Temporal effects of milk protein palate cleansers on capsaicin mouth burn reduction

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Abstract

Sucrose and fat are established palate cleansers that can reduce capsaicin-induced mouth burn. Studies have suggested that milk may also reduce capsaicin mouth burn but individual milk components, namely protein, have not been addressed. New membrane technologies now allow these milk protein fractions (micellar casein concentrate (MCC) and serum protein isolate (SPI) to be manufactured. The objective of this study was to compare water, 10% sucrose solution, skim milk, heavy cream, MCC and SPI as palate cleansers for reduction of capsaicin mouth burn using time-intensity (TI) and a trained panel. A gradient permeability ceramic microfiltration system was used to generate 95% serum protein removed micellar casein concentrate (MCC) and serum protein isolate (SPI) from pasteurized skim milk. A trained descriptive analysis panel (n=8) evaluated the TI of the mouth burn of a 1.3 ppm capsaicin solution (10 ml) using a computer interface and the following protocol: the stimulus was placed in the mouth for 15s and expectorated, then 20 ml of one palate cleanser was placed in the mouth for 15s and expectorated. The intensity of mouth burn was recorded for 5 min. A10 min rest was enforced between samples. The entire experiment was replicated three times. Relative mouth burn reduction (RMR) was calculated to compare the difference in palate cleansers. Heavy cream, MCC, and SPI were more efficient in reducing capsaicin burn than water ($p<0.05$). Sucrose solution and skim milk were also more effective than water ($p<0.05$), but not to the extent of heavy cream, MCC or SPI. This study provides new insights on the role of milk proteins in reducing the oral burn sensation of capsaicin.

Introduction

- Capsaicin burn sensation is related to the activation of transient receptor potential vanilloid 1 (TRPV 1) on the trigeminal nerve fibers. The capsaicin binding mechanism has been studied at the molecular level since the 1990s for the interest of pain treatment and desensitization (Jara-Oseguera et al., 2008, Numazaki et al., 2003). Due to the lipophilic properties of the capsaicin molecule, capsaicin passes through the phospholipid bilayer cell membrane of the tongue and oral surface and acts on binding sites on the intracellular surface of TRPV1 to generate burn signals (Caterina et al., 1997, Tominaga and Tominaga, 2005; Yang et al., 2015). The single stimuli induced capsaicin burn reduction observed with oral rinses could due to the unbinding of capsaicin from the TRPV1. Limited research has been done on the unbinding of capsaicin.
- Capsaicin consumption showed positive effects on weight management, vascular and metabolic health (Diepvens et al., 2007; McCarty et al., 2015). However, the oral burn of capsaicin is an unpleasant sensation to many consumers. Previous studies have demonstrated that sucrose solutions and fat can reduce capsaicin burn (Lawless et al., 2000; Lee and Kim, 2013). Fat reduces capsaicin burn (Govindarajan. 1979; Lawless et al., 2000). Other studies have reported that skim and whole milk may also reduce capsaicin mouth burn, however, the role of individual milk proteins has not been addressed (Nasrawi and Pangborn, 1990). New membrane technologies allow specific milk protein fractions (micellar casein concentrate (MCC) and serum protein isolate (SPI)) to be isolated (Zulewska et al., 2009) and bring new options for evaluation of capsaicin burn reduction and hypothesis of a potential unbinding mechanism.

Objective

- To compare water, 10% sucrose solution, skim milk, heavy cream, micellar casein concentrate (MCC) and serum protein isolate (SPI) as palate cleansers for reduction of capsaicin mouth burn using TI and a trained panel.

Materials

- **Stimuli:** 1.3ppm capsaicin solution was prepared and stored at 4°C. (Lawless et al., 2000). Capsaicin powder was purchased from Sigma, St. Louis, MO. 10ml of the 1.3ppm capsaicin solution at 20°C was served as the stimulus for training and evaluation of capsaicin burn and burn reduction.
- **Palate cleanser:** 20ml of each palate cleanser was dispensed into 2 oz. soufflé cup with lids. Palate cleansers were served at room temperature.

