Bulgarian Chemical Communications, Volume 51, Issue 1 (pp. 103 – 112) 2019

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

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Received November 11, 2017; Revised November 30, 2018

Samples from pericarp, placenta, seeds, and stalk of red hot pepper (*Capsicum annuum L.*), obtained by extraction with *n*-hexane and supercritical carbon dioxide were characterized using ATR-IR and NMR spectroscopy. It was shown that both NMR and IR spectroscopy provide useful information on the triacylglycerols composition, degree of unsaturation and capsaicin content of hot red pepper extracts as IR spectroscopy could serve as a fast tool for identification of capsaicinoids in the extracts, whereas NMR analysis could be successfully applied for determination of the proportion triacylglycerols : capsaicinoids.

Keywords: *Capsicum annuum L.*, red hot pepper, IR, NMR, triacylglycerols, capsaicinoids.

INTRODUCTION

Pepper is an excellent source of fibres, vitamins, minerals, proteins, lipids, phenolic compounds, aromatic substances and other biologically active compounds [1]. The importance of red hot pepper varieties and their oleoresin extracts in the food and pharmaceutical industries is due to the characteristic compounds, capsaicinoids and carotenoids [2]. Mainly, for determination of the compounds in the sweet or hot pepper varieties, chromatographic (TLC, HPLC and GC) and UV-VIS spectrometric methods were used [3, 4]. On the other hand, ^{1}H NMR spectroscopy has been extensively employed to provide information about the composition and relative content of fatty acid residues in triacylglycerols [5-7]. Moreover, NMR spectroscopy is one of the most informative methods applied for analysis of the capsaicinoids and carotenoids [8]. The degree of unsaturation of vegetable oils can be effectively studied also 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 [9, 10]. Thus, ^{1}H NMR and ATR-IR can be used as simpler, more rapid and less expensive methods requiring no sample pretreatment compared to the chromatographic methods. In continuation of our studies on the composition of red hot pepper (*Capsicum annuum L.*) [11, 12], the aim of this study was to evaluate the possibility of applying spectroscopic techniques (NMR and IR) in the characterization of extracts obtained from red hot pepper.

MATERIALS AND METHODS

Plant material and oils

Pericarp, placenta, seed, and stalk separated were obtained from red hot pepper (*Capsicum annuum* L. ssp. *microcarpum longum conoides* convar. Horgoshka), grown in the area of Markova Česma (geographical location: +41°21'36" N latitude, +21°33'36" E longitude and 640 m altitude), Prilep, Republic of Macedonia, in the year 2015. The dried samples with 12% moisture content determined using AOAC method no. 925.10 [13] were grounded using Retsch ZM1 mill (Haan, Germany) with sieve diameter of 0.25, 0.5 mm and 1.0 mm. Rose hip oil used as a reference was bought in a drugstore in Sofia (Bulgaria). Olive, soybean, sunflower, corn, walnut and linseed oils were from the local market.

Chemicals

Analytical grade solvents: acetone, ethanol, and *n*-hexane, as well as, phosphomolybdic acid, boron trichloride (BCl_3), 2,2-dimethoxypropane (DTP), and anhydrous sodium sulfate (Na_2SO_4) were supplied from Merck (Darmstadt, Germany).

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Carbon dioxide (CO_2, > 99.5% purity) and helium (He, > 99.9% purity) were purchased from Messer (Ruše, Slovenia). The standards of *β*-sitosterol, oleic acid, 1,3-diolein, 1-monoolein-rac-glycerol and reference standard mixture of fatty acids methyl esters (FAME Mix RM-6) were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). Deuterated chloroform ($CDCl_3$, 99.8% D) with tetramethylsilane (TMS) were purchased from Deutero GmbH (Kastellaun, Germany).

Extraction of plant material

The samples of pericarp, placenta, seed and stalk from red hot pepper were extracted with *n*-hexane and supercritical CO_2 (Table 1). Soxhlet procedure no. 920.85 [13] was used for the extraction of samples of red hot pepper with *n*-hexane. Five grams of sample (0.0001 g accurately weighted) were extracted in the presence of 10 boiling glass regulators with 200 mL *n*-hexane for 5 h. The solvent was removed from the extract by using rotary vacuum evaporator type Devarot (Ljubljana, Slovenia) at 40 °C and 200 mPa, and vacuum dried in a Heraeus Vacutherm VT 6025 (Langenselbold, Germany) at 40 °C and 105 mPa, followed by cooling in a desiccator and weighing. The steps of drying, cooling and weighing were repeated until the difference between two consecutive weights was smaller than 2 mg. The extraction of plant samples with supercritical CO_2 at 40°C and 400 bar was performed on an apparatus manufactured by the company UHDE-GmbH-Hagen from Hagen (Germany), according the procedure reported by Škerget and Knez [14]. The obtained extracts were evaporated to dryness under a steam of nitrogen. The extract samples accurately weighed (± 0.0001 g) were dissolved in *n*-hexane to 20 mg/mL stock solution concentration, prior to analysis.

Table 1. Extracts of red hot pepper (*Capsicum annuum* L.) obtained with *n*-hexane (A) and supercritical carbon dioxide (B).

