

Preliminary Analysis of *Capsicum Annuum* L. Extracts

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ABSTRACT: The aim of this study was to investigate *Capsicum Annuum* L. ultrasonicated extracts and dry powder. The percentage of water, dry matter and ash were determined. FT-IR analysis confirmed the presence of important classes of secondary metabolites in the extracts and by GC-MS a large number of important pharmaceutical compounds were identified, including capsaicin.

KEYWORDS: capsaicin, FT-IR analysis, GC-MS, *Capsicum Annuum*

Introduction

Peppers contain water, cellulose, vitamins (vitamin C, provitamin A, vitamin B, vitamin B2 and secondary metabolites). It was found that raw pepper contains five to seven times more vitamin C than a lemon. Specific red color of pepper varieties, and especially those used in the manufacture of paint is due capsanthin.

Capsaicin (Fig.1) is a secondary metabolite found in chili peppers of various species including the species *Capsicum annuum*, responsible for burning sensation that is generated at the contact with the oral mucosa, in case of ingestion [1]. Although its effects have been observed since ancient times, only now modern research gives it a deserved place among substances with great healing potential [2].

Capsaicin has a weak acid character due to phenolic group, is readily soluble in alcohol, ether and benzene, is sparingly soluble in water and petroleum ether.

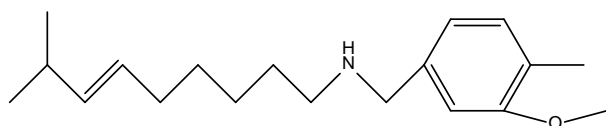


Fig.1. Structure of capsaicin

Research on capsaicin and its effects in the body shows that this substance is very useful for chronic pain caused by chronic rheumatic diseases.

Another role of capsaicin is to stimulate the immune system, helping to destroy bacteria and inactivate toxic substances resulting from cell damage and allergic reactions [3].

In order to identify compounds contained in various species, the most used methods are coupled techniques.

Chromatographic techniques have developed rapidly and are in a continuous process of improvement of their performance and are used in all branches of research: from the analysis of drugs in biological fluids to analysis of secondary metabolites in plant extracts.

Material and Methods

Preliminary analysis

In order to determine the quality of the investigated products the quality parameters were determined (moisture, dry matter, ash). They provide information on the possibility of keeping the *Capsicum annuum* powder (dried plant) for a certain period of time.

Determining the percentage of water, dry matter and ash

3-5 g of dried plant material were weighed and were placed in a Termodry DO oven heated initially at 20 to 40°C, and then gradually from 60°C to 105°C for the first hour, and then the samples were dried at constant temperature for 3 hours.

The resulted dried plant material was introduced into the crucible and was burned at 500 °C in a Pyrotherm oven for 4-5 hours.

Capsicum Annuum ultrasonicated extracts

In order to achieve the extracts, 1 g of powdered sample was extracted with a Bandelin Sonorex bath for one hour using as extraction solvents ethanol and water in the ratio 1:20 (m/v). The extracts (Fig.2) were subjected to FT-IR analysis and the residues to GC-MS analysis.



Fig.2. Ultrasonicated extracts: 1- *Capsicum annuum* (red variety, ethanolic extract); 2- *Capsicum annuum* (red variety, aqueous extract); 3- *Capsicum annuum* without seeds and ribs (red variety, ethanolic extract); 4- *Capsicum annuum* without seeds and ribs (red variety, aqueous extract); 5- *Capsicum annuum* (green variety, ethanolic extract); 6- *Capsicum annuum* (green variety, aqueous extract);

FT-IR analysis

FT-IR (Fourier Transform Infrared Spectroscopy) spectra for the extracts of *Capsicum annuum* were recorded on an Avatar Nicolet spectrophotometer in KBr pellets, within the range 4000–400 cm⁻¹.

GC-MS analysis

An Agilent 7890 A GC System – 5975C VL-MSD gas chromatograph was used with nonpolar capillary column Agilent 1909, 433 (5% - phenyl methyl siloxane, 30.0 cm × 0.25 mm × 0.25 μm).

Carrier Gas: He (99.99%), flow 1 mL/min. The furnace temperature was set at 70 °C for 2 minutes and then programmed to 270 °C at a rate of 20 °C/min. The injector temperature was 280 °C. Injection mode: splitless. injected volume: 4μL.

