ORIGINAL PAPER

CHARACTERIZATION, PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF THREE TYPES OF HOT PEPPER (*CAPSICUM ANNUUM L.*)

IOANA-RALUCA SUICA-BUNGHEZ[1], RODICA-MARIANA ION[1,2]

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Abstract. *Capsaicin is the most preponderant and naturally occurring alkamide found in hot peppers. Since its discovery in the 19th Century, the therapeutic roles of capsaicin have been well tested and characterized, due to the potential applications of capsaicin from a large area, from food flavorings, cosmetics, to maintaining the health of the body, like analgesic, anti-obesity, anti-pruritic, anti-inflammatory, anti-apoptotic, anti-cancer, anti-oxidant, neuro-protective properties. In this research are presented two extraction methods (maceration and ultrasonication) and characterization of extracts using analytical techniques (FTIR, UV-VIS). Also, are presented phytochemical results and antioxidant activity of three hot pepper types (red, yellow and green).*

Keywords: *antioxidant activity, phytochemical compounds, Capsicum annuum L.*

1. INTRODUCTION

Hot peppers are an important source of capsaicin, which is a natural protoalkaloid, member of the vanilloid family of compounds such as vanillin (extracted from vanilla tree), eugenol (derived from bay leaves and cloves), zingerone (present in ginger), and it is the major active pungent ingredient of hot chili peppers (*Capsicum annuum L.*) [1-3]. Chili pepper is generally used as a flavoring spice and is prominent in diets of various communities and cultures worldwide since 7000 BC, with a long history of flavoring, coloring, preserving food as well as medication [4-6]. More than 200 active components identified in hot peppers, play multiple roles in the whole human organism, by presenting numerous treatments for pain inflammation (capsaicin prevents the brain from receiving the impulses that would otherwise feel pain), rheumatoid arthritis, vasomotor rhinitis, psoriasis, diabetic neuropathy [7, 8], fight against high cholesterol levels [9, 10], or for being an effective anti-cancer agent (new studies from the American Association for Cancer Research related that capsaicin (Fig. 1) is able to kill prostate cancer cells by causing them to undergo apoptosis [11, 12]). It is found in cosmetic industry (creams, lotions, gels), patches, nasal or defensive sprays [13, 14]. When it is used a capsaicin ointment, a slight burning or itching sensation is common but temporary. So it is necesary to wash the hands thoroughly after application to avoid spreading the ointment to sensitive areas such as the eyes.

The capsaicin melting point is between 62-65 °C, it is not water soluble, but soluble in ethanol, acetone and fatty oils [4]. It is demonstrated the fact that oil extracted from *Capsicum*

[1] National R&D Institute for Chemistry and Petrochemistry – ICECHIM, 060021 Bucharest, Romania. E-mail: raluca_bunghez@yahoo.com;
[2] Valahia University of Targoviste, 130004 Targoviste, Romania. E-mail: rodica_ion2000@yahoo.co.uk.

annuum L. has an antimicrobial activity [14]. The genus Capsicum provides antioxidant compounds, such as vitamins, phenolics and carotenoids [15, 16].

