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Full Length Research Article

EVALUATION OF CAPSAICIN CONTENT IN PARTS OF SOME PEPPERS GROWN IN NIGERIA

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ABSTRACT
The capsaicin content in parts (placenta, seeds and periderm) of some peppers grown in some parts of Nigeria was evaluated. Capsaicin was extracted from the pepper parts was carried-out via the solvent extraction technique using methanol. A Gas Chromatograph-Mass Spectrometer, (GCMS QP-2010, Shimadzu Analytical Instruments) was used for determination of capsaicin content. Capsaicin content was highest in the placenta, followed by the seeds for all the pepper samples utilized in the study. The outer flesh (periderm) had the lowest content of capsaicin. The capsaicin content of the placenta ranged from 1.1652±0.0002 in the Yellow pepper from Nsukka (*Capsicum chinense*) to 0.3226±0.0002mg/g in the "Atarugu" pepper (*Capsicum annuum var*) obtained from Zaria. The capsaicin content of the outer flesh ranged from 0.4619±0.0050 in the Yellow pepper from Nsukka (*Capsicum chinense*) to 0.0070±0.0002mg/g in the "Atarugu" pepper *(Capsicum annuum var*) obtained from Zaria. The parts of the yellow pepper (*Capsicum chinense*) had higher capsaicin content than the respective parts of the remaining peppers analyzed in the study. Also, the pepper parts of *Capsicum chinense* variety had higher capsaicin contents than those of *Capsicum annuum*, with parts of the large-fruited *Capsicum annuum var* having the least capsaicin content.

Keywords: Pepper, Capsaicin, Solvent Extraction, Gas Chromatograph-Mass Spectrometer.

INTRODUCTION

Fruits of chili pepper plants that belong to the family Solanaceae, genus Capsicum are among the most consumed spices throughout the world and are very important commercially (Sganzerla *et. al.,* 2014). Capsicum constitutes the world's second most important crop after tomato (Yoon *et al.,* 1989). The genus Capsicum has approximately 27 species, out of which five are domesticated: *Capsicum annuum, Capsicum baccatum, Capsicum chinense, Capsicum frutescens* and *Capsicum pubescens. Capsicum annuum*, which is cultivated mainly as a vegetable is the most widespread. 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) (Sganzerla *et. al.,* 2014). The consumption of chili peppers is due mainly to their very pungent flavor. The pungency is caused by capsaicinoids and is proportional to the combined concentrations of the various vanillyl amides that are collectively referred to as capsaicinoids (Sganzerla *et. al.,* 2014).

Pungency, a commercially important attribute of peppers, is due to the presence of six chemically related compounds; capsaicin, dihydrocapsaicin, norcapsaicin, nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin which constitute the "capsaicinoids" group (Perucka and Materska, 2001). The two most abundant capsaicinoids in peppers are capsaicin and dihydrocapsaicin, both constituting 90%, with capsaicin accounting for ~71% of the total capsaicinoids in most of the pungent varieties (Kosuge and Furuta, 1970; Ryu *et. al.,* 2017). Capsaicin content of peppers is one of the major parameters that determine its commercial quality.

Capsaicin, a major alkaloid among capsaicinoids produced only in *Capsicum* fruits, has wide application in the food, medicine, and pharmaceutical industries. It has been used as an analgesic against arthritis pain and inflammation (Deal *et al.,* 1991; Ryu *et. al.,* 2017). It has been reported to show anticancer effect, as it kills certain cancer cells (Moore and Moore, 2003, Ryu *et. al.,* 2017) and neurogenic inflammation (burning and stinging of hands, mouth and eyes) (Szolcsanyi, 2004, Ryu *et. al.,* 2017). The latter property is the reason capsaicin finds application in the formulation of defensive pepper sprays. Capsaicin has also been reported to show protective effects against cholesterol and obesity (Kempaiah *et al.,* 2005, Ryu *et. al.,* 2017).
However, the Scoville organoleptic test has been replaced with chromatographic methods which are considered more reliable and accurate (Ryu *et. al.,* 2017). High Performance Liquid Chromatography has been the method of choice as most workers have reported the need for derivatization when using Gas Chromatography (Sganzerla *et. al.,* 2014; Ryu *et. al.,* 2017).
The aim of this work was to employ the use of a simple method to extract and determine the capsaicin content in the parts of varieties of peppers grown in some parts of Nigeria using Gas Chromatography-Mass Spectrometry with a view to establishing the part with highest content. This work also seeks to carry-out quantitation without the need for derivatization of the samples before analysis.

