EXTENDED ESSAY
-Chemistry-

How does the measured capsaicin content in Scoville heat units of dried chili peppers extrapolated from oleoresins vary for the species *Capsicum annuum* ‘Bird’s eye’, *Capsicum chinense* ‘Habanero’, *Capsicum chinense* × *Capsicum frutescens* ‘Bhut jolokia’, and *Capsicum chinense* ‘Trinidad moruga scorpion’ using spectrophotometric analysis with a sweet *Capsicum annuum* ‘Bell pepper’ used as a control and a purity test using column chromatography?

Word Count: 3939

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Abstract

In this experiment, I have investigated the research question: "How does the measured capsaicin content in Scoville heat units of dried chili peppers extrapolated from oleoresins vary for the species *Capsicum annuum* 'Bird's eye', *Capsicum chinense* 'Habanero', *Capsicum chinense* × *Capsicum frutescens* 'Bhut jolokia', and *Capsicum chinense* 'Trinidad moruga scorpion' using spectrophotometric analysis with a sweet *Capsicum annuum* 'Bell pepper' used as a control and a purity test using column chromatography?"

Capsaicin was extracted from dried, granulated chili samples by means of a Soxhlet extractor followed by concentration to an oleoresin via distillation. The concentration of capsaicin in the oleoresin was then calculated through spectrophotometric analysis at 280nm following extrapolation from a curve developed from standard solutions. In order to test for purity in the oleoresin, one sample was further purified using column chromatography with silica as the adsorbent and hexane as the eluent and absorbance at 280nm was also measured for a sweet *Capsicum annuum* 'Bell pepper'.

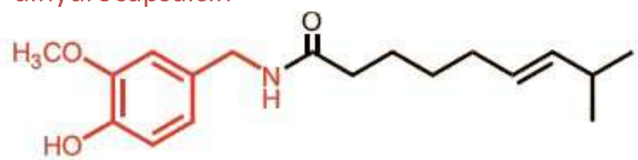
Final calculations indicated that *Capsicum chinense* × *Capsicum frutescens* 'Bhut jolokia' contained the most capsaicin with a Scoville heat rating of 10688 SHU while *Capsicum annuum* 'Bird's eye' contained the least with a rating of 3856 SHU. However, since each of the column's samples all measured as absorbing at 280nm and the *Capsicum annuum* 'Bell pepper' was calculated as having significant capsaicin content,

these results are flawed. This would suggest that spectrophotometric analysis is an inappropriate means to gauge the capsaicin content of chili peppers as has been done in a number of other studies, evidencing the necessity for other means such as gas or high performance liquid chromatography.

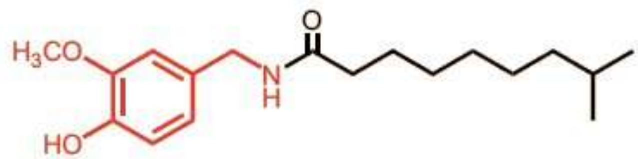
Word Count: 267

Introduction

Figure 1[2]
Skeletal formulas for capsaicin and dihydrocapsaicin



Capsaicin (**22**) (16 Mio SHU)



Dihydrocapsaicin (**24**) (16 Mio SHU)

Since their arrival from the Americas in the 1500s, capsicum fruits or chilies have become a staple in diets globally for their pungent flavor[1]. The pungency of the fruits results from a family of compounds known as capsaicinoids with capsaicin and dihydrocapsaicin (figure 1) being the most common among them[3]. When ingested, capsaicinoids bind to receptors in the mouth lining known as vanillloid receptors subtype 1 (TRPV1). Once bound, calcium ions flood into the receptor triggering the release of neurotransmitters. TRPV1 is activated in the same fashion by temperatures over 43°C, therefore the neurotransmitters are interpreted by the brain in the same way as excessive heat leading to the burning sensation[4]. Besides their culinary uses, capsaicinoids are a key component in pepper sprays, have been shown to be a potent analgesic used to lower the sense of pain for arthritis and other painful chronic conditions, and can facilitate weight loss through thermogenesis[5].

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1 Robinson, Simon. "Chili Peppers: Global Warming." Time. June 14, 2007. Accessed September 25, 2016. http://content.time.com/time/specials/2007/article/0,28804,1628191_1626317_1632291,00.html.

2 Roth, K. The structural formula and SHU of capsaicin and dihydrocapsaicin. Digital image. ChemistryViews. May 6, 2010. Accessed September 25, 2016. http://www.chemistryviews.org/SpringboardWebApp/userfiles/chem/image/2010May/Chiuz_scharf.jpg.

3 Bellringer, Matthew. "The Chemistry of Chilli Peppers." The Scoville Scale. Accessed September 25, 2016. http://www.chm.bris.ac.uk/motm/chilli/scoville.htm.

4 Rohrig, Brian. "Hot Peppers: Muy Caliente!" ChemMatters, December 2013, 6-8.

5 Ibid

I became interested in capsaicinoids after discussing the topic for a presentation on chemistry in the world around us. While I was able to learn much about capsaicin through background research, I was more interested in investigating its concentration in (and thus pungency of) different chili variants, which will be the topic of this paper. Historically, the pungency of specific varieties of chilies has been measured using the Scoville scale. The Scoville scale originally involved the tasting of an increasingly dilute solution of sugar water and chili extract (oleoresin) until the heat was no longer detectable. A bell pepper with no detectable heat even without dilution would have a Scoville heat rating of zero while chili peppers such as habaneros could have a rating of 300,000 requiring a concentration of less than one part in 300,000 to no longer be tasted. The Scoville scale is often extremely inaccurate due to its subjectivity and sensory fatigue resulting from the desensitization of the tasters' palettes after a few samples. As a result, more accurate methods such as high performance liquid chromatography and spectrophotometric analysis have been employed to measure pungency today[6].

Spectrophotometric analysis can be used to measure the concentration of capsaicin in a chili oleoresin obtained through means such as vacuum filtration or organic solvents. By measuring the absorbance of the solution at capsaicin's max absorbance of 280nm, the concentration can then be calculated using a calibration

[6] Rohrig, Brian. "Hot Peppers: Muy Caliente!" ChemMatters, December 2013, 6-8.

curve developed from known concentrations of pure capsaicin in accordance with the Beer-Lambert Law[7].

For this investigation, I will be analyzing Capsicum chinense ‘Trinidad moruga scorpion’, Capsicum chinense × Capsicum frutescens ‘bhut jolokia’, and Capsicum chinense ‘Habanero’ purchased through Amazon as well as Capsicum annuum 'Bird's eye’ and Capsicum anuum ‘Bell pepper’ purchased from Carrefour.

[7] Liljana, Koleva G., Maksimova Viktorija, Serafimovska D. Marija, Gulabovski Rubin, and Ivanovska J. Emilija. "THE EFFECT OF DIFFERENT METHODS OF EXTRACTIONS OF CAPSAICIN ON ITS CONTENT IN THE CAPSICUM OLEORESINS." *SCIENTIFIC WORKS* 60 (October 18, 2013): 917-22. Accessed September 25, 2016. http://eprints.ugd.edu.mk.