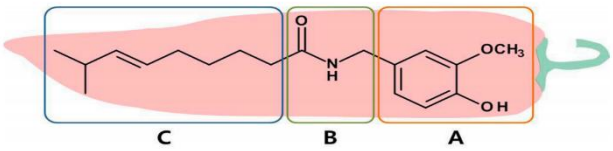


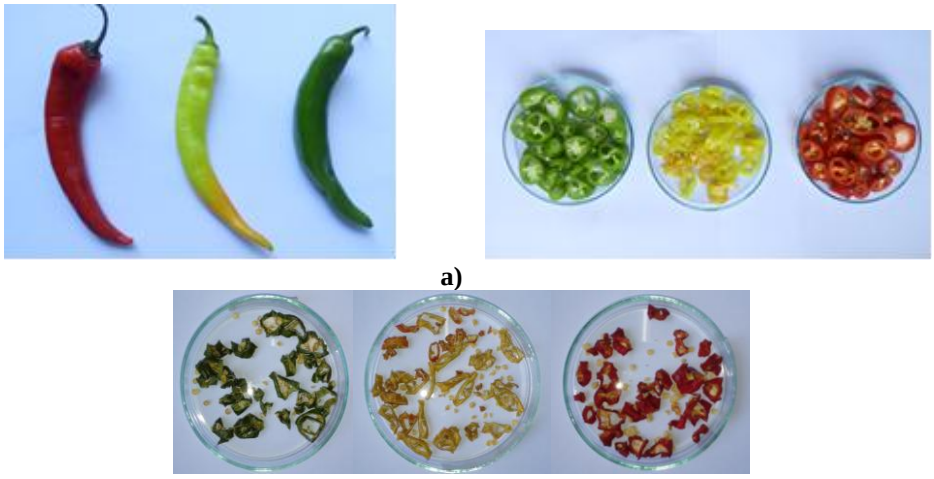
Figure 1. Chemical structure formula of capsaicin, the principal ingredient of chili pepper, and its three important regions: A - aromatic head, B - amide linkage and C - hydrophobic tail [10].

2. MATERIALS AND METHODS

2.1. MATERIALS

In this research, we present the results in the field of antioxidant activity and phytochemical analysis, using extracts obtained from three types of pepper native plants (red, yellow and green) extracted on two method types (maceration and ultrasound). The sample extracts were studied, using analytical methods (UV-VIS, FTIR). The antioxidant property was determined using DPPH method. It were used two types of methods maceration and ultrasound.

The peppers were cutted and let dry at the room temperature for 36 hours, then in the oven at 60 °C for 8 hours (Fig. 2). After drying, the samples were weighed.



a)

b)

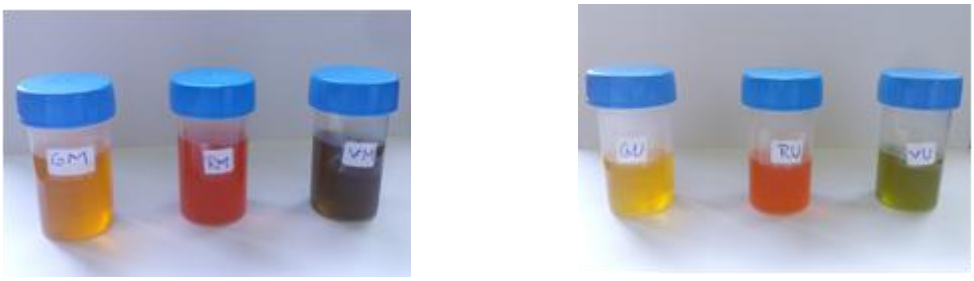
Figure 2. Red, yellow and green hot peppers a) before and b) after drying in the oven.

2.2. METHODS

Extraction method by maceration / ultrasound of samples in ethanol

2 g of each sample were weighed and 20 mL etanol and 5 mL distilled water were added. The samples obtained by maceration method, were left to macerate at room temperature for 24 h. The next day, all samples were stirred using a stirring plate at 50 °C for

2 h, 200 rpm. Then they were left for 5 days at room temperature and filtered (Table 2 and Fig. 3a). All the samples obtained by the ultrasonication method, were stirred for 90 minutes at 50 °C at ultrasonic power 100. Then they were left for 5 days at room temperature and filtered (Table 2 and Fig. 3b).



a) **b)**

Figure 3. Red, yellow and green hot peppers extract obtained by a) maceration and b) ultrasound methods

Table 2. Extraction methods used for the *Capsicuum annuum L.*

Samples	**Method**	
Hot pepper colours	*Maceration (M) in EtOH*	*Ultrasound (U) in EtOH*
Red	RM	RU
Green	VM	VU
Yellow	GM	GU

Characterization methods

UV-VIS Spectroscopy

The absorption spectra of the samples were recorded on a double beam M400 Carl Zeiss Jena UV-VIS spectrophotometer from 250 to 550 nm, at the resolution of 1 nm, with 1 nm slit width and 0.3 nm/s scan rate.

FTIR Spectroscopy

For Fourier transformed IR spectroscopy, the spectra were collected using an Interspec Spectrum GX instrument. Scans in the range of 400–7000 cm^{-1} were accumulated for each spectrum at a spectral resolution of 1 cm^{-1}.

Antioxidant activity (AA%)

The utilization of DPPH method gives an easy and rapid result to antioxidant activity against free radicals. The principle of AA % method consists in reducing in the presence of an antioxidant molecule, giving rise to colored methanol solutions [5].

