



Determination of capsaicinoid profile of some peppers sold in Nigerian markets

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ABSTRACT

The capsaicinoid profile of six different peppers sold in Nigerian markets was determined by Gas Chromatography-Mass Spectrometry. The capsaicinoids were extracted from the peppers using methanol as extractant and analyzed without need for derivatization. A total of eight (8) capsaicinoids were identified and quantitated: Capsaicin, Dihydrocapsaicin, Dihydrocapsaicin 1, Dihydrocapsaicin 2, Norcapsaicin, Nordihydrocapsaicin 1, Nordihydrocapsaicin 2 and Nornordihydrocapsaicin though, not fully present in all the varieties. Dihydrocapsaicin 1, Dihydrocapsaicin 2, Nordihydrocapsaicin 1, Nordihydrocapsaicin 2 and Nornordihydrocapsaicin are isomers. Seven were identified in the Cameroun pepper variety, six in "Zaria atarugu" and Miango, and five in each of the remaining varieties. In all the peppers analyzed, capsaicin had the highest relative concentration, which ranged from 27.3% in the Cameroun variety to 49.38% in the "Zaria atarugu" variety. The sum of the relative concentrations of capsaicin and dihydrocapsaicin ranged from 47.03% in the "Miango" variety to 87.3% in the "Zaria atarugu" variety.

Keywords: Capsaicinoids, Gas Chromatography-Mass Spectrometry, Methanol, Pepper

INTRODUCTION

Peppers are widely used in many parts of the world as a result of their valued sensory attributes; colour, pungency and aroma. Capsicum fruits which vary mainly in colour, size, shape, flavor, and pungency find greatest application in the food industry; as a coloring and flavoring agent in sauces, soups, processed meats, snacks, candies, soft drinks, and alcoholic beverages either in the ground form, or as an oleoresin (concentrated extract).

Capsaicinoids, also known as the phenylalkylamide alkaloid group, are the group of compounds responsible for the "heat" sensation in Capsicum fruits (peppers). The compounds are found in many foods and seasonings. They are also used as pest repellants in agriculture, and there is interest in using them as synergists with organophosphate insecticides [10]. Because of their wide use, determination of the capsaicinoid profile of peppers is desirable. Capsaicinoids are present in varieties of peppers in different amounts. Pure capsaicin is a white crystalline compound that melts at 65 °C and is volatile above this temperature. It produces a dangerous and irritating vapor. In commercial capsicums, capsaicin generally comprises 33- 59%, dihydrocapsaicin accounts for 30-51%, Nordihydrocapsaicin is 7-15%, and the remainder is less than 5% of the capsaicinoids [9].

The Scoville organoleptic test was used for the determination of pepper pungency, but has since been replaced with chromatographic methods which are considered more reliable and accurate. In recent times, High Performance Liquid Chromatography has been the method of choice for the quantitation of capsaicinoids by most workers [1, 2, 3 and 11]. Gas Chromatographic methods reported in the literature employ the use of packed chromatography columns and require sample cleanup step prior to analysis, and in most cases, there was need for derivatization [5, 8 and 7]. Recently, a group of workers, Thomas [10] carried-out an investigation on the capsaicinoid content of some peppers and reported the detection of isomers for five capsaicinoids. However, the isomers could not be quantitated due to non-availability of analytical standards.

The object of this work was to identify the capsaicinoids present in some peppers sold in retail markets in Nigeria and determine the relative concentration of the capsaicinoids by the percentage area of the peaks associated with the deprotonated molecular ion [M-H].

EXPERIMENTAL SECTION

Peppers used for this study were purchased from local retail markets in the region where they were grown, except the Cameroun pepper. The peppers used in the study include “Atarugu”, Zaria (*Capsicum annuum* var), “Tatase” Zaria (*Capsicum annuum*), Yellow pepper, Nsukka (*Capsicum chinense*), “Atarugu”, Miango (*Capsicum annuum* var) and “Atarugu”, Makurdi (*Capsicum annuum* var). After removal of the stalk, the fresh pepper samples were washed with distilled water to remove adhering soil and dirt and then dried with a clean piece of cloth before each analysis. The Cameroun dry pepper samples were dried in an oven at 60°C for 6hrs, ground using a mortar and pestle and passed through a sieve (1mm) and stored in a desiccator until required for analysis. Extraction of capsaicin from the pepper samples was done using the method described by Collins [2] with slight modifications.

