Utilization and Characterizations of Olive Oil Industry By-Product

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Abstract—A considerable amount of lignocellulosic by-product could be obtained from olive pulp during olive oil extraction industry. The major constituents of the olive pulp are husks and seeds. The separation of each portion of olive pulp (seeds and husks) was carried out by water flotation where seeds were sediment in the bottom. Both seeds and husks were dignified by 15% NaOH followed by complete lignin removal by using sodium chlorite in acidic medium. The isolated holocellulose, α-cellulose, hydrogel and CMC which prepared from cellulose of both seeds and husk fractions were characterized by FTIR and SEM. The present study focused on the investigation of the chemical components of the lignocellulosic fraction of olive pulp. Biofunctionlization of hydrogel was achieved through loading of silver nanoparticles AgNPs in to the prepared hydrogel. The antimicrobial activity of the loaded silver hydrogel against G-ve, and G+ve, and candida was demonstrated.

Keywords—Antimicrobial hydrogel, carboxymethyl cellulose, cellulose, grafting, olive pulp.

I. INTRODUCTION

OLIVE oil extraction represents an economic and social industrial activity that is highly relevant in the Mediterranean countries. The extraction process has quite large environmental impact due to the creation of highly polluted waste water and/or solid residue, depending on the olive oil extraction process. The solid wastes generated in the olive oil extraction are alperujo, from the two- phase system, and orujo from the three- phase system [1]. The world olive oil and olive production estimated to be 2.58 and 1.73 million tons/year respectively [2]. After extraction of oil from olive, huge quantities of by-products are present. These by-products may cause environmental problems if not effectively handled and used.

Olive stone is a lignocellulosic material, with hemicellulose, cellulose, and lignin as main components. Many current studies [3] aim to develop methods of recovering the lignocellulosic material or biomass to produce solid, liquid or gas biofuel. Therefore, widespread olive stone or its derivatives are used as a renewable source of energy. Despite the environmental benefits of using this biomass as fuel, some problems remain such as air pollution (carbon monoxide, nitrogen oxides, and particulates such as soot and ash (produced by combustion).

Olive solid wastes have been reported to be used for removing of pollutants from water due to their availability and lowing cost [4]. For example, olive solid wastes from different countries have been used for heavy metal ions removal [5]. In addition, olive solid wastes have been converted to activated carbon for removing of different pollutants from water [6].

Earlier studies showed that olive stones contain a considerable amount of cellulose in addition to hemicellulose and lignin. Also, other minor ingredients such as protein, fat, phenols, free sugars and polyols were also noticed in the stones [7]. As a result, olive stones could be considered as an economic source for cellulose production that used in different applications. A recent study investigated the isolation of cellulose from olive stones by Kraft alkali pulping followed by bleaching using elemental-free chlorine bleaching method. The produced cellulose powder was known by its molecular structure, microscopic characteristics and viscosity [8].

Cellulose, the major constituent of olive stone, is a linear homo-polysaccharide composed of -D-glucopyranose units linked together by -1,4-linkages. The cellulose chains consist of about 10,000 monomer units, grouped together in bundles called microfiber, which form either ordered (crystalline) or less ordered (amorphous) regions. Microfibers build up fibrils and finally cellulose fibers. Chemically, cellulose is very stable and extremely insoluble polymer. Cellulose has no taste, odorless, hydrophilic, insoluble in water and in most organic solvents, chiral, and is biodegradable [9].

CMC is a biocompatible and biodegradable polymer. It is, therefore, often used in the biomedical field [10]. Recently, CMC was first prepared by Jansen in 1918. It was suggested as a substitute for some products such as gelatin, glue, gum etc. [11].

Carboxymethyl cellulose (CMC), the water-soluble anionic polysaccharide, is one of the most important cellulose derivatives having an annual worldwide production of about 300,000 tons which have an immense importance to the industry and in our everyday life [12]. CMC is used in detergent, food, textile, pharmaceutical, and paint industries and high purity grades are employed as food additives [13]. CMC is often used for improving paper quality as it can participate in the development of the bonds between cellulosic fibers. A film coated on the fibers by CMC gel tends to enhance the physical properties of paper for printing purposes [14]. Several studies concerned with the production of CMC

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from different fibers and chemical surface modification of cellulose fibers have been published and the effect of this treatment on the properties of cellulose fibers has been considered by different analyzes [15].

