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Extraction of Natural Cellulose and Zein Protein from Corn Silk: Physico-Chemical and Biological Characterization

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Abstract: The aim of the present study is to extract natural cellulose and zein protein from Egyptian corn silk using two different protocols being; filtration ($NaOH/H_2SO_4$) and Solubility (NaOH/Urea). The extracted cellulose and zein via the two protocols are characterized using XRD, FTIR, and FESEM with EDAX. The biological activity of the extracted zein was assessed. Results proved the successful extraction of cellulose and zein via the two applied protocols. However, significant structural variations had recorded for the extracted cellulose and silk. The solubility protocol produced more crystalline cellulose when compared with the filtration one. The extracted zein via both protocols presents promising antioxidant activity.

Keywords: corn silk; cellulose; zein; total phenols; XRD; FT-IR; FESEM-EDAX.

1. Introduction

In most developing countries, waste source from agriculture is one of the most crucial environmental problems that contribute significantly to global pollution. Recently, several types of research are focused on re-using such valuable wastes, opening the door for cleaner, promising (green) products that are used in several industrial and medical fields.

Corn silk (CS) is quite rich in dietary fibers with a characteristic set of smooth, soft stigma having thread or yellowish hair shape that is collected from female flowers [1]. CS contains several chemical constituents as protein, carbohydrates, fibers, vitamins (B, C, and K), essential oils, and mineral salts (Na, Fe, Si, Zn, K, Ca, Mg and P). Moreover, CS has phytochemical compounds of sitosterol, stigmasterol, hesperidin derivatives, and quercetin [2, 3]. Trace amounts of phenols, terpenoids, and glycosides are also present in it [4]. Additionally, CS contains maysin, β carotene, beta-sitosterol, geraniol, hordenine, limonene, menthol, and viteskin [5, 6]. Jianwei Dong *et al*. extracted cornhusk, corncob, and stigma maydis with water, aqueous ethanol, aqueous methanol, and ethyl acetate [2]. Yilmaz *et al*. mentioned that chemical parameters like alkali concentration and treatment time influenced the properties of extracted cornhusk fibers [7].

Cellulose is the main component of most plant's cell walls. Reddy&Yang extracted natural cellulose fibers from cornhusk in 2007 [8]. In 2013, Larissa *et al*. explored the utilization of corn Stover as a raw material to produce cellulose nano-crystals that possessed great potential as strengthening agents in nano-composites [9]. Micro-crystalline cellulose was extracted and optimized as a potential excipient in the production of pharmaceutical dosage forms due to its low cost.

Zein is a composite of dissimilar peptides of various molecular sizes, solubility, and charge, which represents 45–50% of the corn proteins. Two major fractions of Zein, A, and B, were first described by McKinney 1958 [3]. A plethora of attempts have been synthesized to extract zein from corn silk. Recently, L.C Dikkey extracted pure zein from maize (Zea mays) for possible applications as film production using ethanol [10].

The goal of the present study is to perform comparative extraction of Zein and Cellulose from Corn Silk using two protocols to explore the most efficient one. The filtration method in which cellulose powder and zein were obtained and is based mainly on the cellulose dissolution, sonication and centrifugation to separate zein from cellulose and any other minor ingredients and the Solubility protocol. A dual extraction of zein and cellulose in one technique, which limits the waste of such valuable byproduct, is represented.

2. Materials and Methods

2.1. Materials and methods.

The Egyptian corn silk fibers were dried in the oven for three days at an average temperature of 60˚C. The dried fibers were ground into powder using agitate mortar (Janke & Kunkel GmbH Co., Germany). Cellulose and zein were extracted via two protocols of a) filtration and b) solubility.