GCMS Analysis Conditions

A Gas Chromatograph-Mass Spectrometer, (GCMS QP-2010, Shimadzu Analytical Instruments) was used for analyzing the samples. Injections were performed by AOC-20i Auto injector (Shimadzu, Japan). A Rtx-5MS column (5% diphenyl, 95% dimethylpolysiloxane stationary phase), 30m × 0.25mm i.d. (Restek, USA) was used. The column temperature program is as follows 60 °C for 5min, 15 °C/min to 140 °C for 0min, 25 °C/min to 280 °C for 10min (25.93 min total). The carrier gas used was helium with a flow rate of 1.61ml/min. the detector was a quadrupole mass spectrometer (MS) with EI ionization at 70eV in full scan mode.

RESULTS AND DISCUSSION

Identification of other capsaicinoids present in the peppers analyzed was only by comparing the mass spectra generated by the peak associated with the deprotonated molecular ion peak [M-H] with literature due to the non-availability of standard compounds. The relative concentration of the other capsaicinoids detected was determined by % area of the peaks associated with the deprotonated molecular ion [M-H]. The relative concentrations of the capsaicinoid analogues in the pepper varieties analyzed are summarized in Table 4.4. Also, Figures 3 to 8 show the number and relative concentrations of the capsaicinoid analogues identified in each of the varieties.

The results reveal the identification of a total of eight (8) capsaicinoids, capsaicin, dihydrocapsaicin 1, dihydrocapsaicin 2, norcapsaicin, nordihydrocapsaicin 1, nordihydrocapsaicin 2, homodihydrocapsaicin and normordihydrocapsaicin based strictly on their mass spectra. All the capsaicinoids are commonly reported in literature except normordihydrocapsaicin, which was only reported by Thomas [10]. Identification of normordihydrocapsaicin is based on the fact that the difference between it and nordihydrocapsaicin is CH₂, i.e. 14 amu which is in agreement with report by Thomas [10]. Two peaks which correspond to Dihydrocapsaicin 1 and dihydrocapsaicin 2; and nordihydrocapsaicin 1 and nordihydrocapsaicin 2 were identified for dihydrocapsaicin and nordihydrocapsaicin respectively. Similar mass spectral patterns were observed for each pair. These pairs correspond to isomers as the occurrence of isomers of capsaicinoids has been reported by several workers [2, 4 and 10]. The three (3) major capsaicinoids; capsaicin, dihydrocapsaicin, and nordihydrocapsaicin were identified in all the pepper varieties used in the study. They contribute over 90% of the total capsaicinoids in all but the miango variety (Table 1). In the “Zaria tatashe” variety, the contribution was approximately 100% as no other capsaicinoid was identified. The minor capsaicinoid, norcapsaicin identified in this study has been previously reported by other workers [12]. Seven capsaicinoids were identified in the Cameroun variety, six in “Zaria atarugu” and Miango, and five in each of the remaining varieties. Differences in capsaicinoid profile within a given variety are well established

and can be caused by variations in growing conditions or maturity [10]. Even peppers from the same plant can vary in their capsaicinoid profiles due merely to differences in post-harvest ripening conditions [10].

The mass spectra generated for each of the capsaicinoids generally agree with literature [10]. The capsaicinoids all had similar fragmentation patterns as the most stable portions of the molecules are identical. These fragments include $m/z = 41, 94, 110, 122, 136, 150$ and 194 . The seven (7) capsaicinoids identified in miango pepper are shown in the chromatogram labeled Figure 1. The spectra for the capsaicinoids identified in miango pepper are shown in Figure 2 below, where the molecular ion peak in each spectrum, corresponds to the deprotonated molecular ion peak [M-H].