Due to the importance of its multiple applications, CMC and its sodium salts are commercially produced in higher amounts than any other cellulose ether. These products are prepared by the reaction of monochloroacetic acid or sodium monochloroacetate with alkali cellulose [16]. CMC has been synthesized from raw cellulose, wood, paper, sludge, cotton linter fibers, banana plants, and sago pulp [12], [14]. Superabsorbent hydrogels are three-dimensional crosslinked hydrophilic, linear or branched polymers. They have the ability to absorb large quantities of water, saline or physiological solutions compared with general absorbing materials [17].

Hydrogels can be formed by conventional cross linking methods or free-radical polymerization processes. The latter is initiated by thermal and redox systems or by the use of free radical initiator activated by irradiation in the form of E-beams, microwaves, X-rays, or light (including UV, visible, or near- infrared light) [18]. The excellent hydrophilic properties of hydrogels along with their high swelling ratio, and biocompatibility, promote their widely usage in agriculture [19], biomedical area as antibacterial materials [20], tissue engineering [21], biosensors [22], sorbents for the removal of heavy metals [23] and drug delivery [24].

II. Experimental

A. Raw Material and Chemicals

Olive stones (olive oil by-products) used in this study were collected from Agriculture by-product factories, Egypt. The stones were washed with water in order to separate the husks from the seed and the ratio between them was found to be 1.3: 1.0 (Seed: husk). These wastes were dried under natural conditions (average relative humidity=65%; average temperature=25°C). $NaClO_2$ was purchased from ROTH Chemi, NaOH, ethanol was purchased from SHAM LAB, Monochloroacetic acid was purchased from Acros Belgium, Isopropanol was purchased from Alpha chemical, n-hexane was purchased from SDFCL chemical, Methanol was purchased from Adwic chemical and Ethyl acetate was purchased from Adwic chemical, Acrylamide (AM) was purchased from Alpha Chemika, N, N-methylene bis-acrylamide (MBAM) was purchased from Acros Organic and Ceric ammonium nitrate (CAN) was purchased from Merck whereas, Silver nitrate was purchased from SRL

B. Characterization of Raw Material

The chemical composition of olive stone and husk was determined. The evaluation of extractive substances was carried out in different solvents namely, methanol, methanol mix benzene, hexane and ethyl acetate. The amount of lignin, holocellulose, and cellulose was determined by using the respective standard methods [25] in accordance to Tappi standard, whereas the ash contents were estimated by igniting the material in a muffle furnace in a porcelain crucible first at 400 ˚C for 30 minutes, then at 850 ˚C for 45 minutes, and after cooling at room temperature and the weight was gravimetrically estimated [26].

C. Pulping and Bleaching

Fiber has been obtained from soda pulping by treating of raw materials with 10% sodium hydroxide with consistency of 10% in a stainless-steel mini-high reactor (2.0 L). The temperature was raised gradually through 30 min to reach a maximum of 160 °C and left at this temperature for 2 hrs. At the end of the pre-hydrolysis stage, the obtained pulps were washed several times through a cheese cloth until obtaining a clear filtrate. The bleaching of the pulp was carried out through chlorite standard methods [27].

D. Determination of Total Phenolic Content

1. Removing Oil from the Sample

All materials of plant origin, according to their nature, contain varying amount of resins, fats, oils, and additives. For determination of these products, selective organic solvent extraction accomplished by Soxhlet is usually employed. 24g of each air-dried sample (seed, husk) was extracted by a 500 ml n-hexane for 18 hours. The extracted substances were air-dried and resins and waxes were gravimetrically calculated.

(a) Quantification of Total Phenols:

Five grams of an extracting sample were mixed with 150 ml acetone and stirred for 1.5 h. then filtrated and this process has been repeated for 30 min. The filtrate was collected and the total phenols are quantified by a colorimetric procedure using the Folin-Ciocalteu reagent [28].

2. Phenol Extraction from the Pulp

The liquid portions obtained after pulping was extracted with hexane to remove the lipid fraction by mixing 1 L of the liquid fraction with 500 ml of hexane. The mixture was vigorously shaken, and phases were separated by decantation and the process was repeated twice. Extraction of phenolic compounds was performed with ethyl acetate (500 ml per 200 ml of sample). The liquid–liquid extraction was performed with hot ethyl acetate refluxed at 77 °C in a continuous extractor for 8h. The ethyl acetate phase was rotary evaporated under vacuum at 70 °C producing a phenol rich extract [29]. Total phenol content was determined by the Folin–Ciocalteu spectrophotometric method using Gallic acid as a reference standard.