Operating parameters: mass detector - ionization potential of 70 eV; interface temperature - 200 °C; The acquisition of spectra was between 50-800 m/z.

Results and Discussion

Determining the percentage of water, dry matter, ash

Measurements were performed in triplicate for each sample, and the results (average of three determinations) are shown in Table 1. The results were considered valid when difference between the three calculated measurements was not more than 0.5%.

Table 1. The percentage of water, dry matter and ash from *Capsicum annuum*

No.	Sample	Weight (raw material)	Weight (dried material)	Humidity (%)	Ignition residue	Ash (%)
1.	<i>Capsicum annuum</i> (red variety)	5.0013	4.467	10.68322	0.256	5.73
2.	<i>Capsicum annuum</i> without seeds and ribs (red variety)	4.989	4.3241	13.32732	0.236	5.45
3.	<i>Capsicum annuum</i> (green variety)	5.0025	4.578	8.485757	0.198	4.32

The amount of water from the tissue is variable, depending on a variety of factors such as environment conditions, development stage at the time of harvest, region, etc. From the results it was found that the products can be dried and kept for a period of at least 2 years.

Ultrasound assisted extraction

In contrast to microwaves extraction which requires a certain temperature to which certain metabolites may degrade as they can be thermally labile, ultrasound-assisted extraction can be carried out at ambient temperature [4].

The required time is up to one hour; in this method can be processed simultaneously high amounts of sample, which is why this process began to be used on an industrial scale [5].

FT-IR analysis

All spectra show intense absorption bands in the range 3355-3345 cm⁻¹ for the characteristic stretching vibrations of -H, O-H and N-H from amino acids [6].

In all samples intense bands were observed at 2924-2930 cm⁻¹ due to ν_{stretch} of the -CH (from -CH₃ and -CH₂ groups) from the structure of

carboxylic acids. The presence of polyphenols by FT-IR can be identified by the presence of characteristic absorption bands of C-O and -OH groups in the range 1250 - 1500 cm^{-1} [7].

All samples show a characteristic band situated around 1720 - 1745 cm^{-1} ($\nu_{\text{C=O}}$). Two vibrations were observed also, one between 1220- 1260 cm^{-1} ($\nu_{\text{C-O asym}}$) and one at 1005-1045 cm^{-1} ($\nu_{\text{C-O asym}}$). Medium intensity band at 1454 cm^{-1} is characteristic to bending vibration of C-H [8].

According to Goodacre, the most important class of secondary metabolites of plants, polyphenols, can be recognized by FT-IR by the presence of an intense band in the region of 1180-1260 cm^{-1} due to stretching vibration (C-C-O) and by the presence of low intensity bands bending out of the plane (C-H) [9]. All spectra are similar regardless of sample. In figures 3-4 are shown two of the spectra obtained for *Capsicum annuum* red and respectively green variety.