Background Information

2.1 Soxhlet Extraction[8]

A Soxhlet extractor allows for a solvent to be circulated through a sample repeatedly in order to extract the maximum amount of soluble components in a time efficient manner. Especially when components have limited solubility in the solvent, repeated runs will lead to a significantly greater degree of dissolution. A Soxhlet extractor works by boiling a solvent, which is then condensed over a sample (figure 2)[9]. The solvent then mixes with the sample allowing the soluble components to go into solution. Once the level of the solution reaches the siphon, it is drained back in to the distillation flask. This process is then continuously repeated until all the desired components have been extracted into the distillation flask as shown in figure 3.

Without the use of a Soxhlet extractor, the sample would need to be in solution for days in order to dissolve a similar amount. Larger amounts of solvent would also be necessary. Therefore, its use is cost effective and time efficient.

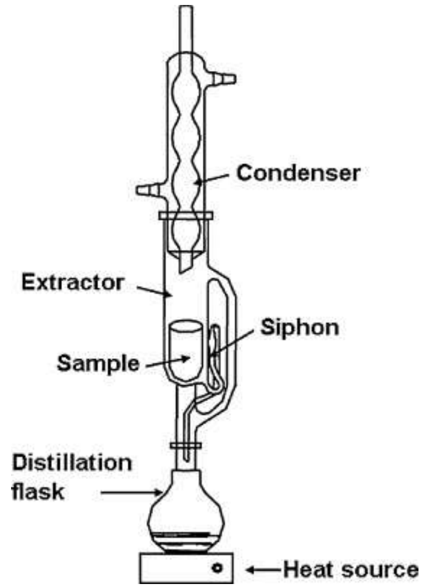

Figure 2[9]
A diagram showing the workings of a Soxhlet apparatus

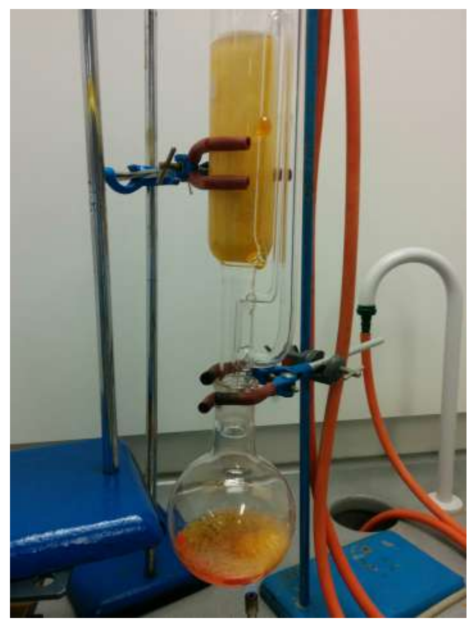

Figure 3
A nearly completed Soxhlet run where the desired components are in the flask

[8] Sella, Andrea. "Classic Kit: Soxhlet Extractor." Chemistry World. August 28, 2007. Accessed September 25, 2016. https://www.chemistryworld.com/opinion/classic-kit-soxhlet-extractor/1014879.article.

[9] Kumar, Manoj. A labeled diagram of a Soxhlet apparatus. Digital image. STU'DESTINATIONs. March 30, 2013. Accessed September 25, 2016. http://1.bp.blogspot.com/-qBElc-iz_ZQ/UVcPI_DqziI/AAAAAAAAA5c/UuX5dW0oroA/s640/1-s2.0-S0021967309016884-gr1.jpg.

2.2 Distillation

Distillation allows for the concentration of the solution and recycling of the solvent used. Distillation works by boiling a solution continuously and condensing the vapors in a separate container (figure 4)[10]. Components in solution will be left in the distillation flask while the solvent will be distilled.

In typical cases, a rotary evaporator would be significantly more effective since it would allow the solvent to be removed until dryness whereas this is impossible with distillation since it would cause them to burn. However, a rotary evaporator was not available for this experiment. In a similar vein, there is an additional concern that the rolling solution will deposit components on the flask, which can burn to the sides affecting the composition of the solution as shown in figure 5.

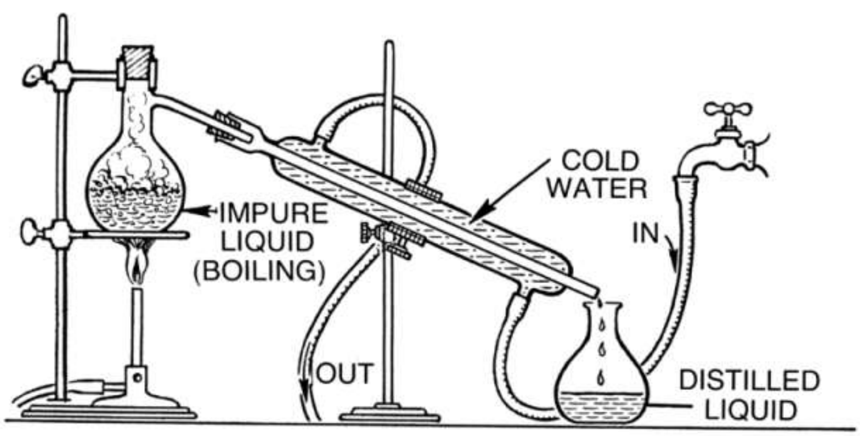

Figure 4[10]
A diagram displaying the workings of a distillation apparatus

Figure 5
A trial where solid components burned to the side of the flask during distillation

[10] Foresman, Pearson Scott. This is an example of a simple setup for distillation. Digital image. How To Set Up Distillation Apparatus. Accessed November 11, 2016. http://f.tqn.com/y/chemistry/1/S/l/n/1/distillation-setup.png.

2.3 Column Chromatography[11]

Column chromatography, like most other absorption-based chromatographic methods, is based upon a stationary and mobile phase. The stationary phase consists of a glass column packed with a solid such as silica gel or alumina (figure 6). This is then saturated with the same solvent as the one used for the sample to be separated. The impure sample will consist of a variety of compounds with greater or lesser solubility in the solvent. As a result, when the sample travels down the column the individual components will begin to separate over time since more soluble components will move through the column faster (figure 8). In this manner, the desired component can be separated in a pure form from the mixture's other constituents. If the desired component is colorless then it is necessary to collect a series of samples of defined volume, which will be analyzed to find which contains the compound.

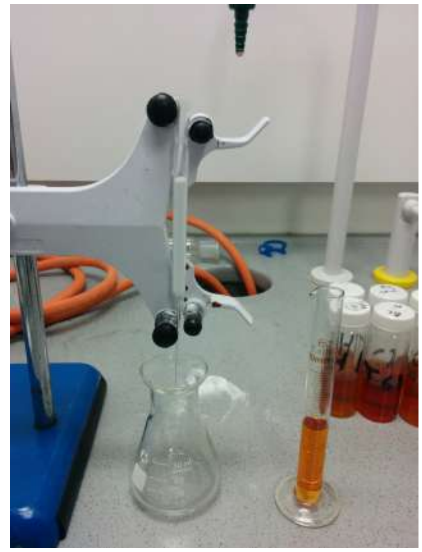

Figure 6
The column with alumina as the adsorbent

Figure 7
The orange layer of carotene is visible at the top of the column

Dedicated column chromatography equipment was not available for this experiment; as a result, a method using a Pasteur pipette as a column was used in its place (figure 8).

