RESTEK food & flavor

Applications note

Analyzing the Heat Level of Spicy Foods Using an Ultra C18 HPLC column

Introduction

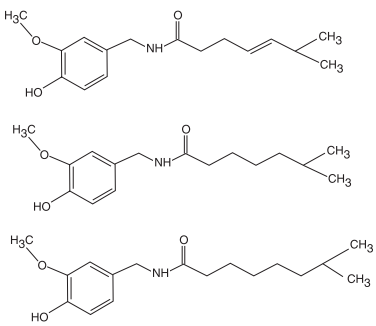
Spicy foods and sauces can be used to add zest to bland foods and to build more complex flavors in a variety of food types. They provide a nutritious way to add flavor to "healthy foods," such as low-salt, low-fat alternatives. Chili pepper extracts also are being explored for their medicinal uses, including rubdown liniments, arthritis pain lotions, and counter-irritant balms. As society's taste in spicy foods grows, so does the need to test and classify products and raw materials for their pungency (i.e., heat levels). Pungency is defined as the hot, sharp, stinging sensations experienced by the nasal and oral cavities and the tongue when certain flavor compounds contact them.[1] The trigeminal nerves in these areas are stimulated, tricking the body into thinking it is in pain. Then the body releases endorphins, which give a pleasant sensation.

In chili peppers, black pepper, and ginger, the pungent species are not volatile. Flavorings such as mustard, garlic, and horseradish, on the other hand, contain volatile compounds that contribute aroma as well as pungency. Pungency can differ both in intensity and duration, depending on the chemical species present.

There are over 200 chili pepper varieties, with a wide range of pungency levels and flavor profiles.[2] Chili peppers belong to the genus Capsicum, which contains five domesticated species. In chili peppers, pungency is a result of capsaicinoids, or vanillylamides of monocarboxylic acids. The capsaicinoid content, and thus the heat level, depends on the type of pepper, maturity, growing conditions, and processing methods. Most of the capsaicinoids can be found in the inner layer of the plant, including the placenta where the seeds attach. Only small amounts of capsaicinoids are found in the seeds themselves. There are seven generally recognized capsaicinoids in chili peppers, all of which evoke different responses in the consumer: The most famous and the hottest is capsaicin (CAP). In chili peppers, CAP and dihydrocapsaicin (DHC) are present in the greatest amounts and are the largest contributors to the heat level (Figure 1). The minor capsaicinoids include nordihydrocapsaicin (NDC), homocapsaicin, norcapsaicin, nornorcapsaicin, and homodihydrocapsaicin.

Figure 1

CAP and DHC are considered major contributors to the heat level. NDC is a minor capsaicinoid, which also contributes to the heat level.



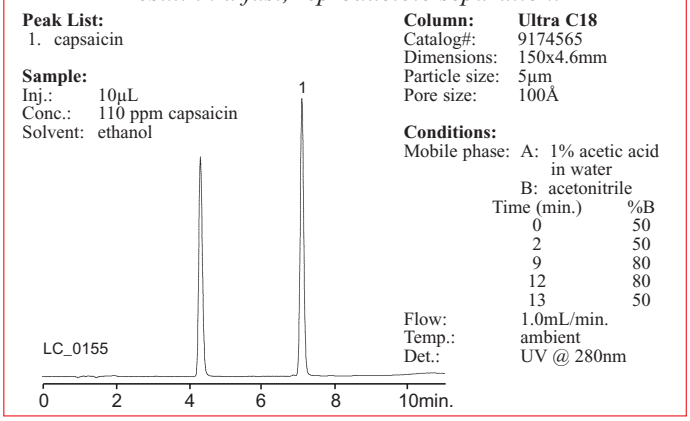
capsaicin (CAP)

dihydrocapsaicin (DHC)

nordihydrocapsaicin (NDC)

Figure 2

The Ultra C18 column and a gradient elution program result in a fast, reproducible separation.



Historical Perspectives

In 1912, Wilbur Scoville developed a method for determining heat levels. In this organoleptic (i.e., affecting the qualities of substances that stimulate the sense organs) procedure, a Scoville Heat Unit (SHU) is defined as the number of parts sugar water needed to neutralize the heat of one part sample extract. For example, if the heat of a cayenne pepper is 30,000 SHU, that means 30,000 parts of sugar water are needed to dilute one part of cayenne pepper extract to the last point that hotness can be detected. However, there are several problems with the organoleptic procedure for determining heat levels. The test is somewhat subjective because it relies on the tasters' palates and sensitivity. The geographic area or culture from which the taster originates can affect the results—different groups of people have different physical perceptions of heat. In addition, tasters can handle only a limited number of samples at one time, before "fatiguing of the palate" occurs.[3] This can make it difficult to process a large number of samples in a reasonable amount of time, such as in a quality control (QC) environment, and can affect the reproducibility of the tests.

