Control of Pungent Active Principles from Indigenous Peppers, by Chromatographic Techniques Coupled with Mass Spectrometry, as a Method of Identifying and Confirming the Separated Compounds

DELIA NICA-BADEA*
Constantin Brancusi University of Târgu – Jiu, Faculty of Medical and Behavioral Sciences, 30 Way Heroes Str., 210135, Târgu - Jiu, Romania

For analysis of pungent active principles (capsaicinoids) and other major components in peppers (Capsicuum annuum), chromatographic and spectroscopic techniques are used for separation, detection and quantification. In this experiment, is presented opportunity to apply of a method (TLC and GC-MS) for the detection of capsaicin in varieties of peppers, fresh or as paprika from northern Oltenia. The active components have been extracted from peppers and commercial peppers powder, separated by thin layer chromatography (TLC) silica gel 60 plates with concentrating zone (Merck), mobile phase toluene-acetone-chloroform mixture (45:30:25, v / v), detection by exposure to iodine vapor. The spots assumed to be generated by capsaicin, was extracted and analyzed by gas chromatography (GC) coupled with mass spectrometry (MS). To confirm the separate components and analyzed were compared to the spectra in the library data (Wiley Library 275). By combining the three methods carry out the separation and identification of these active substances without the use of standards, the result is similar. The discovery in the analyzed samples of synthetic or natural substances added to foods based on commercially peppers (coloring, fatty acids etc.), is a problem of food security.

Keyword: Capsicum annuum, capsaicin detection, TLC, GC/MS, Mass library spectra

Genus *Capsicum* is a member of family Solanaceae and has five species that are commonly recognized as domestic. Spicy taste of pepper varieties of *Capsicum annuum* is due capsaicinoids content [1]. The intracellular localization of capsaicinoids is first of all, in the vacuole [2]. Capsaicinoids is a series of very pungent vanilla-amide alkaloid: a) (E)-N-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methylnon-6-enamide (Capsaicin); b) N-[(4- hydroxy-3-methoxyphenyl)methyl]-8-methylnonan-amide (Dihydrocapsaicin); c) (E)-N-[(4-hydroxy-3-methoxy-phenyl)methyl]-7-methyloctanamide (Nordihydro-capsaicin); d) E)-N-[(4-hydroxy-3-methoxy phenyl) methyl]-9-methyldec-6-enamide (Homodihydro-capsaicin and Homocapsaicin).

The active principle responsible for the pungent taste, hot, spicy, exciting, determined by capsaicin compounds showing percentage varies by species, varieties, area: 0.1-0.9% of vegetable fruit; 0.005 powder paprika to 1.3% in very hot chili pepper [3]. Capsaicin is the major capsaicinoid in hot peppers, followed by dihydrocapsaicin determines 90% of the total pungency of pepper fruits, compared with the minor capsaicinoids: nordihydrocapsaicin, homo-dihydrocapsaicin and homocapsaicin [2, 4]. *Capsicum annuum* species, contained a wide range of nutritional components and pharmacologically active metabolites [5]. The concentrates of the capsaicinoid, in the form of creams concentration of 14% , oleoresin with a concentration between 67 to 78% are used for the preparation of various food products, food, drinks, making pungent levels of up to 150,000 Scoville Heat Units (SHU). Defensive sprays used for animals, people end up creating concentrations of over 430,000 to 2000,000 Scoville Heat Units Oleoresin Capsicum SHU OC [3].

Capsaicin and its analogues were used topically to treat chronic pain syndromes musculoskeletal pain, osteoarthritis, rheumatoid arthritis, post-herpetic neuralgia and diabetic neuropathy [6, 7]. Control of these active pungent ingredients in the medical, food, shopping, and in the defensive sprays is a matter of ensuring food safety and health [3]. Growing interest in capsaicin has led to characterization methods and analysis such as spectrophotometry UV-VIS [8, 9] and chromatography. Chromatographic methods, in particular Thin-Layer Chromatography/High-Performance Thin-Layer Chromatography (TLC/HPTLC) and High-Performance Liquid Chromatography (HPLC), are used extensively for qualitative and quantitative determination of this principle from the hot chili peppers, food and pharmaceutical products. [10-14, 1].