[11] Clark, Jim. "Column Chromatography." ChemGuide. June 2016. Accessed October 05, 2016. http://www.chemguide.co.uk/analysis/chromatography/column.html.

This allows for a micro scale separation where approximately only 0.1mL of solution is needed. Typically column chromatography wastes a large amount of solvent since it must be continuously added to prevent the stationary phase from drying. Through the use of a Pasteur pipette, the separation would progress at a much faster rate with less solvent required.

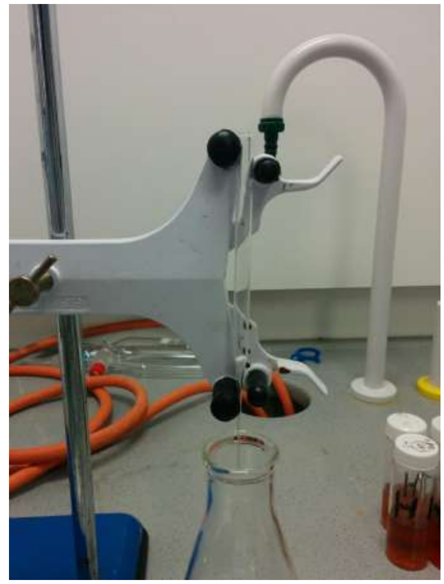

Figure 8
The column chromatography set up made using a Pasteur pipette

2.4 *Spectrophotometric Analysis*

Spectrophotometric analysis allows for the quantification of components in solution based upon their absorbance of certain wavelengths of ultraviolet or visible light. Using a specified wavelength to test, absorbance is measured by comparing the intensity of incident light to that measured by a detector on the opposite side of the solution (figure 9). In order to calibrate the system properly, a blank solution must be analyzed beforehand.

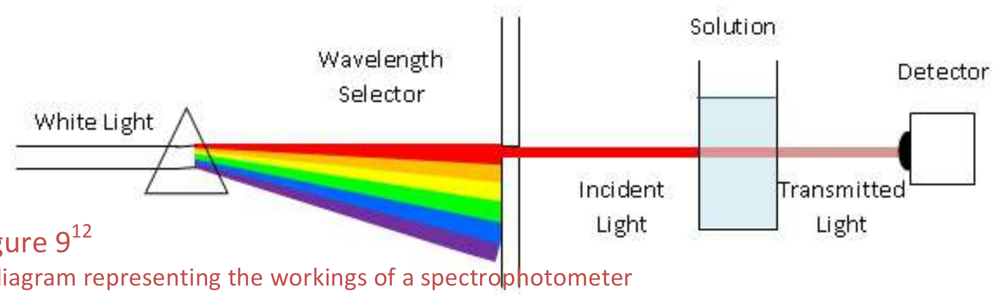

Figure 9[12]
A diagram representing the workings of a spectrophotometer

Absorbance correlates with concentration in a linear relationship according to the Beer-Lambert law. By measuring absorbance for a series of standard solutions and plotting their relationship, the absorbance of a solution can be used to extrapolate its unknown concentration.

Compounds with an ability to absorb light have regions known as chromophores, which can absorb the wavelength. In the case of organic compounds, this results from a repeating structure of conjugated double and single bonds (figure 10). When a molecule

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[12] Flaherty, Margaux. A schematic diagram depicting how a spectrophotometer measures absorbance of a sample solution. Digital image. IdeaExchange@UAkron. Spring 2015. Accessed September 25, 2016. https://encryptedtbn3.gstatic.com/images?q=tbn:ANd9GcTCw2VkBsF_ZtOxvluddv4xjsyA8RjH00hxquD2MYB8mOxd6p35.

[13] Samal. A comparison of conjugated and non-conjugated double bonds. Digital image. People.chem.umass. Accessed November 11, 2016. https://people.chem.umass.edu/samal/269/color.pdf.

absorbs UV/Vis light, it excites electrons from a ground state to higher energy levels. Depending on the difference in energy of the excited and ground state, more or less energetic light is required. Conjugation decreases the difference in energy between the excited and ground states in a molecule. If the degree of conjugation is high the molecule will absorb visible light whereas if it is small it will absorb UV. In either case this allows for absorption to be measured at the specified wavelength. For this experiment, spectrophotometric analysis was undertaken at the UCB pharmaceutical lab since the spectrophotometer readily available was limited to the visible spectrum.

Figure 10[13]
A diagram depicting the difference in structure of conjugated (left) and non-conjugated (right) double bonds

[14] Samal. "A Brief Discussion of Color." People.chem.umass. Accessed November 9, 2016. https://people.chem.umass.edu/samal/269/color.pdf.

Research Question

How does the measured capsaicin content in Scoville heat units of dried chili peppers extrapolated from oleoresins vary for the species Capsicum annuum 'Bird's eye', Capsicum chinense 'Habanero', Capsicum chinense × Capsicum frutescens 'Bhut jolokia', and Capsicum chinense 'Trinidad moruga scorpion' using spectrophotometric analysis with a sweet Capsicum annuum 'Bell pepper' used as a control and a purity test using column chromatography?

Hypothesis

I expect the capsaicin content to fluctuate between the different chili pepper variants and to be between 0 and 125,000ppm (the content of the highest pepper on record) since 16 Scoville heat units are equal to approximately 1ppm[15]. Capsicum annuum 'Bell pepper' should ideally have a capsaicin content of 0ppm but as a result of the accuracy of the spectrophotometer this will be unlikely[16].

I think that the capsaicin content will correlate with the published Scoville units, however, capsaicin content is subject to a large variety of variables including the climate in which they are grown as well as their age. Especially considering that the Capsicum annuum 'Bird's eye' I used are grown in Belgium, the capsaicin content could potentially differ greatly from the value listed. As a result, I foresee a possibility that the Capsicum

15 "Scoville Unit." Sizes. December 28, 2011. Accessed September 25, 2016. https://sizes.com/units/scoville_unit.htm.

16 "The Scoville Scale." Pepperheads For Life. Accessed September 25, 2016. http://pepperheadsforlife.com/the-scoville-scale/.

chinense ‘Habaneros’ could contain less capsaicin than the Capsicum annuum ‘Bird’s eye’ contrary to what is listed. The same could occur for the Capsicum chinense ‘Trinidad moruga scorpion’ and Capsicum chinense × Capsicum frutescens ‘Bhut jolokia’ since capsaicin content varies even more for chili variants where the chemical is typically more concentrated[17].

I predict that after further purification beyond the extraction and distillation, the measured capsaicin content will differ. Considering the large number of other organic compounds that are extracted along with the capsaicin, I expect there to be a noticeable but not significant difference[18].

Procedural write-up[19]

The method used involved the extraction of capsaicin using ethanol in a Soxhlet extractor followed by concentration through distillation of the ethanol. The concentrated solution was then spectrophotometrically analyzed. In order to check for potential issues with the measured concentration, a Capsicum annuum ‘bell pepper’ was used as a control and a sample of Capsicum annuum ‘Bird’s eye’ was further

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17 Tainter, Donna R., and Anthony T. Grenis. Spices and Seasonings: A Food Technology Handbook. 2nd ed. New York: Wiley, 2001. Accessed September 25, 2016. https://books.google.be/books?id=dfp4b3F0598C&pg=PA30&redir_esc=y&hl=en#v=onepage&q&f=false.