  1) Dairy protein palate cleansers:

  High purity MCC and SPI were manufactured at the NC State dairy processing pilot plant from pasteurized skim milk the day before evaluation. A 0.1 um, 3 stage 3X Ceramic Gradient Permeability (GP) Microfiltration membrane was used (Cheng et al., 2018). MCC and SPI were stored at 4°C, then diluted with DI water to 10% total solids before evaluation. Compositions were confirmed by a LactoScope FTIR Advanced milk composition analyzer (Delta instruments, the Netherlands).

  2) Other palate cleansers:

  Skim milk, heavy cream and sucrose were purchased locally. Ten percent sucrose solution (w/v) and deionized water were prepared at the Sensory Service Center at NC State University. All samples were stored at 4°C before evaluations.

Methods

- **Temporal profiling:**
- TI of capsaicin burn was evaluated by trained panel (n=8, 3 replications).
- Panelists were instructed to move a cursor to the appropriate intensity level for the perceived capsaicin burn using a 0 to 15-point intensity scale using iPads. Data was collected using Compusense Cloud (Compusense, Guelph, Canada).
- The training and evaluation protocols were adopted from Nasrawi & Pangborn (1990).

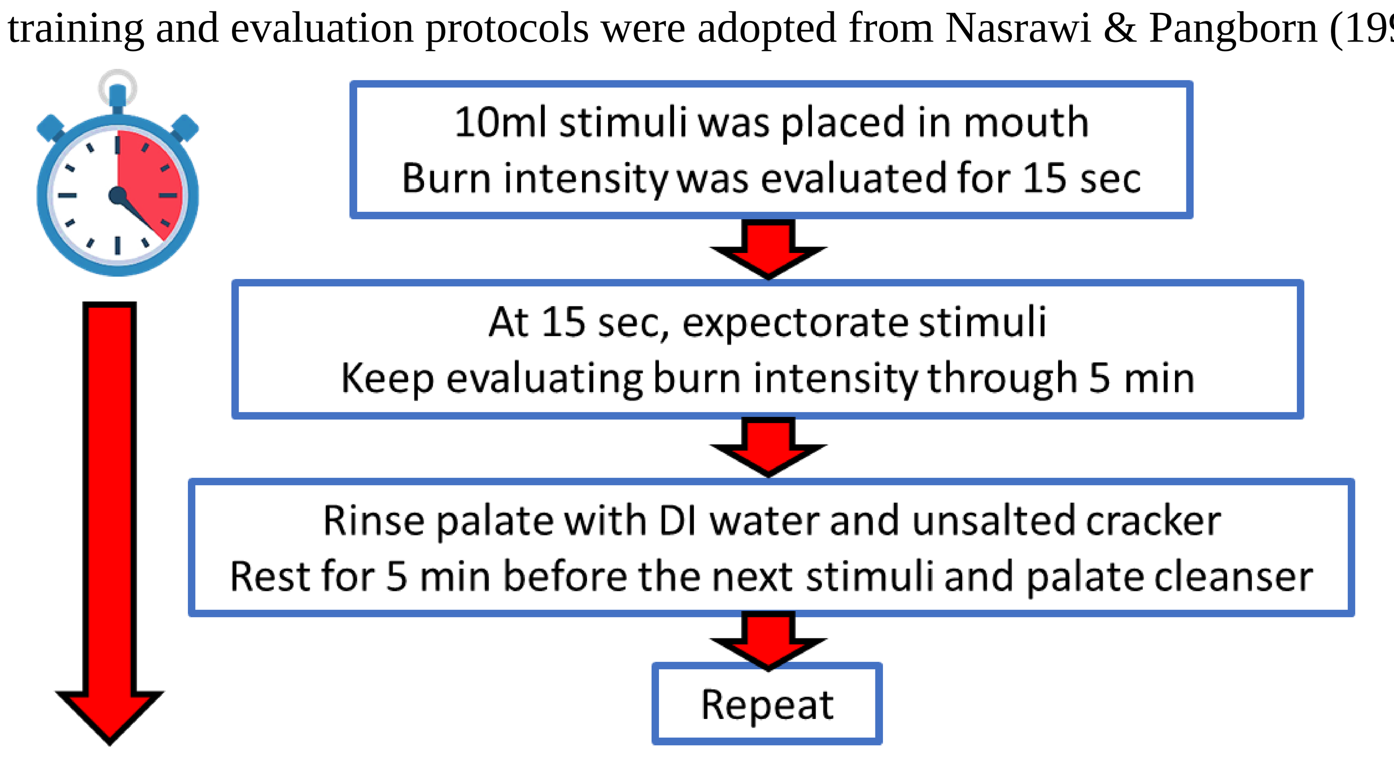

Figure 1. Protocol flow chart for capsaicin burn reduction evaluation with different palate cleansers.

Methods

- **Statistical analysis:** Relative mouth-burn reduction (RMR) (Nasrawi and Pangborn, 1990) was calculated by using area under the curve for each palate cleanser divided by area under the curve of stimuli within rinse. ANOVA (XLSTAT, Pairs, France) on the mean RMR of each palate cleanser was used to determine differences in palate cleansers for capsaicin burn reduction. The smaller the RMR, the more efficient the palate cleanser.