Due to the prevalence, diversity, physiological role and pharmacodynamics action of the active components of peppers is necessary to achieve control of active principles from existing plant samples. This purpose, we have developed an original method for the separation and identification of capsaicin from Romanian peppers, using thin layer chromatography and gas chromatography coupled with mass spectrometry as a way of confirmation and quantification by using libraries spectra analysis of active substances excited (Wiley Library) [15, 16].

Experimental part

Capsicum annuum pepper varieties were collected from the cultivated flora in north and north-west Oltenia, Romania. In the first phase, capsaicin derivatives analysis was performed an exploration of their extraction from peppers of different varieties grown in the country, fresh or

* email: nicabadeadelia@yahoo.com; Tel.: 0762684144

in the form of powder obtained by drying and grinding. [17]. The stationary phase used: UV 254 silica gel (Macherey-Nagel); RP-18 silica gel (Macherey-Nagel); silica R (Cluj-Napoca); silica gel 60 with concentrating zone Merck. Mobile phase used: toluene - acetone - chloroform (45/30/25); petroleum ether - acetone (42.5/7.5); methanol - water (1/1) plus 1.5% silver nitrate. The development was done by ascendant technique, one-dimensional unsaturated normal rooms. Visualization by spraying and exposure to iodine vapor 0.1% solution in ethyl alcohol.

In the second part of the experiment, extractions were made from two samples of commercially available paprika and from hot pepper (Oltenia), green and red, in this order: a) red hot pepper - powder; b) red hot pepper ; c) sweet pepper- powder ; d, e, f) green hot pepper in different concentrations. They were extracted each time 10 g of vegetable finely divided by 200 mL solvent. Extraction was performed by Soxhlet and four-hour from the start of reflux. The chloroform was used as solvent, solvent extraction credited as optimal [17, 18]. The solutions resulted after the extraction, was concentrated in a rotary evaporator up to one volume of about 5 mL. The obtained solutions were filed in flasks of 10 mL to which was added the solutions of the flushing, followed by addition of chloroform up to the mark. From the extracts were applied volume of 3 mL/ spot paprika and red pepper samples and 1, 3, 6 mL green peppers on silica gel 60 plates with concentrating zone Merck. The plates were developed in the unsaturated normal chromatography chamber, using as a mobile phase mixture of toluene-acetone-chloroform (40:35:25 or 45:30:25, v / v). Visualization was achieved by spraying with 0.1% alcoholic solution of 2, 6-dichloro-quinone-4-diclorimidã and exposure to iodine vapor.

Spotlights with the same R_f value with standard of capsaicin were cropped from the plates, visualized with iodine vapor and the organic compound was extracted with chloroform. The extract was analyzed by a Hewlett Packard 5890 gas chromatograph equipped with a mass detector 5972 MS. Operating conditions: capillary column MP5-MS (30 x 0,.2 x 0.25), helium carrier gas, temperature program 60^0 C at 3 ° C / min. The temperature of the transfer line was 240^0 C.

Rezults and discussions

The analysis results of the separation and identification of the active principle: capsaicin and other compounds of different varieties of peppers by TLC coupling GC-MS are shown in figures 1-6.

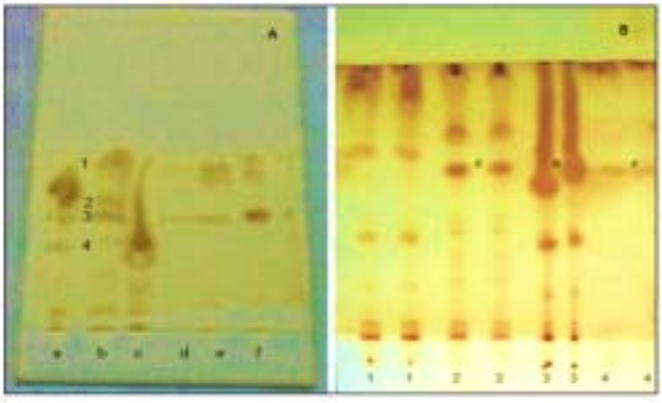

Fig. 1.Cromatograme of the compounds from the extracts of Capsicum annuum.A): a- red hot pepper, powder; β- red hot pepper; c- sweet peppers, powder; d,e,f- green hot pepper. Order spots: 1) β-carotene; 2) dihidrocapsaicina; 3) capsaicin; 4) norhidrocapsaicina