18 Liljana, Koleva G., Maksimova Viktorija, Serafimovska D. Marija, Gulabovski Rubin, and Ivanovska J. Emilija. "THE EFFECT OF DIFFERENT METHODS OF EXTRACTIONS OF CAPSAICIN ON ITS CONTENT IN THE CAPSICUM OLEORESINS." *SCIENTIFIC WORKS* 60 (October 18, 2013): 917-22. Accessed September 25, 2016. http://eprints.ugd.edu.mk.

19 Liljana, Koleva G., Maksimova Viktorija, Serafimovska D. Marija, Gulabovski Rubin, and Ivanovska J. Emilija. "THE EFFECT OF DIFFERENT METHODS OF EXTRACTIONS OF CAPSAICIN ON ITS CONTENT IN THE CAPSICUM OLEORESINS." *SCIENTIFIC WORKS* 60 (October 18, 2013): 917-22. Accessed September 25, 2016. http://eprints.ugd.edu.mk.

purified using column chromatography with alumina for the stationary phase and hexane for the mobile phase.

5.1 Variables

Independent Variable: Species of chili used for capsaicin extraction

Dependent Variable: Capsaicin content of chili in Scoville heat units

5.2 Materials

- Pestle and mortar
- 4 Ziploc bags
- 4 Extraction cartridges
- 4 Glass sample containers
- 1 Graduated cylinder 500ml (±5ml)
- 1 Graduated cylinder 50ml (±0.5ml)
- 2 Microburners
- 1 Funnel
- 4 Paper filters
- Anti bumping granules
- 2 Round bottom flasks 500ml
- Digital scale (±0.01g)
- Cotton sheet
- 4 Clamp stands

- 4 Clamps
- Safety glasses
- Disposable gloves
- 1 Beaker 250ml
- 1 Scraper
- 200mL Soxhlet extractor
- Condenser
- Distillation apparatus
- Spectrophotometer
- 4 Plastic cuvettes
- Dropper
- Tubing
- 3 Porcelain Tablets (for drying)
- 10 Small test tubes
- 2 Pasteur pipettes
- Burette clamp

5.3 Chemicals

- Ethanol (CH_3CH_2OH) 96%
- Hexane ($CH_3(CH2)_4CH_3$)
- Aluminum Oxide (Alumina) (Al_2O_3)

5.4 *Safety Instructions*

- Wear eye protection, a lab coat, and gloves
- The Soxhlet extractor and distillation must be operated in a fume cupboard to avoid risk from gaseous ethanol
- Ethanol – highly flammable in cases of open flames and sparks. Hazardous as an irritant in cases of skin or eye contact. Toxic to blood, the reproductive system, liver, upper respiratory tract, and central nervous system[20].
- Hexane – highly flammable in cases of open flames and sparks. Hazardous in cases of skin contact (permeator), ingestion, and inhalation. Toxic to the skin, peripheral, and central nervous systems[21].
- Alumina – hazardous as an irritant in cases of eye and skin contact and when inhaled or ingested[22].
- Capsaicin – hazardous as both an irritant and sensitizer in cases of eye and skin contact as well as ingestion and inhalation. Severe over exposure can result in death by an accumulation in one or many organs[23].

5.5 *Method for Preparation and Soxhlet Extraction*

The majority of the chilies were purchased dried and others were dried using an oven:

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[20] "Ethyl Alcohol 200 Proof MSDS." Material Safety Data Sheet. May 21, 2013, accessed October 5, 2016, http://www.sciencelab.com/msds.php?msdsId=9923955.

[21] "Hexanes MSDS." Material Safety Data Sheet. May 21, 2013. Accessed October 5, 2016. http://www.sciencelab.com/msds.php?msdsId=9927187.

[22] "Alumina MSDS." Material Safety Data Sheet. May 21, 2013. Accessed October 5, 2016. https://www.sciencelab.com/msds.php?msdsId=9925577.

[23] "Capsaicin, Natural MSDS." Material Safety Data Sheet. May 21, 2013. Accessed October 5, 2016. http://www.sciencelab.com/msds.php?msdsId=9923296.

- Cut chilies into slices and separate seeds
- Place chilies and seeds on a porcelain tablet in order to be heated
- Set the oven to 50°C and heat the chilies
- After two hours, flip the chilies over to prevent them from getting stuck to the tablets
- Leave the chilies overnight and remove them from the oven in the morning

Before the extraction, the chilies must be properly prepared:

- Weigh out a desired amount of chili (this varied between species due to their cost)
- Remove the stems and add the peppers to a Ziploc bag
- Crush the chilies in the bag focusing on releasing the seeds
- Weigh the chilies once more before refining them further with a pestle and mortar
- Move the chili particulate to an empty extraction cartridge and cover the contents with a wad of cotton

Afterwards, the extraction

- Place the cartridge in the Soxhlet chamber
- Measure approximately 300ml of ethanol and add it to a round bottom flask
- Attach the filled flask to the bottom of the Soxhlet extractor and the condenser to the top
- Attach tubing from the condenser to the tap, start the water, and ignite a microburner beneath the flask of ethanol

- Bring the ethanol to a rolling boil while moving the microburner around the flask to heat evenly. The ethanol will condense, dropping into the Soxhlet chamber and mix with the contents. A red-orange solution will begin to form in the chamber whose level will rise as more ethanol condenses. Once the solution in the chamber breaches the height of the siphon, the solution will run back into the round bottom flask emptying the chamber completing one run
- Allow this process to repeat, subsequent runs will have increasingly clear solutions. Once a run is devoid of a color, the extraction is complete

The solution was then concentrated through distillation

- Move the solution to a new round bottom flask and add anti bumping granules
- Attach the flask to a distillation apparatus and place a beaker beneath the end of the condenser
- Make sure the water for the condenser is running and ignite a microburner beneath the solution
- Move the microburner constantly in order to thoroughly heat the solution. When the solution begins to collect on the sides of the flasks, move the microburner away from the location to prevent the solution from burning to the inside of the flask
- Distill as far as possible without burning the contents
- Allow the solution to cool
- Filter the solution and record the volume

5.6 Method for Column Chromatography of One Sample[24]

- Add 1mL of water to a small test tube and mark the water level
- Using a straight edge, mark the same level on four other small test tubes
- Stuff a small wad of cotton into the end of a Pasteur pipette
- Setup a stand and burette clamp and attach the pipette
- Fill the pipette with alumina to where it is three centimeters below the top
- Place a beaker below the pipette and add hexane until the pipette is full
- Once the hexane has passed the top of the silica, add 0.5mL of the sample
- Once the sample has passed the top of the silica, fill the pipette with hexane and place one of the test tubes below the pipette
- When the solution dripping into the test tube reaches the marked level replace it with an empty one and cork it. Repeat this process four more times making sure to keep the level of hexane in the pipette above that of the alumina.
- Repeat the whole process a second time for a second 0.5mL portion of the sample and a new set of five small test tubes

5.7 Method for Spectrophotometric Analysis

- Calibrate the spectrophotometer to the appropriate blank (96% ethanol for the chili extracts and pure hexane for the column samples)
- Fill cuvette three quarters of the way with sample to analyze

[24] Lehman, John W. Operational Organic Chemistry: A Problem-solving Approach to the Laboratory Course. 3rd ed. Upper Saddle River, NJ: Prentice Hall, 1999.

