

## Research Article

### Comparison between Chemical Senses Thresholds for Capsaicin and Dihydrocapsaicin in Aqueous Solutions and Identification of the Area of Burning Sensation

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**Abstract:** In the present study, the pungency detection thresholds for capsaicin and dihydrocapsaicin in aqueous solutions containing an emulsifier (polysorbate 80) were determined and compared. Thresholds were measured for 21 students (12 chili “users” and 9 “non users”) using a 3-Alternative Forced Choice sensory test with ascending concentrations of capsaicin and dihydrocapsaicin (0.025, 0.045, 0.090, 0.180 and 0.360 ppm, respectively). In addition, the panelists were asked where the irritation occurred (throat, tongue or both). The group Best Estimate Thresholds were 0.080 and 0.049 ppm for capsaicin and dihydrocapsaicin, respectively and differed significantly. Chili “users” and “non users” did not differ significantly in their perception of capsaicin and dihydrocapsaicin, suggesting that no desensitization effects occur. In accordance with previous studies, in most cases the first irritation was experienced in the throat.

**Keywords:** 3-AFC, burning localization, chili consumption, pungency, sensory thresholds

## INTRODUCTION

Capsaicinoids are pungent compounds found in plants of the genus *Capsicum*. The primary sensory responses to these compounds, especially capsaicin, include burning, tingling and pain (Cliff and Heymann, 1992).

All capsaicinoids consist of vanillylamides of fatty acids. The main differences between these compounds are the length of the aliphatic side chain, the number of double bonds and their relative pungencies (Krajewska and Powers, 1988). Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) and dihydrocapsaicin (8-methyl-N-vanillylnonanamide), which are the two major capsaicinoids in chili peppers, are responsible for approximately 90% of the total pungency (Barbero *et al.*, 2006). These two compounds differ with respect to only one double bond.

The most common method for the determination of pungency attributed to capsaicinoids was developed by Scoville (1912).

There have been very few investigations of the pungency threshold for capsaicin and no reliable information exists on the absolute pungency threshold for dihydrocapsaicin.

Jurenitsch (1981) reported that capsaicin and dihydrocapsaicin were not equally pungent. Krajewska

and Powers (1988), however, measured the effect of each compound on the total pungency of *Capsicum* fruits and concluded that both capsaicin and dihydrocapsaicin have pungency thresholds within a range of 0.039 and 0.078 ppm and that they do not differ significantly. This result indicates that both compounds feel equally hot. These results are consistent with those of Todd *et al.* (1977), who reported the same pungency for dihydrocapsaicin and capsaicin.

Some studies have reported different thresholds for capsaicin. When investigating concentrations between 0.060 and 4.000 ppm in 0.5 log<sub>2</sub> unit steps, Rozin *et al.* (1981) reported a threshold concentration of approximately 0.310 ppm in aqueous solutions. The threshold was defined as the lowest concentration at which the panelist was able to detect piquancy. Lawless *et al.* (2000) compared thresholds in aqueous solutions and oil-based systems. The concentrations of capsaicin presented in water were 0.03125, 0.0625, 0.125, 0.250 and 0.500 ppm, respectively. The mean group threshold was 0.310 (+/-0.030) ppm in water, which is consistent with the results of Rozin *et al.* (1981). However, the observations of Rozin *et al.* (1981) and Lawless *et al.* (2000) conflict with the results of Sizer and Harris (1985), who reported threshold scores between 0.090 and 0.350 ppm; most panelists required a concentration

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of 0.180 ppm capsaicin for recognition. Karrer and Bartoshuk (1991) agree with these findings because, in their study, a concentration of 0.100 ppm did not evoke a sensation in all subjects. Green (1989) and Schneider *et al.* (2011), however, reported lower thresholds of approximately 0.100 and 0.088 ppm, respectively. A possible explanation could be the existence of different thresholds depending on the location of the sensation in the mouth. Rentmeister-Bryant and Green (1997) reported threshold concentrations of 0.098 ppm for the throat, 0.180 ppm for the roof of the mouth and 0.299 ppm for the tongue. The latest studies report an absolute threshold concentration of 0.050 ppm for the total amount of capsaicinoids, defining the total capsaicinoid content as the sum of capsaicin and dihydrocapsaicin in the tested sample (Orellana-Escobedo *et al.*, 2012). The varied thresholds could perhaps be explained by the different methods and different stimuli used.