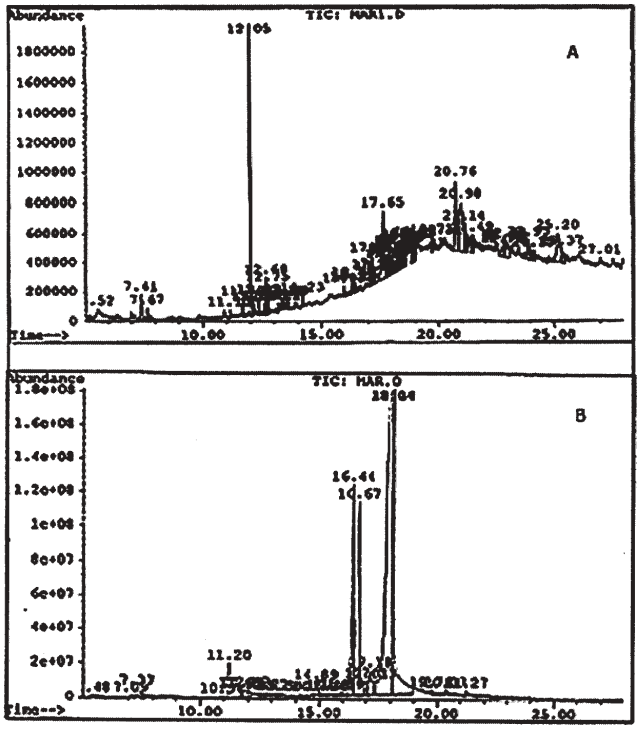

Fig. 2. Gas chromatography of peppers extracts: A) green hot pepper; B) sweet peppers

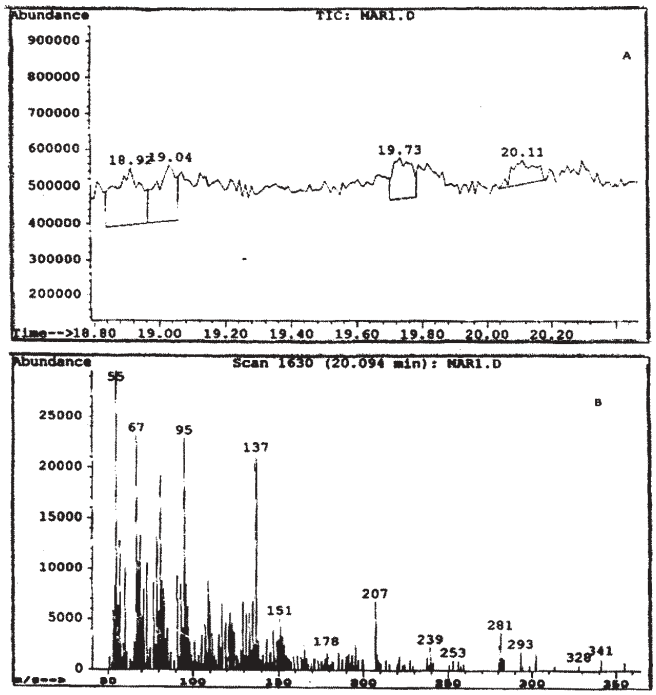

Fig. 3. TLC chromatogram of purified substance: A) analyzed by GC; B) mass spectrum of the most important component

Analyzing the chromatograms of figure 1, it can be noticed that the spot by the number 3, corresponding capsaicin (fig.1-A), is present in all the samples, except for the sample extracts paprika sweet pepper (c-position). The spot position (1-a), in the chromatogram of figure 1 A, is β-carotene, which is not found in the green pepper, is found in high concentration in the paprika, has a tail and is not the even front. Also, in the same figure it can be seen that the paprika sweet pepper trade (c-position) from the chromatogram of 1A, is an addition , because is not β-carotene. Order spots in fig.1-A is: 1) β-carotene; 2) dihidrocapsaicin; 3) capsaicin; 4) norhidrocapsaicin.

For a better evaluation was performed a thin layer chromatogram, containing capsaicin standard (fig. 1B).

Analyzing the two chromatograms (fig. 1), capsaicin spot sample position 3 of chromatographic plate of figure 1-A (a- red hot pepper, powder; b- red hot pepper; d,e,f- green hot pepper), corresponding to the spot 2-2 a- green pepper extract and 4-4 a- standard capsaicin (fig. 1-B).The chromatogram which contains standard capsaicin (fig. 1-B) and mass spectrum subsequently achieved, confirms the chromatogram data without standard (fig. 1-A).