- Run the spectrophotometer and record the absorbance of the solution at 280nm
- Repeat for all the samples

Experimental Data

6.1 Quantitative Results

Pepper sample	Mass of dry, granulated chili in grams (±0.01g)	
	Trial 1	Trial 2
Capsicum anuum 'Bird's eye'	7.24	7.46
Capsicum chinense 'Habanero'	7.24	7.37
Capsicum chinense × *Capsicum frutescens* 'Bhut jolokia'	3.79	3.29
Capsicum chinense 'Trinidad moruga scorpion'	4.98	4.27

Table 1-mass of dry, granulated chili samples

Pepper sample	Volume of pepper extract in mL (±0.5mL)	
	Trial 1	Trial 2
Capsicum anuum 'Bird's eye'	15	7.5
Capsicum chinense 'Habanero'	16	13
Capsicum chinense × *Capsicum frutescens* 'Bhut jolokia'	17	13
Capsicum chinense 'Trinidad moruga scorpion'	11.5	9.5

Table 2-volume of chili oleoresins post distillation

Pepper Sample	Scoville Heat Unit Rating (SHU)
Capsicum anuum 'Bird's eye'	150,000
Capsicum chinense 'Habanero'	350,000
Capsicum chinense × *Capsicum frutescens* 'Bhut jolokia'	1,041,427
Capsicum chinense 'Trinidad moruga scorpion'	2,009,231

Table 3-SHU for each pepper variant[25]

Pepper Sample	Mass of granulated pepper in grams (±0.01g)	Volume of pepper extract in mL (±0.5mL)
Capsicum anuum 'Bell pepper' Control	7.67	14.5
Capsicum anuum 'Bird's eye' for Column	7.56	12.0

Table 4-Extraction data for additional samples

Since purchasing a pure sample of capsaicin proved to be difficult as well as costly, I instead used data from a past experiment. The capsaicin sample used was purchased from Sigma Aldrich with greater than 95% concentration.

Concentration (mg/ml)	0.0156	0.0312	0.0625	0.125	0.25
Absorbance (λ = 280)	0.1639504	0.3119008	0.60875	1.2015	2.387

Table 5-absorbance measured for standard solutions of capsaicin[26]

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25 "The Scoville Scale." Pepperheads For Life. Accessed September 25, 2016. http://pepperheadsforlife.com/the-scoville-scale/.

26 Liljana, Koleva G., Maksimova Viktorija, Serafimovska D. Marija, Gulabovski Rubin, and Ivanovska J. Emilija. "THE EFFECT OF DIFFERENT METHODS OF EXTRACTIONS OF CAPSAICIN ON ITS CONTENT IN THE

Chili sample	Absorbance of chili extract at 280nm (±0.0001)	
	Trial 1	Trial 2
Capsicum anuum 'Bird's eye'	1.5023	1.4890
Capsicum chinense 'Habanero'	1.5154	1.5263
Capsicum chinense × *Capsicum frutescens* 'Bhut jolokia'	1.5229	1.5156
Capsicum chinense 'Trinidad moruga scorpion'	1.4823	1.4903

Table 6-absorbance of chili extracts read against 96% ethanol as a blank

Pepper Sample	Absorbance of pepper extract at 280nm (±0.0001)
Capsicum anuum 'Bell pepper' Control	1.5195
Capsicum anuum 'Bird's eye' for Column	1.5360

Table 7-absorbance of bell pepper for control and Thai chili used for column read against 96% ethanol as a blank

1mL Column Sample	Absorbance of pepper extract at 280nm (±0.0001)	
	Trial 1	Trial 2
First	0.2478	0.2182
Second	0.9419	1.301
Third	0.6906	0.728
Fourth	0.5442	N/A
Fifth	0.3937	N/A

Table 8-absorbance of 1mL chili extract samples gathered from column measured against hexane as a blank

CAPSICUM OLEORESINS." *SCIENTIFIC WORKS* 60 (October 18, 2013): 917-22. Accessed September 25, 2016. http://eprints.ugd.edu.mk.

6.2 *Qualitative Results*

Over the course of the extraction using the Soxhlet extractor, the solution in the distillation flask moved from clear (figure 10) to a shade of red or orange (figure 11) depending on the pepper variant. Before filtration, some solid particulate was also present in the distillation flask (figure 12). After distillation, every sample was distinctly darker in shade and when left overnight there was an oily film on the surface of each (figure 13).

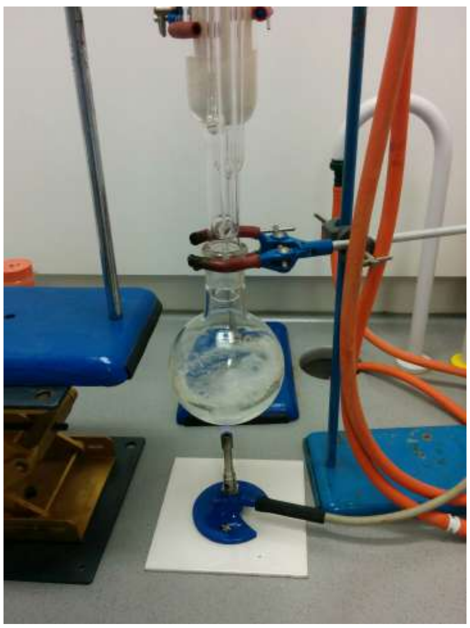

Figure 10
Pure ethanol boiling in the distillation flask

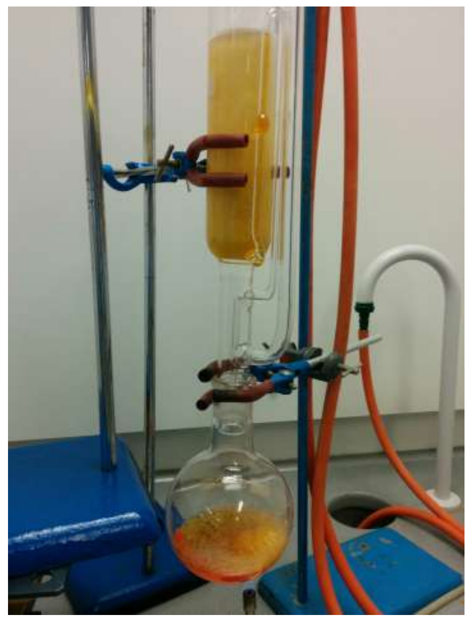

Figure 11
An extraction near completion, soluble components are now in the flask

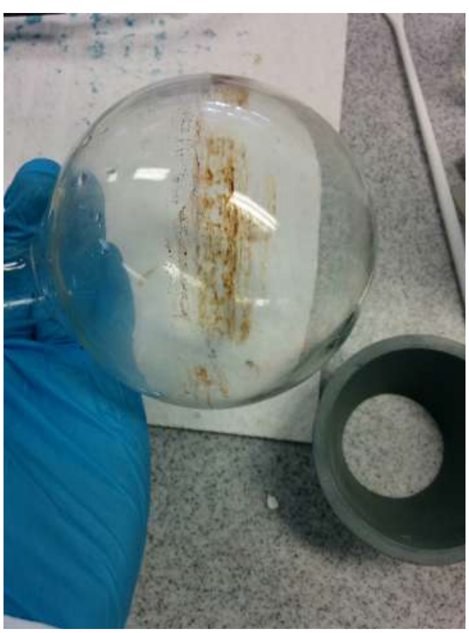

Figure 12
Trace of solid components of extract

Figure 13
Samples post distillation; an oily film can be seen on the surface

When the column was run there was a visible but dilute region of orange travelling through as seen in figure 14. The first, third, fourth, and fifth 1mL samples collected were clear, while the second had a distinct orange tint (figure 15).

