

PHYSICOCHEMICAL CHARACTERIZATION AND BIOACTIVE COMPOUNDS OF STALK FROM HOT FRUITS OF *CAPSICUM ANNUUM* L.

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In stalk of red hot pepper (*Capsicum annuum* L.) with 0.25 mm, 0.5 mm and 1.0 mm particle sizes, the quantities of nutritive and volatile compounds, bioactive capsaicin and carotenoids were determined. Furthermore, the stalk was characterized using TGA, DSC and FTIR spectroscopy. The influence of the particle size on the content of proteins, ash and cellulose is insignificant. Compared to 0.5 and 1.0 mm, stalk with 0.25 mm particle size was darker with a deeper yellow-brown color, and richer in microelements. Among the quantified volatile compounds, 6 were esters, 2 terpenoids, and 1 acid. The highest quantity of extract rich fats was obtained from stalk with 0.25 mm particle size by using ethanol, which is more efficient for capsaicin extraction, while *n*-hexane is efficient for extraction of carotenoids. Stalk with 0.25 mm particle size was characterized with a higher degradation temperature and residual weight.

Keywords: red hot pepper stalk; physicochemical characterization; nutritive value; bioactive compounds; thermogravimetric analysis

ФИЗИЧКОХЕМИСКА КАРАКТЕРИЗАЦИЈА И БИОАКТИВНИ КОМПОНЕНТИ ВО ДРШКАТА ОД ЛУТИ ПЛОДОВИ НА *CAPSICUM ANNUUM* L.

Во дршката од црвена лута пиперка (*Capsicum annuum* L.) со големина на честички од 0,25 mm, 0,5 mm и 1,0 mm се утврдени количествата на нутритивните и испарливите компоненти, на биоактивниот капсаицин и каротеноидите. Дршката беше карактеризирана и со помош на TGA, DSC и FTIR–спектрометрија. Влијанието на големината на честичките врз содржината на протеини, пепел и целулоза е незначително. Во споредба со 0,5 mm и 1,0 mm, дршката со големина на честички од 0,25 mm е потемна, со изразито жолтокафеава боја и побогата со микроелементи. Од испарливите компоненти кои беа квантифицирани, 6 беа естери, 2 терпеноиди и 1 киселина. Најголемо количество екстракт богат со масти беше добиено од дршката со големина на честички од 0,25 mm со користење на етанол, кој е поефикасен за екстракција на капсаицинонот, додека *n*-хексан е ефикасен за екстракција на каротеноидите. Дршката со големина на честички од 0,25 mm се карактеризира со повисока температура на деградација и повисок масен остаток.

Клучни зборови: дршка од црвена лута пиперка; физичкохемиска карактеризација; нутритивна вредност; биоактивни компоненти; термогравиметриска анализа

1. INTRODUCTION

Pepper (*Capsicum annuum* L., *Solanaceae*) as a widely distributed vegetable crop in the world is included in nutrition either fresh or processed, in various specialities or as spices and extracts. Pepper is rich in proteins, fats and oils, minerals, vitamins, ascorbic acid, phenolic compounds, aromatic substances and other biologically active compounds [1]. The characteristic compounds, capsaicinoids and carotenoids, explain the importance of the red hot pepper varieties and their oleoresin extracts in the food and pharmaceutical industry [2, 3].

Pungency, a commercially important pepper characteristic, is defined by the capsaicinoids, *i.e.* a mixture of seven homologous branched-chain alkyl vanillylamides. Capsaicin and dihydrocapsaicin are the most abundant, while norcapsaicin, nordihydrocapsaicin, nomordihydrocapsaicin, homodihydrocapsaicin and homocapsaicin are found in relative low quantities [4]. Interest in determining and confirming the activities of capsaicin has increased in the last twenty years. The action of capsaicin in pain relief [5], chemoprevention [6], body weight regulation through fat thermogenesis [7], cardiovascular and gastrointestinal system maintenance [8], glucose level reduction in blood [9] as well as in haematuria treatment [10] has been studied. The application of capsaicinoids as natural product-based food additives, antimicrobial agents [11] and self-defense product constituents [12] is of high importance. The positive effects of capsaicin on carotenoid deposition in egg yolk, stimulating chicken egg production and improving broiler body weight [13] has been confirmed also. The yellow-orange colors of pepper fruits are mainly due to the accumulation of α - and β -carotene, zeaxanthin, lutein and β -cryptoxanthin. Capsanthin, capsorubin and capsanthin-5,6-epoxide are responsible for the deep red colors [14]. Carotenoids possess a range of important biological activities. They are potent antioxidants acting as scavengers of singlet molecular oxygen, peroxy radicals and reactive nitrogen species [15]. The consumption of carotenoid-rich foods reduces the incidence of several disorders such as cancers, cardiovascular diseases, age-related macular degeneration, cataracts, diseases related to compromised immune function, and other degenerative diseases [16].