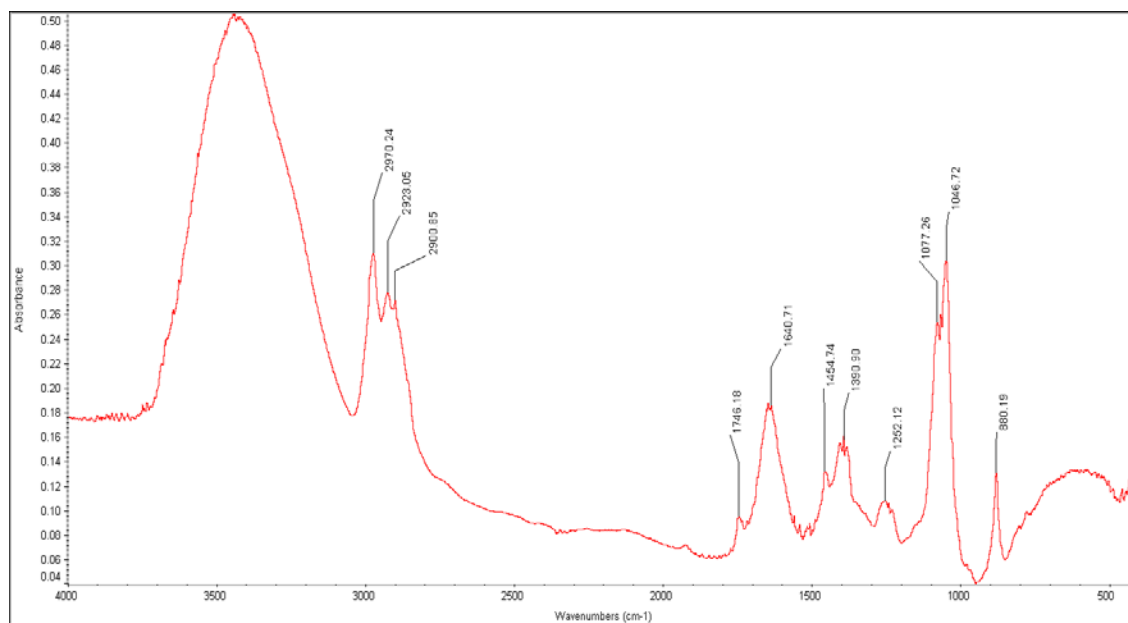


Fig.3. FT-IR spectrum of *Capsicum annuum* without seeds and ribs (red variety)

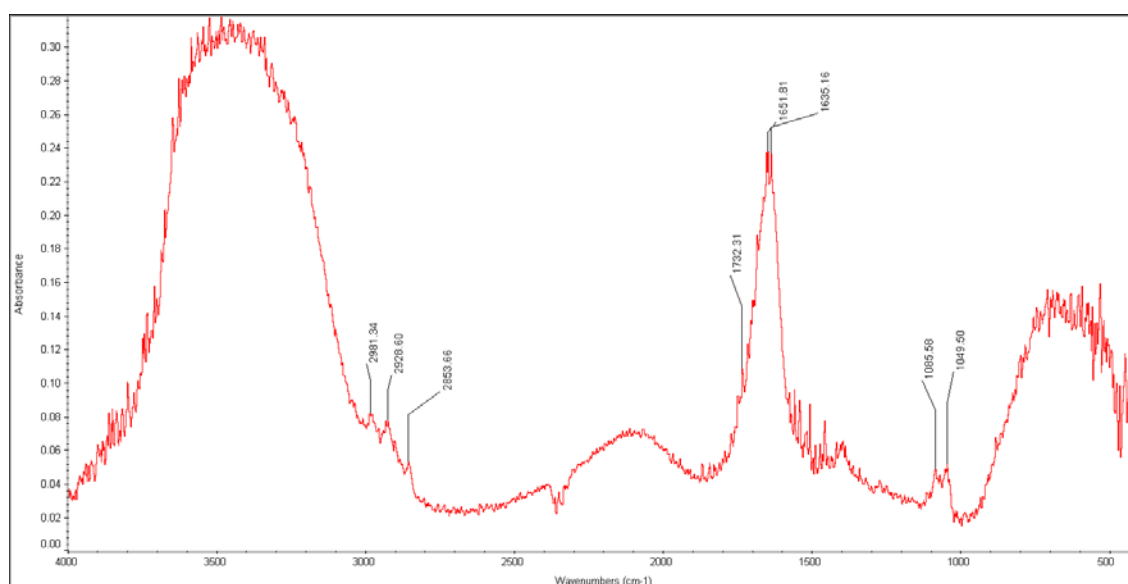


Fig.4. FT-IR spectrum of *Capsicum annuum* (green variety)

GC-MS analysis

Gas chromatograms obtained for the extracts of *Capsicum annuum* present numerous peaks, without clear separation that could not be interpreted without coupling to mass spectrometry. The easiest way to analyze the plant products is by full scan method or TIC (Total Ion Count). In this case it is possible that in the chromatogram the peaks with low resolution not to be properly separated.

Assigning chromatographic peaks was accomplished by comparing the mass spectra obtained with reference compounds of known structure stored in the spectra library and by comparison to those reported in the literature [10]. It may be noted that this species is rich in secondary metabolites, similar to other

medicinal plants [11]. Among these secondary metabolites identified in *Capsicum annuum* exercising a wide range of biological activities on humans, may be listed: palmitic acid (hexadecanoic acid), stearic acid (octadecanoic acid), 9Z, 12Z-octadecadienoic and linolenic acid (docosanoic acid) unsaturated fatty acids, etc. They were tested for their antimicrobial, anti-inflammatory, antioxidant, anti-cancer and hepatoprotective activities [12].

Among these, octadecanoic acid (stearic acid) was found to lower LDL cholesterol in humans [13] (fig.7, 8).

The analysis allowed the identification of large numbers of compounds in *Capsicum annuum* with a high probability.