Analytical Methods

Starting in the 1970s, several analytical methods for heat level measurement were introduced to overcome the limitations of the organoleptic procedure.[4] These included wet chemical methods; spectrometry; and paper, gas (GC), and liquid chromatography (HPLC). Of these, the HPLC procedures have provided the greatest specificity while requiring the least amount of sample preparation. The American Spice Trade Association (ASTA) and the Association of Official Analytical Chemists (AOAC) have published methods for the determination of capsaicinoids by HPLC. AOAC Method 995.03[5] specifies the separation of three target capsaicinoids using reversed phase HPLC on a C18 column, and quantitation using either UV or fluorescence detection. This method is performed isocratically with a 1% acetic acid:acetonitrile (60:40) mobile phase. Standardization is performed using synthetic capsaicin, N-vanillyl-*n*-nonanamide; and the relative amounts of CAP, DHC, and NDC are calculated by applying the specified factors. Using the appropriate calculations, the heat index can then be calculated, where 1ppm of total capsaicinoids is approximately equal to 15 SHU.

food & flavor

The selectivity and lot-to-lot reproducibility of the Restek Ultra C18 column make it an excellent choice for performing heat level measurements by HPLC. The analysis of capsaicinoids using an Ultra C18 column and a gradient elution program results in an efficient separation that can be performed in less than 8 minutes (Figure 2). In addition, the Ultra C18 column achieves good resolution of the three target capsaicinoids in an extract of habanero nuggets (Figure 3). The high percentage of organic at the end of the run helps to elute any strongly absorbing species present in the samples and improves analysis reproducibility.

Several brands of hot sauce were analyzed to determine their heat levels using this HPLC procedure. The samples were extracted in ethanol, filtered, and injected onto the analytical column. After determining the amounts (in ppm) of the three target capsaicinoids, the SHUs were calculated as described in AOAC Method 995.03 (Table 1). Dried habanero nuggets also were analyzed, as seen in Figure 3. This extract had a sizzling hotness rating of 348,000 SHU.

Figure 3

The Ultra C18 achieves good resolution of three target capsaicinoids in less than 9 minutes.

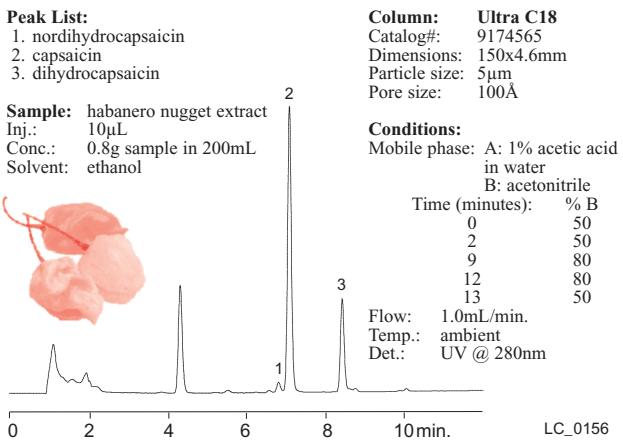


Table I

Comparison of five commercially available hot sauces.

Sample	SHU	Hotness Ranking
sauce #1	8,530	Super Hot
sauce #2	380	Mild
sauce #3	400	Mild
sauce #4	1,100	Hot
sauce #5	1,870	Hot
habanero nuggets	348,000	Off the chart

Conclusion

With the rapidly increasing popularity of Capsicum-based products, there is a need to quantitatively evaluate the heat content of both raw materials and finished products. The HPLC analysis described above gives an objective measurement of the heat level of a wide range of samples. By using a Restek Ultra C18 column and a gradient elution program, the analysis can be performed quickly and reproducibly with only minimal sample preparation.

References

1. Fennema, O. R. Food Chemistry (1996), 3rd edition, pp. 736-738.
2. Uhl, S. "Fire and Spice" in Food Product Design (1996), editorial archives.
3. Bensinger, M. "How Hot is that 'Devil' Sauce?" in Fiery Foods Magazine (1997), Sept/Oct.
4. Chiang, G. H. J. Food Science (1986), 51(2), pp. 499-503.
5. AOAC Official Methods of Analysis (2000), Method 995.03.

Contact Restek for other column dimensions available 800-356-1688

Ultra C18, 5µm Columns

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9174531	9174532	9174533	9174535
50mm	9174551	9174552	9174553	9174555
100mm	9174511	9174512	9174513	9174515
150mm	9174561	9174562	9174563	9174565
200mm	9174521	9174522	9174523	9174525
250mm	9174571	9174572	9174573	9174575

Ultra C18, 5µm Columns with Trident™ Inlet Fitting

Length:		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9174532-700	9174533-700	9174535-700
50mm	—	9174552-700	9174553-700	9174555-700
100mm	—	9174512-700	9174513-700	9174515-700
150mm	—	9174562-700	9174563-700	9174565-700
200mm	—	9174522-700	9174523-700	9174525-700
250mm	—	9174572-700	9174573-700	9174575-700