The pericarp is the edible part of pepper fruits, while the seed, placenta and stalk are waste obtained during pepper processing. Although treated as environmental pollutants, they are recognized as a source of valuable compounds also. As report-

ed in the literature, the seed and placenta of hot red pepper are characterized by a valuable content of proteins and micro- and macroelements. The placenta, in comparison to the pericarp and seed, contains over 60% of the total quantity of capsaicinoids, capsaicin and dihydrocapsaicin [17]. A strong antioxidant and cytostatic activity, as well as anti-inflammatory effects were determined for stalk extracts due to the high content of phenols, flavonoids and capsaicin [18–20].

Nowadays, plant origin waste, which is accumulated in agro-industrial fields or discarded into rivers causing environmental problems, has no significant industrial or commercial use even though it is rich in biologically active compounds. The re-utilization of agricultural waste as a bioresource for the production of novel products is related to its chemical composition and properties. In general, the chemical composition is dependent on the crop species and variety, geographic origin, and conditions during growing, storage and processing [21]. Agricultural wastes are heterogeneous materials that need to be reduced into small particles before processing. The particle size fractions obtained from grinding can differ in their chemical characteristics. In ground samples, the extractives were preferably present in the smaller particle sizes, while higher proportions of lignin, cellulose and hemicelluloses were determined in the largest particle sizes [22, 23].

In the case of pepper stalk, no physicochemical characterization and determination of the content of nutritive and bioactive compounds has been carried out. Therefore, the objective of this paper was to characterize red hot pepper stalk regarding its nutritive values, surface color parameters, volatile compounds, biologically active capsaicin and carotenoids, and thermogravimetric characteristics. The influence of particle size on the physicochemical characteristics and content of biologically active compounds was also studied with the aim of evaluating the potential use of stalk in value-added products.

2. EXPERIMENTAL

2.1. Plant material

The peppers, *Capsicum annuum* L. ssp. *microcarpum longum conoides* convar. Horgoshka, were grown in the locality of Markova Česma, Prilep (geographical location: +41°21'36" N latitude, +21°33'36" E longitude and 640 m altitude),

Republic of Macedonia, in the year 2014. The fruits were harvested at the fully ripe stage (red color, 10–11 cm long) and dried in a dry and ventilated place. After drying, the stalk was cut manually with a knife, and ground using a Retsch ZM1 mill (Germany), with sieve sizes of 0.25 mm, 0.5 mm and 1.0 mm. The stalk samples were placed in dark glass bottles and stored at 4 °C in a refrigerator. The weight fraction of the stalk as a constituent part of the red hot pepper fruit was determined gravimetrically with 0.0001 g accuracy as an average value of 100 red hot pepper fruits. The average weight of the stalk was 0.19 ± 0.044 g or 10.26 % of the whole red hot pepper fruits. The red hot pepper stalk fractions with particle sizes of 0.25 to 1.0 mm presented clear differences in color and shape. Stalk with the 1.0 mm particle size contained yellow-brown cylindrical structures with spherical peduncles. The stalk fractions with 0.5 and 0.25 mm particle size were predominantly formed of rounded particles with light brown and dark brown color, respectively.

2.2. Chemicals and standards

Reagent grade chemicals used for plant material characterization were purchased from Alkaloid AD (R. Macedonia) and Merck (Germany). For the extraction of plant material and determination of carotenoids and capsaicinoids, analytical grade solvents: ethanol, acetone and *n*-hexane supplied from Merck (Germany) were used. The capsaicin standard (65% pure) was supplied by Fluka (Switzerland). Standards: 1-propanol, hexyl hexanoate, hexyl isopentanoate, heptyl butanoate, benzyl benzoate, hexyl isobutanoate, hexyl 2-methyl butanoate, α -ionone, β -ionone and tetradecanoic acid were purchased from Fluka (Switzerland), with a purity >98 %.