Sample code	Plant material		Extraction method	Yield of extract (g/100 g DM*)
	Type	Particle size (mm)		
N1	seed	0.5	A	24.37
N2	seed	0.5	B	37.43
N3	seed	1	A	19.38
N4	seed	1	B	34.91
N5	pericarp	0.5	A	7.05
N6	pericarp	0.5	B	3.05
N7	pericarp	1	B	2.06
N8	placenta	0.25	A	8.87
N9	placenta	0.25	B	6.23
N10	placenta	0.5	A	7.05
N11	stalk	1	A	3.97
N12	stalk	0.25	B	9.95

*Calculated according to the corresponding dry matter (DM).

Table 2. Degree of unsaturation (A_1 / A_2 and A_1 / A_3) of the studied *Capsicum annuum* extracts and various edible oils calculated based on the peak heights for C-H stretching of the *cis*-double bonds (A_1), asymmetric (A_2) and symmetric (A_3) C-H stretching the methylene bonds.

Sample	Peak position for ν(=C-H) (cm^{-1})	Peak height for ν(=C-H) (A_1)	Peak height for $\nu^{as}(CH_2)$ (A_2)	Peak height for $\nu^{s}(CH_2)$ (A_3)	Degree of unsaturation (A_1 / A_2)	(A_1 / A_3)
Capsicum annuum seed extract						
N1	3009.2	0.022	0.130	0.089	0.169	0.247
N2	3009.5	0.012	0.066	0.046	0.182	0.261
N3	3009.1	0.027	0.159	0.108	0.170	0.250
N4	3009.3	0.027	0.159	0.108	0.170	0.250
Olive oil	3005.0	0.016	0.177	0.119	0.090	0.134
Soybean oil	3008.9	0.022	0.155	0.103	0.142	0.214
Sunflower oil	3008.3	0.023	0.157	0.104	0.146	0.221
Corn oil	3008.7	0.024	0.162	0.109	0.148	0.220
Walnut oil	3009.2	0.027	0.138	0.093	0.196	0.290
Linseed oil	3010.4	0.035	0.133	0.090	0.263	0.389

Attenuated total reflectance infrared spectroscopy

Attenuated total reflectance infrared (ATR-IR) spectroscopy was performed on a Bruker Tensor 27 FT spectrometer. The spectra were acquired in the range of 4000–600 cm^{-1} at a resolution of 2 cm^{-1} by accumulation of 64 scans. The samples were

directly deposited on diamond crystal ATR accessory.

Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE II+ 600 spectrometer at ambient temperature. About 15 mg of each sample were dissolved in 0.6 mL $CDCl_3$. TMS was used as an internal standard. The spectral data were reported in ppm. A 30 degree pulse of 3.6 μs and a relaxation delay of 5 s were used in quantitative analysis. Window functions Exponential Multiplication (LB = 0.2) and manual Base Line Correction were applied in the spectra processing.

Thin layer chromatography

Thin layer chromatography (TLC) for qualitative characterization of the main neutral lipid classes in the extracts was performed on aluminum sheets with 0.25 mm thick layer of silica gel 60 (Merck, Darmstadt, Germany). Samples were applied as spots (5 μL of each stock solution) with references applied nearby. After development with *n*-hexane:acetone (100:7, v/v) mixture as a mobile phase, the plates were air dried, sprayed with 10% ethanolic phosphomolybdic acid and heated at 120°C to visualize the separated components.

Gas chromatography

Fatty acid composition of the extracts was determined by gas chromatography (GC) of methyl esters (FAME) in accordance with the AOCS Official Method Ce 1–62 [15] using a Shimadzu GC 2010-Plus (Tokyo, Japan) gas chromatograph equipped with a flame ionization detector and a ZB-FFAP column (30 m × 0.25 mm × 0.25 μm, Phenomenex, USA). Methyl esters were prepared by derivatization of 0.1 g extract in 1 mL *n*-hexane with 2 mL 12% methanolic BCl_3 and 0.5 mL DTP. After heating at 60°C for 10 min the mixture was cooled and 1 mL Milli-Q water (PURELAB classic system, 18.2 MΩ-cm, ELGA, USA) and 1 mL *n*-hexane were added. The organic layer was dried by anhydrous Na_2SO_4 and then diluted to 10 mL with *n*-hexane. Aliquots of 1.0 μL were injected into the column. The carrier gas was He at 3.0 mL min^{-1}, split ratio 1:50. The oven temperature was set at 180°C (3 min) and increased by 2°C min^{-1} to 240°C (25 min). The injector and detector temperatures were 250°C and 260°C, respectively. Identification was according to the retention times of reference FAME standards.

RESULTS AND DISCUSSION

Thin layer chromatography

Samples were subjected to qualitative thin layer chromatography for characterization of their main components (Fig. 1). The results revealed that all extracts contained triacylglycerols (R_f about 0.7) as main component and small amounts of sterols, partial acylglycerols and other concomitants as minor components. The extracts from seeds (samples **N1 – N4**, Table 1) had the profile typical for plant seed oils (rose hip oil as a reference) and contained significantly lower quantities of polar compounds than the extracts from pericarp, placenta and stalk.