Result & Discussion

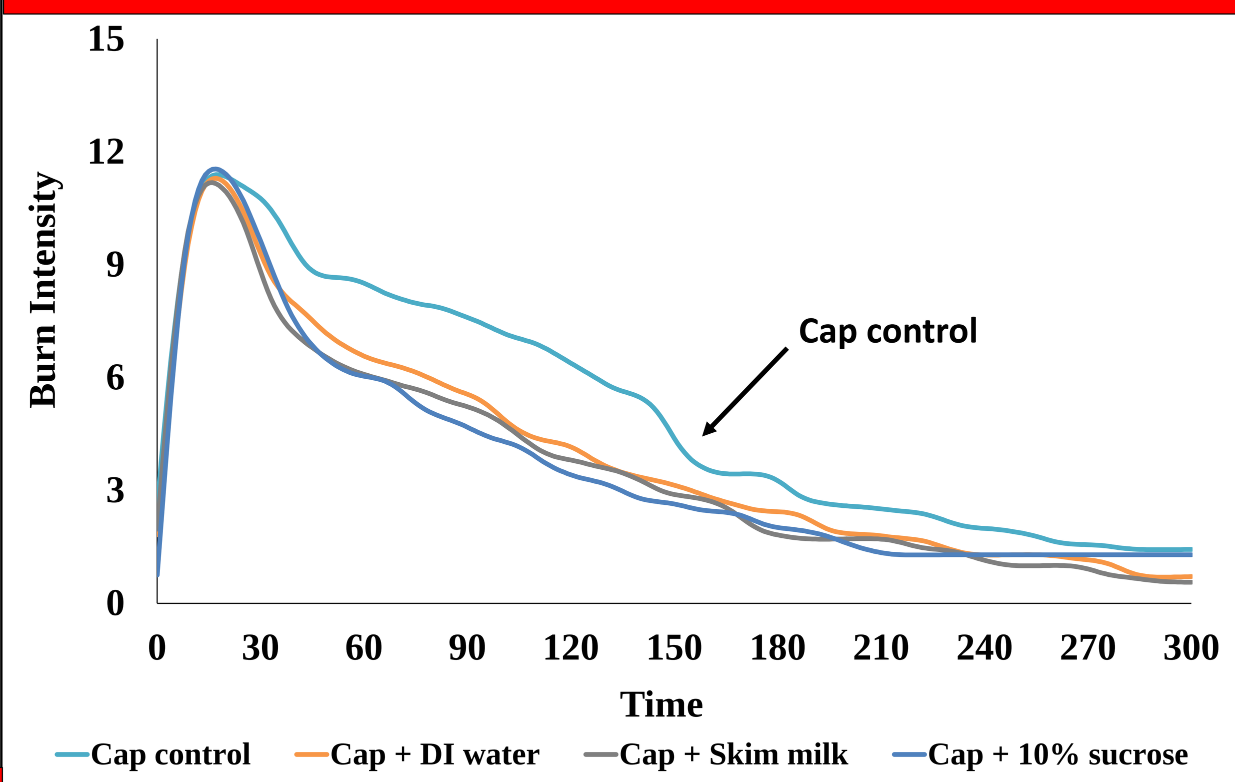

Figure 2. TI profiles of capsaicin burn and capsaicin burn reduction by DI water, skim milk and 10% sucrose solution.

Table 1. Mean area under the curve and relative mouth-burn reduction (RMR) for different palate cleansers for capsaicin (Cap) burn.

Sample	Area Under Curve	RMR	Decrease Angle
Cap control	1385.54a	1.00a	2.03c
Cap+DI water	1233.39ab	0.89b	2.48b
Cap+10% sucrose	1154.57bc	0.83bc	2.47b
Cap+Skim milk	1107.57bc	0.8bc	2.57b
Cap+SPI	1062.32cd	0.77cd	2.70ab
Cap+MCC	921.39de	0.67de	2.86ab
Cap+Heavy Cream	792.39e	0.57e	3.21a

Different letters in a column indicate significant differences ($p<0.05$).
The lower the area under curve and RMR, the more efficient the palate cleanser is.