2.3. Plant material composition analysis

The nutritional chemical composition of the red hot pepper stalk was determined through standard procedures given by AOAC [24]: moisture content by drying at 105 °C until constant mass (925.10), total proteins based on the nitrogen content ($N \times 6.25$) determined by using the Kjeldahl method (978.04), crude fats by extracting with petroleum ether (40–60 °C) using the Soxhlet method (920.85), ash by mineralization at 900 °C (923.03), macro- and microelements by atomic absorption spectroscopy (985.29), and crude fiber

with the gravimetric procedure (985.29). Total and reductive sugars were determined using the Bertrand method [25]. Energy content was calculated according to the food–energy conversion factors [26] by the following equation: energy (kcal) = 2.44 (g proteins) + 3.37 (g total carbohydrates) + 8.37 (g fats).

2.4. Surface color determination

The color parameters, L^* (lightness–darkness), a^* (red–green), b^* (yellow–blue), C (chroma), and Hue angle (Ho) of the red hot pepper stalk were measured using a Dr. Lange spectra colorimeter (Chelmsford, UK). The samples were placed in a 1 cm cell. L^* , a^* , b^* , C , and Ho values were determined using Illuminant D65 and 10° observer angle. The standardized values for a white plate were $L^* = 95.93$, $a^* = -0.19$, and $b^* = 3.12$.

2.5. Volatile compound analysis

The types and concentrations of volatile flavor compounds were determined using a headspace gas chromatography system (HS-GC), *Shimadzu GC 2010-Plus* (Japan), equipped with a flame ionization detector, and capillary column ZB-5 (30 m \times 0.25 mm i.d., 0.25 μ m film thickness, Phenomenex, USA). *Chromatographic conditions*: FID detector temperature 290 °C; helium carrier gas, flow rate 1.0 ml min^{-1} ; *Column temperature program*: initial 40 °C, rate 5 °C min^{-1} to 280 °C, held at 280 °C for 5.0 min, split ratio = 1:40. *Shimadzu GC 2010-Plus Headspace* (HS-20) system, oven: 150 °C; needle: 150 °C; transfer line: 150 °C; sample equilibration: 20.0 min at 100 °C; pressurization: 3.0 min; sample transfer time: 0.5 min; withdraw needle: 1.0 min. Identification of the volatiles was based on comparison with the GC retention time of reference standards (RS). The volatile compounds determination was done in duplicate for each stalk sample.

2.6. Extraction of red hot pepper stalk

Soxhlet procedure no. 920.85 [24] was used for the stalk extraction. A 5 g stalk sample (0.0001 g accurately weighed) was extracted in the presence of 10 boiling glass regulators with 200 ml *n*-hexane or ethanol. After 5 h extraction, the solvent was removed from the extract using a rotary vacuum evaporator (40 °C, 200 mPa). The solvent traces were removed by drying 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 less than 2 mg. The yield of extract was calculated based on the dry matter (DM) weight of the stalk used.

2.7. Determination of total carotenoids

The absorbance of the stalk extract (50 mg) dissolved in acetone (10 ml) was measured at 460 nm on a Varian Cary Scan 50 spectrophotometer (Switzerland) in 1 cm quartz cells, at 25 °C. The carotenoid content was calculated using the extinction coefficient of the major carotenoid capsanthin in acetone, $^{1\%}E_{460\text{nm}} = 2300$ [27].

2.8. Determination of capsaicin

The capsaicin was quantified by measuring the absorbance at 282 nm of the stalk extract dissolved in ethanol. The equation of the calibration curve prepared with standard capsaicin solutions in ethanol was used, $A = 0.0144 \gamma - 0.0091$ ($R^2 = 0.9995$), where γ is μg capsaicin in ml extract and A is absorbance [28].

2.9. Fourier transform infrared ray (FTIR) spectroscopy

Attenuated total reflectance (ATR) spectra were collected by a Varian 660 FT-IR spectrometer (CA, USA) with a MIRacle ZnSe ATR module (PIKE technologies) with low pressure micrometer clamp. The FTIR spectra were acquired in the range of 4000–550 cm^{-1} at a resolution of 4 cm^{-1} by accumulation of 32 scans.

