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

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ABSTRACT

This study shows an ultrasonic assisted extraction procedure of steroids from corn silk (CS). The total steroids contents were positively correlated with the ultrasonic assisted extraction time. The extracted steroids contents varied according to the different maturity stages of CS. There were three tested CS maturity stages: silking stage (CS-S), milky stage (CS-M) and mature stage (CS-MS). The β – sitosterol standardization method with 530 nm wavelength colorimetric measurements were applied to determine the content of extracted steroids. Measured steroids concentrations range were from 38.3×10^{-3} mg.mL^{-1} to 368.9×10^{-3} mg.mL^{-1} in different extraction time and CS maturity stages. The highest concentration of steroids, 368.9×10^{-3} mg.mL^{-1} was found in CS-MS sample with the 75 minutes ultrasonic extraction time. The fluorescence mapping techniques were used to confirm the existence of steroids. The thermal analysis illustrated a typical multistep decomposition process for the CS-S, CS-M and CS-MS samples. Two endothermic peaks were found: The first one was 54.3 °C for CS-S and CS-MS, 60.2 °C for CS-M, the second one, 397 °C (CS-MS), 415.1 °C (CS-M) and 419.7 °C (CS-S) attributed to the total thermal decomposition. The observed exotheric process found at 524 °C corresponded to CS-MS sample decomposition. The optimal ultrasonic-assisted extraction time for all samples under study CS-S, CS-M and CS-MS was about 75 minutes and the optimal steroids extraction contents obtained were 92.8×10^{-3} mg.mL^{-1} (CS-S), 124.2×10^{-3} mg.mL^{-1} (CS-M) and 368.9×10^{-3} mg.mL^{-1} (CS-MS) respectively.

Keywords: corn silk; ultrasonic extraction; maturity stages; steroids; UV-VIS

INTRODUCTION

Corn silk (CS) can be defined as the dried thrum and stigma of *Zea mays* L. (corn) which is cheap and high yielding. The by-product of agriculture is the common use of CS to be abandoned, burned or applied as fodder. Corn silk is remarkablely functional to the clinical diseases as diabetes, nephritis and hypertension etc **(Jin, 1980)**. Additionally, morden researches also revealed the healing functions 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. Corn silk has been used as a cure to urinary tract infection, malaria and heart disease by aboriginal American Indians. Furthermore, the properties of cooling blood, purging heat and removing the damp and heat from human body of CS were also used as weight-losing products in many countries. It was found by the acute toxic test on rats, that cosrn silk extracts were not causing any mortality and was non-toxic at the dose of up to 5 g.kg^{-1} body weight. However at the doses about 1000 mg.kg^{-1} in long term treatments, toxic effects in liver were observed **(Ikpeazu et al., 2018)**.

The previous studies of the improvement of chicken meat nutrition quality by the fermentated corn fodder have made a success **(Angelovičová and Semivanová, 2013; Mačanga et al., 2017; Štenclová et al., 2016)**. Aside from that, corn products were also contributed to the improvement of the sensory quality of crackers **(Kuchtová et al., 2016)**. The alchohol from the fermentation technology of CS has been used in food and chemical industry **(Krejzová et al., 2017; Süli, Hamarová and Sobeková, 2017)**.

Corn silk steroids (CSS) are significantly valuable in the research of its nutrients, which is the cause of the functions of anti-cancer, anti-oxidant, cholesterol – reducing of the corn silk extracts **(Li and Lapcik, 2018)**. The total steroids extractive technology includes solvent crystallization, complexometry, saponification, distillation (simple distillation, molecular distillation), adsorption (column adsorption, high pressure fluid adsorption), supercritical carbon dioxide extraction, enzymic method etc. **(Hossain et al., 2014; Ren et al., 2015)**. **Ren et al. (2015)** extracted total steroid saponins from *Dioscorea zingiberensis* by means of microwave-assisted technique, the optimal extracting conditions were established as 75% ethanol as solvent, ratio of solid/liquid 1:20 (g.mL^{-1}),

