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PHYSICO-CHEMICAL STUDY OF FLAVONOIDS FROM DIFFERENT MATURENESS CORN SILK MATERIAL

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ABSTRACT

There was tested a simple extraction procedure of flavonoids separation from the original corn silk (CS) material. It was found, that the total flavonoids content differs with the extraction time and extraction temperature. There were found different flavonoids contents in extracts prepared from different maturity stages of the original corn silk material (silking stage (CS-S), milky stage (CS-M)). Extracted flavonoids content was quantified by the lutin standardization method by means of colorimetry at 510 nm wavelength. Obsreved flavonoids concentration was ranging from 2×10^{-3} mg.mL^{-1} to 7×10^{-3} mg.mL^{-1} dependent on the extraction time period and extraction temperature. The highest flavonoids concentration of 7.5×10^{-3} mg.mL^{-1} was found for CS-M after 20 minutes extraction time and 80 °C extraction temperature. There was confirmed the presence of flavonoids by fluorescence mapping experiments. There was found a typical multistep decomposition process for both CS-S and CS-M materials by TG analysis. There was found a melting temperature of flavonoids of 54.3 °C for corn silk silking stage material exhibiting 58.9 J.g^{-1} heat of fusion and 60.2 °C for corn silk milky stage material with 112.9 J.g^{-1} heat of fusion. The optimal conditions of corn silk flavonoids extraction were 40 °C, 50 minutes for CS-S, the optimal flavonoids extraction content was $(6.8\pm2.1)\times10^{-3}$ mg.mL^{-1}, 80°C, 20 minutes for CS-M and the optimal extraction content was $(7.2\pm0.3)\times10^{-3}$ mg.mL^{-1}.

Keywords: corn silk; silking stage; milky stage; flavonoids; UV-VIS

INTRODUCTION

Corn has a widespread application as a domastic animals feed, food additives and material of alcohol through fermentated or unfermentated technology **(Ivanišová et al., 2017; Michalová and Tančinová, 2017)**. Corn silk (CS) is the dried thrum and stigma of Zea mays L. (corn), cheap, high yielding and usually considered as a by-product to be abandoned, burned or used as fodder **(Zhang, 1998)**. It is one of the Chinese traditional medicine recorded in many classics. According to the Southern Yunnan Material Medicine and Chinese Medicine Dictionary, corn silk is non-poisonous, also diuretic, cholagogic and resolutive. Corn silk could be used to cure many kinds of diseases clinically, such as diabetes, nephritis and hypertension etc **(Jin, 1980)**. In addition, in terms of the results from the worldwide scientists, corn silk also has the effects of anti-fatigue **(Hu et al., 2010)**, anti-depression **(Mahmoudi and Ehteshami, 2010)**, anti-free radical, anti-cancer **(Ebrahimzadeh, Pourmorad and Hafezi, 2008; Maksimović and Kovačević, 2003)** and anti-radiation **(Bai et al., 2010)**. Native American Indians usually use corn silk to cure urinary tract infection, malaria and heart disease **(Hasanudin et al., 2012)**. In many countries, corn silk is applied to sell in markets as tea and weight-losing products for its good effect of cooling blood, purging heat and removing the damp and heat in human body.

The previous researches have successfully applied the fermentated corn fodder to improve the nutrition quality of chicken meat **(Angelovicová and Semivanová, 2013; Macanga et al., 2017; Štenclová et al., 2016)**. In addition, corn was also used to improve the sensory quality of crackers **(Kuchtová et al., 2016)**. The corn fermentated alcohol has a broad usage in food and chemistry industry, veterinary, pharmaceutical and manufacturing industry for its nutritional value and anti-oxidant properties **(Krejzová et al., 2017; Süli, Hamarová, and Sobeková, 2017)**.