2.10. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA/DTA)

DSC analysis was performed with a NETZSCH DSC 204 F1 instrument, in a temperature range from 0 to 250 °C with a heating rate of 10 °C min^{-1} . The measurements were carried out under a dynamic nitrogen atmosphere (30 ml min^{-1}) in pierced aluminum pans. TGA/DTA based on EN ISO 11358 standard [29] was performed using a Perkin Elmer DIAMOND system (Japan) in the nitrogen atmosphere, at a temperature range from 25 to 1000 °C, with a heating rate of 20 °C min^{-1} .

2.11. Statistical analysis

STATISTICA 8 software (StatSoft, Inc., USA) was applied in the statistical analysis of the results. The data were analyzed with one-way ANOVA followed by multiple comparisons with Tukey's honest significant difference (HSD) test at the 5 % significance level ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Chemical composition

The summative chemical composition of the separated stalk from dried fruits of red hot pepper is presented in Table 1. Generally, the influence of the particle size on the difference in the chemical composition is insignificant. The content of proteins expressed in relation to the corresponding DM varied from 17.73 to 17.78 %. The fat content increases with decreasing particle size. The highest value for fat (3.70 % DM) was determined in stalk with 0.25 mm particle size. The determined quantity of ash and cellulose was around 10 and 26 % DM, respectively. The content of reductive sugars decreased from 6.31 to 6.16 % when increasing the particle size from 0.25 mm to 1.0 mm. The energetic contribution for red hot pepper stalk with particle sizes of 0.25 mm, 0.5 mm and 1.0 mm expressed in kcal/100 g DM was 195.80, 188.47 and 180.63, respectively.

Krstic *et al.* [30] reported a higher content of cellulose in the stalk of hot pepper varieties (20.29% DM) than in sweet varieties (17.13% DM). In comparison to the pericarp and the seed from the same variety of hot red pepper [17], the stalk is richer in proteins (17.77% DM) and ash (3.76% DM). The cellulose content in stalk samples is higher than the cellulose value in the seed (22.66 % DM) and placenta (15.05% DM). The stalk has a higher content of reductive sugars than the seed (2.96 %). The highest contents of cuprum (Cu), zinc (Zn), manganese (Mn) and iron (Fe) were found in stalk with a particle size of 0.25 mm. In comparison to the pericarp and seed, the placenta was characterized by the highest quantities of Fe (916.7 mg kg^{-1} DM), Mg and Ca (4572.9 and 1767.8 mg kg^{-1} DM, respectively). The stalk has a lower content of Cu, Zn and Fe and higher content of Mn than the pericarp, placenta and seed.

Table 1

Chemical composition and surface color data of the red hot pepper stalk with different particle sizes

