SEPARATION AND IDENTIFICATION OF NORMAL HYDROCARBON, NONADECANE IN THE CABBAGE VEGETABLES SAMPLES (*Brassica oleracea VAR. CAPITATA F. ALBA*) BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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ABSTRACT

Toxic normal hydrocarbon (NH), nonadecane in the methanolic extract of the whole of cabbage samples collected from different districts of Bangladesh was analyzed by GC-MS. It was observed that NH deposition on the samples takes place in different morphological parts of the biological materials. The NH, nonadecane, was found in the cabbage samples collected from the highway road side by the extraction of methanol. The identification and quantification of the title compounds have not been reported previously in the cabbage sample.

Keywords: *Cabbage;* brassica oleracea *var. capitata f. alba; quantification; normal hydrocarbon, nonadecane, GC-MS.*

INTRODUCTION

It is well to known that most of the polynuclear aromatic hydrocarbons, polychlorinated biphenyls and cyclic organochlorines are toxic, carinogenic and mutagenic [1-9]. These organocompounds are produced worldwide on an enormous scale. The common features of these organic chemical pollutants are high hydrophobicity and resistance to environmental degradation [10]. These pollutants can be easily found in the samples of environmental interest, e.g. in the atmospheric air, water, soil and sediments [1,4,9-17] and in foodstuffs [18-22].

Toxicological data are not yet available for the organic contaminants generally found in water. Of the 298 volatile organic compounds identified in drinking water, the United States National Academy of Sciences selected 129, including 55 pesticides, as being of concern to the public health [23].

Normal hydrocarbons, e. g. n-decane, n-undecane, n-dodecane, n-tetradecane found in drinking water have been recognised as potent promoters in carcinogenesis [24]. Normal hydrocarbons of higher molecular weight such as $C_{19}H_{40}$-$C_{35}H_{72}$ present in the atmosphere of the working place of Paraffin Extraction Industries have shown the carcinogenesis effect. For example, in this industrial area, 1393 out of 100,000 workers and 340 inhabitants out of 100,000 population in the surrounding area were found to suffer from skin-cancer disease [25]. These findings on high molecular weight hydrocarbons indicate that hydrocarbon contamination of food articles should be carefully evaluated in order to find its health implications.

Scientific data are very much lacking on the concentration level that may cause acute toxicity and the maximum admissible level of the individual constituents of petroleum oils in the environment, which are not harmful with long term or continuous exposure. Smith reported [26] that the phytotoxic effect of hydrocarbons on terrestrial plants increases in the order: straight chain paraffins, olefins, cycloparaffins, aromatics, etc.

The aim of the present study is to identify and quantify the highly toxic normal hydrocarbon, nonadecane in the methanolic extract isolated from the whole cabbage sample by GC-MS.

EXPERIMENTAL

Chemicals

Methanol and dichloromethane (Merck, Germany), solvents used in this experiment were of HPLC grade. Anhydrous sodium sulphate (Merck, Germany) was cleaned by heating at 200 °C before use. Silica gel (60-120 mesh, Merck, Germany) activated at 400 °C for 12 h prior to use. Nonadecane of (Sigma-Aldrich) was used as standard in the present study.

Plant material

The cabbage samples were collected from the nearest different districts of Dhaka Metropolitan City (DMC), Dhaka, Bangladesh in January 2006 and initially identified by morphological features and database present in the library at the herbarium of the Department of Biology, University of Dhaka, Dhaka, Bangladesh.

Isolation and preparation of crude extracts

The collected whole cabbage samples were washed by tap and de-ionized water to removed dusts and any other foreign particles. After having washed,

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the cabbage samples were cut into small pieces and dried either by sunlight or oven below temperature 40 °C. The dried cabbage samples were pulverized into powder form. The dried powder (50 g) was extracted three times with methanol (200 mL x3) at 120 °C for 1 h. It was then filtered and the filtrate was evaporated near to dryness by Kuderna-Danish evaporator.