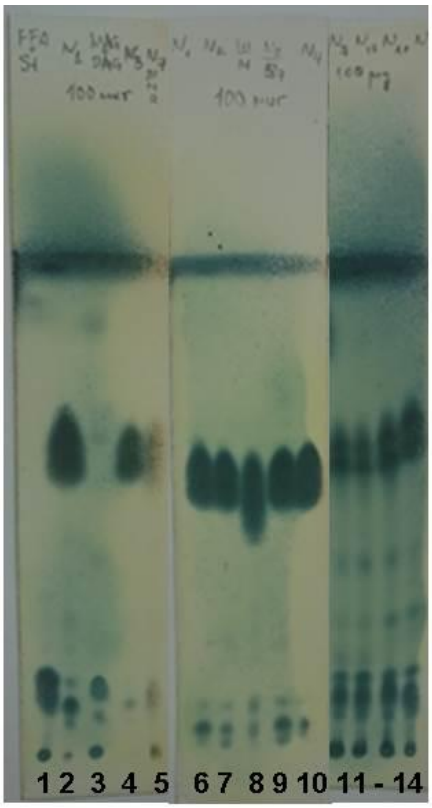


Fig. 1. TLC plates for qualitative characterization of extracts. Tracks: 1 – reference free fatty acids and sterols; 2 – sample **N1**; 3 – reference mono- and diacylglycerols; 4 - sample **N3**; 5 - sample **N7**; 6 - sample **N1**; 7 - sample **N2**; 8 – reference rose hip oil; 9 - sample **N3**; 10 - sample **N4**; 11 - sample **N9**; 12 - sample **N10**; 13 - sample **N11**; 14 - sample **N12**.

The official method for determination of the fatty acid composition of oils is by gas chromatography (GC) [15]. Predominant acyl residues in the triacylglycerols (TAG) of red hot pepper are those from saturated, oleic, linoleic and linolenic acids (Scheme 1).

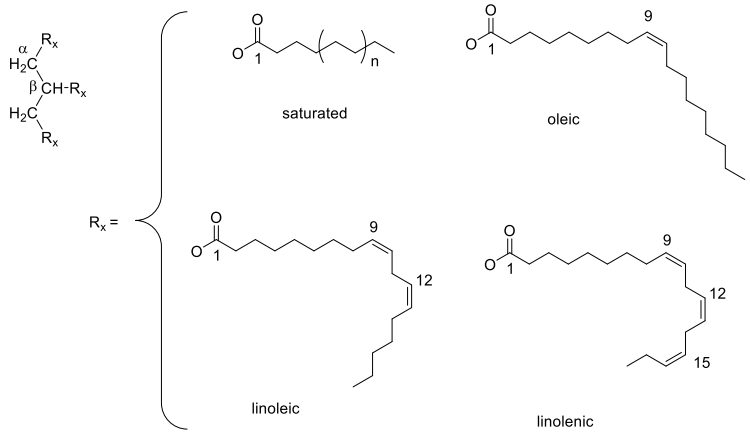
IR spectroscopy

The IR spectra of the extracts obtained from seed, pericarp, placenta, and stalk, of hot fruits of *Capsicum annuum* are presented in Fig. 2. However, the differences in the extract composition resulted in variations of the positions and intensities of the main IR bands as well as in the presence of additional bands in some of the cases.

The ATR-IR spectra of the seed extracts **N1 – N4** are very similar to each other. As illustrated in Fig. 2, the most characteristic bands appeared at the following positions: 3009 ν(=C-H), 2923 $\nu^{as}(CH_2)$, 2853 $\nu^{s}(CH_2)$, 1743 ν(C=O), 1464 $\delta(CH_2)$, 1377 $\delta^{s}(CH_3)$, 1237 ν(C-O), 1160 ν(C-O), and 722 cm^{-1} $\rho(CH_2)$. All of them originate from the molecular vibrations of triacylglycerols [10, 16]. It is known that the exact position of the band for the C-H stretching of the *cis*-double bond is sensitive to the oil composition, and it is shifted to higher frequency when the oil has higher content of polyunsaturated acyl groups [9, 10]. In the ATR-IR spectra of the seed extracts **N1 – N4** the band for ν(=C-H) was found at ca 3009 cm^{-1} (Table 2). This is near to the value measured in our laboratory using the same ATR technique for walnut oil which is rich in polyunsaturated acyl groups. In the ATR-IR spectrum of linseed oil the band is shifted to 3010 cm^{-1}. Likewise, for olive oil, which contains predominantly monounsaturated acyl residues, the band was found at 3005 cm^{-1}.

Furthermore, the ratio of the absorbance of the bands responsible for *cis*-double bond and methylene groups could be used for quantitative estimation of the degree of unsaturation of the oil [9, 10]. For this purpose, either the ratio of the peak heights A_1 and A_2 [10] or the peak heights A_1 and A_3 [9] can be used as a measure. The respective bands are denoted in Fig. 2. The ratios A_1 / A_2 and A_1 / A_3 calculated for extracts **N1 – N4** are compared with the ratios obtained under the same conditions for olive, soybean, corn, walnut and linseed oils (Table 2) which degree of unsaturation has been confirmed by their fatty acid composition [17]. Both ratios represent a uniform trend – the extracts from *Capsicum annuum L.* seeds show a degree of unsaturation much higher than the olive oil. The degree of unsaturation of extracts **N1 – N4** falls in between the values obtained for soybean, sunflower and corn oil on the one hand and walnut and linseed oil on the other hand.