Different authors (Green, 1989; Karrer and Bartoshuk, 1991, 1995) have reported desensitization effects due to regular chili consumption. Desensitization could lead to different thresholds between chili “users” and “non users”.

The main objective of this study was to determine absolute thresholds for dihydrocapsaicin and to compare them to the thresholds of capsaicin in aqueous solutions to find out if both substances were equally pungent.

Aqueous solutions were chosen to allow better comparability of our results with the results of previous studies. The second objective was to determine the location of the first sensation elicited by capsaicin and dihydrocapsaicin and to determine if chili “users” and “non users” differ with respect to capsaicin and dihydrocapsaicin perception because of desensitization effects. The 3-Alternative Forced Choice (3-AFC) method was used because the probability of a correct randomly selected guess is only one-third (ISO 13301, 2002) and therefore, the reliability is better.

## MATERIALS AND METHODS

**Subjects:** Twenty-one healthy subjects (4 males, 17 females) between the ages of 20 and 25 participated in the study. They were all students from the Department of Food Technology at the University of Applied Sciences Fulda. They were selected because of their availability, their basic experience in sensory methods and evaluation and their willingness to participate. Chocolate and book vouchers were offered as incentives to participate.

Subjects filled out a simple questionnaire on the frequency of ingestion (7-point scale; more than once a day, daily, two or three times a week, once a week, once a month, less than once a month, once a year or less), preferred intensity (10-point scale; not pungent to extremely pungent) and time of last chili consumption (5-point scale; today to longer than one month ago).

The subjects were divided into “users” and “non users” of chili peppers. Those who consumed chili at least once a week and who scored 5 or higher for preferred intensity were classified as high-frequency “users”; those with an intensity score lower than 5 and a chili consumption of less than once a week were classified as “non users”. In this study 12 subjects were classified as “users” and 9 as “non users”. Persons with a low chili-consume were also classified as “non users” in this study because no sensitization or desensitization effects can be expected.

**Stimuli/sample preparation:** The two primary piquant components of chili pepper were the irritant compounds capsaicin (Sigma Aldrich,  $\geq 95\%$  purity from *Capsicum* sp.) and dihydrocapsaicin (Sigma Aldrich,  $\sim 90\%$  from *Capsicum* sp.). Capsaicin and dihydrocapsaicin were not hydrophilic and therefore, the food-grade emulsifier polysorbate 80 (Carl Roth GmbH) was used to solve both compounds in water. The polysorbate 80-water mixture was served as the reference so that the panelists would not be able to use the bitter taste of polysorbate 80 to identify the sample containing capsaicin or dihydrocapsaicin. The clear emulsions of sample and reference sample did not differ in appearance.

Stock solutions of capsaicin and dihydrocapsaicin (1000 ppm) were prepared by mixing 10 mg of each irritant with 10 mL of deionized water containing polysorbate 80 (12 ppm). The mixtures were heated to 40°C and sonicated to ensure the complete dissolution of the irritants.

Experimental water-based samples were prepared by diluting the stock solutions with deionized water and sonicated again to ensure a homogeneous sample. The threshold concentrations of the sample solutions were 0.025, 0.045, 0.090, 0.180 and 0.360 ppm, respectively capsaicin or dihydrocapsaicin; the concentrations of the series increased in two-unit steps and were chosen based on pilot work.

In compliance with the guidelines of the ASTM E 679-04 (2011) total number of presentations was 105 for capsaicin and 210 for dihydrocapsaicin (one replication to determine the reproducibility of the panel).

**Experimental procedure:** According to the guidelines in ISO 13301 (2002) and ASTM E679-04 (2011), the 3-AFC test was used to evaluate the threshold values of capsaicin and dihydrocapsaicin.

Before determining the thresholds for dihydrocapsaicin and capsaicin a training session was conducted with sugar to familiarize the panelists with the procedure of a 3-AFC-test.

Each sample (5 mL) was coded and presented to the panelists in a laboratory glass. All samples were presented at room temperature. Five rows of three samples in ascending concentrations-one test sample and two reference samples (polysorbate 80-water mixture)-were presented to the panelists on a tray. To

avoid positional bias three combinations of orders of presentation (AAB, ABA, BAA) were balanced across the panelists. The panelists were instructed to taste each sample in the presented sequence, from left to right. They were requested to put the whole sample into the mouth, swirl it around and finally swallow it all at one time.