Clean-up procedure

The cleanup column (i. d. = 1 cm) was filled with cotton in the bottom. An activated silica gel (17 g) soaked with dichloromethane was loaded into the cleanup column (5 cm), which was then topped with 1.5 cm of anhydrous sodium sulfate. Ten milliliters of dichloromethane was added to wash the sodium sulfate and the silica gel. The dried 1 mL sample was then transferred into the column, the vessel was rinsed twice with 2 mL dichloromethane, which was also added to the column. Fifty milliliters of dichloromethane was added to the column and allowed to flow through the column at a rate of 3–5 mL/min, and the eluent was collected. The collected eluent from the cleanup procedure was reconcentrated to 1 mL with K-D concentrator.

GC-MS analysis

The GC-MS analysis of the methanolic crude extract of cabbage samples was performed using a Varian GC-MS (Model Varian CP 3800) equipped with a VF-5 fused silica capillary column (30 m x 0.25 i. d., film thickness 0.25 μm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. Injector and mass transfer line temperature were set at 250 and 300 °C, respectively. The oven temperature was programmed from 50 to 200 at 8 °C/min, and then held isothermal for 20 min. and finally raised to 300 °C at 10 °C/min. Diluted samples (1/100, v/v, in methanol) of 1 μL were manually injected in the split less mode. Identification of compounds of the methanolic crude extract was based on GC retention time on VF-5 capillary column, computer matching of mass spectra with standards (Mainlab, Replib and Tutorial data of GC-MS systems) and, whenever possible, by co-injection with authentic compounds [27].

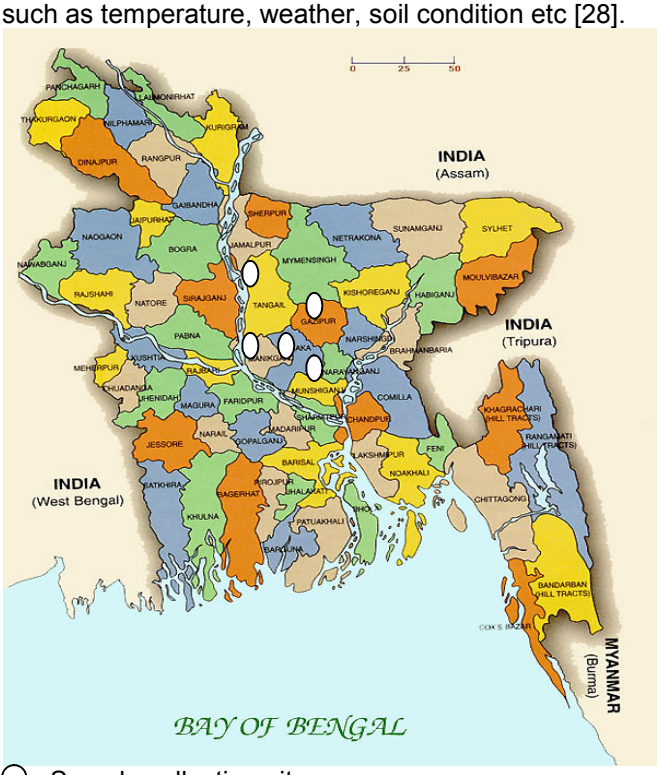
Preparation of standard

Calibration graphs for the samples treated according to the described analytical procedure were made using the SIM mode. Different concentrations of nonadecane (0.5, 1, 5 and 100 ng/L) were used for calibration curve.

RESULT AND DISCUSSION

Bangladesh is an agricultural country. Vegetables, crops and fruits are grown here in plenty, mainly in the winter season. Cabbage is one of the most commonly used as vegetables because it is cheap and available all over the Bangladesh throughout the winter season. Again, to use it for other seasons, sometimes villagers harvest the cabbage samples, cut it into small pieces and dry it under sunlight for storage. This harvested and dried product is also used as animal feed. So considering the fact, the need for checking any toxic compounds contaminating it or not cannot be overlooked. For this reason, the objective of this work is to check the level highly toxic normal hydrocarbons (NHs) in the methanolic extract isolated from the whole cabbage sample by GC-MS.