In all the peppers analyzed, capsaicin had the highest relative concentration, which ranged from 27.3% in the Cameroun variety to 49.38% in the "Zaria atarugu" variety. The sum of the relative concentrations of capsaicin and dihydrocapsaicin ranged from 47.03% in the "Miango" variety to 87.3% in the "Zaria atarugu" variety. These values do not entirely corroborate the report [6] that capsaicin and dihydrocapsaicin account for ~ 91% of the total capsaicinoids in pepper fruit. This may be due to the fact that quantitation in this case, was based on relative concentrations. The chromatograms obtained for each of the pepper samples also clearly reveal that the capsaicinoids eluted according to the length of their aliphatic side chain. That is, the capsaicinoids elute according to increasing molecular weight; which is also in agreement with reports by other workers [4, 2 and 10].

Table 1: Relative Concentrations of Capsaicinoids in the Peppers (% Area of Peak)

Variety	NNDHC	NDHC 1	NDHC 2	CAP	DHC 1	DHC 2	HDHC	NCAP	CAP + DHC+ NDHC
"Zaria atarugu"	1.24	5.72	3.54	49.38	34.08	3.84	2.18	N.D.	96.56
Makurdi	N.D.	15.86	10.85	43.47	22.24	4.53	3.05	N.D.	96.95
Miango	9.24	27.15	10.05	31.67	12.9	2.46	6.53	N.D.	84.23
"Zaria tatase"	N.D.	16.88	10.56	34.26	10.73	27.56	N.D.	N.D.	99.99
Nsukka Yellow	N.D.	10.45	8.84	42.58	22.21	7.81	8.12	N.D.	91.89
Cameroun	N.D.	23.42	11.78	27.3	21.82	6.21	8.69	0.77	90.53

NNDHC – Nornordihydrocapsaicin

NDHC – Nordihydrocapsaicin

CAP – Capsaicin

HDHC – Homodihydrocapsaicin

NCAP – Norcapsaicin

ND – not detected

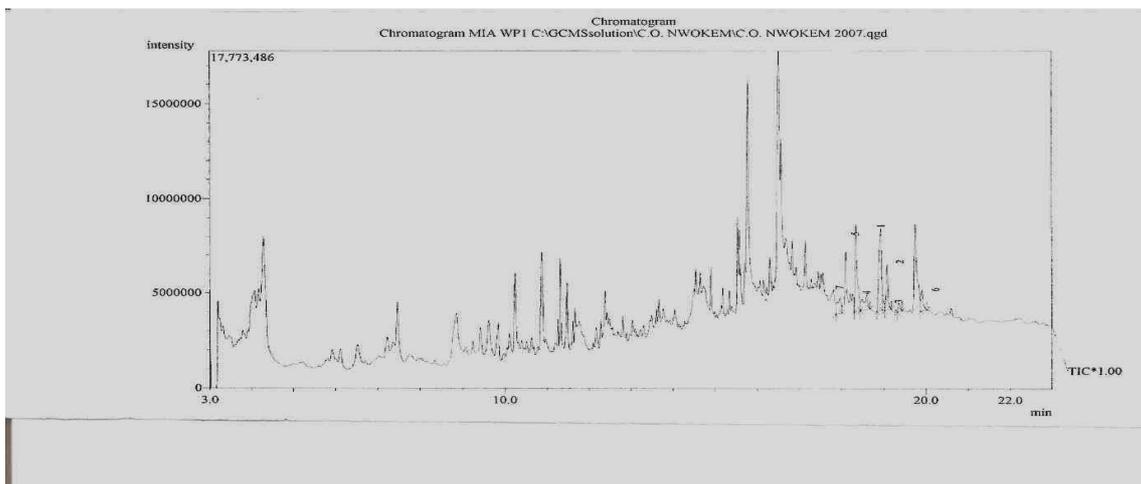


Figure 1: Chromatogram of Miango pepper with peaks representing capsaicinoids labeled 1 to 7