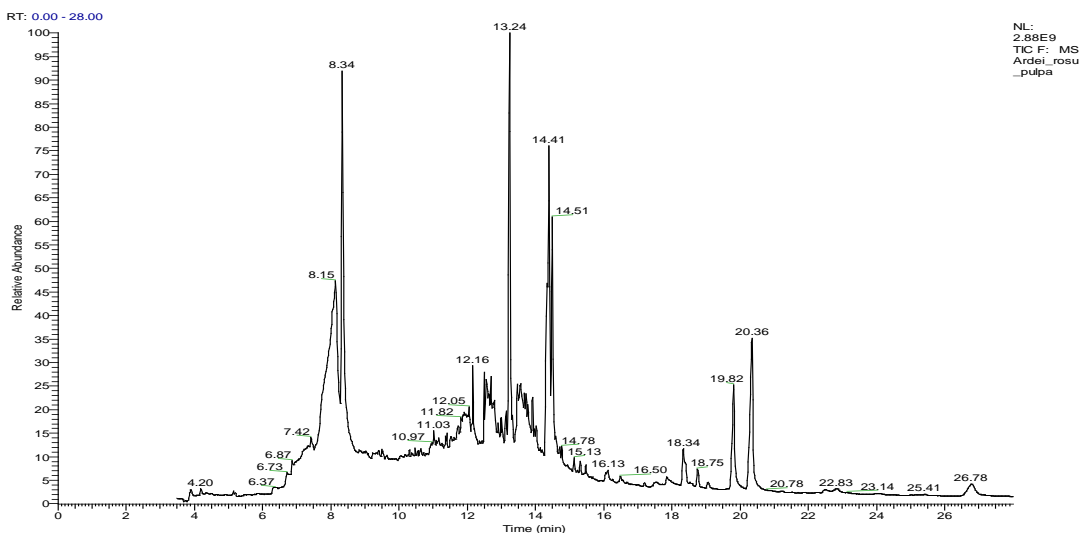


Fig.5. Chromatogram of *Capsicum annuum* (red variety)

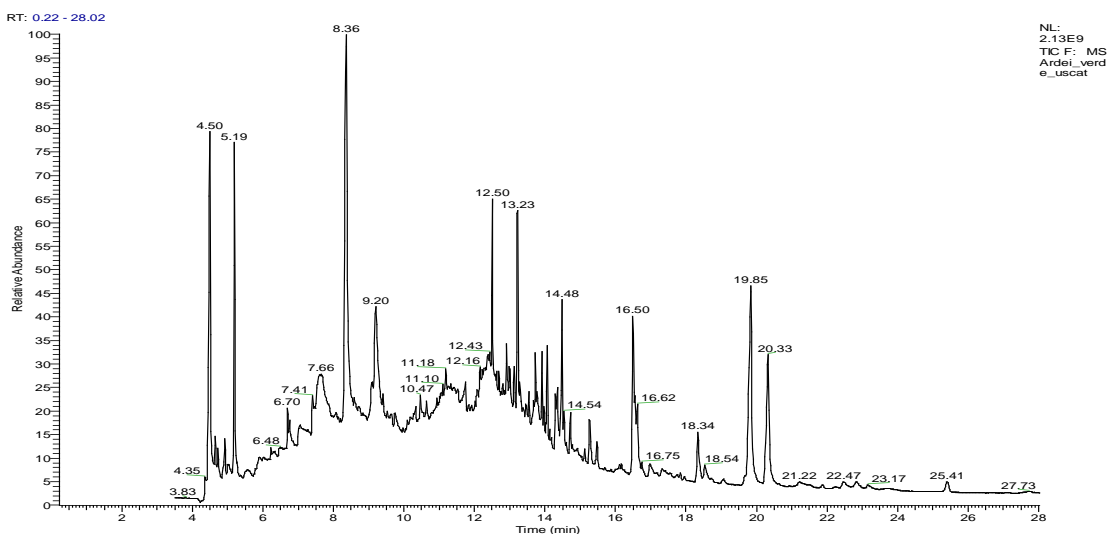


Fig.6. Chromatogram of *Capsicum annuum* (green variety)

