Red Hot Chili Peppers: Isolation, Cleanup, and Determination of Capsaicin

Chemistry 126 Laboratory

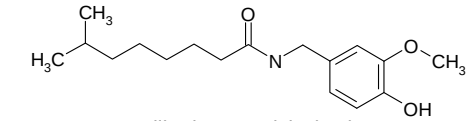
Western Washington University

Last modified 01/05/04 by Steven Emory (Chem 333)

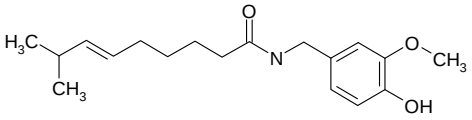
Introduction

Capsainoids are the group of compounds (Figure 1) responsible for the pungent flavor of chili peppers and hot sauces. Capsaicin is the primary ingredient of the red pepper (*Capsicum annum*) and is widely used in food preparation. Capsicum fruits generally contain between 0.1 and 1.0 % of capsaicin. Capsaicin is actually a neurotoxin and observed toxic responses to excess capsaicin are nerve damage, gastric ulcers, and liver cirrhosis. The LD50 (i.e., the "Lethal Dose that kills 50% of test subjects") for Tabasco sauce is reportedly 24 mL/kg body weight.

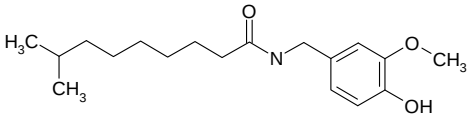
More novel uses of capsaicin have been its utilization as a herpes treatment, a tear gas or rodent repellent, arthritis cream, and a deterrent to dog fights and swine cannibalism. In humans, the limit for taste is approximately 10 ppm which is 70 times lower than that for piperine, the active spice in black pepper, and 1000 times lower than for zingerone, the active ingredient in ginger. Although puzzling, the popularity of this "masochistic gustatory phenomenon" seems to be rising. In 1992, 165.5 million pounds of red chili peppers were purchased for or processed into food products in the United States.



Nordihydrocapsaicin (NC)



Capsaicin (CP)



Dihydrocapsaicin (DC)

Figure 1. Chemical structures of capsainoids.

Capsaicin targets pain receptors in the mouth. The desired response of "hotness" appears to originate from the physiological placement of the pain receptors in the same location as the receptors for high temperatures; thus one feels hot when consuming this incendiary substance. Contrarily, some people appear to tolerate or "enjoy" larger amounts of hot peppers than others. Apparently capsaicin causes a desensitization of the nerve fibers containing the pain and heat receptors upon repeated ingestion.

The Scoville Heat Unit (SHU) Scale is the industry standard by which the heat of chili peppers is measured and compared. This scale is named after the man who developed it, Wilbur Scoville, who was a pharmacologist with Parke Davis. In 1912, he developed a method which used a panel of five taste testers. They took exact weights of chili peppers and dissolved the capsaicin in alcohol. This solution was then diluted with sugar water until it was no longer detectable to the palate. For example, if the dilution required was 1,000 units of water to 1 unit of alcohol solution then the sample was said to measure 1,000 Scoville Units. At least three panel members had to agree before a value was assigned. Although the development of this

method was innovative in classifying the chili peppers according to heat, it was highly subjective and imprecise because it involved human testers.

Today high-pressure liquid chromatography (HPLC) is used to measure capsaicin content in peppers. This method is much more accurate and precise. It measures the capsaicin levels in parts per million which is then converted to SHU using the information provided in Table I. By determining the amount of each capsainoid (g capsainoid/g sample) and multiplying by the SHU for the pure compound.

Table I. Scoville Heat Units for Pure Capsainoids

Capsainoid	SHU for Pure Capsainoid
NC	9.3×10^6
CP	16.1×10^6
DC	16.1×10^6

The purpose of this lab is to acquaint you with the active ingredient of hot peppers, and the extraction, clean-up, and analysis of capsaicin as an analytical exercise. Please bring a bottle of your favorite hot sauce to class. Two "awards" will be made for this lab: (1) to the person who provides the "hottest" pepper sauce, as determined by the HPLC quantification of capsaicin, and (2) to the person who does the best job correlating the ranking of hotness of the sauces by taste to the capsaicin content from the HPLC data. In both cases the award will be a pint of Mallard's ice cream (to help put out the fire…).

Procedure

CAUTION: Wear gloves and goggles at all times! Do not get concentrated capsaicin on your skin and wash hands when complete. Capsaicin is a skin irritant. Getting capsaicin in your eyes can cause serious harm and is easy to do if any of the material gets on your hands or clothes.

1. Weigh 15 g of hot sauce in a plastic weighing boat. Record the mass and quantitatively transfer its contents to a labeled 125-mL Erlenmeyer flask. Use 95% ethanol to wash the sauce from the weighing boat. Bring the total volume in the flask to approximately 50 mL using ethanol. Note: some sauces may require considerable washing to completely remove them from the weighing boat.
2. Place a Teflon stir bar in the flask and then place the flask on a hotplate/stirrer. **Perform this extraction in a hood!**
3. Heat with stirring until a slow boil is reached. Note: if sample "bumps", increase the rate of stir. Boil for 30 minutes. Remove from heat and allow to cool for a few minutes. Remove the stir bar and wash with ethanol, collecting washes in the extraction flask.
4. While the flask is cooling, prepare the filter apparatus. Place one disc of Whatman's No. 41 filter paper and place it in a glass funnel. Wash the filter paper three times with ethanol, catching the waste in a waste container.
5. Once the flask has cooled and the filter paper has drained, filter the sample into a labeled 100-mL volumetric flask. Be sure to get all of the contents out of the extraction flask. Wash the filtrate three times with ethanol, but be careful not to overfill the volumetric flask. Once filtered, bring the solution to volume with ethanol, cap, and mix well.

6. Be sure wash hands after transferring liquids. The capsaicin could be strong enough to irritate the eyes.
7. Once the samples is diluted and mixed, the solution must be filtered with a 0.45-micron pore size syringe filter cartridge to remove minute particles. The solutions are filtered into labeled 2-mL autosampler vials. These vials are capped, placed in the autosampler, and the HPLC is set to run by the instructor. More information about the operation of the HPLC will be given in the laboratory by the instructor.
8. If you want a chance to win some ice cream you will need to rank order the sauces by hotness. The instructor will provide chips for you to use in tasting the sauces. Please wash your hands thoroughly before tasting any sauces, and do not use a chip more than once to obtain a sample. Record your observations.

Lab Report Due 3/7 by 3pm

1. Turn in your rankings of the hotness of the sauces (from step 8 above) before the HPLC data is released to the class.

2. Calculate the retention time (t_r) for each analyte (NC, CP, and DC).

3. You will be given raw data for the preparation of a standard calibration curve for capsaicin concentration. Prepare the calibration curve (integrated absorbance vs. concentration) for CP. If two wavelengths were examined, prepare a calibration plot for both. Which curve is better to use for further calculations? Why?

4. Determine the Scoville Heat Units (SHU) for your sample. If the sample was analyzed more than once, report the SHU with 95% confidence limits.

5. What color was the extracted sample? Is there a relationship between the color and the SHU? Why or why not?

6. Did your HPLC results correlate with your expectations? In other words, given your perception of how hot *your* sauce is, how did it rank in capsaicin content (by HPLC) vs. other sauces you tasted. How do you explain discrepancies between the HPLC results and your expectation from tasting the sauces?