



THE EFFECT OF DIFFERENT METHODS OF EXTRACTIONS OF CAPSAICIN ON ITS CONTENT IN THE CAPSICUM OLEORESINS

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Abstract

Several types of capsaicinoids can be present in the oleoresin extracted from hot peppers *Capsicum annuum* L. (Solanaceae). A major component of this group is capsaicin (69%). The extraction of the oleoresin from the fruit of hot pepper can be made in many ways. The most appropriate, in our experiments is extraction by Soxlet, and this procedure was compared to extractions with vacuum filtration. We have used an ethanol as appropriate for extraction and quantification of capsaicin for food and pharmaceutical grade.

Content of capsaicin in three different varieties of hot peppers with Macedonian origin was measured spectrometrically and results were compared with the sweet pepper variety as a control. Results of quantification measurements made for Soxlet oleoresins were differing in a really special way from the results for vacuum filtration oleoresins, and it's due to conditions used for the procedure of extraction. These results are showing that for extraction of capsaicin in different aims, conditions should be always adjusted.

Key words: Capsaicinoids, capsaicin, oleoresin, Soxlet extraction, spectrometrically.

Introduction

Capsicum fruits or hot peppers have been used for a long time ago in the food industry, in traditional medicine, in agricultural industry and for many other aims. These fruits contain large group of alkaloids capsacinoids, which are characteristic only for the genus *Capsicum*. These alkaloids are responsible for the pungency of hot peppers. The great diversity of the genus *Capsicum*, always complicated the taxonomic classification of peppers, and thus the extraction and identification of the exact number of capsaicinoids [1, 2].

Capsaicinoids are synthesized in a condensation reaction between an aromatic moiety and an unsaturated acyl chain (C9-C11 branched fatty acid). From biogenetic point of view, capsaicin is acilyted degraded phenilpropane, whereby the aromatic ring and acyl radicals are the result of the unique metabolic processes that take place only in the placenta of the fruit of hot pepper. So far, in the literature are known 17 isomers, with the differences mainly between their acyl-group. This difference may refer to: the length of the alkyl chain (C8-C13), the way it ends, and the presence or absence of ω -3 or ω -4 carbon atom. The main component in this group is capsaicin. Capsaicin is presented with 69-72 % of the

content of capsaicinoids, and is the most responsible component for pungency of the peppers together with dihydrocapsaicin. The degree of pungency depends on the *Capsicum* species and cultivars, because capsaicin and dihydrocapsaicin content can be affected by different factors such as developmental stage of the fruit, and the environmental growth conditions. The first test developed to measure pungency was the Scoville test, first developed in 1912 by Wilbur Scoville and it was an organoleptic test. Nowadays, however, this test has been largely replaced by spectroscopic and chromatographic methods which are considered to be more reliable and accurate. The pungency of pepper is expressed as a Scoville heat units [3, 4].

One of the most common hot pepper products is the pungent capsicum oleoresin that presents an organic oily resin derived from dried ripe fruits of different pungent varieties of *Capsicum annuum* L. by means of solid – liquid extraction. Generally, the most commonly employed and a preferred method for extraction of compounds present in plant matrices is the conventional solid-liquid extraction using organic solvents. In later studies, these conventional methods were improved, modified or rationalized by varying different operating parameters. There are several techniques that can be used for solid – liquid

extraction of capsaicin like vacuum filtration, percolation, Soxlet technique, supercritical fluid extraction etc [5, 6].

Quantification of capsaicin, according to the later studies can be made by many conventional methods who can always be improved, modified or rationalized by varying different operating parameters. Spectrophotometric UV/VIS, fluorescence techniques, HPLC, HPLC-MS, and many other are methods are still developing in order to offer fast and reliable methods for separation and quantification of capsaicin and its analogues [7, 8, 9 and 10].

Materials and methods

Four different genotypes of hot pepper of *Capsicum annum* L., fam. *Solanaceae* were taken, in the experiments we performed. We have used three species of hot peppers and one mild as a control. Traditional names in the region where they were cultivated are taken as names for the samples in the experiment. *Capsicum annum* var. *microcarpum*, genotype *Bombonka*, genotype *Vezena* and genotype *Feferona*, were taken as hot varieties. The mild one, genotype *Sivrija*, was used as a control. The fruits of these peppers were picked up in phase of botanical maturity [11, 12]. They were cut on pieces and dried on a room temperature, in a dark and dry place for about two weeks. Their weight was measured before and after the process of drying to constant weight, and the percent of water in them was counted. Dried fruits of the peppers were grounded and kept in an exsiccator. For the extraction of oleoresin

we have used two different methods: vacuum filtration and Soxlet extraction procedures [13].