Corn silk contains many sorts of nutritional and functional ingredients, including sterols, polysaccharides, alkaloids, flavones, cryptoxanthins, polyphenols, organic acids, vitamins and allantoins etc. **(Li and Lapcik, 2018)**

Corn silk flavonoids (CSF) are one of the most important sorts of nutrients in corn silk, which is not only a role of pigment but also the cause of the corn silk extracts antioxidant activity conditions **(Li and Lapcik, 2018)**. The total flavonoids extractive technology includes hot water, alkaline water or alkaline dilute alcohol and organic solvent extraction. Additionally, microwave, ultrasonic extraction, supercritical fluid extraction, enzyme, aqueous two-phase,

semi bionic extraction, membrane separation, thermal fluid extraction and high pressure liquid extraction **(Jing et al., 2016; Ko, Kwon and Chung, 2016; Shan et al., 2012; Wang et al., 2016; Wei et al., 2013; Weiz et al., 2016; Xie et al., 2017; Yang et al., 2017; Zhang, Shan and Gao, 2011; Zhou et al., 2011)**. **Peng et al. (2016)** applied 80 ℃ hot water extraction, the parity of CSF was 10.45% **(Peng, Zhang and Zhou, 2016)**; **Liu et al. (2005)** applied 50 ℃ 95% ethanol to extract CSF, the highest total CSF content was 69.4 ±5.1 μg RE.g^{-1} DCS (RE = rutin equivalents; DCS = dry mass of corn silk) **(Liu et al. 2011)**; **Liu et al. (2011)** used supercritical fluid to extract CSF and used BBD response surface methodology to analyse and optimize the extractive conditions. The maximal yield of CSF was approximately 4.24 mg.g^{-1}, the optimal conditions were 50.88 ℃, 41.80 MPa, 2.488 mL.g^{-1} water content in ethanol co-solvent, 120 min extractive time, 0.4 mm particle sizes and 20% aqueous ethanol as the co-solvent **(Liu et al., 2011)**.

UV-VIS method has been used to measure the content and determine the kinds of flavonoids in plants **(Liu et al., 2011)** but the research of extraction conditions optimization, flavonoids properties comparison and analysis and the determination of the flavonoids kind in different mature stages of corn silk is rarely reported. As found in the studies mentioned above applied extraction procedure has an effect on the total flavonoids content of corn silk material. We expect, that the maturity stage of the corn silk used as a source material for extraction will affect the total flavonoids content obtained as well. That is why, this research focuses on the evaluation of the extraction procedures, kinetics and stage of the corn maturity effects on the final composition of the extracted materials, allowing in more detail knowledge for the on going development of the novel types of nutrition or health care products both for human and veterinary applications.

Scientific hypothesis

In the current research, the content and sorts of flavonoids in plants have been measured and determined by UV-VIS and fluorescence techniques. However, the extraction conditions optimization, flavonoids properties comparison and analysis and the determination of the flavonoids kind in different mature stages of corn silk are rarely reported. We expect the maturity stage of the corn silk will be a significant effective factor for the total extracted flavonoids content. That is why, this research is focused on the quantification of the total flavonoids content extracted from different maturity stages of the corn silk at defined extraction conditions such as extraction time and temperature.

MATERIAL AND METHODOLOGY

Corn silk samples were collected from the corn kernels type dent produced in a field in Southern Moravia agricultural region (Uherské Hradiště County, Czech Republic). Fresh corn silk fibers were first 14 days dryied on air in the shade and then final drying was done in a thermostatic hot air-drying oven (Hot air sterillizator Stericell 55 Standard, BMT Medical Technology, Czech Republic), pulverized and sifted through a 80mesh sieve (Analysette 3, Fritsch, Germany) to obtain the final product powder samples. There were collected two types of corn silk materials, dependent on the growth stage. The first one was silking stage (assigned as CS-S), the second one was the milky stage (assigned as CS-M) **(Rahman and Wan Rosli, 2014; Sarepoua et al., 2015)**.

All reagents and chemicals used in this research such as rutin, ethanol, sodium nitrite, aluminium nitrate and