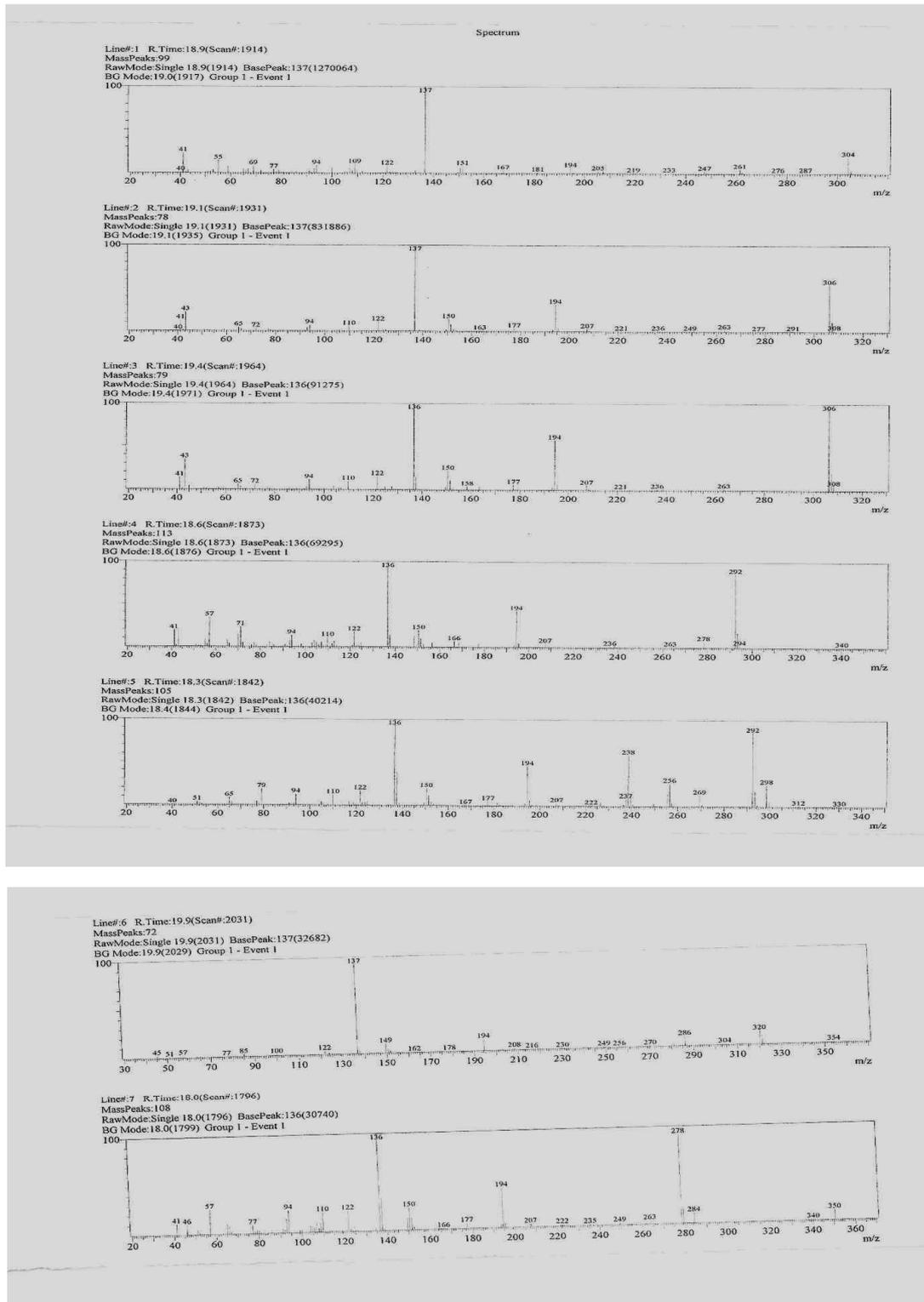


Figure 2: Mass spectra of the various capsaicinoids identified in Miango pepper

Molecular weights of Capsaicinoids and the deprotonated molecular ion peak [M-H]