Phytochemical Analyses

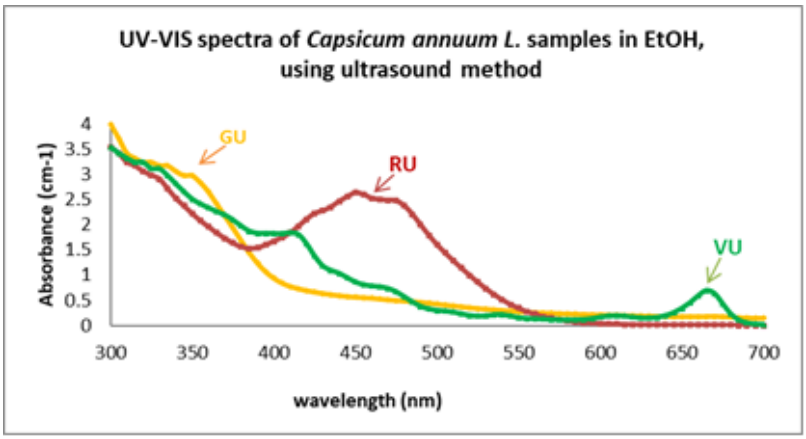
The phytochemical quantification procedures were used for the determination of total tannins, total flavonoids, total pholyphenols and total terpenoids existent in the hot peppers extracts. The assays are presented in Table 1.

Table 1. Phytochemical assays [5] effectuated for *Capsicuum annuum L.*

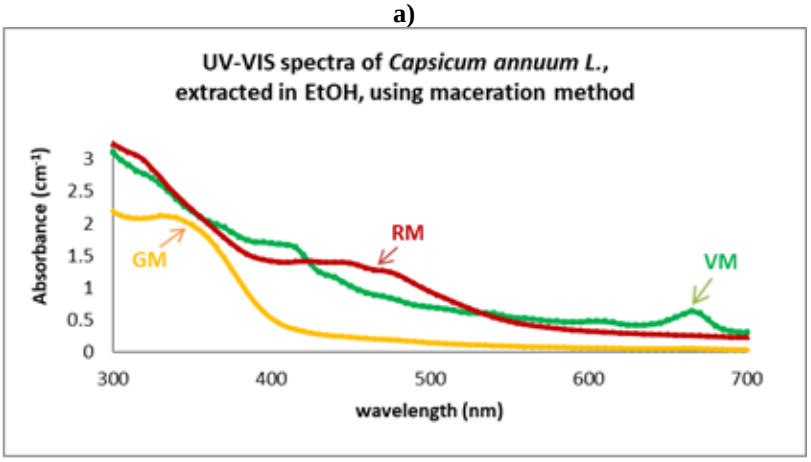
Assay	Reagents	Conditions	Monitoring system and standard curve
Total tannins	0.5 mL extract + 3 mL 4% vanillin: MeOH + 1.5 mL HCl	15 minutes incubation at room temperature	Absorbance = 500 nm; standard curve calibration of Catechin
Total flavonoids	1 mL extract + 4 mL distilled water + 0.3 mL $NaNO_2$ (5%); After 5 min: 0.3 mL $AlCl_3$ (10%) After 5 min: 2 mL 1M NaOH + 2.4 mL distilled water	30 minutes incubation at room temperature	Absorbance = 510 nm; standard curve calibration of Catechin
Total polyphenols	1 mL diluted extract + 5 mL Folin Ciocalteau reagent; After 8 min: 4 mL Na_2CO_3	60 min incubation at room temperature	Absorbance = 765 nm; standard curve calibration of Gallic acid
Total terpenoids	2 mL extract + 1 mL 2% vanillin: H_2SO_4	heated at 60ºC/20 min; cooled at 25ºC/5 min	Absorbance = 608 nm; standard curve calibration of Linalool

3. RESULTS AND DISCUSSION

The components and phytosynthesis of extracts were confirmed by modern analytical techniques (UV-VIS, FTIR). The UV-VIS analysis (Fig. 4) presented the UV-VIS spectra obtained from the *Capsicuum annuum* L. hidroalcoolic extracts. The wavelength spectrum was made between 250-550 nm.



a)



b)

Figure 4. Red, yellow and green hot peppers extract obtained by a) maceration and b) ultrasound methods.