The IR spectra of the pericarp extracts **N5** and N6 resemble very much that of the seed extracts. They show all characteristic bands for triacylglycerols content and the respective band positions are very similar to the above-mentioned ones. The presence of a small amount of capsaicin in extract **N7** was identified by the appearance of two weak bands at 1647 and 1515 cm^{-1} (Fig. 2). The positions of these bands correspond to the frequencies of the amide ν(C=O) and δ(N-H) vibrations of capsaicin (or other capsaicinoid compounds) [18, 19]. The ATR-FTIR spectra of extracts **N8 – N10** obtained from placenta show different IR characteristics (Fig. 2). Along with the bands corresponding to the vibrations of the triacylglycerols there are several bands originating from the capsaicinoids vibrations: 3332 cm^{-1} overlapped ν(O-H) and ν(N-H), 1647 ν(C=O), 1516 δ(N-H), 1273 $\gamma(CH_2)$, 1036 cm^{-1} $\nu(O\text{-}CH_3)$.



Scheme 1. Structure of the main fatty acids residues in TAG of red hot pepper.

(a)

(b)

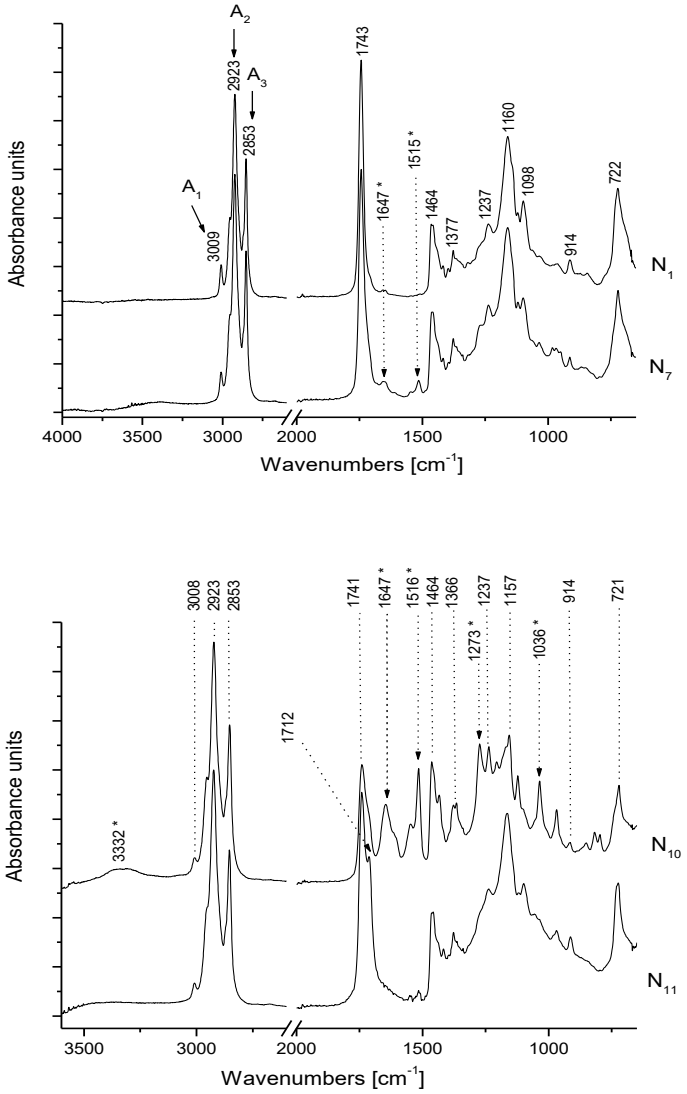


Fig. 2. ATR-IR spectra of *Capsicum annuum L.* extracts: (a) **N1** from seeds and **N7** from pericarp; peak heights A_1 – A_3 are used for quantitative estimation of the degree of unsaturation; IR bands attributed to capsaicinoids are marked with asterisk; (b) **N10** from placenta and **N11** from stalk; IR bands attributed to capsaicinoids

The band for the stretching vibration of the ester carbonyl groups of triacylglycerols is slightly shifted to lower frequencies compared to extracts **N1 – N7** and appears with an asymmetric shape on the low-frequency side. Another specific feature is the considerably increased intensity of the bands for the C-H stretching vibrations which are by far the most intensive in the spectra of **N8 – N10**. Such predominant intensity of the bands of the C-H stretching vibrations and appearance of multiple bands for carbonyl stretching is characteristic for plant waxes [20]. It could be assumed that extracts **N8 – N10** are a mixture of triacylglycerols, capsaicinoids and waxes.

The same is observed in the IR spectra of the stalk extracts **N11** and **N12** where the band for ν(C=O) of the triacylglycerols is at 1741 cm^{-1} overlapped with another one at 1712 cm^{-1} for ν(C=O) of plant waxes (Fig. 2). The amount of capsaicinoids is obviously much lower as only a weak band at 1515 cm^{-1} is present in the spectra of **N11** and **N12**.