The dried cabbage powder sample was extracted with methanol and filtered. The filtrate was cleaned up to remove the vegetable fats and oily or gammy compounds. The methanol solvent was evaporated to dryness by Kuderna-Danish evaporator. From the concentrated extract only 1 μL was injected to the GC-MS. We have collected cabbage samples from some nearest districts of Dhaka Metropolitan City (DMC) in the month of January 2006 **Fig. 1**. We know that the chemical composition of all kinds of fruits, vegetables and plants depends on the geographical distribution such as temperature, weather, soil condition etc [28].



○ Sample collection site

Fig 1. Five cabbage samples collected from the nearest district of DMC in Bangladesh.

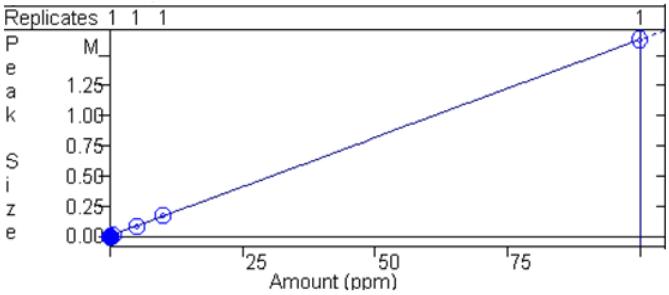


Fig 2. Calibration curve of nonadecane standard (Column: VF-5 (l. 30 m, i.d. 0.25, film thickness 0.25 µm); delay: 5 min; Temperature Program: 50°C(1)—› 200 °C (8 °C/min) —›300°C (10 °C/min); Injector Temperature: 250 °C; Split: 20%; Injection volume: 1 µL; Carrier gas: He; Flow rate: 1mL/min.)

The separation, identification and quantitative determination of nonadecane were done by external calibration curve method. The calibration curve already prepared with known concentration of nonadecane is detailed below **Fig 2**. Standard curve for nondecane generated by plotting the area of four spots vs. the concentration, gave high correlation coefficients.

The nonadecane is identified by comparing their retention time (RT) on the total ion chromatogram (TIC) of the substance in the samples **Fig. 3-8** with that of the respective compound in a standard solution analyzed under the same conditions. The existing GC-MS/MS library database (NIST) shows the RT of nonadecane from the cabbage samples in **Fig. 3-8** as - 22.242 (base peak, 57.1).

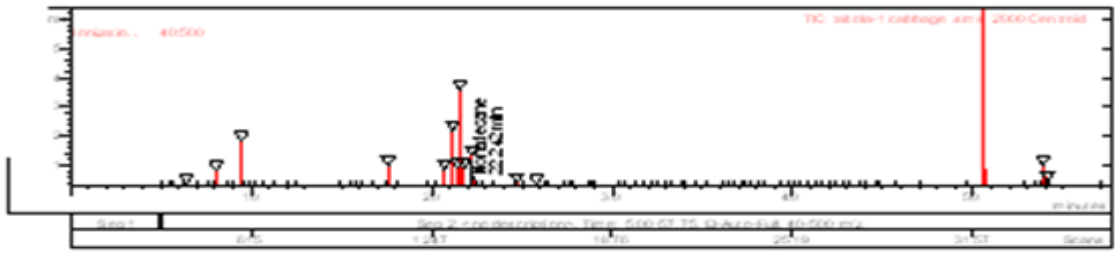


Fig 3. TIC Chromatogram of the cabbage samples from the Talola, Dhaka (Column: VF-5 (l. 30m, i.d. 0.25, film thickness 0.25 µm); delay: 5 min; Temperature Program: 50 °C(1)—› 200°C (8 °C/min) —›300 °C (10 °C/min); Injector Temperature: 250 °C; Split: 20%; Injection volume: 1 µL; Carrier gas: He; Flow rate: 1mL/min.)