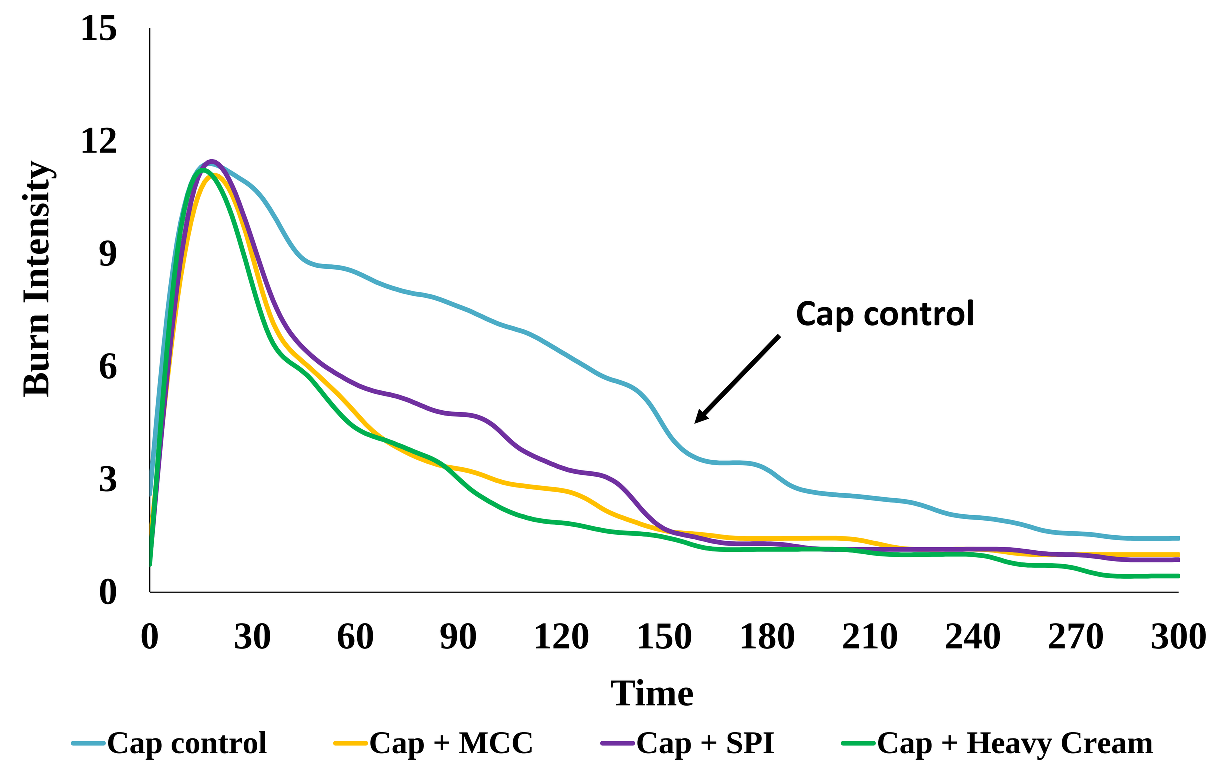

Figure 3. TI profiles of capsaicin burn and capsaicin burn reduction by MCC, SPI and heavy cream.

Table 2. Percent compositions of skim milk, SPI and MCC and heavy cream.

	Fat (%)	Lactose (%)	Protein (%)	Total solids (%)	Total solids non fat (%)
Skim milk	0.07	4.73	3.24	9.16	9.09
SPI	0.14	0.12	9.35	10.23	10.08
MCC	0.36	0.67	7.82	9.93	9.57
Heavy cream	41.10	2.72	1.78	46.51	5.41

- 10% sucrose solution, DI water and skim milk palate cleansers showed small reductions ($p<0.05$) of capsaicin burn by smaller area under the curve compared with no rinse (Table 1, Figure 2).
- Heavy cream, MCC, and SPI reduced capsaicin burn to a greater extent than sucrose solution, DI water or skim milk (Table 1).
- A recent study by Yang et al. (2015) proposed a Tail-up, Head-down configuration of capsaicin in the TRPV1 binding pocket for channel activation, with the aliphatic tail of capsaicin molecule interacting with the channel through non-specific Van der Waals force and the capsaicin vanillyl Head and amid Neck binding the channel (E571 and T551, respectively) by the hydrogen bond. The activation gate on TRPV1 opens after binding and structural rearranging, which generates the Ca^{2+} influx and the signal for burn/pain sensation (Yang et al., 2015, Figure 4). Heavy cream reduces the capsaicin burn primarily because of the hydrophobicity of milk fat (Govindarajan. 1979; Lawless et al., 2000). Due to the serving order of capsaicin and palate cleanser, we hypothesize that this process is due to a higher binding affinity between capsaicin and fat (more soluble in fat) and unbinding of the capsaicin from the TRPV1.
- Compared with skim milk, MCC and SPI had slightly higher residual fat, lower lactose and two to three times more protein (Table 2). The higher protein content could be the reason that MCC and SPI had greater capsaicin burn reduction than skim milk. We hypothesize that the binding affinity between the capsaicin and milk protein may be stronger than the binding affinity between capsaicin with TPRV1.
- Steeper decreasing slopes were observed for heavy cream compared to DI water or skim milk or 10% sucrose solution ($p<0.05$) (Table 2, Figures 2, 3). MCC and SPI also showed steeper decreasing slopes compared to DI water or skim or 10% sucrose solution but not to the extent of heavy cream (Table 2, Figures 2, 3).