The ratios A_1 / A_2 and A_1 / A_3 calculated from the spectra of **N5 – N7** are lower than those for the extracts from seeds. This fact might be due to different proportions of the monounsaturated and polyunsaturated fatty acid residues in the extracts obtained from different parts of *C. annuum L.* [21, 22].

The ratios A_1 / A_2 and A_1 / A_3 calculated for the placenta extracts **N8 – N10** are also lower than those for the extracts from seeds. It is in agreement

with the fact that the band for the C-H stretching of the *cis*-double bond is found at 3008 cm^{-1} in the ATR-IR spectra of **N8 – N10**. However, it should be taken into account that the intensity of the absorption A_2 and A_3 might be significantly increased by the presence of waxes, so the calculated ratio A_1 / A_2 and A_1 / A_3 would not reflect reliably the degree of unsaturation coming from the oil itself. In the ATR-IR spectra of **N11** and **N12** the band for ν(=C-H) is found at 3009 cm^{-1}, but the two extracts show much different degree of unsaturation. The degree of unsaturation of extract **N11** obtained by Soxhlet extraction of stalk with 1 mm, is similar to that of the extracts from pericarp and placenta, while the degree of unsaturation of extract **N12**, obtained by supercritical carbon dioxide extraction of stalk with 0.25 mm, is close to the values shown by the extracts of *Capsicum annuum* seeds.

The frequencies of the amide C=O stretching and amide N-H bending vibration of capsaicinoids do not overlap with the IR bands of triacylglycerols, and therefore might serve for identification of capsaicinoids even at low concentrations. However, the exact determination of the capsaicinoid content requires a calibration curve based on a series of standard mixtures with known amount of triacylglycerols and capsaicin.

NMR spectroscopy

A methodology for determination of the fatty acid composition through ^1H NMR has been proposed as a simpler, more rapid and less expensive method requiring no sample pretreatment. It can be explained briefly by the following [6, 7]:

The protons of the two αCH_2 groups of the glycerol show signals at δ 4.1 - 4.3 ppm with integral for total 4H ($2\times CH_2$), and the signals of the βCH at 5.23 ppm for 1H (Fig. 3). The proton signals at $\delta \sim 2.30$ ppm are due to $CO\text{-}CH_2$ groups. They would appear in the spectra of both saturated and unsaturated fatty acids. The same is valid for the signals of $CO\text{-}CH_2\text{-}CH_2$- groups of saturated and unsaturated fatty acids at $\delta \sim 1.6$ ppm. Each of these signals should show integral for 6H. The above mentioned signals can be used for calibration of the integral areas of the fatty acid signals in the ^1H spectrum. The proton signals of the terminal methyl groups appear at $\delta \sim$ 0.8-1.00 ppm and should be integrated for 9H.

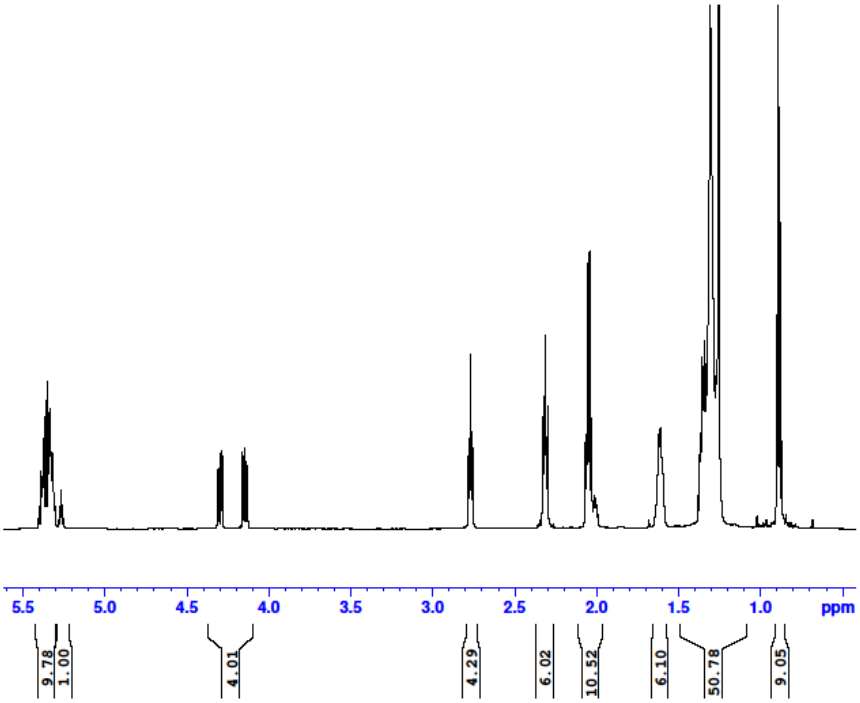


Fig. 3. ^1H NMR spectrum of the extract **N1** from seeds with supercritical carbon dioxide. On the bottom line are depicted the values of the integral areas.