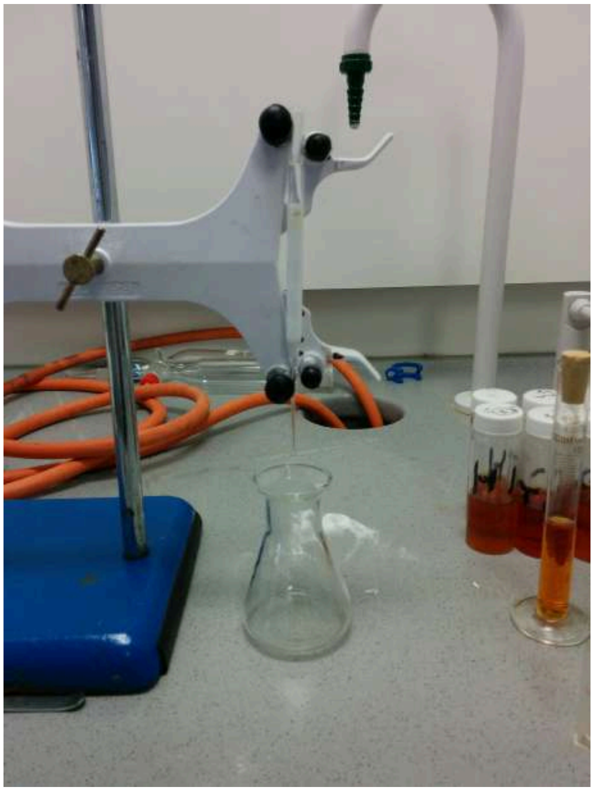

Figure 14
The orange band is slightly visible at the top of the column

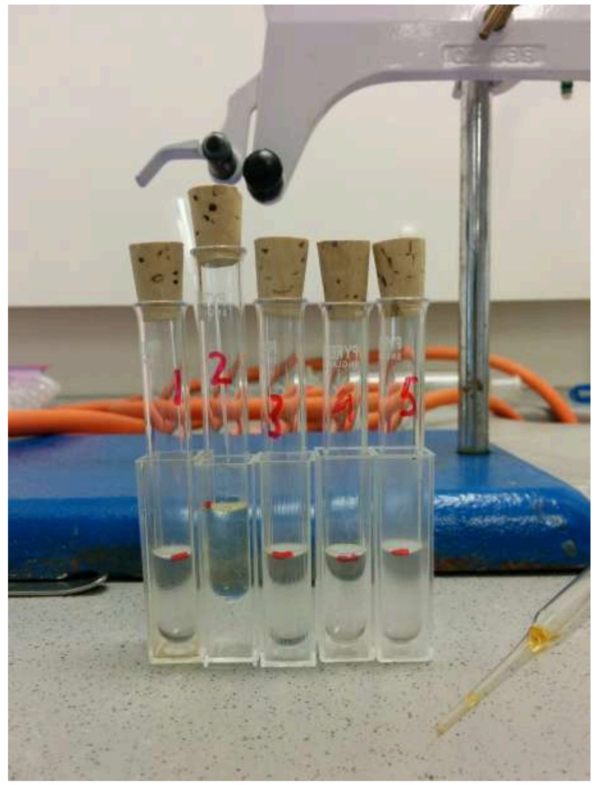

Figure 15
One round of five samples made using the column, the second one has the orange tint

6.3 Processed Results

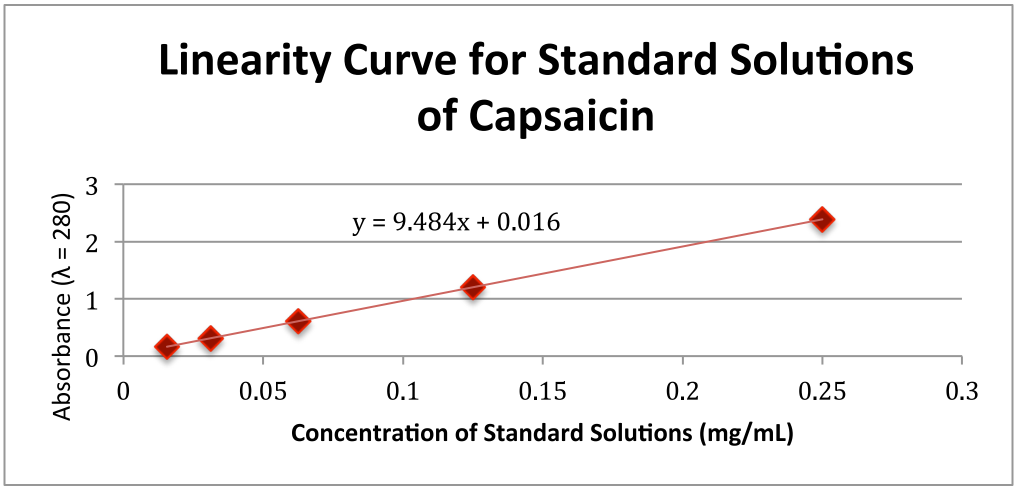

Figure 16

Graph of absorbance at 280nm vs. concentration (mg/ml) for standard solutions of capsaicin

Concentrations of the chili oleoresins were calculated by plugging the values from table 6 into the equation for the trend line from figure 16.

Pepper sample	Capsaicin Concentration (mg/mL)	
	Trial 1	Trial 2
Capsicum anuum 'Bird's eye'	0.157	0.155
Capsicum chinense 'Habanero'	0.158	0.159
Capsicum chinense × *Capsicum frutescens* 'Bhut jolokia'	0.159	0.158
Capsicum chinense 'Trinidad moruga scorpion'	0.155	0.155

Table 9-concentration of capsaicin in chili extracts

1mL Column Sample	Concentration (mg/mL)	
	Trial 1	Trial 2
First	0.024	0.021
Second	0.098	0.135
Third	0.071	0.075
Fourth	0.056	N/A
Fifth	0.040	N/A

Table 10-concentration of capsaicin in 1mL samples from column

To calculate the mass of capsaicin in the oleoresins multiply the concentrations from table 10 by the volumes from table 2. For the column samples the volume used for calculations was 1mL.