temperature 75 °C, irradiation power 600 W and three extraction cycles of 6 minutes each **(Hossain et al., 2014; Ren et al., 2015)**. **Hossain et al. (2014)** applied ultrasonic-assisted technology on the extraction of steroidal alkaloids from potato peel waste, used response surface methodology to optimize the extracting conditions. The optimal ultrasonic-assisted extraction conditions were identified as an amplitude of 61 μm and an extraction time of 17 min which resulted the recovery of 1102 μg steroidal alkaloids per g dried potato peel. In contrast, solid liquid extraction yielded 710.51 glycoalkaloid $\mu g.g^{-1}$ dried potato peel. Recoveries of individual glycoalkoids using ultrasonic-assisted extraction yielded 273, 542.7, 231 and 55.3 $\mu g.g^{-1}$ dried potato peel for α-solanine, α-chaconine, solanidine and demissidine respectively. Whereas for solid liquid extraction yields were 180.3, 337.6, 160.2 and 32.4 $\mu g.g^{-1}$ dried potato peel for α-solanine, α-chaconine, solanidine and demissidine respectively **(Hossain et al., 2014; Ren et al., 2015)**.

UV-VIS method is a common methodology to determine the content and kinds of steroids **(Antonisamy and Eahamban, 2012)**. However, the optimization of extraction conditions, steroids properties comparison and the determination of the steroids kind according to the various maturity stages of corn silk are rarely reported. In this study, we expect the maturity stage will be a vital influential factor in the extracted total steroids content. This study focuses on the extraction procedures evaluation, kinetics and the maturity stage effects on the CS extracts, allowing in more detailed knowledgement for the developing novel nutrition or health care products to contribute to the human and veterinary applications.

Scientific hypothesis

UV-VIS and fluorescence techniques have been applied for the content and sorts of steroids in plants in modern research. However, the extraction conditions optimization, steroids properties comparison and analyzation and the determination of the steroids kind in different maturity stages of CS are rarely reported. We expect the maturity stage will be a significant effective factor for the total extracted steroids content. That is why, this research is focused on the quantification of the total steroids content extracted from different maturity stages of CS at defined ultrasonic-assisted extraction times.

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 dried 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 80 mesh sieve (Analysette 3, Fritsch, Germany) to obtain the final product powder samples. There were collected three 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), the third one was mature stage (assigned as CS-MS) **(Rahman and Wan Rosli, 2014; Sarepoua et al., 2015)**.

All reagents and chemicals used in this research such as β-sitosterol, ethanol, phosphoric acid, sulfuric acid and ferric chloride 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).

β-sitosterol standard curve determination procedure **(Hossain et al., 2014)**: Precisely weigh 10 mg β-sitosterol into 10 mL volumetric flask, use absolute ethyl alcohol dilute to scale. Fetch 1 mL above solution into 10 mL volumetric flask, use absolute ethyl alcohol to dilute to scale as the standard sample solution. Precisely move the standard sample solution 0, 1, 2, 3, 4, 5 mL into 50 mL conical flask, separately add 5, 4, 3, 2, 1, 0 mL absolute ethyl alcohol, then slowly pour the pulfate-phosphate-ferric reagent 5 mL long the cup wall into every conical flask separately, shake up, cool down in room temperature for 20 min. Then was measured the 530 nm absorbance by UV spectrometry. Each experiment was repeated 5×. 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×.

Determination of the sterols content procedure **(Hossain et al., 2014)**: weigh 5 samples 3 g corn silk powder, add 70% ethanol (material: liquid = 1:20), then use 200 W ultrasonic extract for 15 min, 30 min, 45 min, 60 min, 75 min for the silking, milky and mature stages. Then was used 119 W microwave extraction apparatous for 8 min, followed by addition of pulfate-phosphate-ferric reagent to process for 20 min (the same procedure as for standard curve determination), then measure the 530 nm absorbance by UV spectrometry.

Then there was used the standard curve to count the content of sterol in prepared corn silk extracts. Each experiment was repeated 5×.

Statistic analysis

Statistical analysis of the observed data was performed by application of the one way analysis of variance (ANOVA) method (Microsoft Excel, USA). This analysis allowed to detect the significance of the effect of ultrasonic-assisted extraction time and the maturity stage on extracted amounts of steroids. Five ultrasonic-assisted

extraction times and three maturity stages 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 530 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

Figure 1 shows the SEM images of the tested corn silk powders. The rectangular shape of individual particles illustrates the complex microporous structure on the intersection as a typical botanic cellulose based materials. The moisture content and thermoal analysis by TG and DTA of the samples were resulted as in Figure 2, which are the typical multistep decomposition process for all CS-S, CS-M and CS-MS samples as shown in Figure 2. The first step decomposition of CS-S was in the temperature range from 30 to 120 °C with observed weight loss 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 and CS-MS, TG data exhibited the first step decomposition of 5.9% and 10.04% in the same temperature range as CS-S followed by the total weight loss of 65.5% and 83.88% in the temperature range of 30 to 550 °C, which indicated the CS-MS contains the most thermally labile substances compared with CS-S and CS-M.