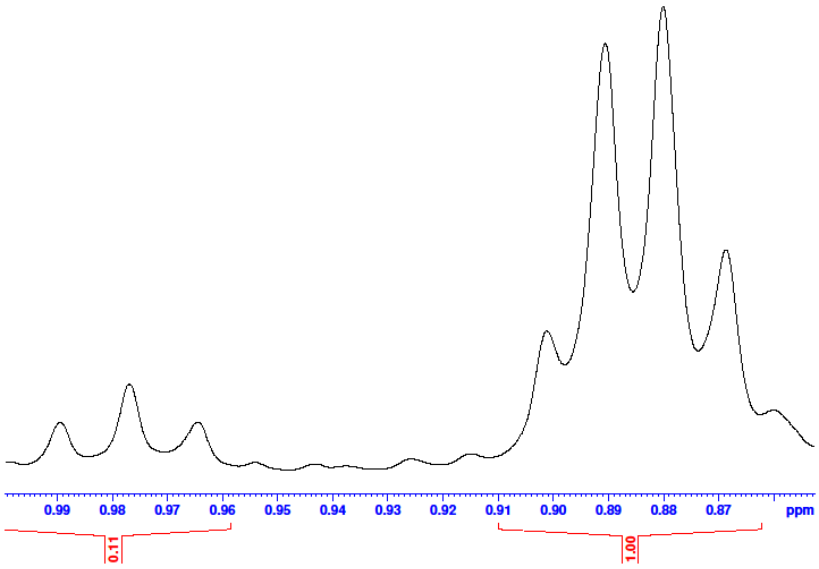


Fig. 4. ^{1}H NMR spectral region 0.86 - 1.00 ppm of extract **N6** from pericarp.

From them the triplet at δ 0.98 ppm gives the percentage of linolenic acid (Fig. 4).

The signals at δ 5.3 - 5.42 ppm correspond to the total amount of olefinic protons in unsaturated fatty acids.

Only polyunsaturated fatty acids would give signals at δ 2.78 ppm corresponding to the chemical shifts of the methylene hydrogens between two double bonds.

^{1}H NMR signals at δ 2.05 ppm are characteristic for the allylic methylene protons of all unsaturated fatty acids, including monoenoic and polyunsaturated and the molar percentage of unsaturated fatty acids could be obtained from the area of this peak.

For triacylglycerols containing virtually no saturated fatty acids residues the ratio of the peak areas at δ 2.05 ppm (allylic methylene protons) and terminal methyl protons $\delta \sim 0.8$ - 1.00 ppm should be 12:9.

The signals in the region 1.2 - 1.4 ppm belong to saturated CH_2 chains.

It should be noted that NMR spectra do not give information about the fatty acid distribution in α and β positions of glycerol units rather their proportion in the sample.

NMR spectroscopy is one of the most informative methods applied for analysis of the capsaicinoids. A large amount of NMR data of capsaicinoids has been published [8]. In capsaicin and all types of capsaicinoids the aromatic signals characteristic for a 1,2,4-substituted phenyl at δ 6.7 - 6.9 ppm are observed (d, $J \sim 8$ Hz; dd $J \sim 8$, 2 Hz and d, $J \sim 2$ Hz.

The protons at the double bonds of carotenoids resonate in the region of δ 5.7 – 6.5 ppm [23].

*Extracts from seeds - samples **N1 – N4***

The samples **N1** (Fig. 3) – **N4** showed similar spectra and fatty acids compositions. The signal at δ 0.98 ppm characteristic for linolenic acid was not observed. The fatty acids composition of N1 determined with GC and NMR analysis is given in Table 3. From the integrals of the peaks at 0.9 ppm and 2.05 ppm approximately 87% of unsaturated acid and 13% saturated, respectively could be calculated. The signal at δ 2.78 which corresponds to the chemical shifts of the double-allylic protons and is characteristic for polyunsaturated fatty acids. It was obviously due to the presence of linoleic acid because linolenic acid could be practically excluded. The results of the NMR analysis of samples **N1 – N4** vary in the range of a few percents (7% - 13%, 8% - 11% and 76%-82% for saturated, oleic and linoleic acids, respectively).

The results of the NMR spectra are in agreement with the results of GC analysis (Table 3) and with previous studies of *C. annuum* [21, 24].

Traces of capsaicinoids were detected. Quantitative determination was not possible.

In this way the composition of triacylglycerols in the seeds was shown to be similar to sunflower and soybean oil [6, 7, 24].

Table 3. Fatty acid composition of extract **N1** from seed obtained with GC and NMR analyses.

Fatty acid		GC	NMR
Saturated		*12.7*	13
Palmitic	C16:0	10.203	
Stearic	C18:0	2.494	
Monounsaturated		*8.7*	8
Palmitoleic	C16:1	0.167	
Oleic	C18:1	8.537	
Polyunsaturated		*78.3*	79
Linoleic	C18:2	78.183	
Linolenic	C18:3	0.145	

*Extracts from pericarp- samples **N5 – N7***

The spectra of the samples **N5 – N7** (Fig. 5) indicated more complex mixtures which made difficult the determination of the triglyceride composition. The main difference between the spectra of **N1 – N4** and the spectra of the samples **N5 – N7** is the signal at δ 0.98 ppm characteristic for the methyl groups of linolenic acid (Fig. 4). This finding is in accordance with the results of Perez-Calvez *et al.* [21] about the composition of fatty acids of the pericarp and seeds of some varieties of *C. annuum L.*

In addition, **N5** and **N6** showed intense signals in the region *of* δ 1.5 - 1.7 where protons from saturated long chains resonate (Fig. 5). In the spectrum of **N7** these signals did not appear. In the spectrum of **N7** a small amount of capsaicin (about 10%) was detected (Fig. 6).