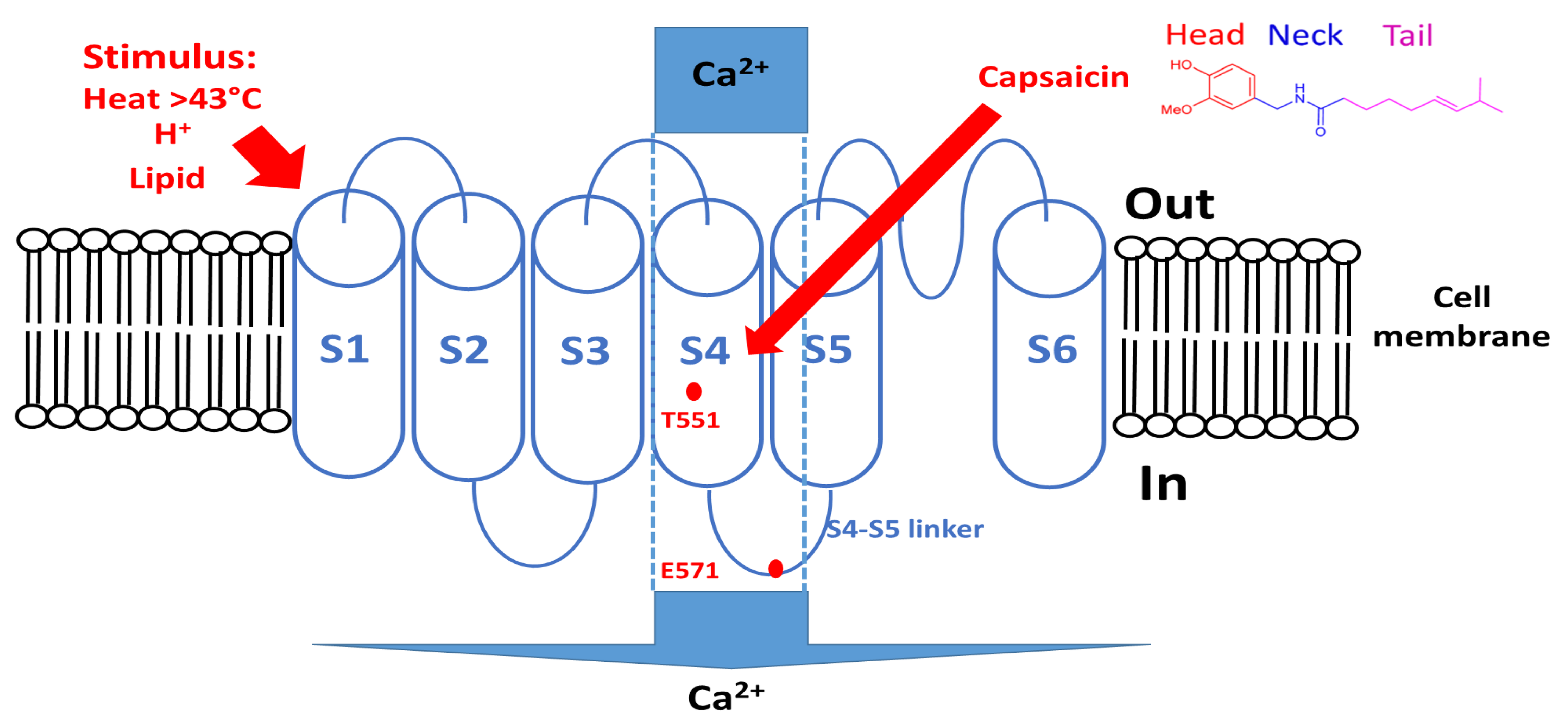

Figure 4. Modified tetrameric structure of transient receptor potential vanilloid 1 (TRPV1) in the plasma membrane with physical and chemical stimulus (Tominaga and Tominaga, 2005; Yang et al., 2015)

Conclusion

- Heavy cream, MCC, and SPI were more efficient in reducing capsaicin burn than water ($p<0.05$).
- Sucrose solution and skim milk were also more effective than water ($p<0.05$), but not to the extent of heavy cream, MCC or SPI.
- These results suggest that milk protein (as well as fat) may be an effective agent for reducing oral capsaicin burn.

Future work

- Formulate palate cleansers to specific protein, fat and lactose loads using milk fat and high purity lactose, MCC, SPI as ingredients and test the proposed mechanism of capsaicin burn reduction, such as switching the serving order of capsaicin stimuli and palate cleanser or serving a mixture of capsaicin and palate cleanser.
- Develop a low fat and low sugar protein beverages/drink to make the consumption of capsaicin enriched food more enjoyable to general population.

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