Capsaicin	305 g/mol	[M-H] = 304
Dihydrocapsaicin	307 g/mol	[M-H] = 306
Nordihydrocapsaicin	293 g/mol	[M-H] = 292
Nornordihydrocapsaicin	279 g/mol	[M-H] = 278
Norcapsaicin	291 g/mol	[M-H] = 290
Homodihydrocapsaicin	321 g/mol	[M-H] = 320

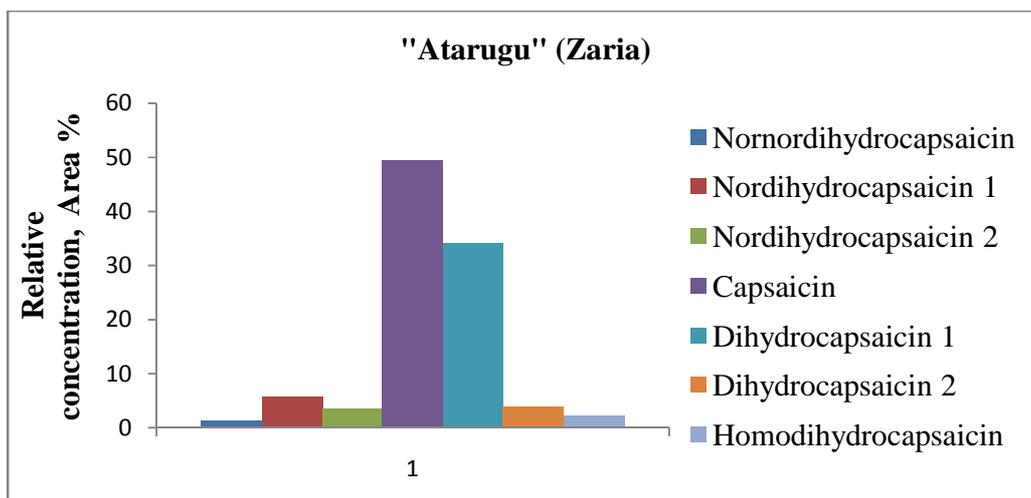


Figure 3. Chart Showing the Relative Concentrations of the Capsaicinoids identified in the "Atarugu" (Zaria) Pepper

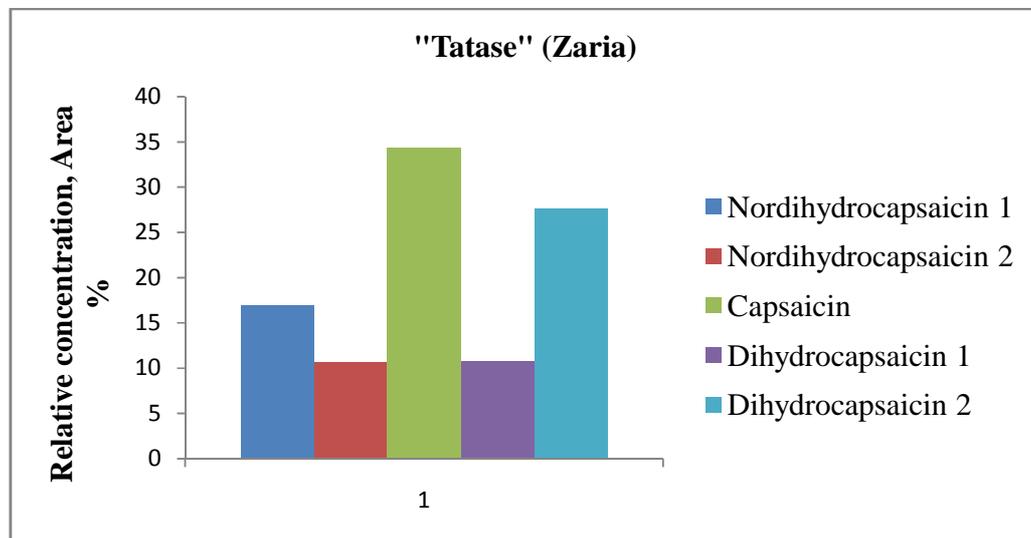


Figure 4. Chart Showing the Relative Concentrations of the Capsaicinoids identified in the "Tatase" (Zaria) Pepper