Subjects were asked to identify the different sample in the sets of three at each concentration. If the panelists felt uncertain, they were told to guess according to the forced-choice principle. The panelists were also forced to identify the region in which there was a burning sensation (tongue, throat or both). The test proceeded for all five series of three samples. The five series had five different concentrations, which increased by a factor two per step. The subjects had to wait for at least 30 sec and to rinse their mouths with polysorbate 80 in water (12 ppm) before evaluating a new row. They were instructed to wait until there was no more burn in the mouth before testing the next series on the tray.

Furthermore the panelists had to determine the location (tongue, throat, tongue and throat) of the first sensation elicited by capsaicin or dihydrocapsaicin at each concentration.

To avoid carry-over-effects, sensitization or desensitization, the thresholds for capsaicin and dihydrocapsaicin were measured on different days with one week between the sessions.

In addition, the threshold determination for dihydrocapsaicin was repeated to determine if the panel yielded reliable and consistent results.

We confirm that all participants involved in the study, which complies with the Declaration of Helsinki for Medical Research involving Human Subjects, provided written and verbal consent and that the study was approved by the ethical committee of the University of Applied Sciences in Fulda.

**Data analysis/statistical analysis:** The Best Estimate Threshold (BET) for each panelist was determined by calculating the geometric mean of the last failed concentration and the first concentration with a correct response except for the following sequence. The BET of the group (group threshold) was determined as the geometric mean of the individuals' BETs (ASTM International, 2011; ISO 13301, 2002).

Two-way (panelists and products) Analysis of Variance (ANOVA) was performed to assess the significance of the differences in the thresholds for capsaicin and dihydrocapsaicin pungency and the differences in the thresholds for chili "users" and "non users".

Furthermore the number of answers for the burning sensation at each concentration was determined. Only the panelists who gave correct answers in the triangle

were accounted for the analysis. The panelists were instructed not to give an answer if they could not localize the burn exactly.

## RESULTS

The group BETs for capsaicin and dihydrocapsaicin were 0.080 (SD  $\log_{10}$  +/- 0.275) ppm and 0.049 (S.D.  $\log_{10}$  +/- 0.323) ppm, respectively. The number of correct answers according to concentration is illustrated in Fig. 1.

The number of correct responses increased with higher capsaicin or dihydrocapsaicin concentrations except for the first two levels, suggesting that the panelists had to guess which samples were the different ones at these concentrations.

Two-way ANOVA (panelists and products) indicated that there was a significant difference between the thresholds for capsaicin and dihydrocapsaicin. The impact of the panelists was not statistically significant ( $p = 0.205$ ). The variance between the thresholds resulted from the substances - capsaicin and dihydrocapsaicin ( $p = 0.018$ ) -and was not due to the panelists.

The results from the second dihydrocapsaicin threshold determination were not significant different from the results of the first determination ( $p = 0.817$ ), demonstrating the reproducibility of this protocol.

Chili "users" had lower thresholds for capsaicin and dihydrocapsaicin than "non users" (Fig. 2). The differences, however, are not highly significant ( $p = 0.090$ ).

The results for the location of burning sensation of capsaicin and dihydrocapsaicin are shown in Fig. 3. Most of the panelists perceived the burn of capsaicin and dihydrocapsaicin predominantly in the throat. However, some of them also noticed a burn on the tongue or both on the tongue and in the throat. Therefore, a general conclusion cannot be made.

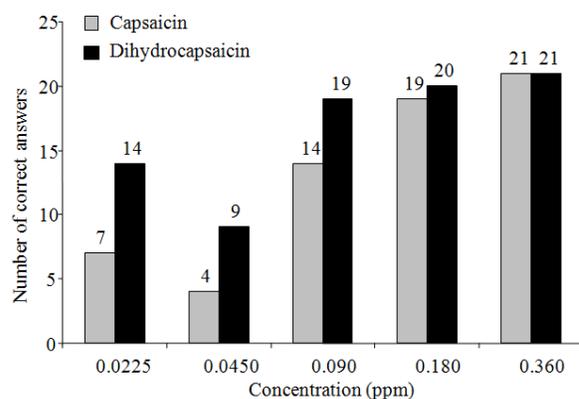


Fig. 1: Number of correct answers (3-AFC-Test) for different concentrations of capsaicin and dihydrocapsaicin (ppm)