There are two endothermic peaks in Figure 2. The first one was located in the temperature of 54.3 °C for CS-S and CS-MS, 60.2 °C for CS-M attributed to the melting point of flavonoids.

The second one was observed at 397 °C (CS-MS), 415.1 °C (CS-M) and 419.7 °C (CS-S) attributed to the total thermal decomposition with the formation of a low quantity carbonaceous residues respectively. Observed exotheric process at 524 °C corresponds to the decomposition CS-MS sample.

The β-sitosterol standard curve is shown in Figure 3, the regression parameters and inset as well. The Obtained data were highly correlated as the correlation coefficient 0.999.

Figure 4 shows the effects of the Ultrasonic time and different maturity stages by the steroids concentration vs. ultrasonic time correlations. All of the CS-S, CS-M and CS-MS were a non-linear character, modeled as a third order polynomial dependencies. However, all of those three stages extraction contrations had a direct proportionality trend with the increase of the ultrasonic processing time. All of the three stages samples had an obvious increasing range of the extraction concentration from ultrasonic extraction time 15 min to 60 min. From 60 min to 75 min, three samples showed a similar steady tendency which means from 60 min to 75 min the increase of the concentration is not conspicuous anymore. Therefore, the 75 min ultrasonic extraction time can be marked as the optimum extraction time to obtain maximum extracted content for all of the three stages. Obtained maximum concentrations were as follows: CS-S 0.09 $mg.mL^{-1}$, CS-M 0.12 $mg.mL^{-1}$, CS-MS 0.37 $mg.mL^{-1}$. It is noteworthy, that the CS-MS had a much higher maximum extraction concentration as well as the increasing rate in comparison to CS-S and CS-M samples. This imply that the CS-MS has much higher content of steroids than CS-S and CS-M. Simultaneously, the ultrasonic assisted technique can be sonsidered to be much more effective to CS-MS sample extraction rather than for CS-S and CS-M. , which is in agreement with the results of UV-VIS and fluorescence excitation-emmision mapping illustrated in Figure 5, Figure 6, Figure 7 and Figure 8.

The UV-VIS as well as fluorescence spectra were measured as shown in Figure 5, Figure 6, Figure 7 and Figure 8. These were typical three major light absorption regions at 350 nm (near ultraviolet region) and visible light region of 500 nm and 650 nm. The absorption of electromagnetic radiation in the visible light region is typical for anthraquinone and phenanthrene compounds such as steroids. All of the CS-S, CS-M and CS-MS exhibited similar UV-VIS spectra.

Results of the fluorescence excitation-emmision mapping of the studied extracts are shown in Figure 8. These are characteristic similarly as the UV-VIS absorption spectra with the three distinct fluorescence enission regions at 300 nm, 430 nm and 680 nm.

There were found three distinct excitation wavelengths regions at about 275 nm, 350 nm and 380 nm. Obtained results indicate the major difference between CS-S, CS-M and CS-MS is in the fluorescence emission centered at the 300 nm and 430 nm regions. The highest intensity of the fluorescence emission at 300 nm was found for CS-MS extracted at 40 °C for 15 minutes in the ultrasonic extraction bath. Furthermore, there was found that the fluorescence emmision intensity region located at 430 nm region was of the highest intensity for CS-MS as well. Observed results were considered as statistically significant ($p \leq 0.05$). However, there was not found any major difference between fluorescence emmission intensity located at 670 nm region for all studied materials.