Pepper sample	Mass of capsaicin in extract (mg)	
	Trial 1	Trial 2
Capsicum anuum 'Bird's eye'	2.36	1.16
Capsicum chinense 'Habanero'	2.53	2.07
Capsicum chinense × *Capsicum frutescens* 'Bhut jolokia'	2.70	2.05
Capsicum chinense 'Trinidad moruga scorpion'	1.78	1.47

Table 11-mass of capsaicin in chili extracts in milligrams

Since the second sample contained the carotene as shown by figure 15, it is assumed that the third sample contains the capsaicin since it had the next to largest absorbance.

1mL Column Sample	Mass of capsaicin in samples (mg)	
	Trial 1	Trial 2
First	0.024	0.021
Second	0.098	0.135
Third	0.071	0.075
Fourth	0.056	N/A
Fifth	0.040	N/A

Table 12-mass of capsaicin in in 1mL samples from column

The total capsaicin mass of the extract based on the concentration of each sample was extrapolated using the volume from table 4. Since 0.5mL of oleoresin was used in the column, the expected total can thus be calculated.

1mL Column Sample	Mass of capsaicin in entire extract (mg)	
	Trial 1	Trial 2
First	0.288	0.252
Second	1.18	1.62
Third	0.852	0.900
Fourth	0.672	N/A
Fifth	0.480	N/A
Total	3.472	2.77

Table 13-mass of capsaicin in entire chili extract from column based on samples

Parts per million weight of capsaicin in the dry chili species was calculated using the dry chili masses from table 1 which were converted into kilograms.

Pepper sample	Parts per million weight (mg/kg)		
	Trial 1	Trial 2	Average
Capsicum anuum 'Bird's eye'	326	155	241
Capsicum chinense 'Habanero'	349	281	315
Capsicum chinense × *Capsicum frutescens* 'Bhut jolokia'	712	623	668
Capsicum chinense 'Trinidad moruga scorpion'	357	344	351
Third column sample	113	199	156

Table 14-parts per million weight of capsaicin in dry chili species

The Scoville rating for each dry chili species was calculated by multiplying the parts per million by 16 as discussed previously.

Pepper sample	Scoville rating (SHU)		
	Trial 1	Trial 2	Average
Belgian Red Hot Chile	5216	2480	3856
Habanero	5584	4496	5040
Ghost Pepper	11392	9968	10688
Moruga Scorpion	5712	5504	5616
Third Column Sample	1808	3184	2496

Table 15-extrapolated SHU of dry chili species

Pepper Sample	Concentration	Mass of capsaicin in extract	Parts per million weight	Scoville
Bell Pepper Control	0.159	2.31	301	4816
Thai Chile for Column	0.160	1.92	254	4064

Table 16-a collection of the previous data for the extracts of bell pepper and Thai chili for column

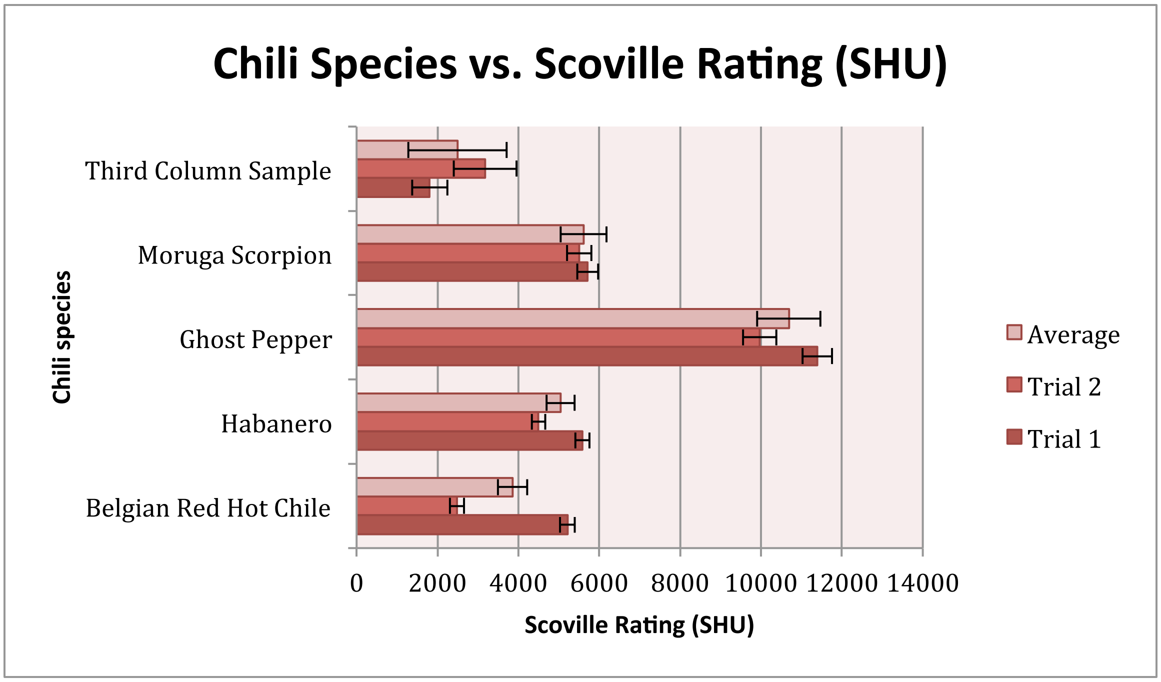

Figure 17-bar graph representing the Scoville rating for two trials and average for each chili species

$$\%Uncertainty\ chili\ mass = \frac{0.01}{chili\ mass}$$

$$\%Uncertainty\ extract\ volume = \frac{0.1}{extract\ volume}$$

$$\%Uncertainty\ absorbance = \frac{0.0001}{absorbance}$$

$$\%Uncertainty\ ppmw = \%U\ peel\ mass + \%U\ extract\ volume + \ \%U\ absorbance$$

$$\%Uncertainty\ ppmw\ for\ column = \%U\ ppmw + \frac{0.1}{0.5}$$

$$\%Uncertainty\ in\ average\ ppmw = \frac{\sum \%Uncertainty\ in\ ppmw}{2}$$

Interpretation of Results

It is immediately apparent that the calculated Scoville ratings for the chili species are significantly off from the literature values considering the degree is of error is as much as 98.9%. Not only are the Scoville ratings for the chilies drastically lower than expected (see table 3), the bell pepper was measured to have a higher Scoville rating than the Thai chilies when it should have measured at 0. Furthermore, the column was unsuccessful in isolating a sole sample, which absorbed at 280nm. Instead, each of the five samples absorbed to a lesser or greater degree at the wavelength. Upon doing further research into the use of spectrophotometric analysis to quantify capsaicinoids in chili extracts, this turns out to be a common issue. In a Sudan University of Science and Technology study, the effectiveness of spectrophotometric analysis was compared to gas chromatography (a more accurate measure). Where using gas chromatography sweet peppers measured as having 0% capsaicin by mass, with spectrophotometric analysis they had 0.33%, which was roughly equal to the measured values for all the other chilies tested using the method[27]. This degree of error results from 280nm being an incredibly common wavelength of max absorption for organic compounds[28]. Aside from certain varieties of carotenoids, which absorb at 280nm and have high concentrations in chilies, aromatic compounds like β-Ionone and even various vitamins all absorb at the wavelength as well. This variety of compounds would account for each of the samples taken from the column absorbing at 280nm since they would all be more