On the UV-Vis spectra (Fig 4a) of the alcoholic solution obtained from ultrasound hot pepper extracts (GU, RU, VU) it is observed the presence of maximum characteristics owing to the functional groups. A maximum level at 450 nm is specific to beta carotene and it is the most evident in RU sample. Also, between 300-350 nm, are specific to compounds with aromatic nucleus, such as flavonoids or polyphenols. In Fig. 4b), the UV-VIS spectrum of the alcoholic solution obtained by maceration method (GM, RM, VM), the maximum wavelengths are found a little below, which means that bioactive compounds are present less amount using extraction method, compared to the extract obtained from ultrasound method. In the 300-350 nm area the peaks which correspond to flavonoids and phenolic acids are observed, more highlighted at yellow pepper (GM sample) [4, 12].

Table 3. Absorbance for capsaicin standard solutions.

Concentration (ppm)	Absorbance
2	0.3271
4	0.3444
6	0.3602
8	0.3794
10	0.4003

For preparation of the calibration curve, it was used capsaicin standard (Table 3). The regression equation for the calibration curve was y = 0.0091x +0.3079 and a good result for R^2 = 0.9971 (Fig. 5).

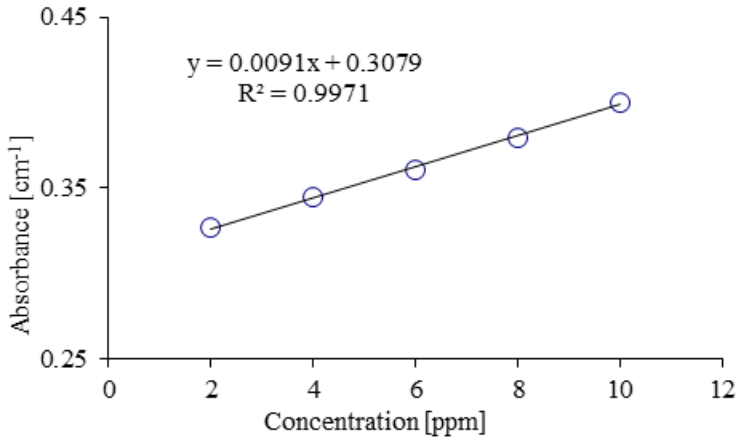


Figure 5. Capsaicin calibration curve.

In the next figures, are presented the photochemical results of the three types of hot pepper (R=red, G = yellow, V = green) samples, extracted by two methods (U = ultrasonated and M = macerated). In the Fig. 6, the graphic reports a verry good antioxidant activity, especially for GU and VU samples extracted by ultasound method and GM and VM samples extracted by the second method, ultrasound.

Total flavonoids (Fig. 7) and polyphenols (Fig. 8) shows that the extract samples (RM, VM and GM) obtained by maceration method have the high values than extract samples (RU, VU and GU) obtained by the other method, ultrasound. Therefore, it is observed that the better values of total flavonoids and polyphenols content are represented by macerated extracts.

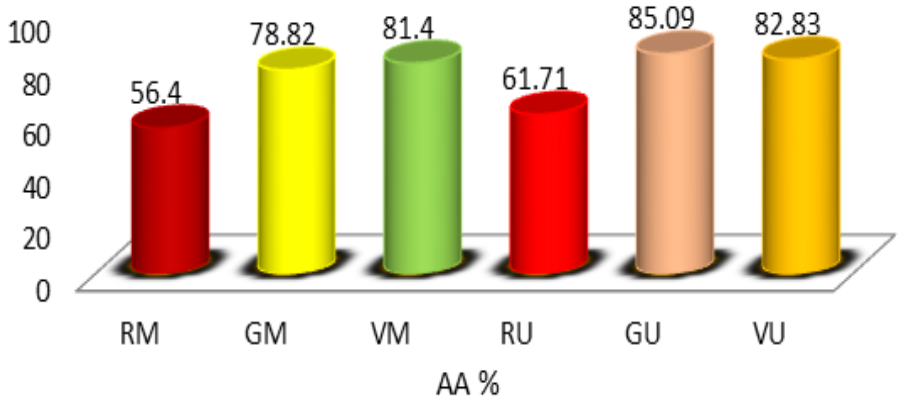


Figure 6. Antioxidant activity of hot pepper samples.