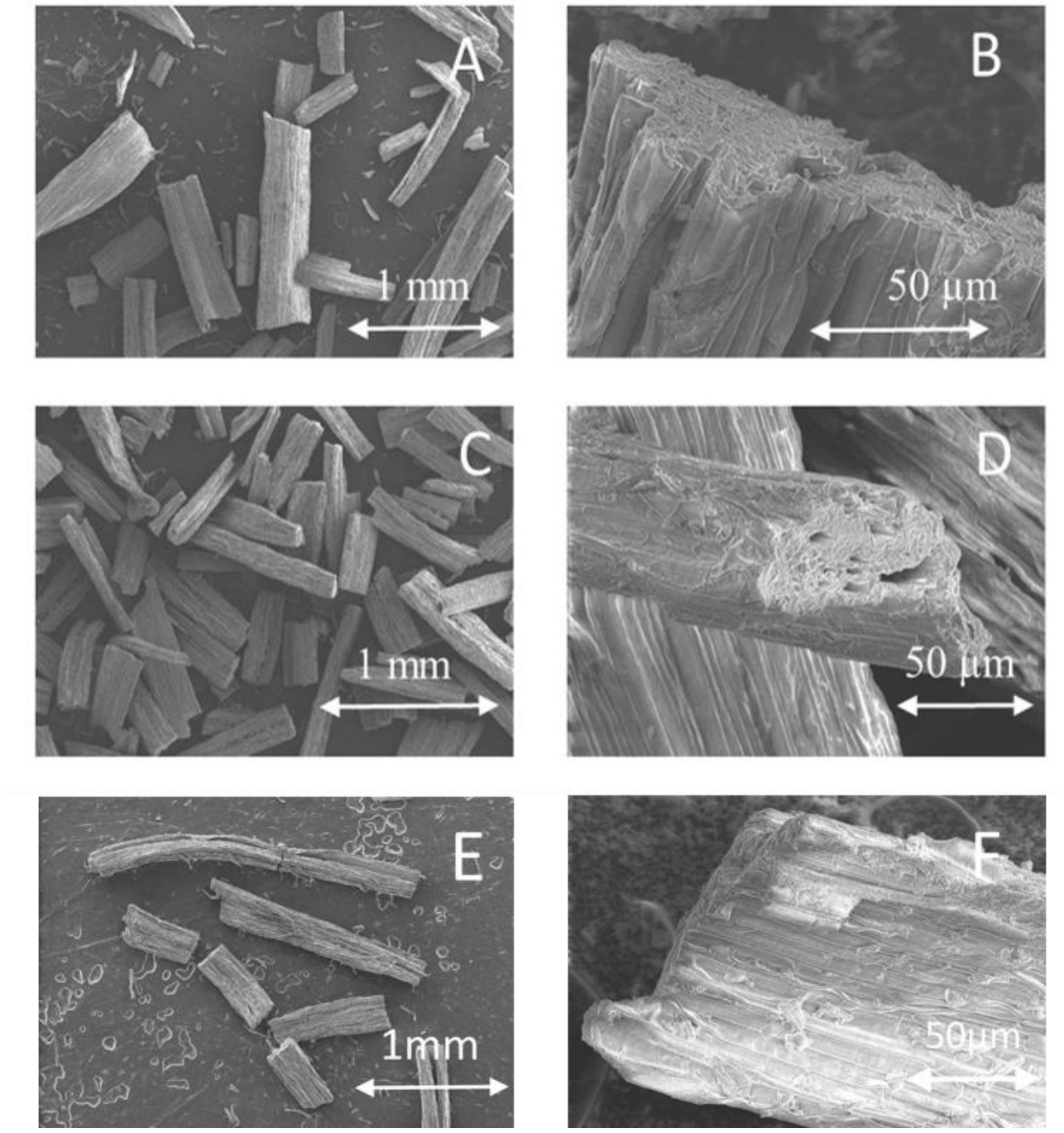

Figure 1 Studied corn silk SEM images. Note: A,B – sample CS-S, C,D – sample CS-M, E,F – sample CS-MS.

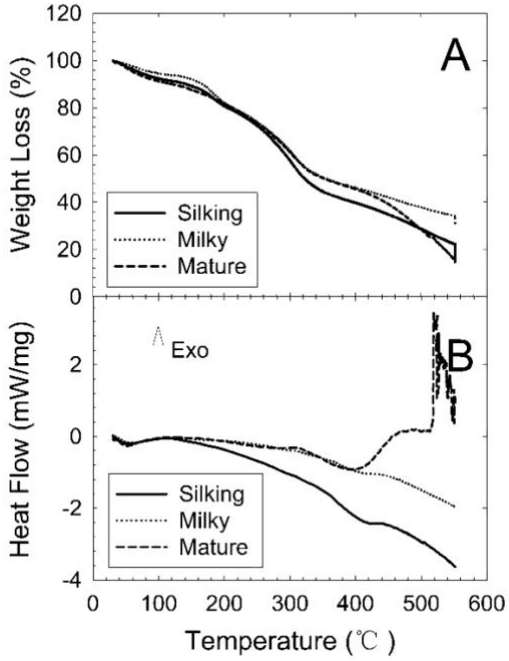

Figure 2 Thermal analysis of corn silk samples. Note: A – Thermogravimetry (TG), B – differential thermal analysis (DTA).