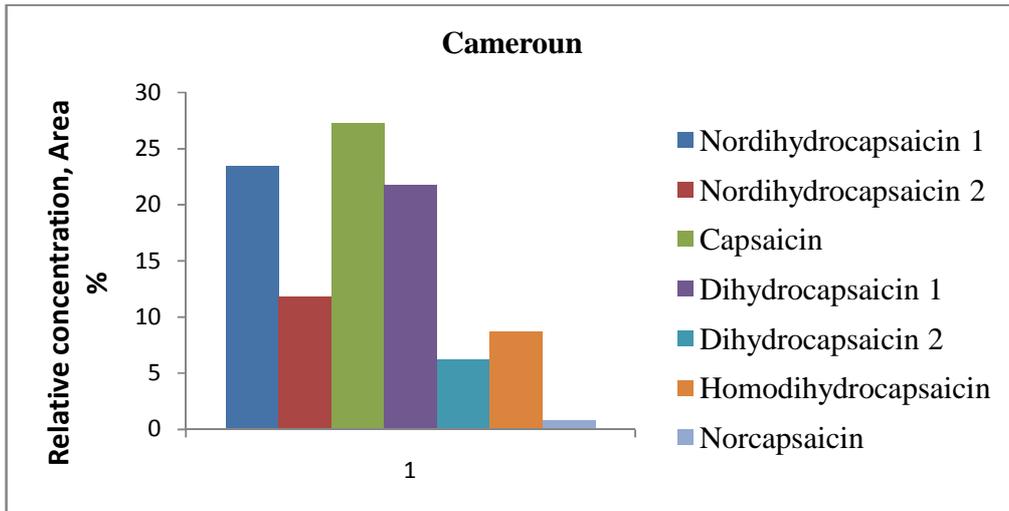


Figure 5. Chart Showing the Relative Concentrations of the Capsaicinoids identified in the Cameroun Pepper

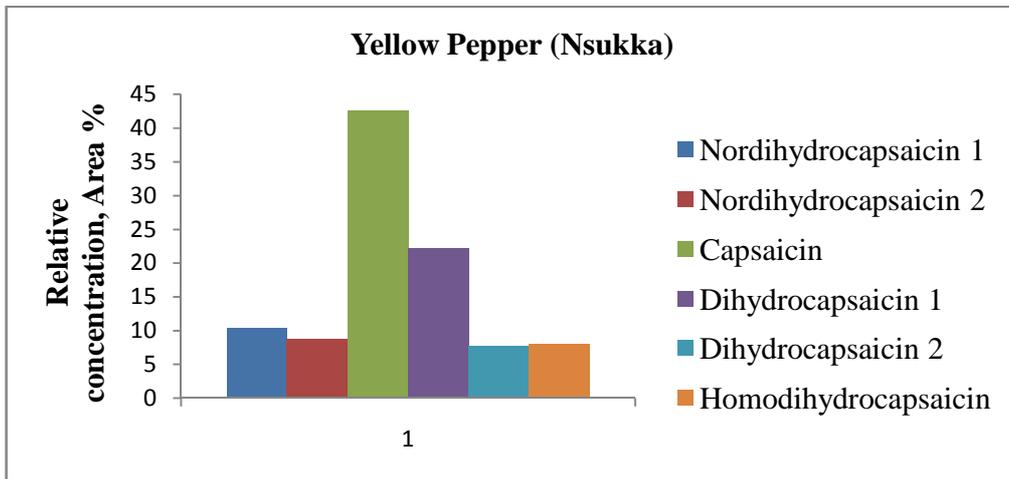


Figure 6. Chart Showing the Relative Concentrations of the Capsaicinoids identified in the Yellow Pepper (Nsukka)