MATERIALS AND METHODS

Peppers used for this study were purchased from local retail markets in the region where they were grown. The samples were authenticated at the herbarium, Department of Botany, Ahmadu Bello University, Zaria. 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).
Extraction of capsaicin from the pepper samples was done using the method described by Collins *et al.* (1995) and Nwokem *et al.* (2010).

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The whole peppers were finely ground in a blender and weighed into 120 cm^3 glass bottles with lid and 20 cm^3 of methanol added. The mixture was placed in a water bath maintained at 80 °C with swirling. The homogenate was filtered into a 50 cm^3 volumetric flask. The supernatant was drawn-out by means of a 5 cm^3 disposable syringe and filtered using a 0.45 µm membrane filter unit into a 2 cm^3 glass sample vial. The vial was capped and stored at 5 °C until analysis.

GCMS Analysis Conditions

Injections were performed by AOC-20i Auto injector (Shimadzu, Japan). An 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.

Standard Curve

The following standard solutions were prepared from a stock solution of capsaicin using serial dilution: 20, 40, 60, 80 and 100mg/dm^3.

The standard solutions were run on the Gas Chromatograph Mass Spectrometer (GCMS) and the standard curve plot of peak area against concentration obtained. Figures 1 and 2 below, show the Chromatogram. The standard curve is shown in the quantitative results.

RESULTS AND DISCUSSION

Although capsaicin is soluble in all organic solvents, methanol was chosen as extraction solvent because it is non-toxic and has been reported to give high extraction efficiency with reduced amounts of pigments and oils extracted with capsaicin as compared to other suitable solvents like acetone (Attuquayefio and Buckle, 1987; Collins *et al.,* 1995). Extraction and quantitation was carried-out in triplicate for each variety.

The standards used for the standard curve were injected at intervals during sample injection to confirm retention time.