a) Filtration protocol (using $NaOH/H_2SO_4$): Five grams of the powdered dried corn silk were treated with 25 ml of 1 N sodium hydroxide 98% to de-lignify the corn silk for thirty minutes. The resulting slurry was filtered using a cotton cloth. Further, the residue on the cotton cloth was treated with 125 ml of 1 N Sulphuric acid 98% at 80°C for 1 h to digest the powdered materials. The pH was adjusted to be 5.5 using de-ionized water. The cellulose slurry (C1) was filtered again. The residue was thoroughly washed with distilled water. Further, water was manually squeezed out to obtain small lumps and consequently dried at 600C for 6 hours. Additionally, 0.96 g of microcrystalline cellulose was added to 48 ml of 1mm sulfuric acid (2 Wt. % loading). The suspension was sonicated at 100% amplitude for 2 h at room temperature. The total treatment time was delivered as 15 s pulses with 15 s breaks while being cooled by an ambient temperature water bath to avoid excessive heating and followed by centrifugation for 15 minutes at 300 rpm [11, 12]. The resulted cellulose Nano-powder (C1) and the remnant solution from filtration (Z1) were stored at 4˚C for further characterization.

b) Solubility method protocol (NaOH/Urea): A mixture of the aqueous solution from alkali hydroxide, urea, and distilled water was used as a cellulose solvent. 0.175 mole alkali hydroxide (7%) /0.2 mole urea (12%) were used. The dried cellulose was immersed immediately in the pre-cooled solvent at -5 to -20℃ and stirred for about 5 min at ambient temperature. The cellulose was considered being dissolved completely when a transparent cellulose solution without any native fibers was observed. The resulting cellulose solution was subjected to ultra-sonication at 500,000 joules for 2 hours with 15 minutes pulsation, followed by centrifugation at 3000 rpm for 30 minutes to separate any other excessive remnants. Both

the formed slurry at the bottoms of the centrifuge tubes, which contain the resultant cellulose solution (C2) and Zein (Z2), were then stored at 4° C for further analysis [13].

The extracted samples were characterized using FTIR, XRD, and FESEM. Total phenolic concentration was investigated. The standard extract (0.1 mL) was mixed with DDW (1 mL). Consequently, F–C reagent (0.1 mL) and 0.8 mL of Na_2CO_3 (75 g L^{-1}) were added and maintained to react for six minutes. The developed solution was dark incubated for 90 min at room temperature to allow for color development. Finally, the sample absorbance was measured at 760 nm against the blank. Gallic acid was used as a standard at 0–300 μgmL^{-1}. The experiments were run in duplicates. Total phenolic concentration is expressed in mg gallic acid equivalent (GAE) g^{-1} sample.

2.2. Characterization.

X-ray powder diffraction (XRD) analyses were achieved using Cu Kα radiation (l=1.5418 Å) at 0.3 S scanning speed (Philips X'pert Pro X-ray powder diffractometer). The applied current and voltage were 40 mA and 40 kV, respectively. Fourier Transform Infrared (FTIR) spectra were recorded using Vertex 70 Bruker optics, Germany spectrometer. The spectra were collected using a spectral resolution of 4.0 $cm^{-1,}$ and 64 scans collected to get an acceptable signal to noise ratio. The surface topology of the samples was investigated by FESEM (Quanta FEG 250-type microscope equipped with an energy dispersive X-ray attachment EDAX/Genesis device).

Total phenolic concentrations were measured following the modified Singleton and Rossi method.

3. Results and Discussion

3.1. Fourier transform infrared spectroscopy (FTIR).

FTIR spectra of the extracted cellulose show an absorption band at 3444.4 cm^{-1} correspondings to the OH stretching of the cellulose hydroxyl group of C1 (Fig. 1 (a)). The CH stretching peak that arises at 2875 cm^{-1} is attributed to the cellulosic CH_2OH groups. However, the absorption spectra of C2 show a peak at 1637 cm^{-1} due to the anti-symmetric deformation of the C-O-C band in cellulose and hemicellulose. Additionally, a 1627 cm^{-1} peak is attributed to the C=O stretching of the carbonyl group. The 1627 cm^{-1} peak in both spectra is assigned to CH_2 symmetric bending. Whereas the shoulder peak at 630.60 cm^{-1} found in cornhusk fiber spectra is interpreted as C-O-C stretching at the ß (1-4) glycoside linkage of cellulose.