E. Carboxymethylation

The synthesis of CMC was carried out based on the procedures of Heidrich and Ullmann with some modification: 2 g of hollow cellulose in 53 ml of isopropanol was stirred vigorously while 10 ml of 40% aqueous sodium hydroxide solution was added during 20min at room temperature. Stirring was continued for another 1 h at 40 °C, an equimolar amount of Monochloroacetic acid 2.4 g (dissolved in 5mL organic solvent) was then added during 20 min. The mixture was allowed to react for 4h at 30°C. After

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carboxymethylation, the mixture was filtered, suspended in methanol (70 %) and neutralized with acetic acid. The product was collected by filtration, washed three times with 70% (w/w) aqueous ethanol and then dried at 45 °C in a vacuum oven [30].

1. Determination of Degree of Substitution

(a) The Purity of Carboxymethyl

Exactly 0.5 g of CMC was dissolved in 10 ml of water with stirring then 10 ml of 1 M hydrochloric acid was added and the mixture was agitated to dissolve completely. Five drops of phenolphthalein indicator were added to the mixture, and then 1 M sodium hydroxide was added dropwise with stirring until the appearance of red color. Ethanol (50 ml, 95%) was slowly added to the mixture with stirring and another 100 ml of 95% ethanol was added and the mixture was left to settle for 15 min. After the solution had settled the mixture was filtered by G3 type glass cylinder and discarded. The precipitate was washed four times with 80% ethanol. The precipitate was then washed again with 50 ml of 95% ethanol and dried in the oven at 105 °C for 4h.

(b) Degree of Substitution

The average values of the degree of substitution (DS) were determined by acidimetric titration. Exactly 0.2 g of CMC was weighed in 250 ml flask, and then 50 ml distilled water was added with stirring for 10 min. pH value of the solution was adjusted up to 8. Then the solution was titrated with 0.05M H_2SO_4 until the pH value of solution decreased to 3.74.

The degree of substitution was calculated based on the equations shown below [31].

$$A = m_0 / m$$

$$B = 2 \times M \times V / A \times m$$

$$DS = 0.132 \times B / 1 - 0.08 \times B$$

where A is the purity of CMC. M_0 and m = carboxymethylated products purified after and before, M= molarity of H_2SO_4 used, V = ml of H_2SO_4 used to titrate sample, B = mmol/g of H_2SO_4 consumed per gram of carboxymethylated products.

F. Preparation of Hydrogels

The preparation of hydrogel was carried out by a free radical polymerization method. Into a graduated bottle of 250 ml, the following components were dissolved in distilled water; CMC, AM, MBAM and CAN (0.1N HNO_3) and the mixture was mixed. The bottle was then kept in a water bath at 25± °C for 2 h. The contents were changed into a gel-like mass. The gel obtained was cut into small pieces and then washed with a suitable amount of ethanol and ether to remove the excess of water. The formed hydrogel was dried overnight at 45 °C.

1. Extraction of Homopolymer

The hydrogel was extracted with hot water in a Soxhlet for 24 h to remove the excess of homopolymer. Different parameters have been monitored to evaluate grafting process, namely: grafting percent (GP%) was calculated as follows:

$$GP\% = [(B-A) / A] \times 100$$

where A is the weight of CMC, B is the weight of grafted polymer after extraction.

2. Water Uptake (Swelling) Experiments

The progress of the water uptake (swelling) process was monitored gravimetrically. In a typical swelling experiment, a piece of hydrogel (0.1 g) was immersed in an aqueous reservoir using distilled water and allowed to swell for a definite period. The swollen piece was taken out after time pressed in between two filter papers to remove excess water and weighed [32].

The water uptake and/or swelling degree percentage of hydrogel. [33] can be determined as a function of time as:

$$\text{Water uptake (g/g)} = m_t - m_0$$

$$\text{Swelling degree \%} = (m_t - m_0 / m_0) \times 100$$

where m_t is the weight of the swollen hydrogel sample at time T and m_0 is the weight of the dry hydrogel sample

3. Swelling in Buffer Solutions

The pH-reversibility of hydrogels has been studied by using two buffers with pH 3 (H_3PO_4/NaOH, 0.1 mol/L of H_3PO_4 was titrated with 0.1 M of NaOH solution) and 10 $NaHCO_3$/NaOH, 0.1 mol/L of $NaHCO_3$ was titrated with 0.1 M of NaOH solution). The pH values were precisely checked with a pH-meter.