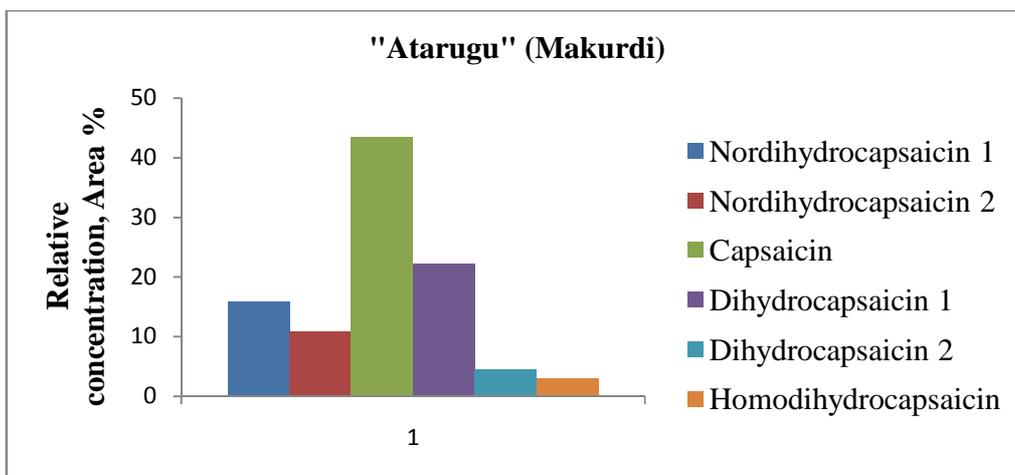


Figure 7. Chart Showing the Relative Concentrations of the Capsaicinoids identified in the "Atarugu" (Makurdi) Pepper

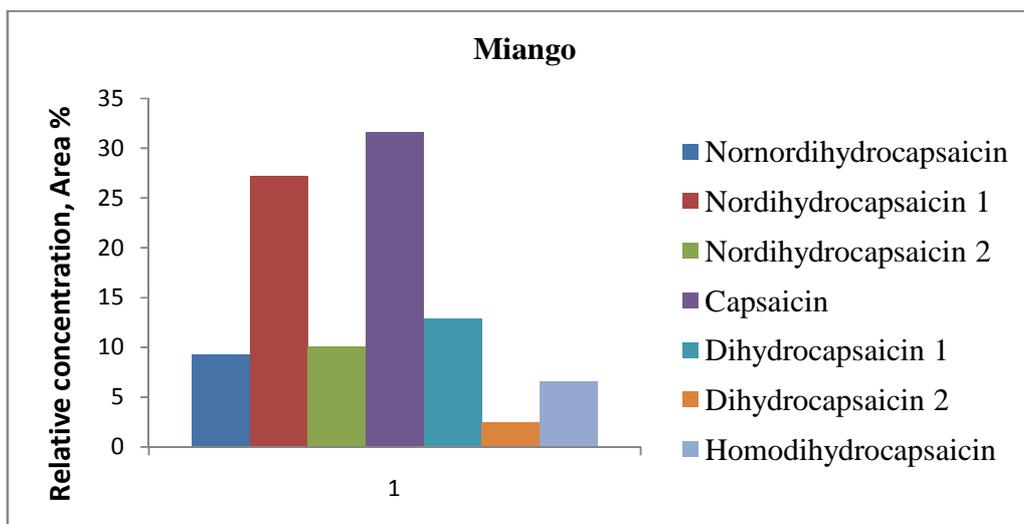


Figure 8. Chart Showing the Relative Concentrations of the Capsaicinoids identified in the "Atarugu" (Miango) Pepper

CONCLUSION

This study reports the identification of eight capsaicinoids in some peppers sold in retail markets in Nigeria. This includes the capsaicinoid Nornordihydrocapsaicin which of all literature consulted, was only reported by Thomas *et al.* These results, though relative, show that capsaicin, is the most abundant capsaicinoid in pepper. However, there is need for further work on the quantitation of individual capsaicinoid based on the use of external standards.

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