*Extracts from placenta- samples **N8 – N10***

The samples **N8**, **N9** and **N10** from placenta (Fig. 7) showed similar spectra. In the region *of* $\delta \sim$ 1.2 - 1.4 ppm intense signals appeared for long CH_2 chains, probably waxes. The proportion of different types of fatty acids could not be estimated because of the overlap with the signals of capsaicinoids.

In the samples from the placenta a substantial amount of capsaicinoids was presented, approximately:

N8 - Capsaicinoids : TAG ~ 5 : 1.
N9 - Capsaicinoids : TAG ~ 2 : 1.
N10 - Capsaicinoids : TAG ~ 3 : 1.

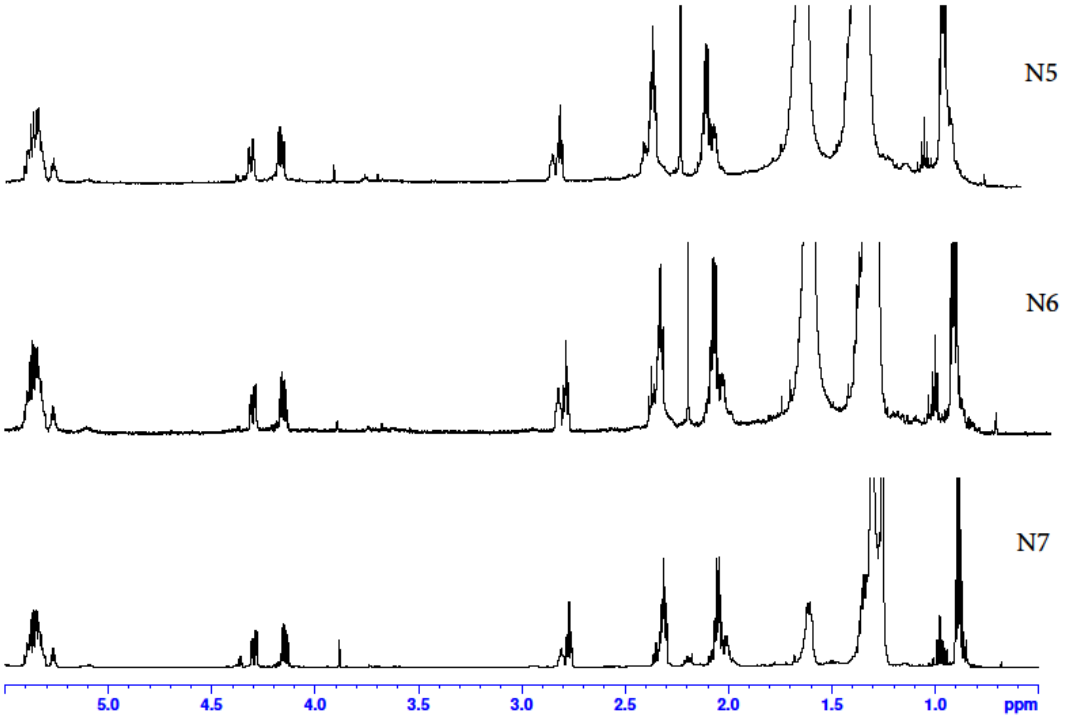


Fig. 5. ^{1}H NMR spectra of extracts from pericarp (**N5 – N7**) in the region 0 – 5.5 ppm.

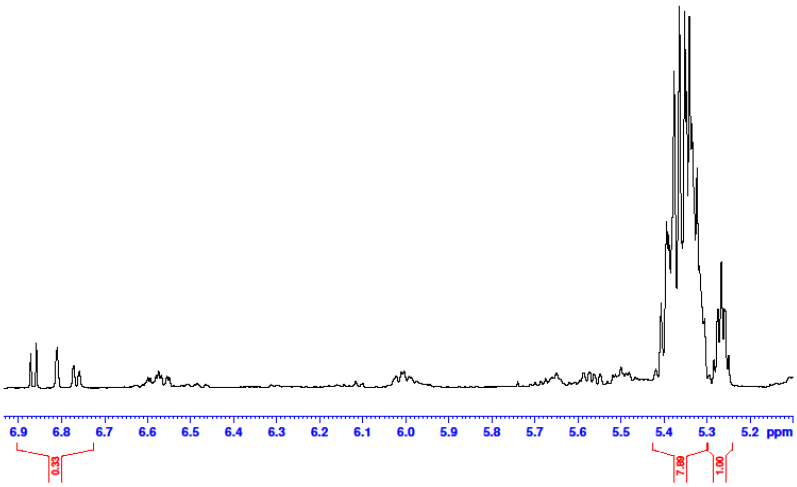


Fig. 6. ^{1}H NMR spectra of extract from pericarp (**N7**). ^{1}H NMR spectral region 5.1 - 7.0 ppm