Characteristic		Particle size (mm)		
		0.25	0.5	1.0
Moisture content (%) ¹		7.84 ^a ± 0.06	8.02 ^a ± 0.08	8.01 ^a ± 0.11
Proteins (%) ^{1,2}		17.77 ^a ± 0.03	17.73 ^a ± 0.05	17.78 ^a ± 0.09
Fats (%) ^{1,2}		3.70 ^a ± 0.12	2.98 ^b ± 0.10	1.97 ^c ± 0.06
Ash (%) ^{1,2}		9.75 ^a ± 0.05	9.68 ^b ± 0.04	9.79 ^b ± 0.02
Cellulose (%) ^{1,2}		25.91 ^a ± 0.08	25.71 ^{ab} ± 0.03	25.87 ^b ± 0.02
Reductive sugars (%) ^{1,2}		6.31 ^a ± 0.02	6.19 ^b ± 0.02	6.16 ^b ± 0.05
Cu (mg kg ⁻¹) ^{1,2}		9.16 ^a ± 0.06	9.06 ^a ± 0.07	8.25 ^a ± 0.08
Zn (mg kg ⁻¹) ^{1,2}		14.05 ^a ± 0.19	13.77 ^a ± 0.21	10.71 ^b ± 0.41
Mn (mg kg ⁻¹) ^{1,2}		90.90 ^a ± 2.57	71.86 ^b ± 1.60	71.01 ^b ± 1.67
Fe (mg kg ⁻¹) ^{1,2}		175.44 ^a ± 2.58	163.55 ^b ± 2.35	132.47 ^c ± 1.42
Extract yield (g kg ⁻¹) ^{1,2}	<i>E</i>	236.77 ^a ± 0.82	112.31 ^b ± 0.55	54.77 ^c ± 0.76
	<i>H</i>	95.89 ^a ± 0.30	63.69 ^b ± 0.46	39.64 ^c ± 0.54
Capsaicin (mg kg ⁻¹) ^{1,2}	<i>E</i>	28.75 ^a ± 0.68	23.00 ^b ± 0.66	22.14 ^b ± 0.46
	<i>H</i>	9.28 ^a ± 0.46	6.16 ^b ± 0.44	2.22 ^b ± 0.116
Total carotenoids (mg kg ⁻¹) ^{1,2}	<i>E</i>	228.15 ^a ± 3.61	272.34 ^a ± 1.36	338.38 ^b ± 5.07
	<i>H</i>	344.19 ^a ± 22.59	443.32 ^b ± 13.10	721.10 ^c ± 15.90
L* ¹		68.42 ^a ± 0.18	65.58 ^b ± 0.22	64.29 ^c ± 0.08
a* ¹		6.47 ^a ± 0.11	6.64 ^a ± 0.28	7.26 ^b ± 0.17
b* ¹		25.07 ^a ± 0.24	23.73 ^b ± 0.43	23.29 ^b ± 0.20
C ¹		24.50 ^a ± 0.20	24.41 ^b ± 0.16	24.93 ^b ± 0.70
Ho ¹		74.43 ^a ± 0.25	74.13 ^b ± 0.58	72.50 ^c ± 0.19

¹Data are expressed as mean ± standard deviation ($n = 3$). ²Calculated according to the corresponding dry matter (DM).*E* – Ethanol; *H* – *n*-Hexane. ^{a,b,c} Values with different superscripts within a row are significantly different (Tukey test, $p < 0.05$).

3.2. Extractives and content of capsaicin and color compounds

The results for the quantities of extractives and the contents of capsaicin and total carotenoids in red hot pepper stalk are presented in Table 1. Concerning the quantity of extractives obtained by ethanol and *n*-hexane, the major proportion corresponds to polar compounds solubilized by ethanol. The highest extract quantity (236.77 g kg⁻¹ DM) was obtained from stalk with a 0.25 mm particle size when ethanol was applied as solvent. Ethanol was more effective in the extraction of capsaicin, while *n*-hexane was better for the extraction of total carotenoids. No data were found in the literature related to the quantity of extractives and carotenoid content in red hot pepper stalk. The published data for the pericarp of the red hot pepper cultivars indicate that methanol and ethanol are more appropriate for the extraction of bigger quantities of extracts richer in capsaicinoids, while *n*-hexane and acetone are more suitable for pigments extraction [31]. Chen *et al.* [20] reported that the

stalk extract obtained with methanol is richer in capsaicin than placenta and seed extract.

3.3. Surface color

As presented in Table 1, the surface color data of the stalk samples ranged from 64.9 to 68.42 (L*), 6.47 to 7.26 (a*) and 23.29 to 25.07 (b*). The color saturation (C*) and hue angle (Ho) decrease with increase of the particle size. The L* and Ho values significantly increase with decreasing stalk particle size ($p < 0.05$). In the case of both solvents used, the quantity of total carotenoids extracted from the stalk samples increases with increasing particle size, which is in agreement with the findings for a*. The stalk sample with 0.25 mm particle size was darker, duller and deeper yellow-brown in color than the samples with particle sizes of 0.5 and 1.0 mm. In the literature no data were found about the color characteristics of pepper stalk. The surface color parameters published are related to the pericarp as an edible part of pepper fruits. The degree of maturity of the pepper [32, 33], the influence of the drying and processing

conditions [34, 35], and the differences in pepper varieties have been studied by using the surface color characteristics. The pericarp is characterized with lower values of L^* (29.95÷31.61) and b^* (6.99÷10.49). The a^* values are higher and varied from 21.81 to 31.10 [33, 34].

3.4. Volatile compounds

In the stalk of hot red pepper 39 volatile compounds were separated by HS/GC analysis. A typical chromatogram of stalk volatiles is shown in Figure 1.