4. Effect of Temperature on Swelling

The progress of the water uptake (swelling) process was monitored gravimetrically as described in different temperature (30 °C, 45 °C & 55 °C). A piece of hydrogel (0.1 g) was immersed in an aqueous reservoir using distilled water and allowed to swell for 24 h.

5. Loading of AgNPs into Hydrogel

The hydrogel prepared was steeped in distilled water for 24 h, then transferred to the silver nitrate solution (0.1 M $AgNO_3$) for another 24 h. The gel was taken out and put in trisodium citrate solution (0.1 M) for another 3 h to reduce Ag+ ions into AgNPs within the swollen gel. The dark brown color of the desk indicated the formation of AgNPs. The hydrogel was dried in an oven at 45 °C [34].

6. Antibacterial Activity

The agar disc diffusion method was employed for the determination of antimicrobial activity of the prepared hydrogel [35]. The tested compounds were steeped in distilled water to get concentrations of 0.25 g of gel in 5 ml. A volume of 0.1 ml of the tested microorganisms grown in Brian heart infusion broth (at 42 °C for 24 h, 108–109 cells/ml), was incubated in Brian heart infusion media, and then spread on the entire surface of the dish using a sterile spatula.

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Subsequently, sterile discs were placed onto agar at certain intervals by passing gently. After the plates were incubated at 42 °C for 24 h, the inhibition zones around the discs where no growth occurred were measured in millimeters, the experiments were repeated in duplicated for all of the test strains

G. Analysis

1. Fourier Transform Infrared Spectroscopy

The extracted cellulose, lignin, and CMC were calibrated by Fourier Transforms IR (FTIR) instrument. To get the spectra, a pellet made from the sample was ground with KBr. The transmission was measured in the wave number range of 800–4400 cm^{-1}.

2. Morphological Properties

The morphology of cellulose in the residues of olive oil, fibers were observed using Scanning Electron Microscope (SEM) (Hitachi High Technologies America, Schaumburg, IL). Samples to be observed under the SEM were mounted on conductive adhesive tape, sputter coated with gold- palladium, and observed under the SEM. The widths of the single cells obtained by maceration were measured from the SEM pictures and the lengths of the single cells were measured using a digital microscope.

III. RESULTS AND DISCUSSION

A. Chemical Characterization

TABLE I
CHEMICAL COMPOSITION OF SEED AND HUSK

Properties	Seed	Husk
Ash	0.56	3.10
Lignin	22	28
α-Cellulose	31	41
Hollocellulose	47	54
Resin	7.6	28

TABLE II
WAX, RESIN AND PHENOL EXTRACTION IN SEED, HUSK AND PULP OF SEED AND HUSK

Type of solvent	Wax and Risen (seed, husk) %	Seed	Husk	Pulp
Ethyl acetate	11	-	-	-
Methanol	13	-	-	-
Hexane	19	-	-	-
Benzene/ ethanol	24	-	-	-
Phenol extraction %	-	4.3	5.0	3.7

The compositional analysis of the raw lignocellulosic material of both seed and husks materials has been illustrated in Tables I and II. Wax content is the main difference between seed and husks, which is higher in husks compared to that of seeds. For determination of this product, selective organic solvent extraction is usually employed. Various organic solvents have been used for such extraction. The phenolic compounds that have been present in the black liquor of pulp were also mentioned.

The cellulose content of husk pulp is higher than that of seed pulp (41% and 31%, respectively). Considering the structural components, lignin for both materials was found to be quite high for husks (28%) than seeds (22%). Comparing to the annual plants it has been found non-wood and hardwood sources have a percent of lignin around 20%. Lignin content of these two materials has been found to be close to that of softwood. In the same way the holocellulose and cellulose contents for both seed and husk raw materials were comparable to those found in wood and non-wood plants.