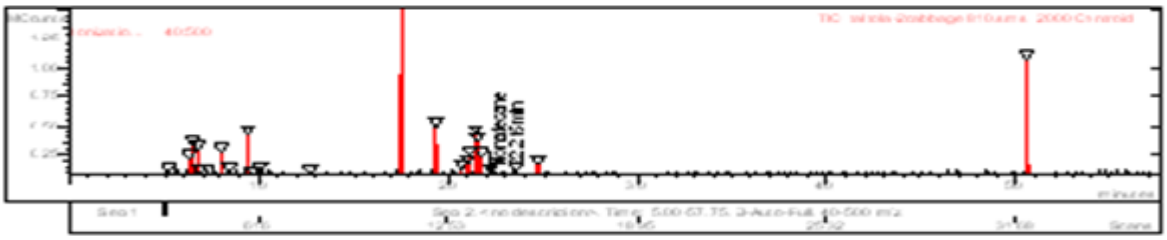


Fig 4. TIC Chromatogram of the cabbage samples from the district of Tangail

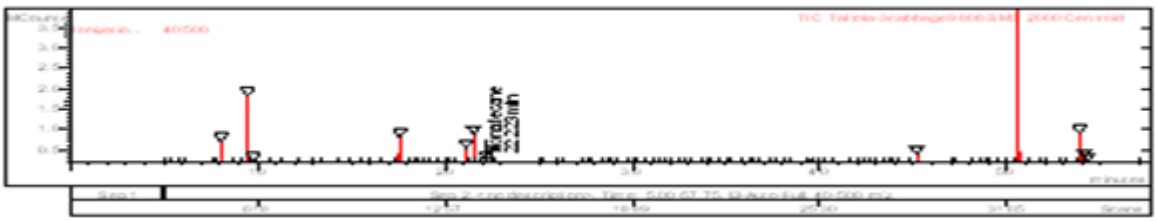


Fig 5. TIC Chromatogram of the cabbage samples from the district of Manikgonj

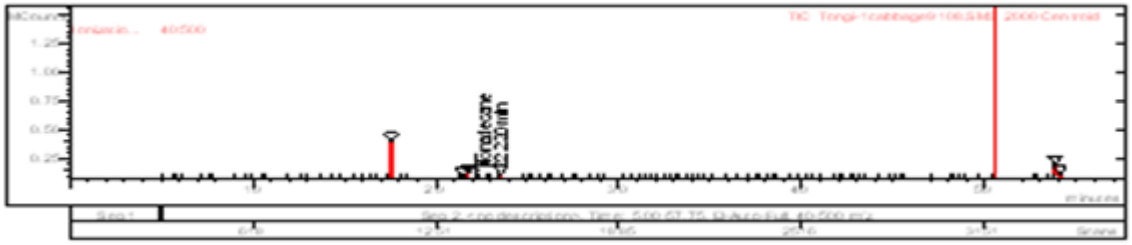


Fig 6. TIC Chromatogram of the cabbage samples from the district of Tongi, Dhaka

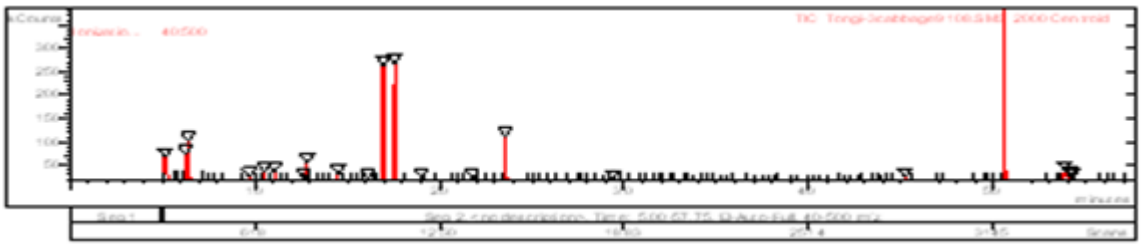


Fig 7. TIC Chromatogram of the cabbage samples from the district of Gazipur

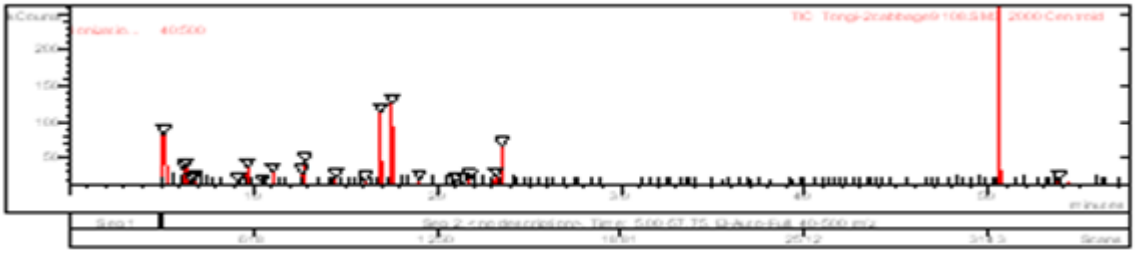


Fig 8. TIC Chromatogram of the blank samples

Table 1. Concentration of nonadecane in the five cabbage samples from the nearest district of DMC, Bangladesh

Sl. No	Cabbage samples, District	Location	Concentration (mg/g)
1	Taltola, Dhaka	Highway road side	7.665 µg/g
2	Tangail	Road side	0.242 µg/g
3	Manikgonj	Highway road side	3.817 µg/g
4	Tongi, Dhaka	Away from road side	ND*
5	Gazipur	Away road side	ND*
6	Blank	-	ND*

*ND=Not detectable

The methanol crude extract contains a complex mixture consisting of mainly flavonoids, alkaloids, caffice acid, oxygenated mono, di and triterpenes, and mono and sesqueterpene hydrocarbons [29].

Firstly for our experiment, we have taken five cabbage samples, which were collected from the nearest district of the DMC. The collected samples were cultivated and harvested during the winter season. The concentration of nonadecane, a toxic normal hydrocarbon, in the five cabbage samples were measured by GC-MS and the results calculated from the external curve method Table-1.