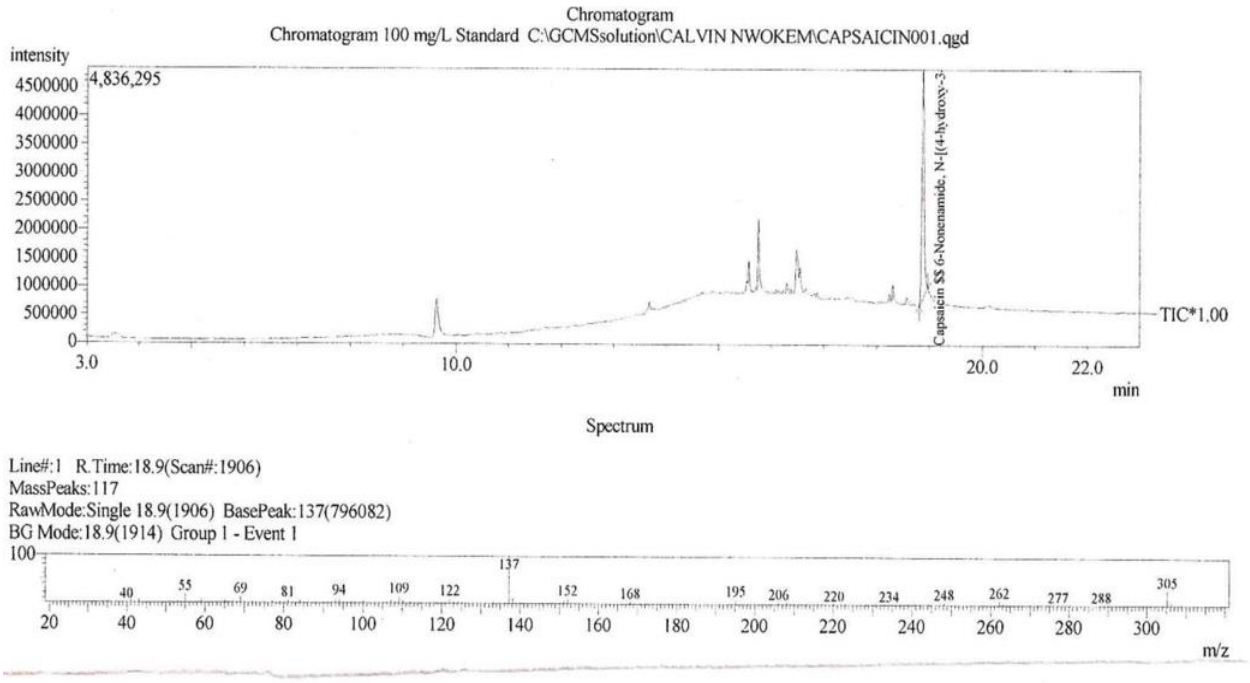

Figure 1: Chromatogram and mass spectrum for 100 mg/dm^3 standard capsaicin

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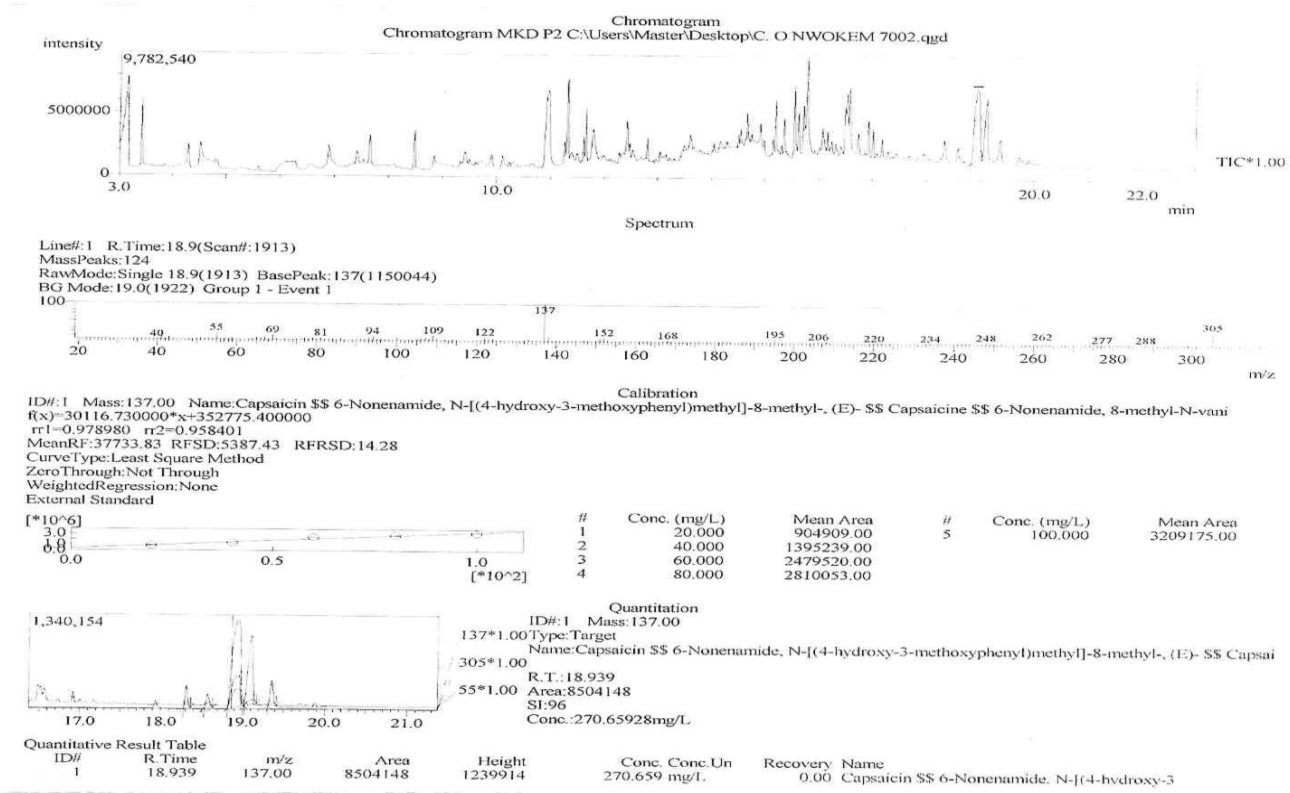

