | 11.31 <sup>a</sup><br>(1.85) | 12.19 <sup>a</sup><br>(2.48) |
|                 | SWS             | 1.63 <sup>d</sup><br>(1.54)                  | 4.19 <sup>c</sup><br>(2.48)  | 5.13 <sup>c</sup><br>(4.03) | 8.75 <sup>b</sup><br>(3.56)  | 11.38 <sup>a</sup><br>(2.50) | 12.00 <sup>a</sup><br>(2.63) |
|                 | WCR             | 1.56 <sup>c</sup><br>(0.81)                  | 3.19 <sup>c</sup><br>(2.16)  | 5.25 <sup>b</sup><br>(3.04) | 7.00 <sup>b</sup><br>(2.80)  | 10.44 <sup>a</sup><br>(3.10) | 10.63 <sup>a</sup><br>(3.34) |
|                 | WCS             | 1.56 <sup>d</sup><br>(0.89)                  | 3.00 <sup>c</sup><br>(2.00)  | 4.38 <sup>c</sup><br>(2.16) | 8.13 <sup>b</sup><br>(2.19)  | 10.94 <sup>a</sup><br>(2.67) | 12.00 <sup>a</sup><br>(2.13) |

<sup>1)</sup>Within a row, means sharing a common lowercase letter are not significantly different at  $\alpha=0.05$ .

<sup>2)</sup>Numbers in parenthesis indicate standard deviations.

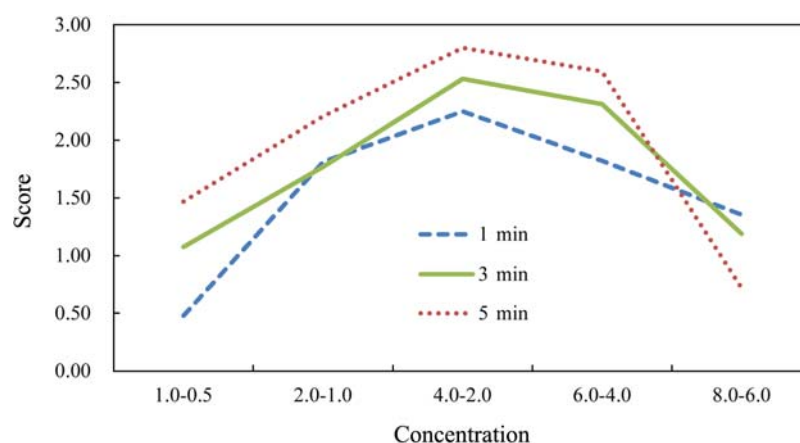
intensity of capsaicin samples was not in order according to the concentration of capsaicin. In 1 min interval, there was no significant difference between 0.5 and 1.0 ppm when SKM or WCS was used; This result was confirmed by Allison *et al.* (26) who reported that an interval length of 2 min or less was not enough to reduce the burning sensation. There might be a possibility that desensitization could be one of the reasons for above result. In 5 min interval, the intensity of the 2 ppm capsaicin sample was slightly higher than the 4 ppm capsaicin sample when SKS was used. This result can also indicate that memory could

be involved when the 5 min interval was used and it is supported by the result of previous studies (27,28). They reported that longer interval time could induce forgetting, thus making panelist more difficult to remember the previous tasted samples.

**Ability of sample discrimination** To determine which palate cleanser in each interval group allowed subjects to more clearly distinguish sample, cases that were not significantly different were counted by each palate cleanser and interval length based on the mean intensity scores

**Table 4.** Number of cases that are not significant different using different palate cleansers in different interval lengths

| Interval length | SKM | SKS | SKW | SWS | WCR | WCS | Total |
|-----------------|-----|-----|-----|-----|-----|-----|-------|
| 1 min           | 3   | 4   | 3   | 2   | 2   | 3   | 17    |
| 3 min           | 3   | 2   | 2   | 3   | 2   | 2   | 14    |
| 5 min           | 3   | 3   | 2   | 2   | 3   | 2   | 15    |

**Fig. 1.** Differences between adjacent concentrations of samples according to the different interval lengths.

shown in Table 3 (Table 4). SKM and SKS which contain a low fat content did not discriminate the samples better than other cleansers. Moreover, the total number of samples that were not significantly differentiated was higher for 1 min interval than for the 3 or 5 min interval. In other words, subjects tended to distinguish more samples when 3 or 5 min interval lengths were used. However, since it was not related sample design but independent sample design between interval lengths, there should be careful consideration for interpreting the results of this study. As shown in Fig. 1, the differences were observed in the burning sensation between the samples with close concentrations. Bigger differences mean greater difference in perception between the 2 samples. For the 1 min interval, the differences were smaller at low and medium capsaicin concentrations and larger at higher concentrations than the other interval lengths. This means that subjects could be confused when evaluating samples with low and medium concentrations due to the lack of recovery time. In contrast, for the 5 min interval, the differences were larger at low and medium concentration and smaller at high capsaicin concentration. This indicates that the 5-min interval allowed people to better distinguish the low and medium capsaicin concentrations, which was difficult at high concentration. Cain (29) and Engen (30) reported that people evaluated samples by remembering the previous sample. This means that subjects could be confused when evaluating high adjacent concentration samples (6.0 and 8.0 ppm) due to memory effects. As indicated above, in this study, different subjects were used between interval lengths to reduce

learning effects from repeated test. Therefore it was not possible to compare the effect of interval length statistically.

Based on the results of this study, fat seemed to be more effective than sucrose and the panelist's ability to discriminate different samples improved when more fat was combined with sucrose. Although using WCS and a 5 min interval was better in regards to discriminating different samples, there is a possibility that memory effects could have played a role in the results since the *F*-values were not consistent and burning intensity of 2 samples (2.0 and 4.0 ppm) was reversed. Subjects were shown to discriminate the samples better when 3 or 5 min interval was used. As indicated in this study, burning sensation was affected by palate cleansers and interval lengths. In order to examine which factors improve panelist discrimination clearly, more levels of fat and sucrose, and same subjects between different interval lengths are recommended.

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