Linear responses were achieved for nonadecane in the concentration range for the cabbage samples with the values 7.665, 0.242, 3.817, ND and ND µg/g respectively. Over this concentration range, the linear regression analysis of peak areas (y) in function of concentration (x), calculated by least square method, leads to the following equations: y = 16,276x +4,168 (r^2 = 0.99) for nonadecane.

From experiment we have found that three cabbage samples out of five contain toxic nonadecanebut the concentration is low to reach the permissible limit [30]. Although the method had the low detection limits, no analytes were detected in the blank mixture samples. We have also seen that the NHs contamination in vegetables, fruits and plants is mainly depended on the sample collection site. In the road side, the cabbage samples are normally contaminated by the NHs, but away from the roadside, no NHs detected by the GC-MS in our experiment Table 1. So from our experiment we conclude that the cabbage sample was contaminated mainly by the vehicle exhaust or coal tar.

CONCLUSION

The method, combination of liquid-phase extraction with gas chromatographic–mass spectrometric towards analysis of trace normal hydrocarbon, nonadecane in cabbage samples, was reported for the first time by the authors in our laboratory. The method has favorable extraction effect and higher enrichment factor, especially to normal hydrocarbons. This reliable, rapid and convenient method was applied successfully to determine other normal hydrocarbons and also polycyclic aromatic hydrocarbons in the cabbage and other biological sample. From the data of nonadecane, it is confirmed that the vehicular emission is the major source of NHs and it has polluted our environment and foodstuff.

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