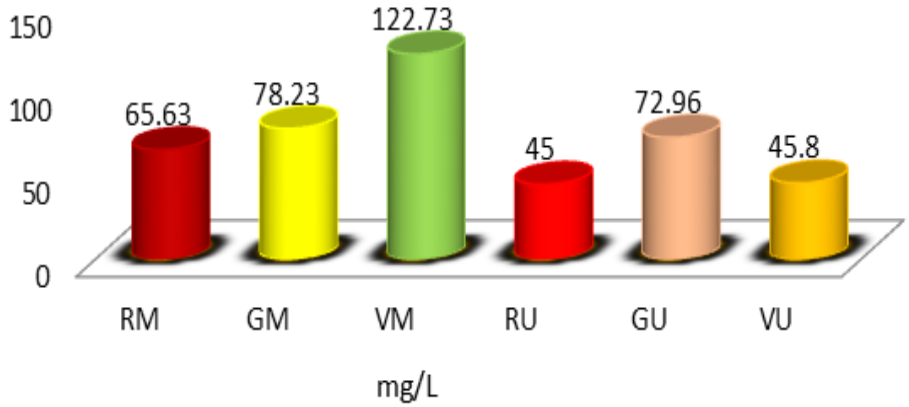


Figure 7. Total flavonoids content (TFC) of hot pepper samples.

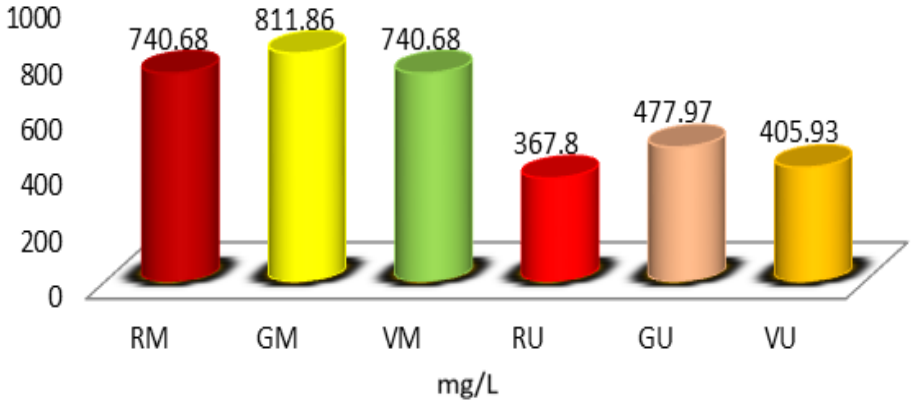


Figure 8. Total polyphenols content (TPC) of hot pepper samples.

Fig. 9, where the total tannin content of the hot pepper samples is presented, confirms that the maceration method offers higher results than the samples extracted by the ultrasonic method. The highest tannin content it is attributed to red pepper extract, obtained by both methods. So, RM sample have 88.2 mg/L and RU have 83.73 mg/L, while yellow pepper extracts present the lowest values (GM = 60.4 mg/L and GU = 45.46 mg/L).

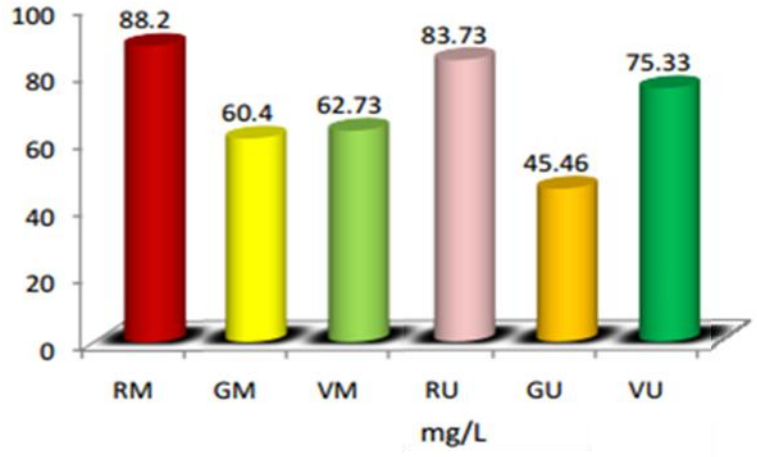


Figure 9. Total tanins content (TPC) of hot pepper samples.

