Characterization of Capsaicinoids and Related Pungent Agents in Chili Peppers by LC/MS/MS

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Introduction

Capsaicin and related compounds are responsible for pungency, the burning sensation associated with chili peppers and other spicy foods. In high concentrations, all capsaicinoids produce burning sensations throughout the mouth, throat and mucous membrane; in low concentrations they affect only specific areas of the mouth and throat. As a result, low level pungent compounds can enhance the food flavor[1-4]. Additionally, capsaicin may have clinical applications, including antioxidant properties[5] and is used as the main ingredient in several over-the-counter topical ointments for the treatment of pain and inflammation.

Because the amount of capsaicin produced by chili peppers can vary due to environmental and weather conditions[6], it is critically important for the food industry to effectively quantitate capsaicinoids. Traditionally, subjective methods were used in this process. In 1912, Wilber Scoville established a scale to demonstrate the pungency of chili peppers based on taste-test[1].

To more accurately quantitate pungency, concentration values of three major capsaicinoids (capsaicin, dihydrocapsaicin, and nordihydrocapsaicin) are determined by HPLC. The concentration (in ppm) is then multiplied by a pungency factor for each species and added together to arrive at a corresponding Scoville Heat Unit value.[2]

In this technical note, capsaicinoids were extracted from chili peppers and analyzed by a triple quadrupole LC/MS/MS system to identify each capsaicinoid. Ion intensities for different chili pepper extracts were then reviewed and evaluated.

Materials and Methods

Reagents and Chemicals

- Capsaicin, dihydrocapsaicin, and nonivaimide were purchased from ChromaDex®, Irvine, CA.
- Methanol, Ethanol and Acetonitrile were purchased from Sigma-Aldrich, St. Louis, MO.
- Thai, Habanero, Serrano and Jalapeno peppers were purchased from various local grocery stores

Experimental Conditions

Extraction Procedures

250 or 500 g of chili peppers (depending on pepper size) were blended for an extended period of time (approximately 5 mins or until all seeds were crushed) with Ethanol to contain approximately 60-70% of alcohol. An aliquot of the puree was removed and filtered using a Phenex™ PTFE Membrane with pore size of 0.45 µm, 25 mm syringe filter, Part No. AF0-1102-12 prior to analysis with LC/MS/MS. The rest of the puree was retained to prepare purified capsaicinoids by Prep Chromatography.

LC/MS/MS Conditions

Column: Kinetex® 5 µm C18
Dimensions: 100 x 2.1 mm
Part No.: 00D-4601-AN
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in methanol
Flow Rate: 0.6 mL/min
Gradient:

Time (min)	B (%)
0.0	30
7.0	90
9.0	90
9.1	30
12.0	30

Injection Volume:: 2 µL
Temperature: Ambient
Detection: API 4000™ triple quadrupole LC/MS/MS
Electrospray ionization (ESI) analyzed in positive mode
System: Agilent® 1200SL LC system (Agilent Technologies, Palo Alto, CA, USA), equipped with a binary pump, Autosampler
Reconstitute: Reconstitute in 500 µL of mobile phase

Precursor Method

Precursor mass:	136.9 amu
Q1 Mass Range:	150 to 350 amu
Scan Time:	2 sec
Scan mode:	Profile @ 0.25 amu step size
Collision Energy:	25 V

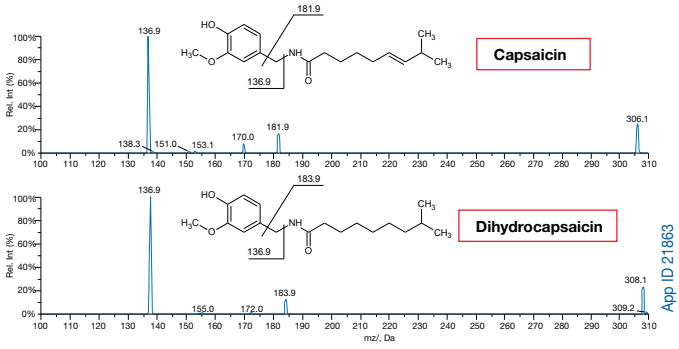
Results and Discussion

Capsaicins Fragmentation Profile

Capsaicin and dihydrocapsaicin were analyzed by infusion to determine their ionization properties and fragmentation patterns. The product ion scan revealed similarities in the fragmentation pattern of the two compounds. More specifically, the bond breakage between the amine group and the benzene ring appears to be predominant and common. The most abundant fragment (136.9 m/z) is displayed (**Figure 1**) and was used in the precursor scan analysis.

APPLICATIONS

Figure 1. Fragmentation Comparison of Capsaicin and Dihydrocapsaicin at CE of 15 V



Analysis of Chili Pepper Extract Comparison

Extracts from Habanero, Serrano, Jalapeno and Thai chili peppers were analyzed by a precursor scan method. In this scan function, the first quadrupole scans a predetermined mass range, in this case, 50-350 m/z. The selected ions enter the collision cell and undergo fragmentation at a specific energy or rolling energy, in this case, 20 V. The resulting ion fragments enter the last quadrupole stage and scanned for a specific ion mass, in this case 136.90 m/z. The detected ions will be reported as corresponding to the mass of the ions that were selected by the first quadrupole. A secondary dynamic product ion scan was collected as further confirmation of capsaicinoid compounds (**Figure 2**). All commonly referenced capsaicinoids are listed in **Table 1**. Surprisingly, based on ion intensities, in our sample set, Thai chili pepper had the highest overall concentration of capsaicinoids. In addition, nordihydrocapsaicon ratio to capsaicin was highest in the Thai chili pepper sample (**Figure 3.**)