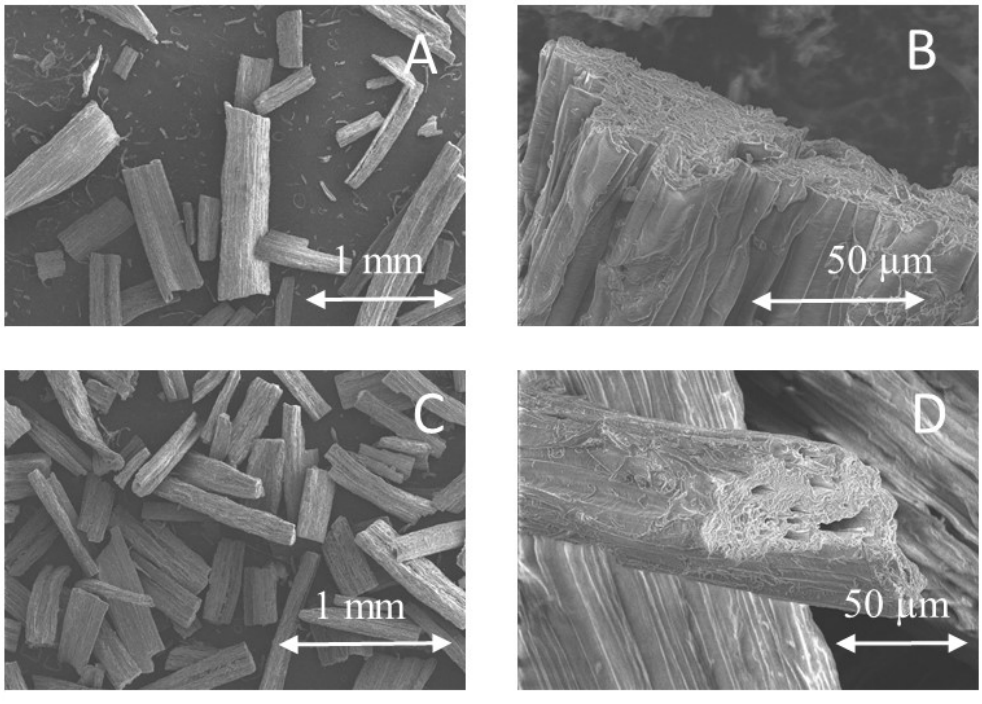

Figure 1 SEM images of the tested corn silk material: A,B – sample CS-S, C,D – sample CS-M.

sodium hydroxide were purchased from Sigma-Aldrich (USA) in an analytical reagents purity grade. As a solvent distilled water was used. Distilled water conductivity was about 0.6 $\mu S.cm^{-1}$).

UV/VIS spectrophotometer used was Lambda 25 (Perkin Elmer, MA, USA). Measurements were performed in the wavelength range from 200 to 700 nm in 1 cm quartz cells (Marques et al., 2013).

Thermogravimetry (TG) and differential thermal analysis (DTA) experiments were performed on simultaneous DTA-TG apparatus (Shimadzu DTG 60, Japan). Measurements were performed at heat flow rate of 5 °C.min^{-1} in the static nitrogen atmosphere (gas flow of 50 mL.min^{-1}) at the temperature range from 30 °C to 550 °C. The apparat was calibrated using Indium as a standard **(Liu et al., 2005; Wu et al., 2008)**.

Fluorescence excitation−emission maps of the different maturity stages corn silk extracts were measured on a FLS980 fluorescence spectrometer (Edinburgh Instruments, UK). Each experiment was repeated 10 times.

Samples were pulverized in a table top blender (Philips HR2170/40, The Netherlands).

Rutin standard curve determination procedure **(Peng et al., 2016)**: disolve 20 mg lutin into 70 v.% ethanol to 50 mL (0.4mg.mL^{-1} lutin solution); separately were brought 0, 1, 2, 4, 6, 8, 10 mL 0.4mg.mL^{-1} lutin standard solutions into 50 mL volumetric flasks, added 70% ethanol 12 mL, then added 2 mL 5 w.% $NaNO_2$, shaked up and placed for 10 min to react. Then into the solutions were added 2 mL 10 w.% $Al(NO_3)_3$, shaked up and placed for 10min to react, then diluted with 20 mL 10 w.% NaOH to the scale of volumetric flask, placed for 5 min. Each experiment was repeated 5 times. There was used 510 nm UV spectrometry to measure the absorbance of the solutions. Obtained absorbance vs. concentration dependency data were used to build up the standard curve. The numerical linear regression analysis was performed to obtain standard curve lineasr regression parameters. Each experiment was repeated 3 times.