The identity and quantity of nine components confirmed by using reference standards are listed in Table 2. Among all the quantified volatiles, ester hexyl isobutanoate is the greatest in quantity. The highest contents of hexyl isobutanoate (3.27 mg kg⁻¹ DM) and tetradecanoic acid (3.05 mg kg⁻¹ DM)

were found in stalk with 1.0 mm particle size. The content of terpenoid β -ionone was almost six times higher than α -ionone. No data about the volatile compounds of pepper stalk have been reported in the literature. Generally, the chili pepper varieties are well-known to be very aromatic and they contain characteristic volatile compounds. Hexyl isopentanoate and hexyl isobutanoate are responsible for a powerful fruity odor note [36]. β -ionone makes a great contribution as an odor-active compound to the pepper aroma according to Zimmermann and Schieberle [37], while the presence of α -ionone, dihydro- β -ionone and β -ionone suggests that β -carotene may be considered to be its precursor [38]. Aromatic esters such as benzyl pentanoate, hexyl benzoate and isohexyl benzoate have powerful fruity flavor notes and they might therefore contribute to the overall flavor of *Capsicum* species [39].

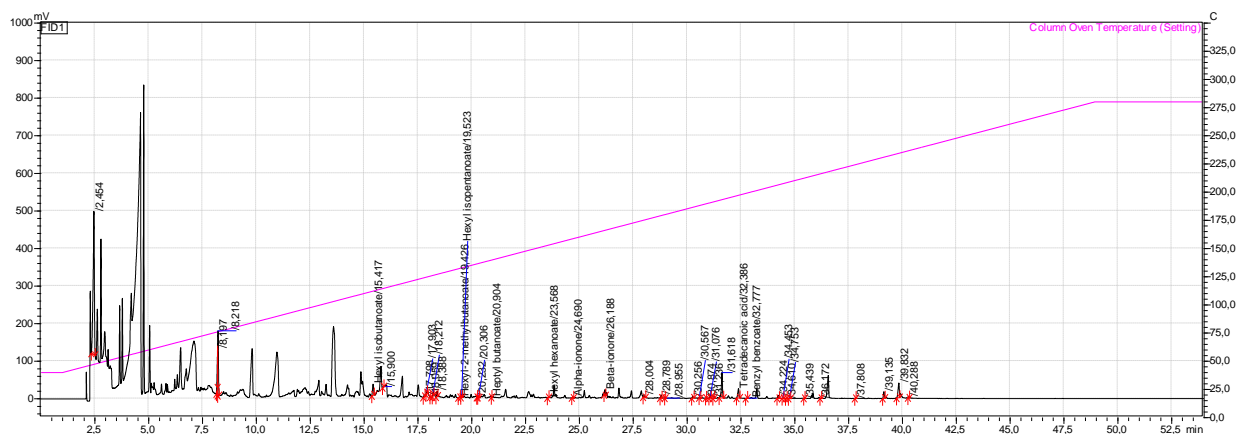


Fig. 1. Typical chromatogram of volatiles of red hot pepper stalk with 1mm particle size

Table 2

Volatile compounds (mg kg⁻¹ DM)* in hot red pepper stalk with different particle sizes

Compound	Retention time (min)	Stalk particle size (mm)		
		0.25	0.5	1.0
Hexyl isobutanoate	15.417	1.38	1.78	3.27
Hexyl-2-methyl butanoate	19.426	0.02	0.02	0.18
Hexyl isopentanoate	19.523	0.20	0.12	0.10
Heptyl butanoate	20.904	0.32	0.28	0.09
Hexyl hexanoate	23.568	0.16	0.13	0.05
α -Ionone	24.695	0.51	0.39	0.29
β -Ionone	26.188	1.02	1.27	1.32
Tetradecanoic acid	32.386	0.02	0.02	3.05
Benzyl benzoate	32.777	1.02	0.09	0.03