Methods of extraction

Process of extraction with vacuum filtration was made using 0.2 g of grounded peppers in 25 ml of extraction solvent. According to the literature a few organic polar and non-polar solvents can be used for capsaicin extraction [14]. We have tried to use acetone and ethanol, but acetone has shown as an inappropriate solvent for spectrophotometric measurements on wavelength of 280nm. Therefore, in the focus of this experiment were taken only the ethanol extracts. Vacuum filtration process was performed in volumetric flasks for 5 hours, on temperature of 50°C. Separation of extracts from the powder was conducted with vacuum filtration using a Buchner funnel and a water vacuum pump. Final extracts were diluted 1:25 with the same solvent (Figure 1). Soxlet extraction was performed using a Soxlet apparatus (figure 2). This extraction was made with the same ratio of solid-liquid phase (0.2:25). In order to assure the appropriate volume of solvent for Soxlet apparatus we had to take 0.8g of grounded peppers in 100ml 96% ethanol. The extraction was performed in 5 hours on temperature of 80-85 °C (ethanol boiling point is 78°C). Final extracts were also diluted in ratio 1: 25.



Figure 1. Phases of vacuum filtration method for extracting the oleoresins (1a maceration; 1b vacuum filtration; 1c and 1d dilution of oleoresins)



Figure 2. Soxlet apparatus for extraction

Method for determination and quantification of capsaicin

Concentrations of capsaicin in the ethanol extracts was measured with one of the cheapest and fastest method for quantification of capsaicin, the UV/VIS spectrophotometric method [15, 16, and 17].

These measurement were made using an appropriate method of standard solutions. Dilutions of capsaicin (> 95%, natural capsaicin, Sigma Aldrich) in its standard solutions in the range of 0.0156mg/ml up to 0.25mg/ml.

Table 1. Standard solution concentrations

Standard dilution	St. sol. 1	St. sol. 2	St. sol. 3	St. sol. 4	St. sol. 5
Concentration [mg/ml]	0.0156	0.0312	0.0625	0.1250	0.2500

Results and discussion

Measurements of the concentration of capsaicin in the extracts were evaluated through their absorbencies measured on $\lambda=280\text{nm}$. Lambert-Beer law, where absorbance (A) is equal to the concentration multiplied by extinction coefficient ($A1\%$), where the extinction coefficient ($A1\%$) is defined as the absorbance of a 1% (10 g/liter) solution of

capsaicin, in a defined solvent, in a 1-cm path-length cuvette, at a specific wavelength (λ) was used. The absorbencies for standard dilutions were also measured and used to prepare the linearity curve presented on figure 3. The results for concentration of capsaicin in the samples were calculated using the linearity equation $y=9.484 x + 0.016$, and they are presented in figure 4 and 5.