Determination of the flavonoids content procedure **(Peng et al., 2016)**: Use the 1/10 solid-liquid ratio of cornsilk powder and 70 v.% ethanol to extract the flavonoids in temperatures of 40°C and 80°C for 20, 30, 40, 50, 60 minutes extraction time intervals. Then the flavonoids extract solutions were centrifuged on Hettich EBA 21 centrifuge (Germany) at 3000 rpm for 10 min to get the supernatant. Then there was used the same methodology as lutin standard curve to measure the flavonoids absorbance and there was used the lutin standard curve to count the given content of flavonoids. Each experiment was repeated 5 times.

STATISIC ANALYSIS

Statistical analysis of the observed data were performed by using one way analysis of variance (ANOVA) method (Microsoft Excel, USA). This analysis allowed to detect the significance of the effect of extraction time, temperature and the maturity stage on extracted amounts of flavonoids. Five extraction times, two maturity stages and two extraction temperatures were considered in this study. Each experiment was replicated 5 times. Differences were considered significant at $p \leq 0.05$. Additionally, the mean values and standard errors were calculated from all measurements by application of the SigmaPlot 8.0 software (SPSS, USA). Differencies between obtained emission peaks located at 450 nm were analyzed by one-way analysis of variance (ANOVA) method (Origin 8.5.0 software was used (OriginLab, USA)). Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

In Figure 1 there are shown SEM images of the tested corn silk powders. They are characteristic with the rectangular shape of individual particles exhibiting complex

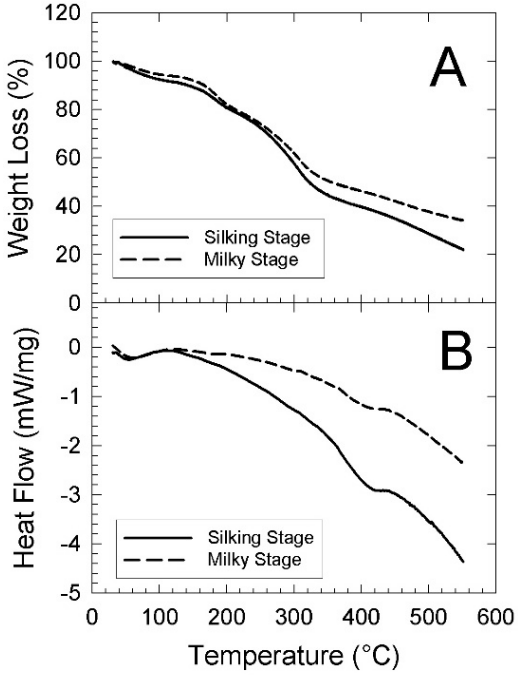

Figure 2 Results of the thermal analysis of the tested corn silk samples: A – Thermogravimetry (TG), B – differential thermal analysis (DTA) data.

microporous structure on the intersection. Such structures are typical for plants cellulose-based materials.

Prior to the extraction, the moisture content and thermoal analysis of the samples was performed. Results of TG and DTA analysis are shown in Figure 2. These are typical of multistep decomposition process for both CS-S and CS-M samples as shown in Figure 2A. The first step decomposition for sample CS-S was found in the temperature range of 30 to 120 °C with observed weight loss being 8.3% attributed to the moisture content. Total decomposition step was about 77.45% in the temperature range of 30 to 550 °C. Similarly, for the sample CS-M TG

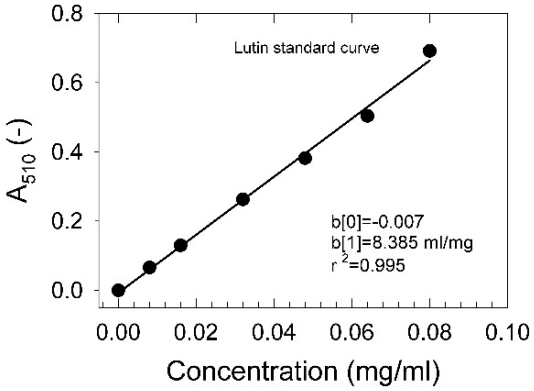

Figure 3 Lutin standard curve. Inset: Linear regression standard curve parameters.