The extracted zein spectra showed a peak at 3435 cm^{-1} correspondings to -NH_2 stretching in zein, which remained unchanged for Z1& Z2. The C–H stretching vibration absorption located at 2925 cm^{-1} and indicated inter and intramolecular interaction of the polysaccharides chains. Amide I, II, and III peaks are located at 1637.26 (C=O stretching), 1458.885 (N-H bending and C-N stretching), and 1268 cm^{-1}, respectively. The presence of a higher amount of β-sheets secondary structure is thus, confirmed by the symmetrical spectral peak at 1637 cm^{-1} for both spectra. However, Z2 spectra possess β-turns shoulder at 1662 cm^{-1}. The peak at 1093 cm^{-1} is indicating the sugar pyranose [14].

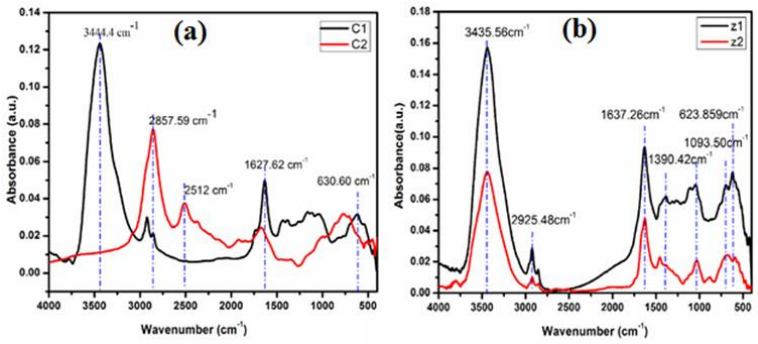

Figure 1. FTIR spectra of a) Extracted cellulose b) Extracted zein.

3.2. X-ray diffractometry (XRD).

X-ray diffractometry was applied to assess the crystallinity features of the extracted cellulose via the two applied protocols (Fig. 2). XRD patterns of C1 and C2 exhibited one major peak positioned at $2\Theta=22.5^{\circ}$ which is attributed to the crystalline cellulose structure. C2 pattern confirms typical crystalline cellulose compared to C1. Therefore, it is concluded that C1 contains a small quantity of hemicellulose with a random, amorphous structure [15].

XRD spectra of Z1 and Z2 possess four major peaks to arise at 32.50°, 34.00°, 36.00°, and 45.50° indicating, therefore, the crystalline nature of the extracted zein via the two applied protocols. The depicted peak at 38° in the Z2 spectrum is referring to a higher β sheet configuration within the extracted zein [16]. XRD results are in good accordance with FTIR inspections.

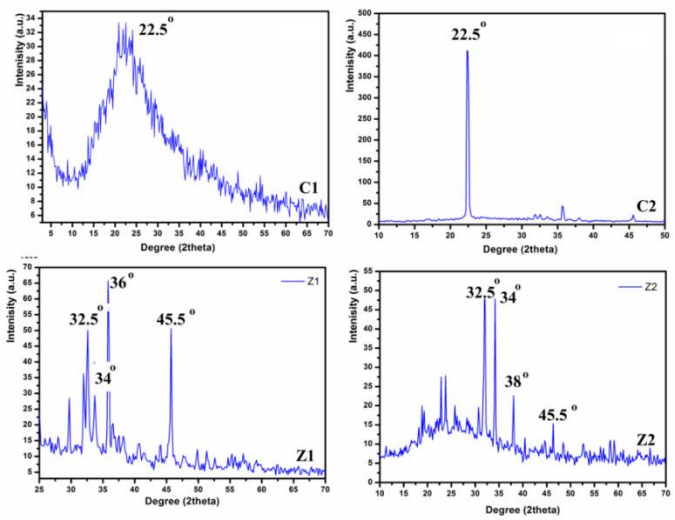

Figure 2. XRD spectra: C1, C2) Extracted cellulose Z1, Z2) Extracted zein via two applied protocols.