Figure 2. Precursor Scan of Nordihydrocapsaicin in Serrano Chili Pepper and MS/MS Spectrum of Nordihydrocapsaicin at CE = 20 V

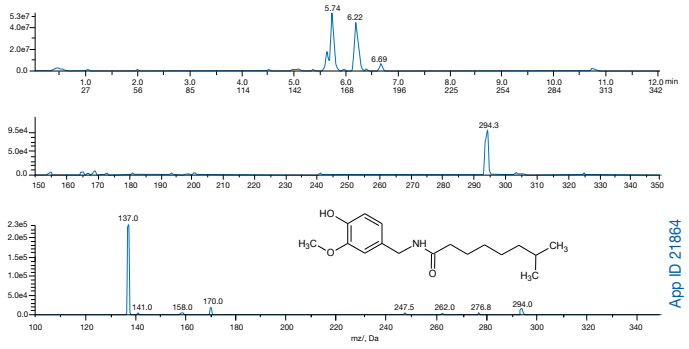


Figure 3. Comparison of Chili Pepper Extracts Using a Precursor Ion Scan, Mass Assignments:

1. Octanoic Acid vanillylamide isomers (280.2 m/z)
2. Nordihydrocapsaicin (294.2 m/z)
3. Capsaicin (306.2 m/z)
4. Dihydrocapsaisin (308.2 m/z)
5. Homocapsaicin (320.2 m/z)
6. Homodihydrocapsaicin (322.2 m/z)z

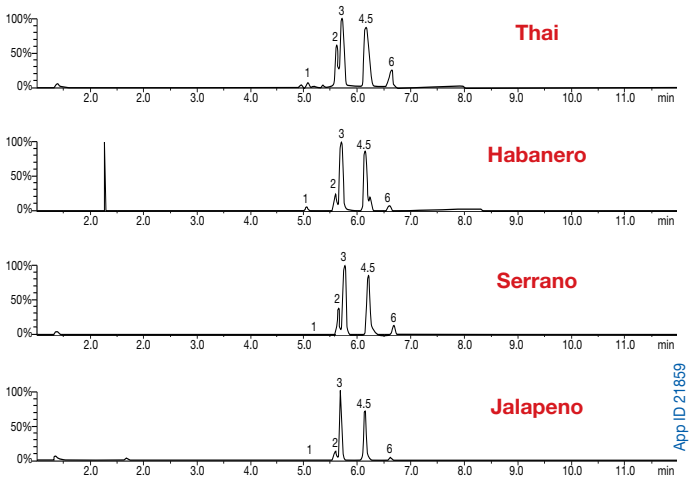
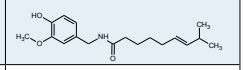
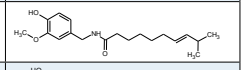
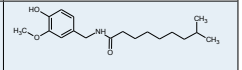
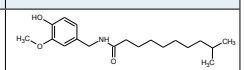
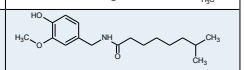
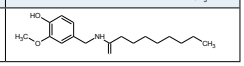
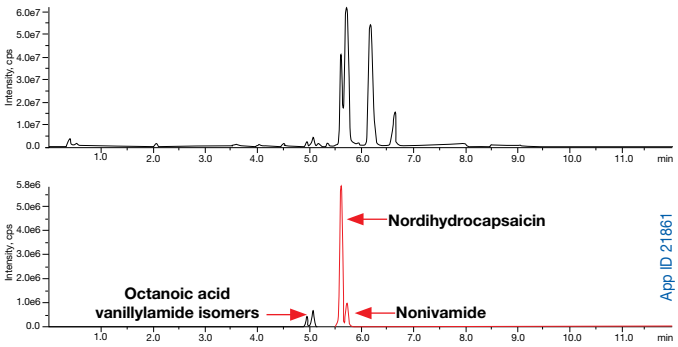


Table 1. Identified Capsaicinoids in Chili Peppers

Compound	Monoisotopic MW, Da	Prec. Mass $(M+H^+)^+$, m/z	Structure
Capsaicin	305.2	306.2	
Homocapsaicin	319.2	320.2	
Dihydrocapsaicin	307.2	308.2	
Homodihydrocapsaicin	321.2	322.2	
Nordihydrocapsaicin	293.2	294.2	
Nonivamide	293.2	294.2	

The presence of multiple peaks for some of these compounds, most notably nordihydrocapsaicin, suggest the presence of isomers, possibly on the fatty acid tail side. The presence of branched (e.g. isopropyl tail) and straight chain acid could possibly be one isomeric form (**Figure 4**). Further confirmation will require additional techniques such as H-NMR data. Such an endeavor will be pursued in future work.

Figure 4. Isomeric Forms of Nordihydrocapsaicin and Octanoic Acid Vanillylamide Isomers in Thai Chili Pepper Extract



Conclusions

In this analysis a precursor scan is an effective way to identify the various capsaicinoids species in plant matrix. A fast LC/MS/MS method can then efficiently separate all capsaicinoids including all isomeric species in both quantitative and qualitative modes. Thai chili peppers contained the highest ratio of nordihydrocapsaicin to capsaicin than other tested chili peppers.

References

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C18	00B-4475-Y0	00D-4475-Y0	AJ0-8775 for 3.0 mm ID

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