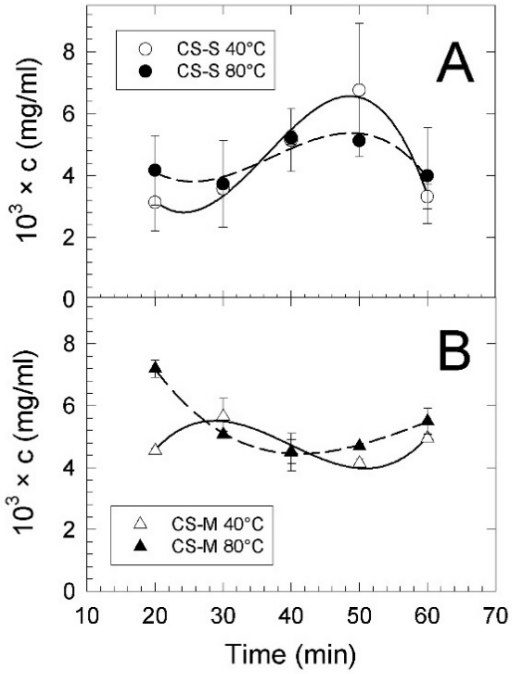

Figure 4 Flavonoids extraction kinetics: A – corn silk silking stage, B – corn silk milky stage. Values for CS-S 40 °C, CS-M 40 °C and CS-M 80 °C were considered significantly different ($p \leq 0.05$). Values for CS-S 80 °C were considered not significantly different ($p \geq 0.3$).

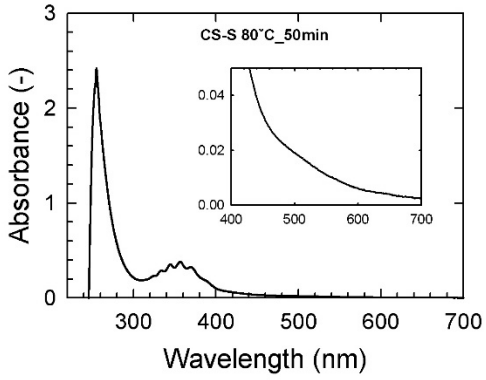

Figure 5 UV VIS spectrum of the CS-S sample extracted at 80 °C temperature after 50 min extraction time. Inset: expanded 400 nm to 700 nm region.

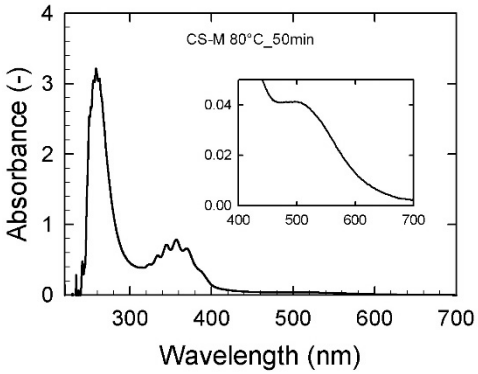

Figure 6 UV VIS spectrum of the CS-M sample extracted at 80 °C temperature after 50 min extraction time. Inset: expanded 400 nm to 700 nm region.