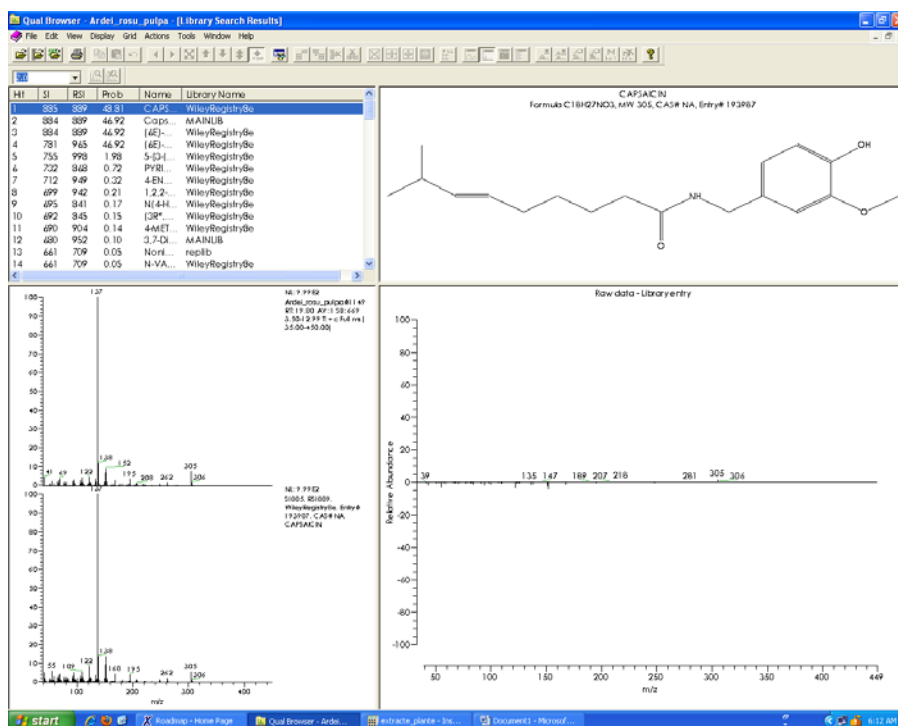


Fig.7. Mass spectrum of capsaicin from *Capsicum annuum* pulp

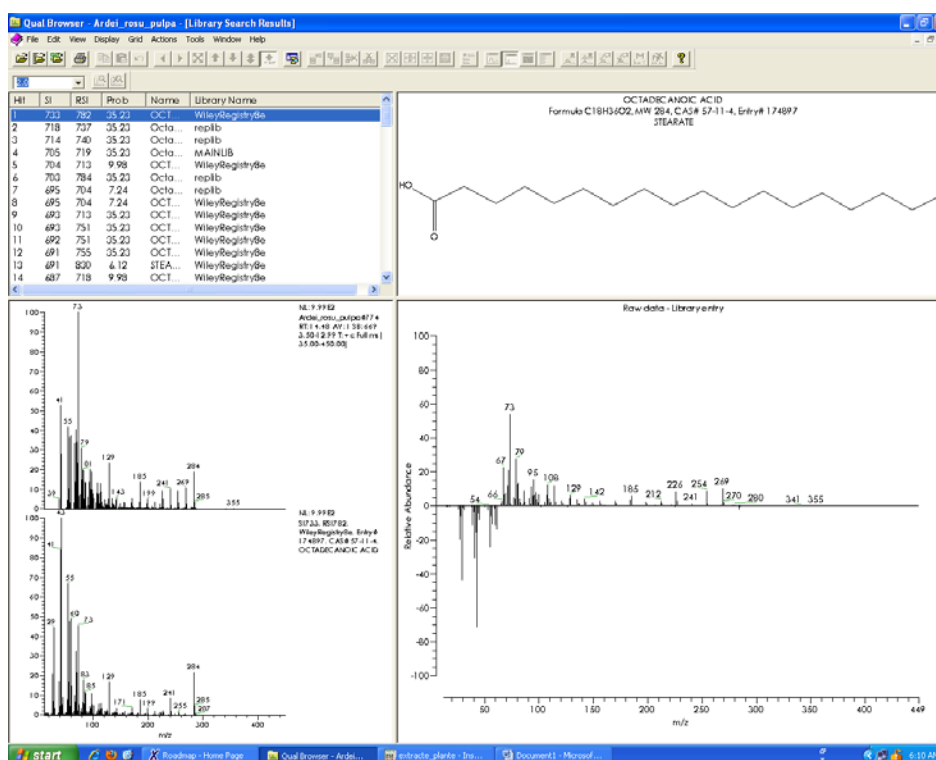


Fig.8. Mass spectrum of octadecanoic acid from *Capsicum annuum* pulp

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