



Capsaicinoid, polyphenol, and carotenoid content of three Zambian grown *Capsicum* spp.



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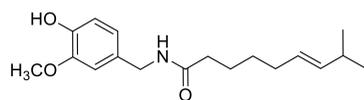
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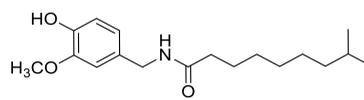
Introduction

Chili peppers are one of the most popular spices consumed for centuries all over the world due to their piquancy and pungency. The ease in their hybridization has led to exciting new variations in flavor, color, shape, and pungency within and between *Capsicum* species and new varieties. In this study, three Zambian grown hot pepper varieties which included Long Slim, Rochelle, and the African Birds Eye Chili variety, Malaga, were analyzed for their capsaicin and dihydrocapsaicin (DHC) content using HPLC with a UV detector. Capsaicin and DHC are the two primary compounds responsible for a pepper's pungency. From the capsaicinoid content, an estimated Scoville rating can be determined which can help match growers with interested buyers. Long slim and Rochelle peppers were very similar in capsaicinoid content with Scoville ratings similar to that of the moderately pungent Manzano pepper. Malaga peppers were much more pungent with higher Scoville rating similar to that of other African Bird's Eye Chili peppers.

Polyphenols and carotenoids are natural products that play an important role in human health and nutrition. These compounds can act as free radical scavengers and as such, may help protect against cancer, heart disease, as well as aging and their presence can enhance the value of these crops. The total polyphenol content was determined by the Folin-Ciocalteu assay. Each of the varieties were found to be very high in total polyphenols, with over 20 times more polyphenol content than blueberries. Carotenoids for the three varieties were determined by a UV-VIS technique. It was found that each variety contained more carotenoids than spinach and kale.



Capsaicin



Dihydrocapsaicin (DHC)

Background

Phytochemical and physical variation among organisms of the same species is a common theme in nature; variations in gene expression as well as external factors such as moisture content, sunlight exposure, and soil quality can have a large impact on the quantity of secondary metabolites produced within a plant. To better understand the quality of Zambian grown hot peppers, and as a strategy to strengthen the marketability of these peppers we measured the capsaicinoid content by HPLC, the total polyphenol content by the Folin-Ciocalteu assay, and the carotenoids content using a UV-VIS technique.



Long slim

Rochelle

Malaga

Plant materials and sample preparation

Each pepper variety was grown at the Mambova Chili Farm in Southern Province, Zambia. After harvesting, samples were allowed to sun dry for several days. Samples were then shipped to Rutgers University and ground to a fine powder. Approximately 300 mg of the ground pepper was extracted with 10 mL of MeOH under sonication for 30 minutes to obtain stock solutions for the samples.

References

1. Handbook of Plant Food Phytochemicals: Sources, Stability and Extraction. ohn Wiley & Sons, 2013
2. University of Maryland Medical Center: <http://umm.edu/health/medical/altmed/supplement/betacarotene>. Accessed April 2015.

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I. Determination of capsaicinoid content

Capsaicin and dihydrocapsaicin contents were quantified using high-performance liquid chromatography with UV detection at 280 nm.

1. Materials and methods

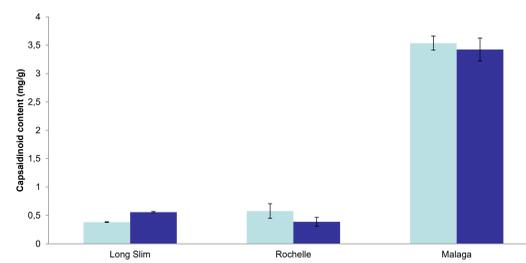
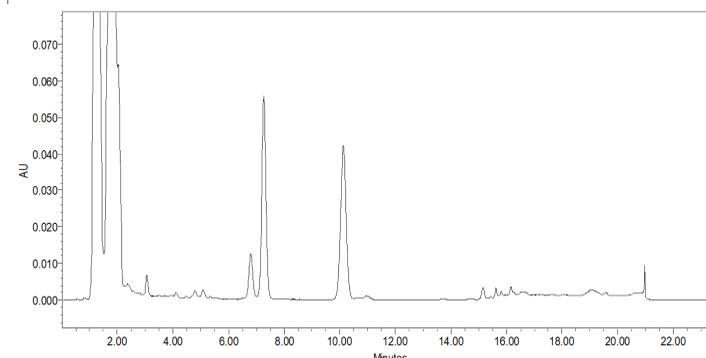
HPLC analysis

1 mL of stock solution analyzed by HPLC/UV. Pure capsaicin and DHC purchased from Sigma Aldrich (St. Louis, MO) were used as reference standards.

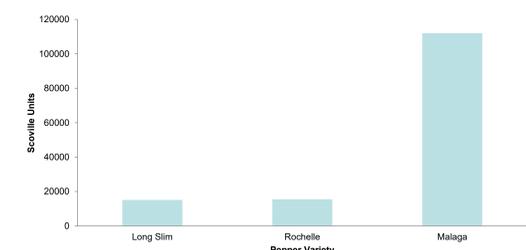
HPLC analysis was performed on a Waters 2695 module equipped with a Waters 2996 photodiode array detector. Separation was achieved on a Phenomenex 5 µm C18 column, 250 x 4.6 mm. The mobile phase contained 50% solvent A (ddH₂O with 0.1% formic acid) and 50% B (CAN with 0.1% formic acid) from 0 to 10 minutes and then increased to 53% B in a gradient from 10 to 13 minutes. Flow rate set at 1.0 mL/min and UV absorbance was measured at 280 nm.