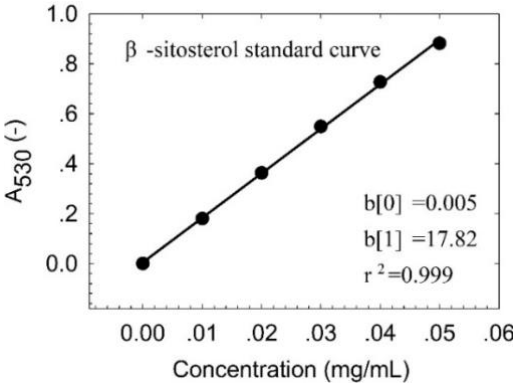

Figure 3 β-sitosterol standard curve. Note: Inset: Linear regression standard curve parameters.

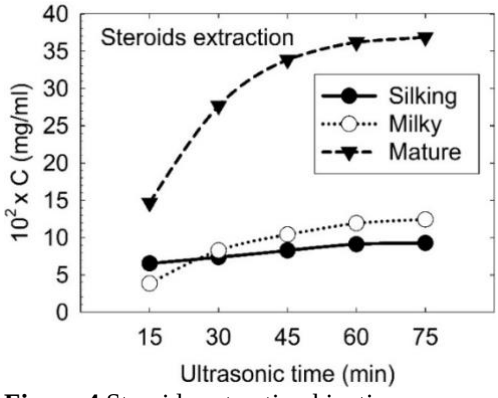

Figure 4 Steroids extraction kinetics.

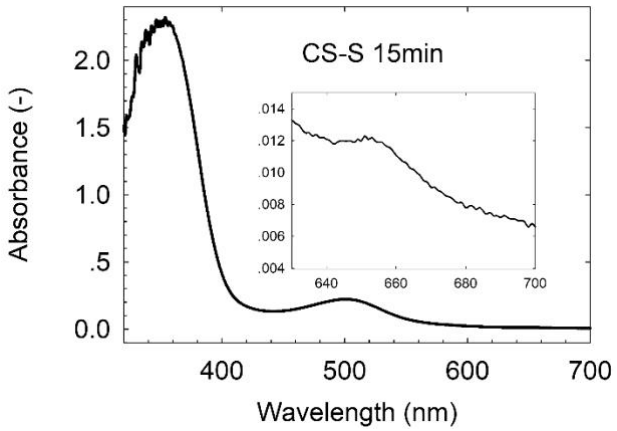

Figure 5 UV-VIS spectrum of the CS-S sample extracted at 15 min ultrasonic-assisted extraction time. Note: Inset: expanded 630 nm to 700 nm region.

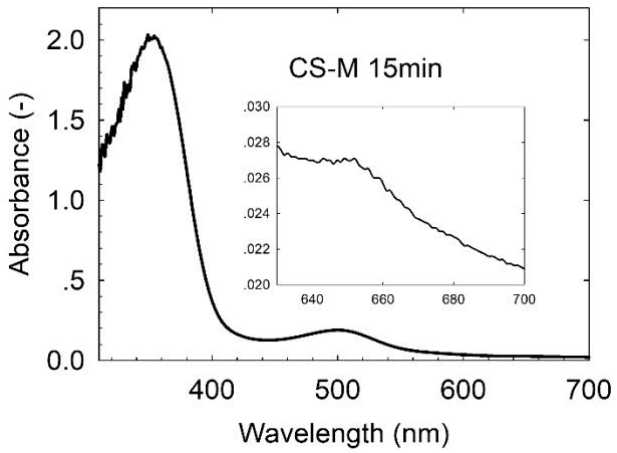

Figure 6 UV-VIS spectrum of the CS-M sample extracted at 15 min ultrasonic-assisted extraction time. Note: Inset: expanded 630 nm to 700 nm region.