B. Carboxymethylation and Degree of Substitution

Table III shows that the degree of substitution (DS) of carboxy-methylation is increased by repeating carboxy-methylation steps (second and third) and this means that the increase of substitution of OH in holocellulose by -COONa that makes it more reactive and more soluble in water. Also, DS is decreased by increasing monochloro acetic acid, which reacts with NaOH to form sodium glycolate

$$CLCH_2COOH + 2NaOH \longrightarrow HOCH_2COONa + NaCL + H_2O$$
Sodium glycolate

TABLE III
DEGREE OF SUBSTITUTION OF CMC PREPARED FROM SEED AND HUSK

Carboxymethylation	DS. Seed	DS. Husk
The first	0.12	0.3
The second	0.16	0.33
The third	0.24	0.34
Acid ratio		
3.5 g	0.20	0.30
7.0 g	0.10	0.10

C. Grafting Process
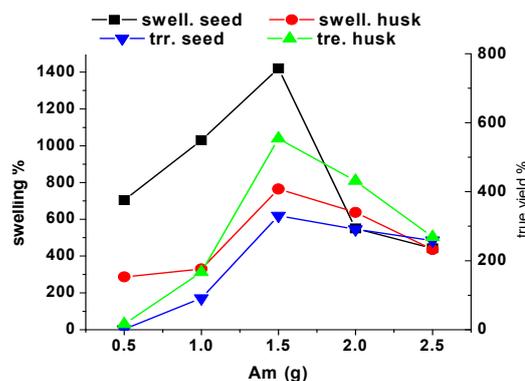
1. Effect of Monomer Concentration

Fig. 1 Effect of monomer concentration on the true yield and swelling (1.0 g CMC, 0.015 g MBAM, 10 ml CAN (0.1 N), 2 h, at 25 °C)

The diffusion ability of monomers into the polymer matrix has a great influence on the grafting percentage. It has been investigated that grafting is directly proportional to the monomer concentration (AM) as illustrated in Fig. 1 and this increment depends on true yield and swelling percentages. The grafting percentage was highest at 1.5 g of AM and then decreased. These observations may be attributed to facilitate

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the diffuse-ability of monomer towards the initiated sites on the cellulose chains in CMC, upon increase the monomer concentration which consequently increases the grafting yield. Increasing monomer than 1.5 g the rate of radical formation on the monomer molecules becomes greater which affected the viscosity of the polymerization medium and also leads to increase the rate of the termination process.
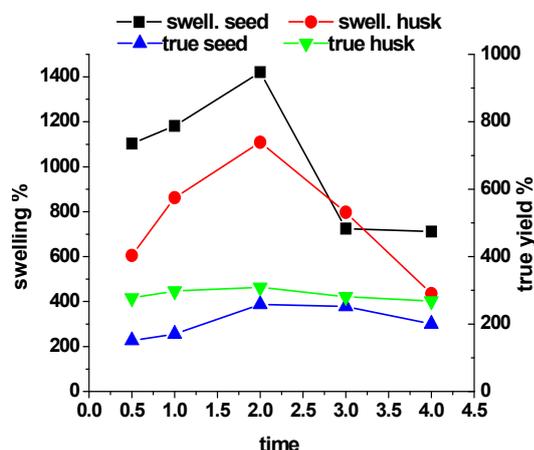
Fig. 2 Effect of time on true yield and swelling (1.0 g CMC, 0.015 g MBAM, 10 ml CAN (0.1 N), 1.5 g Am, at 25 °C)
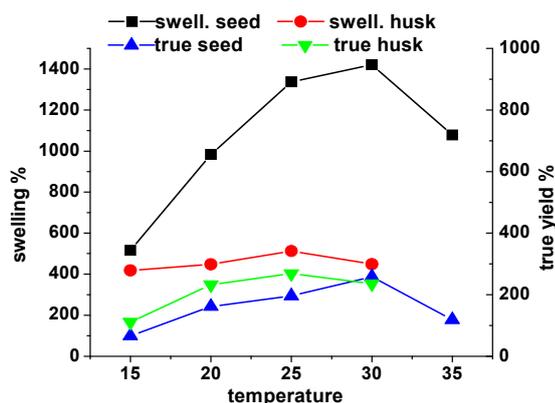
Fig. 3 Effect of temperature on the true yield and swelling (1.0 g CMC, 0.015 g MBAM, 10 ml CAN (0.1 N), 1.5 g Am, 2h)

2. Effect of Grafting Time

The effect of variation grafting time on the studied grafting percentages which depend on true yield is illustrated in Fig. 2. The results show that increasing the grafting time from 0.5 h to 2h increase the grafting percentage to reach maximum value at 2h then tends decrease after that. These observations could be explained as that the increasing the number of formed free radicals in the grafting media with prolongation of polymerization time. Beyond 2 h of grafting time, higher number of formed free radicals accelerates the polymerization process as well as the viscosity of the grafting medium. Moreover, the diffusion rate of the monomer into the polymer chains will be reduced and hence the grafting percentage and swelling.