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[27] Abdelmajid, Asmaa Elrasheed Eltigani. "Extraction and Characterization of Capsaicin from Capsicum Frutescens and Capsicum Annuum." Master's thesis, Sudan University of Science and Technology, 2016.
[28] Philips, G. F. "UV-visible Spectroscopy 3.8.7." Kaye & Laby Tables of Physical and Chemical Constants. Accessed November 08, 2016. http://www.kayelaby.npl.co.uk/chemistry/3_8/3_8_7.html.

or less soluble with the largest sample (the second) signaling the presence of β-carotene[29]. Despite the significant discrepancy in values there is still variance amongst the species with Capsicum chinense × Capsicum frutescens 'Bhut jolokia' having the highest content followed by Capsicum chinense 'Habanero', then Capsicum chinense 'Trinidad moruga scorpion', and lastly the Capsicum anuum 'Bird's eye'. This is generally in line with literature values aside from the Capsicum chinense 'Trinidad moruga scorpion'; however this could possibly still be correct. The reviews for the heat of the Capsicum chinense 'Trinidad moruga scorpion'purchased from Amazon varied considerably[30] [31]. There is a large chance that the particular batch that was purchased was just as hot as the Capsicum chinense 'Habanero'.

[29] Roth, Klaus. "The Biochemistry of Peppers." ChemistryViews. May 06, 2014. Accessed November 08, 2016. http://www.chemistryviews.org/details/ezine/6108461/The_Biochemistry_of_Peppers.
[30] "Disappointed." Review. Amazon.com (blog), October 11, 2014. Accessed November 10, 2016. https://www.amazon.com/review/RUW0YS0VXA8E8/ref=cm_cr_dp_title?ie=UTF8&ASIN=B0092FKVQ2&channel=detail-glance&nodeID=2972638011&store=lawn-garden.
[31] "BEST EXPERIENCE EVER." Review. Amazon.com, July 24, 2014. Accessed November 10, 2016. https://www.amazon.com/review/R3I7J8YT16NV2N/ref=cm_cr_dp_title?ie=UTF8&ASIN=B0092FKVQ2&channel=detail-glance&nodeID=2972638011&store=lawn-garden.

Conclusion

From the results it appears that Capsicum chinense × Capsicum frutescens 'Bhut jolokia' has the highest Scoville heat rating at an average of 10688 SHU while Capsicum annuum 'Bird's eye' had the lowest at an average of 3856 SHU. However since these results are significantly off from the average recorded in literature and the sweet Capsicum annuum 'Bell pepper' was calculated as containing capsaicin, the results provide no reliable information. Despite this, they do prove the ineffective nature of spectrophotometric analysis for capsaicin quantification and its inappropriate usage as a means for a conclusion as done in the Goce Delchev University study[32].

Evaluation

8.1 Random Errors

Quantities of capsaicin in chilies vary significantly between individual peppers, which could be a result a result of a variety of factors including storage, growth climate, and genetics. Any experiment with the aim of quantifying the compound would experience large variance in results as a result of these random factors[33].

[32] Liljana, Koleva G., Maksimova Viktorija, Serafimovska D. Marija, Gulabovski Rubin, and Ivanovska J. Emilija. "THE EFFECT OF DIFFERENT METHODS OF EXTRACTIONS OF CAPSAICIN ON ITS CONTENT IN THE CAPSICUM OLEORESINS." *SCIENTIFIC WORKS* 60 (October 18, 2013): 917-22. Accessed September 25, 2016. http://eprints.ugd.edu.mk.

[33] Tainter, Donna R., and Anthony T. Grenis. Spices and Seasonings: A Food Technology Handbook. 2nd ed. New York: Wiley, 2001. Accessed September 25, 2016. https://books.google.be/books?id=dfp4b3F0598C&pg=PA30&redir_esc=y&hl=en#v=onepage&q&f=false.

Due to time constraints, only two trials were run for each species of pepper, which was far from sufficient to make general conclusions. Further trials would be a necessity for the development of relevant data.

8.2 Systematic Errors

The significant issue with the experiment was the method of quantification. While spectrophotometric analysis is very effective for solutions with a small number of components with varying absorption spectra, it is bound to be inaccurate for organic mixtures containing possibly hundreds of different compounds many of which would have overlapping spectra. To avoid such issues, gas chromatography or high performance liquid chromatography should have been used instead. In gas chromatography, the sample is vaporized and then passed through a liquid stationary phase in order to separate the gaseous components. These components are then detected at the end of the process resulting in a peak for each constituent. Based on the nature of the peak the compound and its concentration can be found in an accurate manner[34]. Such devices are beyond the capabilities of a school but would provide significantly more interesting and better results, which is why they are commonly used for Scoville heat quantification for culinary purposes.

[34] "Gas Chromatography." Faculty of Health and Wellbeing Biosciences Division Chemistry Resources. Accessed November 10, 2016. http://www.webinfobits.com/page/56260e91403a.

Further background research following the experiment showed that the length of the Soxhlet extraction could have been insufficient. In similar papers, the Soxhlet extractor was run for as long as five hours[35]. While a large part of the β-carotene would have dissolved if the siphon ran clear, it is fully possible that a significant amount of the capsaicin was left in the Soxhlet chamber, which could have partially led to the low results. Furthermore, the dried samples themselves were in small pieces rather than as a powder or slurry. As a powder or slurry a larger surface area would have been exposed to the solvent, which would lead to a higher yield.

While the column did evidence the impurity of the sample, the spectrophotometer readings of the extracts were not accurate enough for significant conclusions. In order to be sure that layers of varying compounds in the solution had formed a taller column should have been used and more samples taken. Furthermore, the test tubes in which the samples were stored were not sealed to a sufficient degree. The corks present in the lab obviously allowed air to pass since two of the samples actually fully evaporated. The others lost some volume but were still measurable using the spectrophotometer. A repeated experiment would require a better seal for any meaningful results.

[35] Abdelmajid, Asmaa Elrasheed Eltigani. "Extraction and Characterization of Capsaicin from Capsicum Frutescens and Capsicum Annuum." Master's thesis, Sudan University of Science and Technology, 2016.

8.3 Evaluation of Sources

The majority of the sources used were web-based versions of printed works such as university papers or scientific magazines, which are generally known to be more reliable than webpages. However, especially the papers from the Goce Delchev University and Sudan University of Science and Technology lacked sufficient explanation and development of data. Being the only two papers I found describing the use of spectrophotometric analysis for capsaicin quantification in oleoresins, this raises a significant issue for similar studies. Both papers had conflicting values (18% vs. 0.3% content by mass) presenting the need for a paper, which effectively analyzes the method with sufficient data points and development of results[36] [37].

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[36] Ibid

[37] Liljana, Koleva G., Maksimova Viktorija, Serafimovska D. Marija, Gulabovski Rubin, and Ivanovska J. Emilija. "THE EFFECT OF DIFFERENT METHODS OF EXTRACTIONS OF CAPSAICIN ON ITS CONTENT IN THE CAPSICUM OLEORESINS." *SCIENTIFIC WORKS* 60 (October 18, 2013): 917-22. Accessed September 25, 2016. http://eprints.ugd.edu.mk.

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