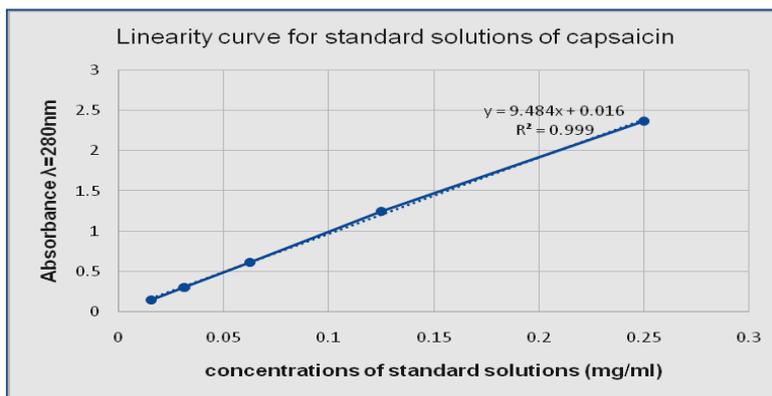


Figure 3. Linearity curve for standard solutions of capsaicin

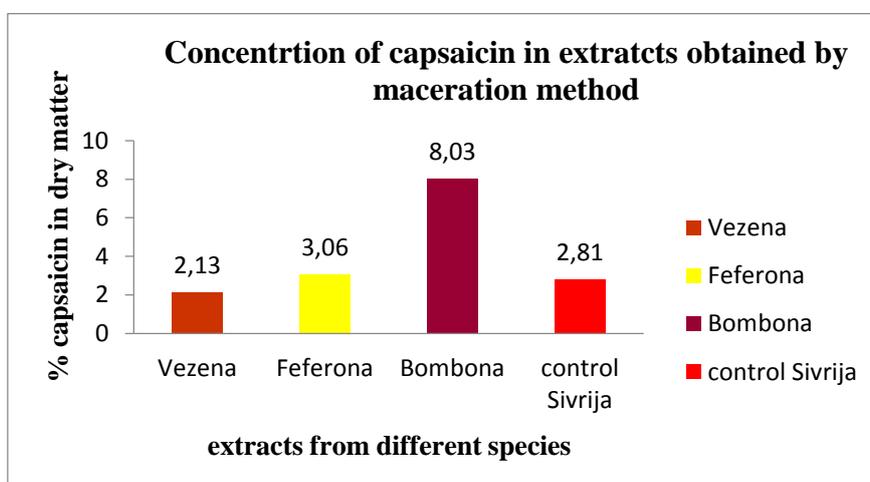


Figure 4. Concentration of capsaicin in extracts obtained by vacuum filtration

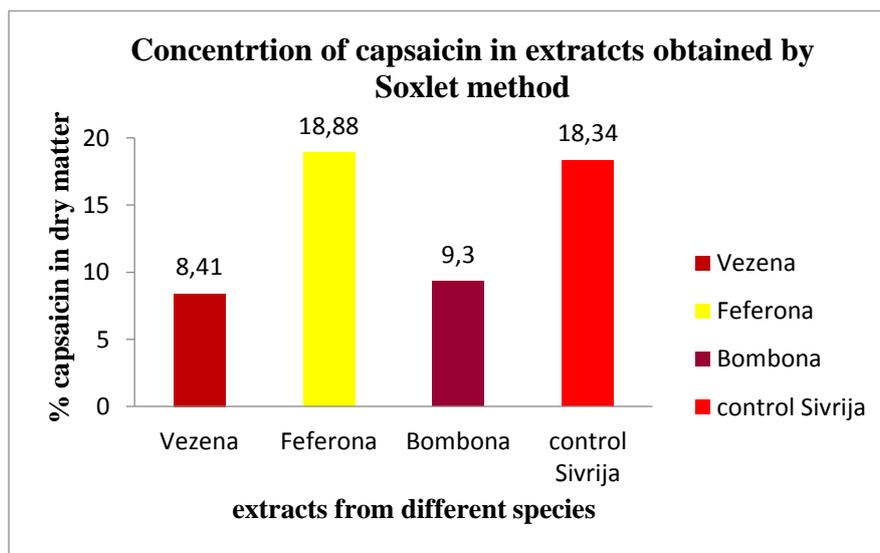


Figure 5. Concentration of capsaicin in extracts obtained by Soxlet extraction



According to the results we obtained, we can say that extraction method which is used has a large influence on the capsaicin content in the extract. They are not raising the concentration of capsaicin in extracts proportionally, but anyway, Soxlet method is giving up to 9 times higher results for the concentrations of capsaicin. Conditions which are allowing recirculation of the solvent through the material for a few times are extracting not only capsaicin but also other components, who may give their maximum absorbencies close to the wavelength of the capsaicin and may give us some fault results. It is known that carotenoids are also present in the oleoresin extract from peppers that we are examining and they could give some fake results. The differences in spectral characteristics of individual carotenoids are often small, but are of great importance in their identification. However, carotenoids having the same chromophore, such as B-caroten and its hydroxy-derivative zeaxanthin have identical spectra. The spectra of *cis* - or *Z* - isomers of carotenoids, while being similar to *all-trans* or *all-E* form, are different, as far as, they exhibit a change in λ max to a shorter wavelength, a decrease in the magnitude of absorbance, a reduction in the fine structure and additional absorption bands in the UV region on 340 and 280nm [18,19]. Therefore, this can mean that absorbancies measured on 280 nm for our extracts can also be due to the presence of these forms of carotenoids, and this fact should be taken in consideration.

Conclusion

Over the years, capsaicin presents a promising molecule with many possible applications. Its medical applications has been comprehensively studied (experimentally, clinically and epidemiologically) owing to its

prominent antioxidant, antimicrobial and anti-inflammatory [20, 21 and 22]. Moreover, considerable interest has developed in expanding the usage of capsaicinoids in other forms such as natural product-based food additive, dietary supplements and as constituent in self-defense products [23, 24].

Our results are showing that Soxlet extraction is much better than vacuum filtration method, and fruits of hot peppers from Macedonian origin can serve as rich sources for capsaicin extraction. Namely using the same conditions (solid: liquid ratio, time for extraction and nearly close temperatures for extraction), much higher percent of extraction on capsaicin can be obtained by the Soxlet method. Also we can say that this spectrophotometric method for identification is not highly specific to the capsaicin, due to the fact that many other capsaicinoids can have similar absorption maxima, as well as some isomers of carotenoids. Assuming that the content of capsaicin in these spices is more than 2%, the daily intake of capsaicin can be estimated. Republic of Macedonia imports a lot of foodstuffs from several countries. These food products may be grown in different areas and exposed to different environmental conditions: soil composition, irrigation water and pesticides; so the objective of this work was to estimate the levels of capsaicin that may be present in some pepper samples available in local markets in Macedonia. These concentrations should allow us to calculate the Scoville heat units (SHU), determine the pungency level of each type of pepper analyzed, evaluate the Macedonian population daily intake of capsaicin, and compare the results with other studies carried out around the world to know whether these levels exceed the accepted international range.

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