2. Results and discussion

HPLC method provided excellent separation of the capsaicinoids. The total capsaicinoid content for Malaga, Rochelle, and Long Slim was 6960 µg/g, 962 µg/g, and 380 µg/g respectively. Each variety was tested in triplicate with the results reported as an average of each replicate. Standard deviation for Rochelle pepper was quite high (~20%). This indicates that capsaicin and DHC concentration can vary greatly within a variety. The Scoville ratings were determined by using the formula [CAP(ppm)+DHC(ppm)] x 16.1. The estimated Scoville ratings for Long Slim, Rochelle, and Malaga were approximately 15071, 15475, and 112047 respectively. Long Slim and Rochelle pepper have a moderate pungency similar to that of the Manzano pepper while Malaga's pungency is closer to that of other African Bird's Eye Chilis.



Graph 1: Capsaicin and DHC content of each variety.



Graph 2: Estimated scoville ratings for each variety.

	Capsaicin content (µg/g)	DHC content (µg/g)	Total capsaicinoids	Approximate scoville rating
Long Slim	380 ± 1.78%	556 ± 2.19%	936	15071
Rochelle	576 ± 22.50%	386 ± 20.05%	962	15475
Malaga	3540 ± 3.53%	3420 ± 5.91%	6960	112047
manzano pepper				~15000
Birds eye chili				~100000

Table 1: capsaicin and DHC concentrations and estimated scoville rating.

II. Determination of polyphenol content and antioxidant capacity

The total polyphenol content was determined by the Folin-Ciocalteu assay with gallic acid as reference standard. Carotenoids were measured by a spectroscopic technique with β-carotene as the reference standard.

1. Materials and methods

Folin-Ciocalteu assay

Approximately 400 mg of pepper powder was extracted with 25mL of 60% MeOH and sonicated for 10 minutes. After sonication, aliquot was centrifuged and 40µL of solution was combined with 900µL of Folin-Ciocalteu reagent and the mixture was allowed to equilibrate for 4 minutes. 400 µL of 15% Na₂CO₃ was added to this mixture which was then allowed to incubate at room temp. for 45 minutes. 200µL of this solution was then transferred to 96 well plate and the absorbance was measured at 765nm.

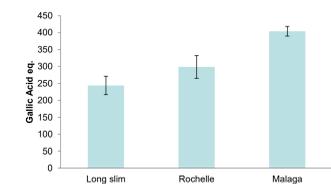
Carotenoid assay

Approximately 10mg of pepper powder was saponified with 0.5mL of 40% KOH at 70C for one hour. After saponification, carotenoids were extracted by adding 0.4mL of cyclohexane and vortexing for 1 minute. 200µL of cyclohexane extract was transferred to a 96 well plate and UV absorbance was measured at 450nm.

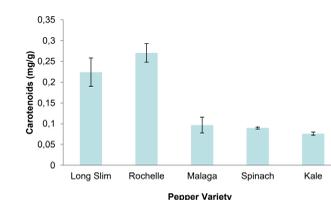
UV/Vis analysis was performed using an Synergy HT spectrophotometer.

Results and discussion

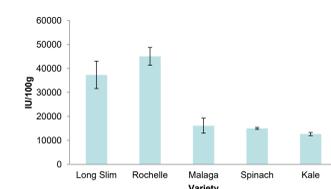
Polyphenol content as measured by the Folin-Ciocalteu assay determined that Malaga, Long Slim, and Rochelle peppers contained approximately 404, 243, and 298 gallic acid equivalence per gram (GAE/g) respectively. In comparison, blueberry extract which is known for its high polyphenol content ranges from 0.44GAE/gram to 7.73GAE/gram¹. It should be noted that capsaicin and DHC are reactive towards the Folin-Ciocalteu reagent thus results may be slightly inflated. Standard deviation for Long Slim and Rochelle was over 11% suggesting large variation in polyphenol content within the fruit of these varieties. The carotenoid content of Long Slim, Rochelle, and Malaga was 37332IU/100g, 45065IU/100g, and 16119IU/100g respectively. In contrast, and for just comparative purposes, store bought kale and spinach contained 12683IU/100g and 15134IU/100g respectively. There is no recommended daily intake of carotenoids, yet some literature suggests recommendations between 10,000IU to 83,000IU of β-carotene per day². These peppers field-grown by communities in southern Zambia resulted in high quality, pungent and highly nutritious fruit.



Graph 3: Estimated polyphenol content of each variety.



Graph 4: Estimated carotenoid content of each variety.



Graph 5: Estimated IU of carotenoids/100grams for each variety

Variety	Polyphenol concentration (GAE/gram)	Carotenoid concentration (mg/g)	Carotenoids IU/100g
Long Slim	243.94±11.13%	0.224±15.32%	37322.91
Rochelle	298.32±11.27%	0.270±8.21%	45065.59
Malaga	403.69±3.58%	0.097±19.40%	16119.32
Spinach	ND	0.090±2.63%	15134.49
Kale	ND	0.076±5.23%	12683.04
Blueberry extract	0.44 to 7.73 ¹	ND	ND

Table 2: Estimated polyphenol and carotenoid content of each variety