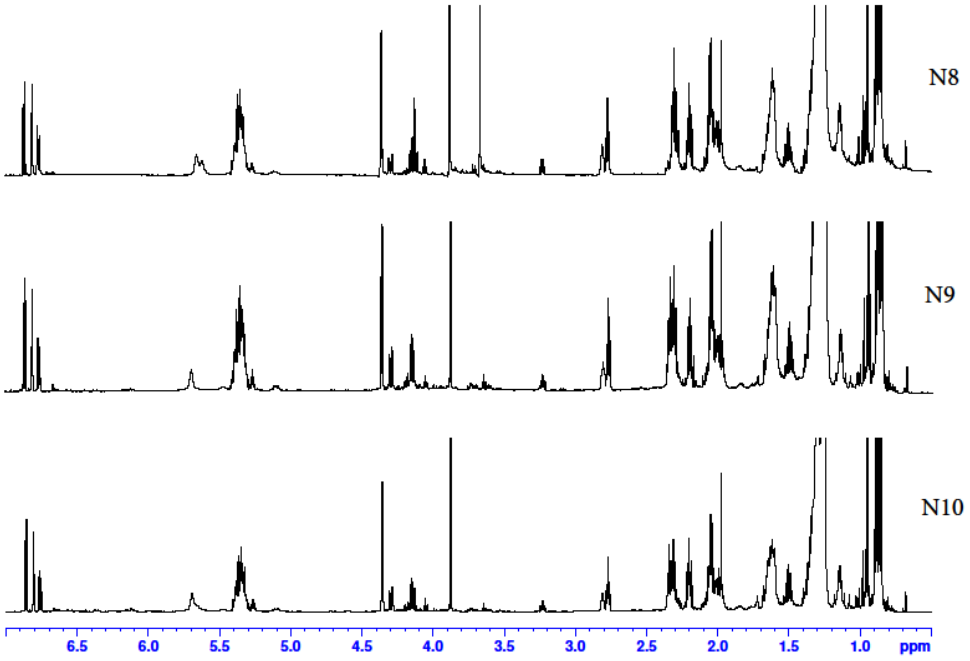


Fig. 7. ^{1}H NMR spectra of extracts from placenta (**N8** – **N10**).

*Extracts from placenta- samples **N8 - N10***

The samples **N8**, **N9** and **N10** from placenta (Fig. 7) showed similar spectra. In the region $\delta \sim$ 1.2 - 1.4 ppm intense signals appeared for long CH_2 chains, probably waxes. The proportion of different types of fatty acids could not be estimated because of the overlap with the signals of capsaicinoids.

In the samples from the placenta a substantial amount of capsaicinoids was presented, approximately:

N8 - Capsaicinoids : TAG ~ 5 : 1.

N9 - Capsaicinoids : TAG ~ 2 : 1.

N10 - Capsaicinoids : TAG ~ 3 : 1.

*Extracts from stalk - samples **N11**, **N12***

The samples **N11** and **N12** showed similar spectra and quantitative proportions. In the region $\delta \sim$ 1.2 - 1.4 ppm intensive signals were obtained probably due to presence of waxes. The proportion of different types of fatty acids could not be estimated because of the overlap with the signals of capsicinoids. The correlation capsaicinoids: TAG was estimated to be ~ 0.15 : 1.

Despite of the characteristic color of the samples carotenoids were not detected in the NMR and ATR-IR spectra.

It should be noted that NMR is less sensitive than the traditional chromatographic methods. The merit of the method is in its simplicity and swiftness but the quantitative measurements depend to some extent on the manually calibration of the integral area. For a more exact quantitative analysis the information from the NMR spectra should be supported with other analysis (GC, HPLC).

CONCLUSIONS

Extracts from the seeds, pericarp, placenta and stalk of hot red pepper (*Capsicum annuum* L., ssp. *microcarpum longum conoides*, convar. Horgoshka) were studied by NMR and IR spectroscopy. It was shown that both spectral techniques can be used to follow the extraction effectiveness in simple, rapid and inexpensive way without sample pretreatment and that both spectral techniques provide useful information on the triacylglycerol content and degree of unsaturation of the hot red 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 triacylglycerols : capsaicinoids.

In summary, the obtained spectral results showed that the seed extracts **N1 – N4** have similar triacylglycerol content with approximate ratio of saturated (7% - 13%), oleic (8% - 11%) and linoleic acids (76% - 82%) and only traces of capsaicinoids. The pericarp extracts **N5 – N7** encompassed more complex triacylglycerol mixtures including linolenic acid, saturated long chain hydrocarbons – presumably plant waxes, and a small amount of capsaicinoids in the case of N7. On the other hand, the placenta extracts **N8 – N10** had predominant capsaicinoid content with capsaicinoids : TAG ratio from 5:1 to 2:1. The stalk extracts **N11** and **N12** contained triglycerols, plant waxes and smaller amount of capsaicinoids. The ratio capsaicinoids : TAG was estimated to be ~ 0.15 : 1.

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