3.3. Field emission scanning electron microscopy (FESEM).

FESEM images of the chemically extracted cellulose showed the presence of arranged bulk micro-cellulosic fibers with an aspect ratio of 6.96 as depicted in figure 3 (a, b). C2 images show comparatively well-arranged smooth, regular surface appearance due to its higher purity than previously confirmed by FTIR results. The arranged fibers structure supports the higher crystallinity depicted by the XRD diffraction pattern. Figure 3 (c, d) shows FESEM of Z1 and Z2. The Z1 protein appears as random distributed flowery flakes and is confirmed by the presence of N in its EDAX analysis. On the other hand, Z2 shows dense plate-like structures

with fine embedded fibers and could be cellulosic remnants, as confirmed by its EDAX analysis.

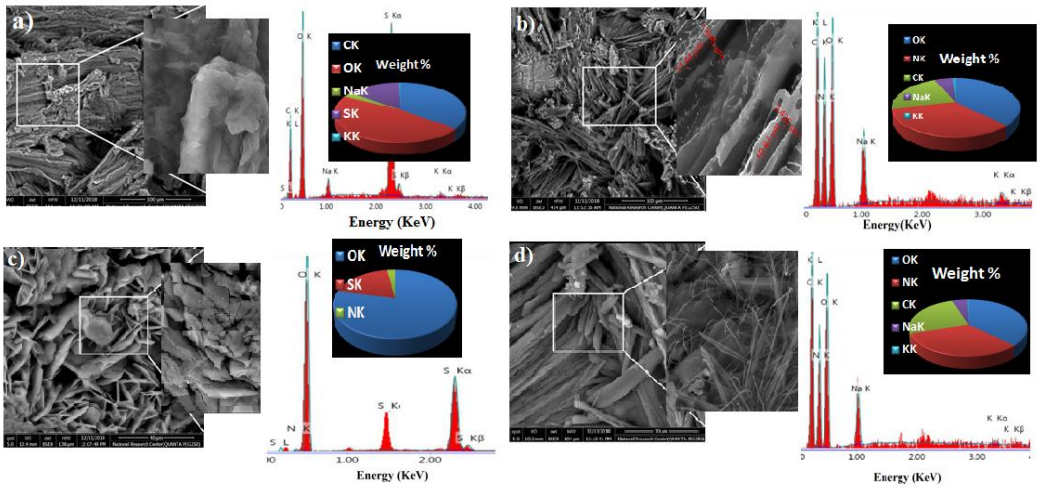

Figure 3. FESEM of a) Extracted cellulose C1, b) Extracted cellulose C2, c) Extracted zein Z1, d) Extracted zein Z2 With EDAX analysis via the two applied protocols.

3.4. Total phenols.

Corn silk pharmacological importance having antioxidant, anti-inflammatory, and diuretic activities are attributed to its high total phenols and flavonoids contents [17]. Total phenols concentration was measured within the extracted Z1 and Z2 solutions. Z2 exhibited a higher value of 76.99 compared to 61.03 µg/20 ul of Z1. The recorded high concentrations exhibit promising antioxidant activity for both zein silk extracts via the two applied protocols making them reliable routes for zein extraction without affecting their antioxidant activities.

4. Conclusions

Corn silk cellulose and zein were successfully extracted, applying either filtration or solubility routes. Physicochemical examination of the extracted cellulose and zein assessed their characteristic peaks. However, the solubility protocol presents highly crystalline cellulose. The investigated morphological features via FESEM depicted cellulosic fibers and zein flower flakes. The extracted Z1 and Z2 possessed high phenolic contents; therefore, their promising antioxidant activity is verified.

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Conflicts of Interest

The authors declare no conflict of interest.

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