*DM

3.5. Thermogravimetric (TG/DTG) and differential scanning calorimetry (DSC) data

Figure 2 shows the thermal stability curve (TGA) and the derivative of the TGA weight loss curve, or the rate of weight loss (DTG) for the red hot pepper stalk with particle sizes of 0.25 mm, 0.5 mm and 1 mm. The similar shaped TGA curves obtained for all samples showed a four-stage weight loss below 600 °C (Fig. 2). The initial sharp slope in the first region, starting from room temperature up to 100 °C corresponds, to the water loss (drying). The moisture content was in the range from 2 % for 1 mm stalk to 3 % for 0.5 mm stalk, which is comparable with the data of Ali *et al.* [40]. In the second rather narrow region from 100 to 170 °C, the stalk samples experienced the first weight loss (about 6 % of the sample), decomposed into volatiles because of the thermal decomposition of low molecular weight components. Generally, the cellulose degradation occurs in the range of 240 to 350 °C [41]. In the third region in the temperature range of 210 to 230 °C,

the cellulose in the stalk starts to decompose slowly with 16 % of the weight loss. At a temperature around 304 °C hemicellulose continues to decompose with a weight loss of 30–33 %. In the temperature range from 392 to 433 °C, the lignin components in the stalk samples decompose with around 50 % weight loss. The corresponding DTG curves (Fig. 2) show a series of maxima related to vaporization of water or to decomposition of stalk compounds. The stalk samples with a smaller particle size (0.25 mm) showed higher thermal stability, with a higher initial and final degradation temperature, as well as a higher residual weight. The DSC curves of the hot red pepper stalk samples with particle sizes of 0.25 mm, 0.5 mm and 1 mm are presented in Figure 3. The characteristic glass transition temperature (T_g) was determined in the range of 49 to 56 °C. It increases with increase of the particle size ($T_g = 56$ °C for 1 mm stalk; $T_g = 52$ °C for 0.5 mm stalk; $T_g = 49$ °C for 0.25 mm stalk). It is evident that T_g slightly decreases with decrease of the particle size of the red hot pepper stalk [42].

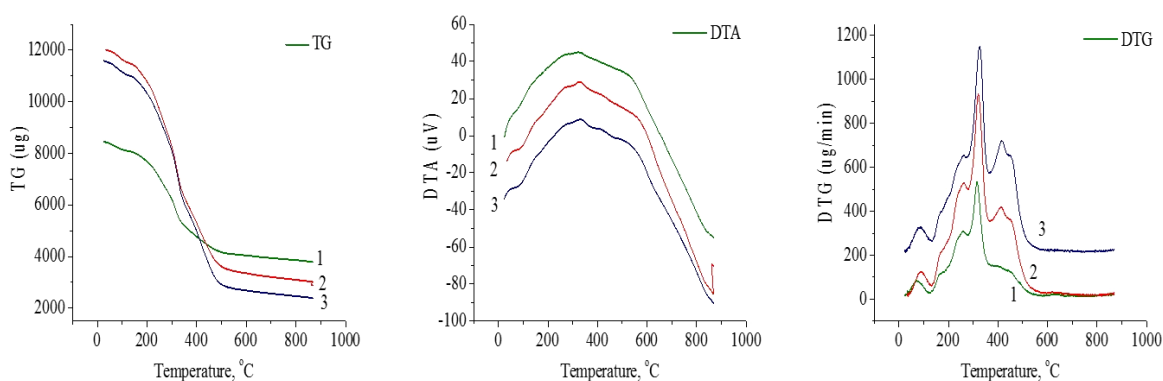


Fig. 2. TGA/DTA curves of red hot pepper stalk with particle sizes of 0.25 mm (1), 0.5 mm (2) and 1.0 mm (3)

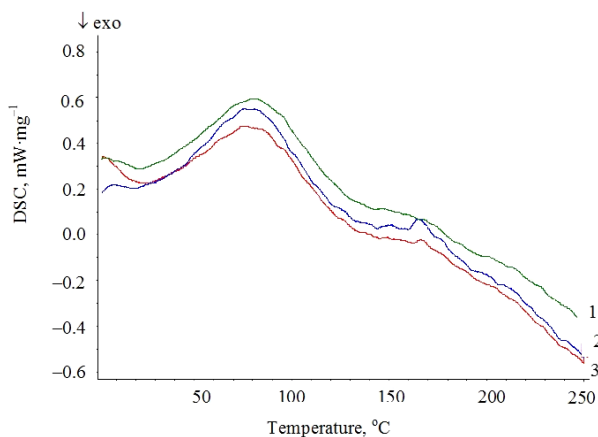


Fig. 3. DSC curves of red hot pepper stalk with particle sizes of 0.25 mm (1), 0.5 mm (2) and 1.0 mm (3).