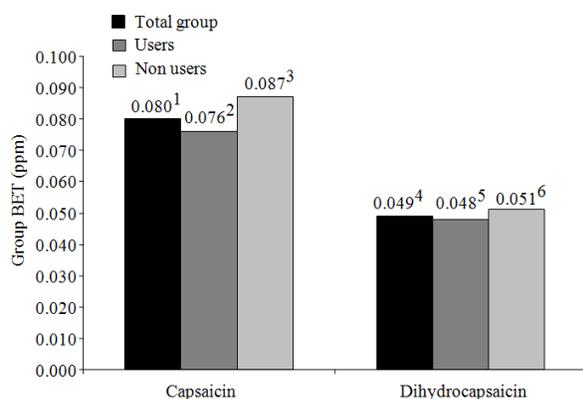


Fig. 2: Comparison of the thresholds (group BET, geometric mean in ppm) for capsaicin and dihydrocapsaicin of “users” and “non users” of chili peppers  
<sup>1</sup>: 0.080 ppm (S.D.  $\log_{10}$  +/- 0.275)\*; <sup>2</sup>: 0.076 ppm (S.D.  $\log_{10}$  +/- 0.318)\*; <sup>3</sup>: 0.087 ppm (S.D.  $\log_{10}$  +/- 0.219)\*; <sup>4</sup>: 0.049 ppm (S.D.  $\log_{10}$  +/- 0.323)\*; <sup>5</sup>: 0.048 ppm (S.D.  $\log_{10}$  +/- 0.415)\*; <sup>6</sup>: 0.051 ppm (S.D.  $\log_{10}$  +/- 0.151)\*; \*: According to ISO 13301 (2002) and ASTM E679-04 (2011)

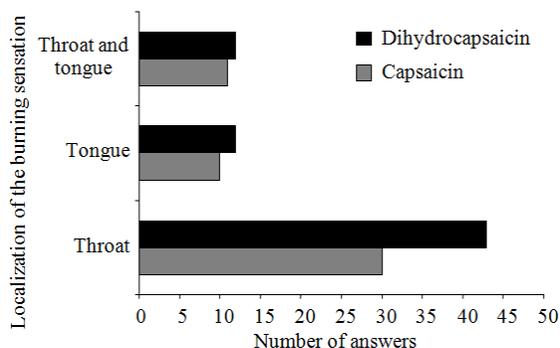


Fig. 3: Localization of the burning sensation (number of answers)

## DISCUSSION

On the contrary to the results of these studies (Fig. 1) other authors (Todd *et al.*, 1977; Krajewska and Powers, 1988) reported the same pungency for capsaicin and dihydrocapsaicin and were consistent with the results of Scoville (1912) who rated both, capsaicin and dihydrocapsaicin, to be 16.000.000 Scoville Heat Units (SHU), but interestingly the thresholds differ significantly. Krajewska and Powers (1988) calculated thresholds for capsaicin and dihydrocapsaicin within a range of 0.039 and 0.078 ppm -thresholds close to those determined in this study.

Nasrawi and Pangborn (1990) stated that the perception of pungency is due to the hydroxy group at C-4 of the aromatic ring. Capsaicin and dihydrocapsaicin only differ in one double bond. Jurenitsch (1981), however, reported different thresholds for capsaicin ( $17.0 \cdot 10^6$  SHU) and dihydrocapsaicin ( $10.8 \cdot 10^6$  SHU). These results were

consistent with the results of this study, which showed a lower threshold for dihydrocapsaicin than for capsaicin. It is possible that the presence of the 3-methoxy-4-hydroxy-benzyl residue is responsible for the perception of burning and pain (Nasrawi and Pangborn, 1990). The structure, however, also has an impact on the binding of the molecule to the receptor.

Our results showed no concurrence with the results of other authors, who reported lower responsiveness to irritants because of desensitization effects evoked by regular chili consumption (Lawless, 1984; Prescott and Stevenson, 1995). Nasrawi and Pangborn (1989) and Orellana-Escobedo *et al.* (2012), however, affirmed that people who regularly consume chili were not less sensitive to capsaicinoids. Mexican judges (Orellana-Escobedo *et al.*, 2012) and German judges (own study) even showed similar thresholds. The only differences in the studies were the methods used (2-AFC vs. 3-AFC) and that, in the study of Orellana-Escobedo *et al.* (2012), the threshold for capsaicinoid content was defined as the sum of the capsaicin and dihydrocapsaicin in natural samples, while in this study, the thresholds for capsaicin and dihydrocapsaicin were evaluated separately.

