

Spectral analysis of extracts from red hot pepper (*Capsicum annuum* L.)

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Introduction

Pepper is an excellent source of proteins, vitamins, minerals, fats and oils, phenolic compounds, aromatic substances and other biologically active compounds (Campos et al., 2013). The importance of the red hot pepper varieties and their oleoresin extracts in the food and pharmaceutical industry is caused by the characteristic compounds, capsaicinoids and carotenoids (Guzman et al., 2011). Mainly, for determination of the compounds in the sweet or hot pepper varieties, the chromatographic methods (TLC, HPLC and GC) and UV-VIS spectrometric method were used (Davis et al., 2008; Othman et al., 2011). On the other hand, ¹H NMR spectroscopy has been extensively used to provide information about the composition and relative content of fatty acid units in triglycerides (Barison et al., 2010). For analysis of the capsaicinoids and carotenoids, NMR spectroscopy is one of the most informative methods applied (Gómez-Calvario et al., 2015). The degree of unsaturation of vegetable oils can be effectively studied by IR spectroscopy based on the changes observed in the frequency data of some bands and in the ratios of absorbances of the IR spectra (Vlachos et al., 2006). Therefore, the aim of this study is to evaluate the possibility of applying spectroscop-

ic techniques (NMR and IR) in the characterization of extracts obtained from red hot pepper.

Materials and methods

Extracts from the pericarp, placenta, seeds, and stalk of red hot pepper (*Capsicum annuum* L., ssp. microcarpum longum conoides, convar. Horgoshka) obtained by extraction with n-hexane using Soxhlet method and supercritical carbon dioxide were analyzed using NMR and ATR-IR spectroscopy. The NMR spectra were run on a Bruker AVANCE II+ 600 spectrometer at ambient temperature. About 15 mg of each sample were dissolved in CDCl₃. TMS was used as an internal standard. The ATR-IR spectra were measured in the middle IR region 600-4000 cm⁻¹ on a Brucker Tensor 27 FT spectrometer. The samples were directly deposited on diamond crystal ATR accessory and spectra were recorded by accumulating 64 scans at resolution of 2 cm⁻¹.

Results and discussion

NMR Spectra. It was shown that the samples of extracts from the seeds contain exclusively triglycerides (TG). The calculated percent of unsaturated fatty acids was estimated about 80%. The estimated linoleic:oleic acid ra-

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tio was 2:1. N-polyunsaturated fatty acids (linolenic acid) were not observed. In this way the composition of TG in the seeds was shown to be similar to the corn and soybean oil. Traces of capsaicinoids were detected, while quantitative determination was not possible. Carotenoids were not detected. The spectra of the samples from pericarp showed similar composition as these from the seeds. Additionally, the presence of about 25% of n-polyunsaturated fatty acids was detected. In the samples from the placenta a substantial amount of capsaicinoids was presented. The proportion of TG:capsaicinoids was 1:2. In the region delta about 1.2-1.4 ppm intense signals appear for long CH₂ chains, probably waxes. In the stalk samples intensive signals were obtained due to presence of waxes. The proportion of the TG:capsaicinoids was approximately 1:0.15.

ATR-IR Spectra. The oil composition affects the exact position of the band for the C-H stretching of the *cis*-double bond, and yields higher-frequency shift when the oil has higher content of polyunsaturated acyl groups (Vlachos et al., 2006). Furthermore, the ratio of the absorbance of the bands responsible for the C-H stretching of the *cis*-double bonds and the asymmetric C-H stretching the methylene bonds could be used for quantitative estimation of the degree of unsaturation (Vlachos et al., 2006). In the ATR-IR spectra of the extracts of *Capsicum annuum* the band for the C-H stretching of the *cis*-double bond typical for vegetable oils rich in linoleic acid, such as soybean and corn oil, was found at 3009 cm⁻¹. According to the ratio of the absorbance at 3009 and 2923 cm⁻¹, the highest degree of unsaturation was found for the seed extracts. Also, the ATR-IR technique provides a fast and reliable identification of capsaicinoids in *Capsicum annuum* extracts. The typical frequencies of the amide C=O stretching and amide N-H bending vibration of capsaicin do not overlap with the IR bands of triglycerides, and therefore allow identification of capsaicinoids even at low concentrations. Among the studied extracts, those obtained from placenta had the highest content of capsaicinoids. However, for exact determination of the capsaicinoids content a calibration curve based on a series of standard mixtures with known amount of triglycerides and capsaicin is required.

Conclusion

Extracts from the pericarp, placenta, seeds, and stalk of red hot pepper (*Capsicum annuum* L., ssp. *microcarpum* longum conoides, convar. Horgoshka) were studied by NMR and IR spectroscopy. It was shown that both spectral techniques provide useful information on the triglyceride content and degree of unsaturation of the red hot pepper extracts. The IR spectroscopy could serve as a fast tool for identification of capsaicinoids in the extracts, while the NMR analysis could be successfully applied for determination of the proportion triglycerides:capsaicinoids.

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