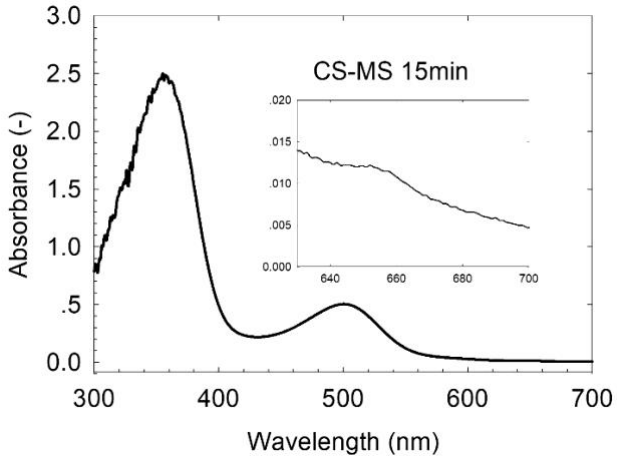

Figure 7 UV-VIS spectrum of the CS-MS sample extracted at 15 min ultrasonic-assisted extraction time. Note: Inset: expanded 630 nm to 700 nm region.

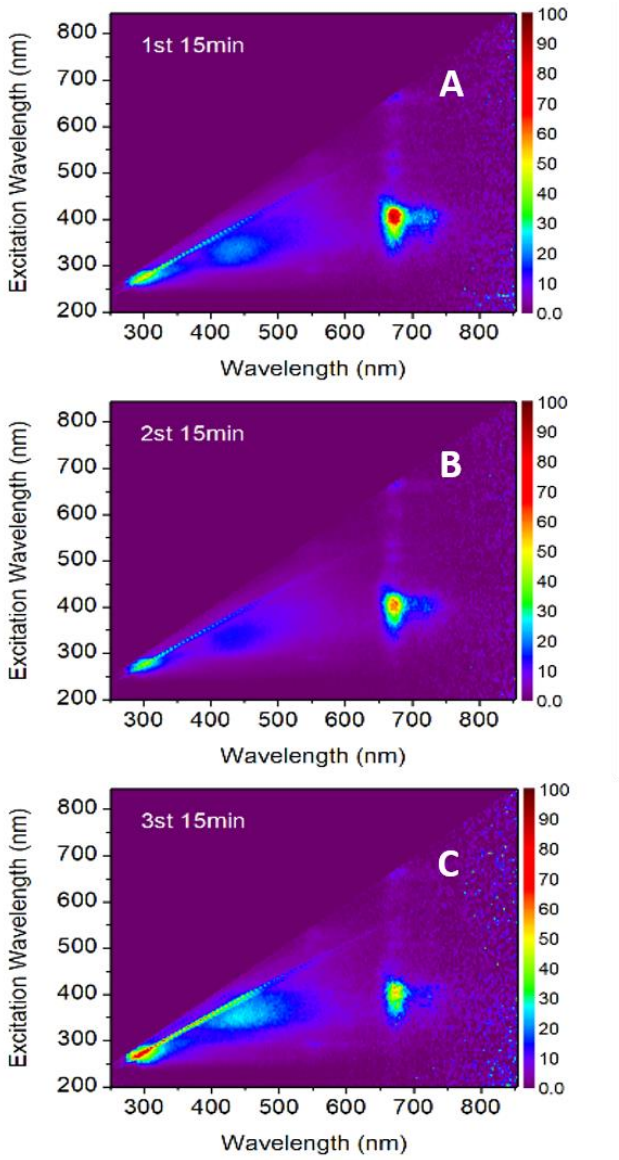

Figure 8 Results of the fluorescence excitation – emmision mapping of the studied corn silk extracts. Note: Inset legend: A – corresponds to CS-S sample, B – CS-M sample, C – CS-MS sample. Extraction temperature (40 °C or 80 °C), 15 min ultrasonic extraction time.

CONCLUSION

The ultrasonic assisted extraction procedure was ascertained to be valid for the CS steroids extraction. The total steroids contents were positively correlated with the ultrasonic assisted extraction time. The extracted steroids contents varied according to the different maturity stages of CS (silking stage, milky stage, mature stage). The β-sitosterol standardization method was applied to quantity the extracted steroids content by the 530 nm wavelength colorimetry measurements. Measured steroids concentrations range were from 38.3×10^{-3} mg.mL^{-1} to 368.9×10^{-3} mg.mL^{-1} in different extraction time and CS maturity stages. The highest concentration of steroids about 368.9×10^{-3} mg.mL^{-1} was observed for CS-MS sample after 75 min ultrasonic processing extraction time. The fluorescence mapping technique confirmed the highest extraction efficiency of steroids by ultrasonic treatment was at 40 °C from the CS-MS samples. A typical multistep thermal decomposition process was found for all of the CS-S, CS-M and CS-MS materials.

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