The present results show a trend of first irritation in the throat and are consistent with the results of Rentmeister-Bryant and Green (1997), who also reported higher thresholds for the tongue than for the throat. Allison *et al.* (1999), however, reported significantly higher ratings for capsaicin heat on the tongue than in the oral cavity or throat. The most likely explanation is that at low concentrations, close to the threshold, pungency is predominantly perceived in the throat, whereas at higher concentrations, the burning sensation on the tongue and in the oral cavity increased.

The question arises as to why different groups detected unequal thresholds. The following factors could have an impact on the results: the presence of sugar, the use of different emulsifiers and the use of different sensory evaluation methods.

As shown in Table 1, three working groups (Todd *et al.*, 1977; Krawjeska and Powers, 1981; Rentmeister-Bryant and Green, 1997) used aqueous solutions containing sucrose as a carrier for capsaicinoids. Several studies have been conducted to evaluate the interaction of chili peppers with other food ingredients such as sucrose. Sizer and Harris (1985) described masking effects of sucrose on capsaicin threshold perception under conditions of simultaneous stimulation with sucrose. These findings are corroborated by Stevens and Lawless (1986), who noted attenuation by sucrose of the irritation evoked by capsaicin. Nasrawi and Pangborn (1989) concluded that sucrose is effective in reducing mouth burn. In contrast, Prescott *et al.* (1984) noted no influence of sucrose on the irritation evoked in the presence of capsaicin. The data in Table 1 also show no clear evidence of higher thresholds when using sugar-based water solutions as carriers.

Table 1: Comparison of the threshold concentrations in the literature and differences in the materials and methods

Author	Threshold (ppm)	Emulsifier	Sugar added	Method
Todd <i>et al.</i> (1977)	0.019-0.625	Ethanol	Yes	Swallow
Rozin <i>et al.</i> (1981)	0.310	Ethanol	No	Swallow
Sizer and Harris (1985)	0.090-0.350	Ethanol	Yes and no	Expectorate
Krajewska and Powers (1988)	0.039-0.078	Ethanol	Yes	Swallow
Rentmeister-Bryant and Green (1997)	0.098 throat 0.299 tongue	Ethanol and polysorbate 80	Yes	Swallow
Lawless <i>et al.</i> (2000)	0.310	Ethanol or polysorbate 80	No	Expectorate
Schneider <i>et al.</i> (2011)	0.088	Polysorbate 80	No	Swallow
Orellana-Escobedo <i>et al.</i> (2012)	0.050	Polysorbate 80	No	Swallow
Present results	0.080 (Cap) 0.049 (DHC)	Polysorbate 80	No	Swallow

The varying thresholds could be explained by the different study methods. In two studies (Sizer and Harris, 1985; Lawless *et al.*, 2000), the panelists were presumably instructed to expectorate the sample after “swirling” the sample around in the mouth. In other studies (Rozin *et al.*, 1981; Krajewska and Powers, 1988; Rentmeister-Bryant and Green, 1997; Schneider *et al.*, 2011; Orellana-Escobedo *et al.*, 2012), the panelists were instructed to swallow the samples after a few seconds. As Rentmeister-Bryant and Green (1997) noted, the threshold for the throat (0.098 ppm) is lower than the threshold for the roof of the mouth (0.180 ppm) and the front and the back parts of the tongue (0.299 ppm). This variation in sensitivity could explain why the results of this study are not consistent with the results of Lawless *et al.* (2000) and Sizer and Harris (1985). The differing threshold found by Rozin *et al.* (1981) may be due to the use of ethanol as an emulsifier. TRPV (transient receptor potential channel)-1 is directly activated both by endogenous factors such as noxious heat (>43°C) and protons (pH<6.0) and by exogenous factors such as capsaicin and ethanol (Caterina *et al.*, 1997; Trevisani *et al.*, 2002). Trevisani *et al.* (2002) found out that ethanol potentiates the response of TRPV1 to capsaicin. In this study, polysorbate 80 was therefore used as an emulsifier, although Lawless *et al.* (2000) found no clear impact on threshold determination when comparing ethanol and polysorbate 80 when used as solvating agents.

### CONCLUSION

The present study using the 3-Alternative Forced Choice method showed different thresholds for capsaicin and dihydrocapsaicin. The absolute thresholds for capsaicin and dihydrocapsaicin were 0.080 and 0.049 ppm, respectively. No significant differences were observed between chili “users” and “non users”, but for substances, capsaicin and dihydrocapsaicin, “users” showed marginally lower thresholds. In most cases, the first irritation was experienced in the throat.

### ACKNOWLEDGMENT

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