Analysis of the active principle of the peppers (capsaicin) using three techniques TLC, GC / MS, revealed the presence or absence in raw or mature hot peppers, sweet, the spicy or sweet paprika. Lack of the capsaicin sweet pepper and sweet paprika is evidenced by thin-layer chromatograms of figure 1-A (position c), figure 1-B (position 1-1). The gas chromatogram of the figure 2-B, that does not contain capsaicin compared with the corresponding peak gas chromatogram of figure 2A, which shows the presence of capsaicin in the corresponding peak.

In order to characterize capsaicin extracts was appealed to the coupling gas chromatography with mass

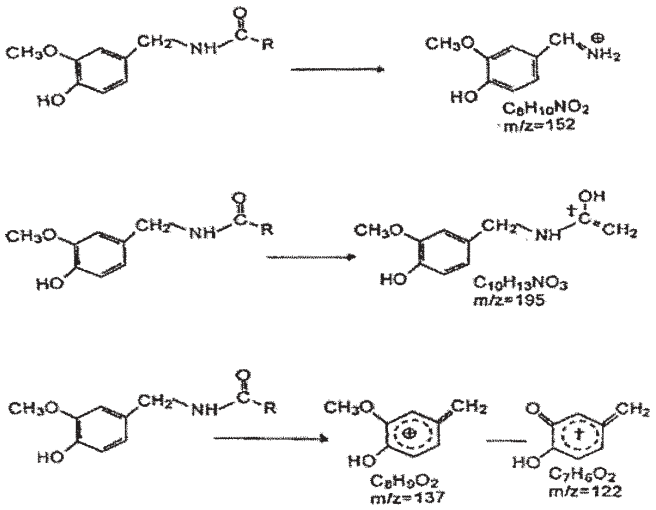

Fig. 4. Mechanism of capsaicinoid cleavage (capsaicin mass peak [M +]

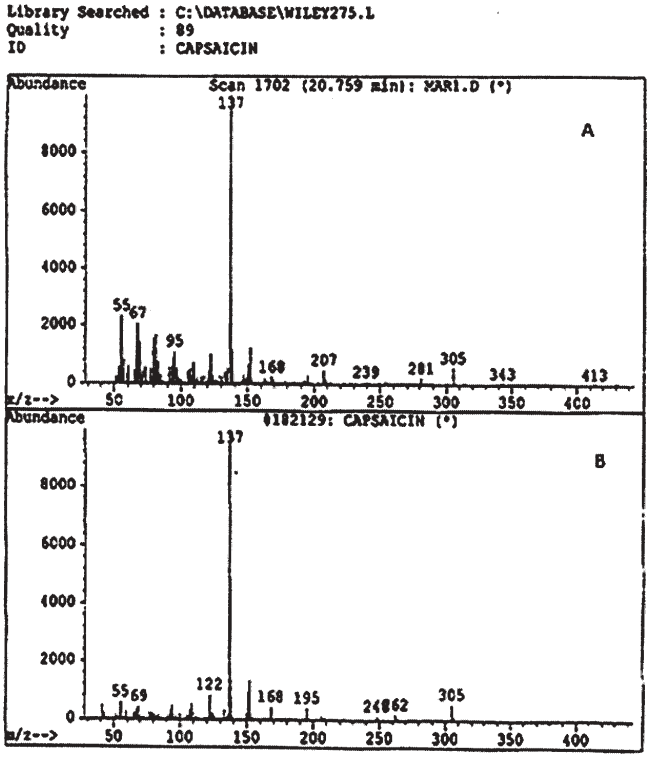

Fig. 5. Mass spectrum of capsaicin: A) data library system; B) Separated by TLC (chromatogram of Figure 1B)