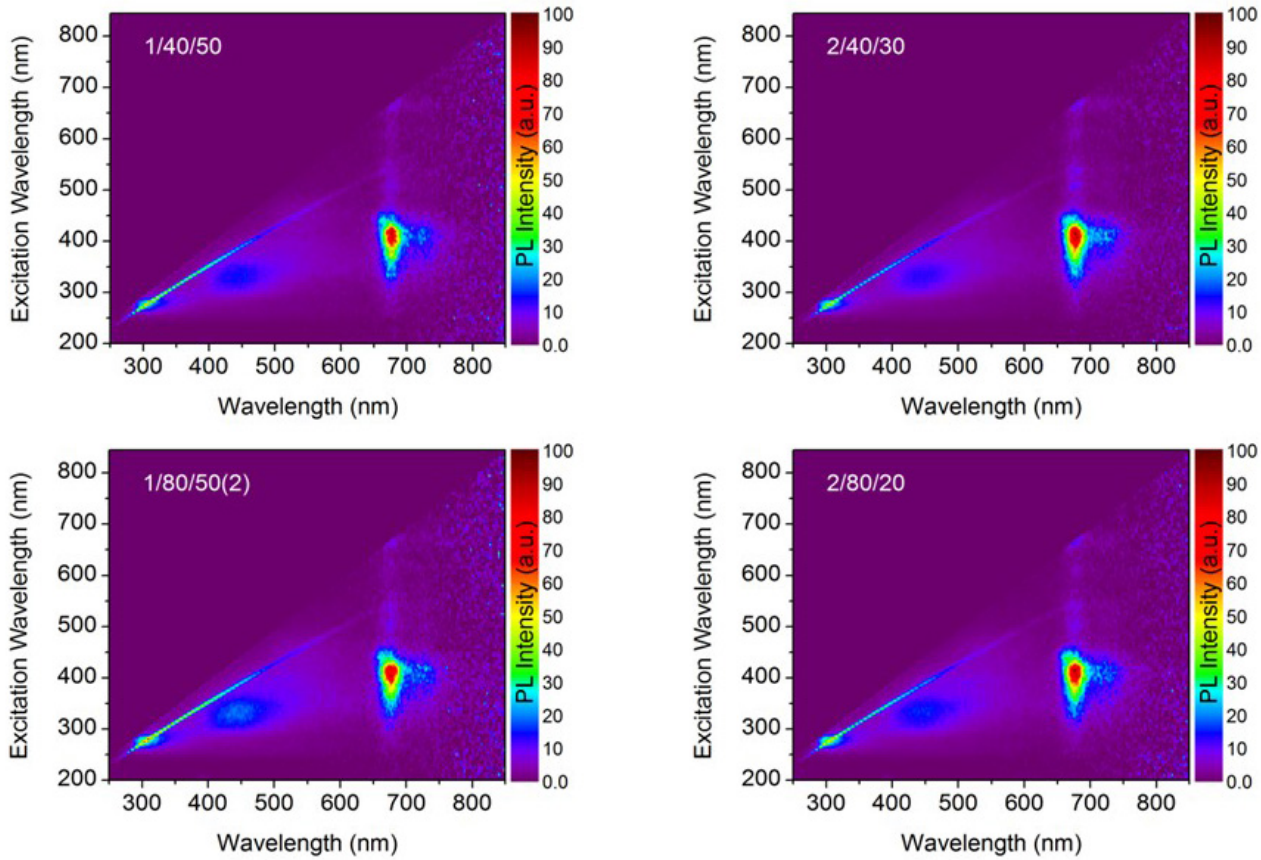

Figure 7 Results of the fluorescence excitation-emmision mapping of the studied corn silk extracts.
Note: Inset legend X/Y/Z: X=1 corresponds to CS-S sample, X=2 corresponds to CS-M sample, Y is the extraction temperature (40 °C or 80 °C), Z is extraction time in minutes (20, 30 or 50 minutes).

data exhibited the first step decomposition of 5.9% attribute to the moisture content in the same temperature range of 30 to 120 °C followed by the total weight loss step of 65.5% in the temperature ramge of 30 to 550 °C indicating, that the CS-S contains more thermally labile substances in comparison to CS-M samples. In Figure 2B there are shown two endothermic peaks. The first one located in the temperature of 54.3° for CS-S and of 60.2 °C for CS-M attributed to the melting point of flavonoids (Miziara et al. 2017). The second endothermic peaks observed at 415.1 °C (CS-M) and 419.7 °C (CS-S) were attributed to the total thermal decomposition with formation of a low quantity carbonaceous residues respectively. The heat of fusion corresponding to the first endothermic peak was of 58.9 $J.g^{-1}$ for CS-S and of 112.9 $J.g^{-1}$ for CS-M samples.

In Figure 3 there is shown a typical lutin standard curve as obtained according to the standard procedure described in detail in the materials and methodology section. Obtained regression parameters are given as the Figure 3 inset as well. Obtained data were of a high correlation as indicated by the correlation coefficient being 0.995.