Figure 2: Chromatogram, mass spectrum and quantitative results for placenta of (*Capsicum annuum*) Makurdi pepper

Table 1: Capsaicin content of the various parts of the peppers analyzed[a]

	Placenta		Seeds		Outer flesh	
Variety	Mean	Std Dev.	Mean	Std Dev.	Mean	Std Dev.
Capsicum annuum var (Zaria)	1.1652	0.0048	0.2821	0.0147	0.2191	0.0002
Capsicum annuum var (Makurdi)	1.3215	0.0005	0.6712	0.0444	0.1097	0.0078
Capsicum annuum var (Miango)	1.0956	0.0338	0.9758	0.0107	0.2368	0.0042
Capsicum annuum (Zaria)	0.3226	0.0024	0.0259	0.0001	0.0070	0.0002
Capsicum chinense (Nsukka)	1.3286	0.0425	0.7334	0.0156	0.4619	0.0050

[a] values in mg/g ± SD on fresh weight basis, n = 3

The parts of the Yellow Pepper from Nsukka, South-East Nigeria had the highest capsaicin content as compared with those of the remaining peppers studied. *Capsicum chinense is* recognized as the most pungent species within the domesticated *Capsicum* species (Guillen *et. al.,* 2018). Also, according to the pungency in Scoville (SHU) categories, fruits of *C. chinense* are classified as very pungent (Guillen *et. al.,* 2018). This is in agreement with findings reported by our research group (Nwokem *et al*., 2010) in which we discovered that the Yellow pepper (*Capsicum chinense*) had the highest capsaicin content of the peppers used in that study.

Therefore, the results of this study show that Capsaicin content of the parts of *Capsicum annuum* peppers is lower than that of *Capsicum chinense* pepper parts. This also can be attributed to the fact that *Capsicum chinense* is recognized as the most pungent of the Capsicum species. This is in also in agreement with previous reports by our research group (Nwokem *et. al,* 2010) and other workers (Sanathombi and Sharma, 2008).

It clearly follows from the results shown in Table 1, that the distribution of capsaicin in the pepper fruit is varied and uneven. In each of the pepper varieties used in this study, the placenta had the highest capsaicin concentration followed by the seeds, whereas the outer flesh had the lowest concentration. This is due to the fact that capsaicinoids are synthesized in the placenta and that the presence of capsaicinoids in other parts of the fruit appears to be the result of leakage and diffusion from the placenta to the seeds and outer flesh. These findings are in agreement with reports by other workers (Estrada *et al.*, 2002; Kozukue *et al.*, 2005; Sanathombi and Sharma, 2008).

Conclusion

The results from this study which employed the use of Gas Chromatography-Mass Spectrometry show that the placenta has the highest concentration of capsaicin in the pepper fruit. Therefore, it is recommended that the placenta be employed for use as a source of capsaicin rather than the seeds and outer flesh which constitute the bulk of the pepper fruit, but have lower capsaicin content.

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