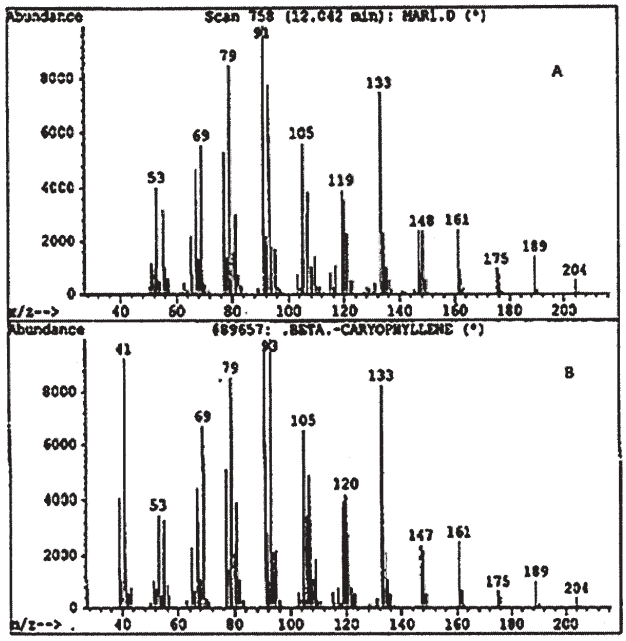

Fig. 6. Mass spectrum of major compound in peppers (B - carotene)

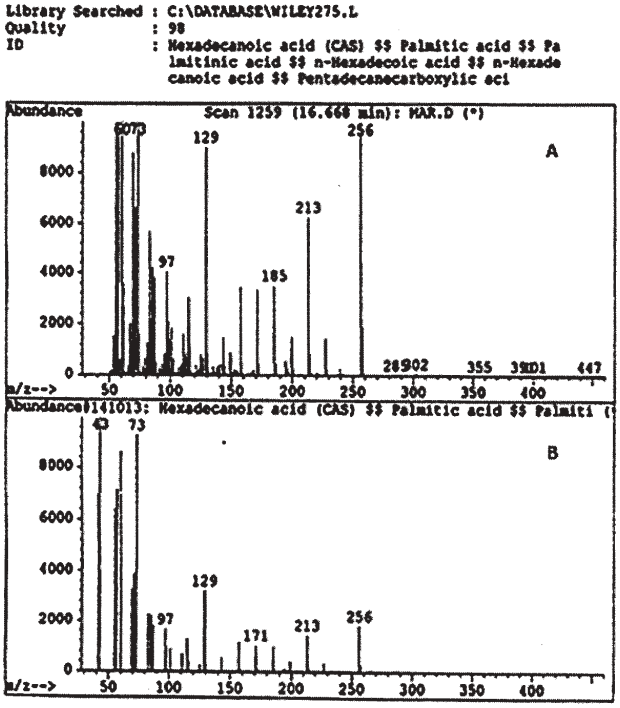

Fig. 7. Major compound in peppers: fatty acids

spectrometer (fig. 3). Considering that compounds elutes at very close retention intervals and that library spectra data contain only the compound capsaicin, the other isomers can be identified by judgments of the interpretation of the mass spectra [19, 20]. In this regard, the presence of capsaicin peaks [M +] reflects the dependence of the weight, stability and ionization level. Specific fragmentation mechanism class of compounds vanilla-amide alkaloid N – groups substituted are described in the side of figure 4. This model presented mechanism can be interpreted as follows:

- fragments of mass m / z corresponding to the peaks 151, 152 caused the connection N-R position B, followed by rearrangement hydrogen;

- the fragments located on the values of m / z 195, 152, 151, 137, 122, 94, represents a series of capsaicin ions

characteristic, considerable weight, it is interpreted as indicating the determined stability of the aromatic nucleus;

- the fragments located on the values m / z small 43, 57, 71, 85, represents alkyl moieties formed by cleavage of the side chain.

Based on this process could be identified that the spot capsaicin (fig. 1) corresponding to the library mass spectra data (fig. 5).

The colorant, major red pepper compound is β-carotene (fig. 6) and a synthetic colorant shown in the chromatograms of figure 1A (sample c position) and in figure 1-B (sample 3-3 position). This synthetic colorant highlighted can be considered a counterfeit from the paprika packaged free trade. Increased fatty acids content in analyzed samples constitutes a sign of the additives introduction in paprika (fig. 7).

Conclusions

Separation and identification of samples of the active ingredient capsaicin of peppers and of peppers preparations were optimally achieved by TLC GG coupled with MS as a possible confirmatory spectra obtained with corresponding data library spectra. Along with major compound capsaicin, responsible for properties pungent of peppers were separated and identified other active compounds: natural colorings (carotenoids), fatty acids, important for flavor and aroma, and synthetic dyes that can falsify the food trade (paprika powder, peppers pastes and creams, spice mixtures). Combining thin layer chromatography separation with gas chromatography and mass spectrometry as a confirmatory method of identifying compounds of capsaicin family, eliminates the use of costly standards, the results are similar.

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Manuscript received: 26.10.2015