3.6. FTIR spectra

The FTIR spectra of different particle size fractions of red hot pepper stalk are presented in Figure 4. The spectra display a number of adsorption peaks indicating the complex nature of this material. The broad peak at around 3300–3400 cm^{-1} is indicative of OH vibration modes. The two sharp peaks at 2920 cm^{-1} and 2880 cm^{-1} correspond to the asymmetric and symmetric vibration, respectively, C-H in the olefinic chains, and the peak at 1743 cm^{-1} is attributed to the carbonyl C-O in ester groups. The presence of lignin was confirmed by the typical lignin bands at 1323 cm^{-1} , 1270 cm^{-1} and 1600 cm^{-1} , the first two bands being

attributed to skeletal vibrations of aromatic rings with CO stretching, and the last one to aromatic skeletal vibrations [43]. The band at 1450 cm^{-1} associated with deformation vibration of C-H in the aromatic ring of lignin moieties is less intense. The typical peaks of polysaccharides appear at 1075 cm^{-1} , 1118 cm^{-1} and 996 cm^{-1} . Comparison among the FTIR spectra of different particle size fractions of red hot pepper stalk shows that the above mentioned peaks are present in all fractions. Slight differences in the 2870 cm^{-1} band intensity can be observed in the finest particle size fraction compared to the other two ones. For the finest fraction (0.25 mm), the most relevant differences are observed in the bands attributed to polysaccharides (1075 cm^{-1} , 1118 cm^{-1} and 996 cm^{-1}) and to lignin (1525 and 1450 cm^{-1}), which show lower peak intensities than the other two fractions.

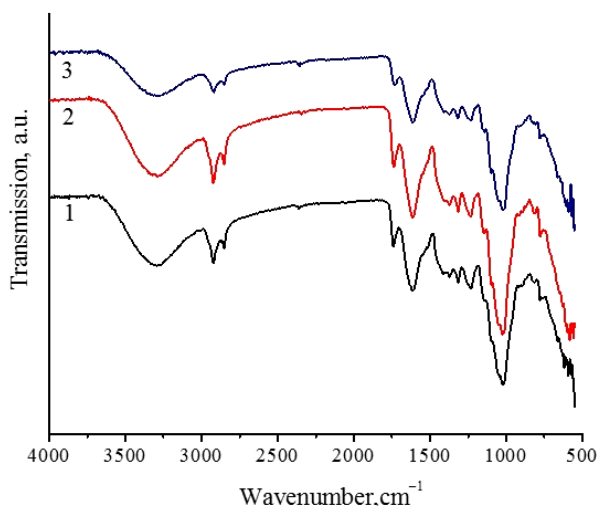


Fig. 4. FTIR spectra of red hot pepper stalk with particle sizes of 0.25 mm (1), 0.5 mm (2) and 1.0 mm (3)

4. CONCLUSION

The results show that stalk separated from the fruits of red hot pepper variety *Capsicum annum* L. ssp. *microcarpum longum conoides* convar. Horgoshka, cultivated in the Republic of Macedonia, can be a promising source of natural valuable products, thus improving the efficiency of resources utilization.

The differences obtained concerning the quantity of extractives, fats and content of capsaicinoids, carotenoids and volatile compounds emphasize the need for reducing the stalk particle size to 0.25 mm.

TGA–DTA/DCS/FTIR analysis shows that ground red hot pepper stalk with different particle sizes has potential as a renewable, cellulose-based,

micro-reinforcing filler in biocomposites for industrial and biotechnology applications. Stalk with higher particle sizes (1 mm and higher) is more convenient for composites with improved mechanical behavior because they will be able to take the load. Smaller stalk particles (0.5 and 0.25 mm) would be suitable for barrier/filtration systems, since they could be incorporated on a macromolecular level into the polymer matrix. The thermogravimetric study of the stalk will be very important to better understand the thermal degradation behavior of natural fibers in relation to polymers obtained by nanocomposite processing.

The findings acquired from this research will be valuable in the future utilization of stalk as feed and food supplements or a polymer matrix ingredient, as well as a renewable energy feedstock.

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