In order to obtain FTIR spectrum of *Capsicum annuum L.* extract samples was recorded in the region 400 - 4000 cm^{-1} (Fig. 10). FTIR spectra have been presented NH stretching appear at 3444 nm, OH, H_2O picks at 3350 cm^{-1}; at 2932-2923 cm^{-1} are found CH groups; 1598 cm^{-1} it is associated to amides I, carboxyl and C=C bounds from alchenes and aromatics groups, at 1397 cm^{-1} methyl groups, C-O bonds appear at 1253 cm^{-1}, 1032 cm^{-1} CO stretching. At 1078 cm^{-1} are found bands of aromatic rings or CH_2, OH and C-O aliphatic groups.

According to FT-IR spectral data in fig 9, revealed that all spectra show absorption bands at 3320 cm^{-1}, due to the presences of characteristic stretching vibrations of (N-H) in amino acids. Moreover, both samples showed an aliphatic (C-H) stretching vibration in the range of (2846-2955 cm^{-1}) also presences of (C=O) stretching vibration at the range (1620-1739 cm^{-1}) for the two samples. Also, the samples showed a range of (1442-1556 cm^{-1}) due to the presence of (C-C) stretching vibration in aromatic ring and bending out-of-plane (C-H) at 755 cm^{-1}.

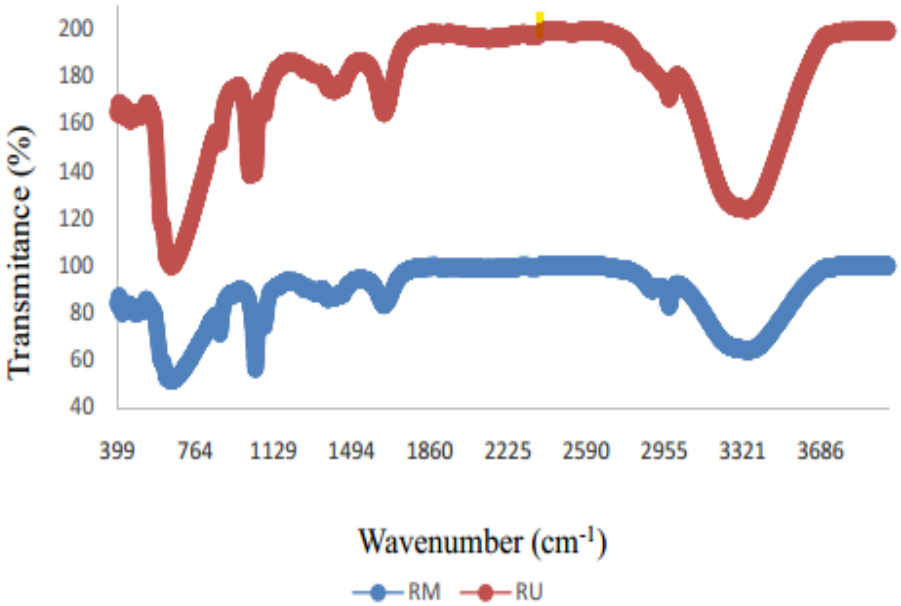


Figure 10. FTIR spectra of *Capsicuum annuum L.* extracts red samples.

4. CONCLUSIONS

Capsaicin has presented enormous interest for several centuries due to its culinary and clinical applications. It is still being used as an active principle in several pharmaceutical formulations for treating various human ailments, like cancer, obesity, skin disorders, cardiovascular diseases.

Red, yellow and green pepper extracts have a very high antioxidant activity (AA%), which denotes the benefits of the compounds existing in these vegetables. The ultrasound method gave the highest values, compared to the second method used (maceration). The color of the peppers is due to the presence of carotenoids, which resulted from the UV-VIS spectrum. In the case of yellow pepper extract (GM), the carotenoids are found in the range 325-425 nm. In red peppers (RM), β-carotenoids peak between 445-478 nm and are more evident using ultrasound method. In the case of terpenoids (TTpC), it can be observed that the extracts of green pepper (VM and VU), obtained by both methods (maceration and ultrasound), have the highest values. Capsaicin was successfully identified by using FT- IR method, this technique is nondestructive, allows for easy identification and less cost.

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