Effects of the extraction time and of the extraction temperature are summarized in Figure 4, where kinetics data of the extraction of CS-S and CS-M samples are plotted

in flavonoids concentration vs. extraction time coordinates. Each extraction was performed at two different temperature, the first one was of 40 °C and the second one at 80 °C. Both, the CS-S as well as the CS-M dependencies were of a complex non-linear character, modeled as a third order polynomial dependency. However, in the case of CS-S, for both extraction temperatures the same characteristic sinusoidal pattern was found exhibiting the maximum extraction efficiency at 50 °C. Observed concentrations were of about 6.5×10^{-3} mg.mL^{-1} for CS-S extracted at 40 °C and of about 5×10^{-3} mg.mL^{-1} for CS-M extracted at 80 °C indicating, that the extracted flavonoids substances are sensitive on thermal history and are undergoing thermal decomposition similarly as reported by **Chaaban et al. (2017)**. In the latter paper, the linear degaradtion pattern was found for rutin at 70 °C degradation temperature. With increasing degradation temperature up to 130 °C the exponential decay pattern was found. In the case of CS-M, the maximum extraction efficiency was found at 30 minutes extraction time being of 5.8×10^{-3} mg.mL^{-1} at 40 °C extraction temperature. However, at higher temperature, the degradation of the flavoinoids content was observed, and only the exponential decay pattenr was found as indicated in Figure 4B. That is why, the maximum extraction was found at 20 minutes extraction time at 80 °C extraction temperature. It was found, that the highest extracted flavonoids content was $(7.2 \pm 0.3) \times 10^{-3}$ mg.mL^{-1} for CS-M 80°C sample. However for the CS-S the highest content was found of $(6.8 \pm 2.1) \times 10^{-3}$ mg.mL^{-1} for CS-S 40 °C sample. To characterize obtained extracts of flavonoids, the UV VIS as well as fluorescence spectra were measured as shown in Figures 5 to 7. Prior to the fluorescence mapping analysis of the studied corn silk extracts, the UV VIS spectra were recorded.

These were typical with three major absorption regions at 260 nm and 360 nm (near ultraviolet region), and at visible light region of 490 nm. The absorption of electromagnetic radiation in the near ultraviolet region is typical for poly-unsaturated and aromatic compounds such as flavonoids. Both CS-S as well as CS-M exhibited similar UV VIS spectra except the visible range region, where a 480 nm shoulder peak occurred for CS-M sample.

Results of the fluorescence excitation-emmision mapping of the studied extracts are shown in Figure 7. These are characteristic similarly as the UV VIS absorption spectra with the three distinct fluorescence enission regions at 320 nm, 450 nm and 680 nm. Emmision region located at 450 nm was ascribed to the flavonoids compounds similarly as observed by **Shan et al. (2017)**, who found, that the flavonols characteristic excitation/emission spectral range is 365 – 390 nm/450 – 470 nm. The excitation/emission spectral range of 480 – 500 nm/510 – 520 nm was ascribed to flavanols.

There were found three distinct excitation wavelengths regions at about 275 nm, 350 nm and 420 nm. Obtained results indicate, that the major difference between CS-S and CS-M is in the fluorescence emission centered at the 450 nm region, where the intensity of the fluorescence emission was highest for CS-S extracted at 80 °C for 50 minutes. Furthermore, there was found that the fluorescence emmision intensity region located at 450 nm region was of higher intensity for CS-S in comparison to CS-M at 80 °C extraction temperature. Observedresults were considered as statistically significant ($p \leq 0.05$). These results are in an excellent correspondence with the TG analysis, where the first step decomposition weight loss was found higher for CS-S in comparison to CS-M. However, there was not found any major difference between fluorescence emmission intensities located at 320 nm region for all studied materials.

CONCLUSION

There was confirmed in this study, that it is possible to obtain flavonoids from the studied corn silk material by a simple extraction procedure. It was found, that the total flavonoids content differs with the extraction time and extraction temperature. There were found different flavonoids contents in extracts prepared from different maturity stages of the original corn silk material (silking stage, milky stage). Extracted flavonoids content was quantified by the lutin standardization method by means of colorimetry measurements at 510 nm wavelength. Obsreved flavonoids concentrations were ranging from 2×10^{-3} mg.mL^{-1} to 7.5×10^{-3} mg.mL^{-1} dependent on the extraction time period and extraction temperature. The highest concentration of flavonoids of 7.2×10^{-3} mg.mL^{-1} was found for CS-S after 50 minutes extraction time and 40°C extraction temperature. By fluorescence mapping experiments, there was confirmed the presence of the flavonoids by the appearance of the characteristic fluorescence emission region at 450 nm. There was found a typical multistep thermal decomposition process for both CS-S and CS-M materials.

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