3. Effect of Grafting Temperature

Fig. 3 shows the grafting percentages as affected by the medium temperature in the range of 15-25 °C. It has been recorded that the grafting was increased with the increment of temperature up to 30 °C for seed and up to 25 °C for husk, then decreased. Temperatures more than 30°C decreased the grafting percentage due to rapid termination step this attributed to the following reasons [36], [37]:

(1) Increase the diffusion of AM from the solution phase to the swell-able CMC phase.
(2) Increase the rate of thermal dissociation of MBAM, hence the rate of free radical formation on CMC backbone will increase.
(3) Increase the solubility of the monomer.
(4) Also, increase formation and propagation of grafted chains.

The net effect of all such factors leads to high grafting with increasing the polymerization temperature. Higher temperatures beyond 30°C may be lead to termination step occurring rapidly resulting in a decrease the grafting percentage.

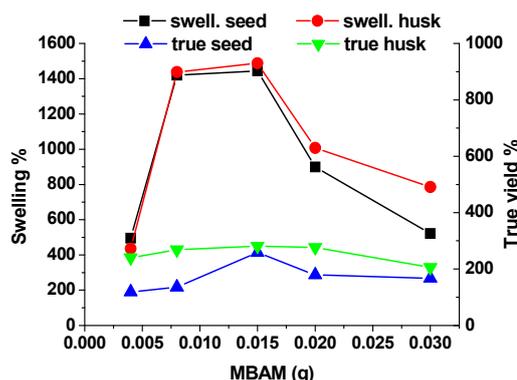4. Effect of Methylene Bis-Acrylamide

Fig. 4 Effect of MBAM on the true yield and swelling (1.0 g CMC, 10 ml CAN (0.1 N), 1.5 g Am, 2 h at 25 °C)

Crosslinking is necessary to form a superabsorbent hydrogel in order to prevent dissolution of the hydrophilic polymer chains in an aqueous environment. In this study, methylene bis-acrylamide (MBA) acts as a cross-linker and also, as a monomer. It was found that with increasing the concentration of MBAM up to 0.015, the grafting percent (true yield and swelling) increased but with further increase of MBA concentration the true yield and swelling decreased as shown in Fig. 4. These results may be attributed to that with increasing MBA concentration up to (0.015g) MBA molecules react as cross-linking grafted AM chains as "grafting from" and grafting AM homopolymer chains to CMC backbone as "grafting to". Further increase of MBA leads to increase the cross-linking density of AM-grafted chains and the cross-linking of AM homopolymers chains. Accordingly, the diffusion of left free AM monomer molecules will find a diffusion barrier which directly influences the grafting percentage and swelling. Also, as the concentration of MBA was increased, the water absorbency of hydrogel decreased.

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This is due to a decrease in the space between the polymer chains as the crosslinker concentration is increased. This decreasing trend is similar to that has been found in this study [38] and other groups for other superabsorbent hydrogels.
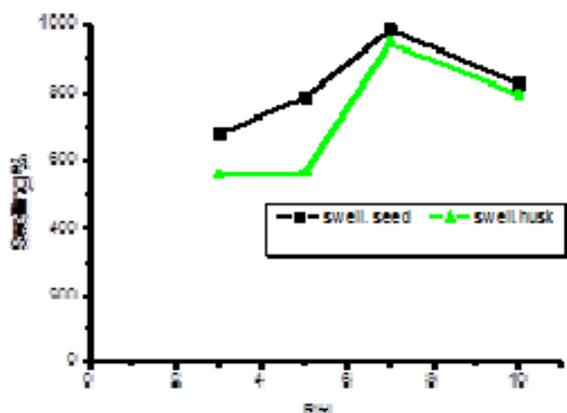
5. Swelling in Buffer Solution

Fig. 5 Effect of pH on swelling: The pH-reversibility of hydrogels has been studied by using two buffers with pH 3 (H3PO4/NaOH, 0.1 mol/L of H3PO4 was titrated with 0.1 M of NaOH solution) and 10 NaHCO3/NaOH, 0.1 mol/L of NaHCO3 was titrated with 0.1 M of NaOH solution). The pH values were precisely checked with a pH-meter
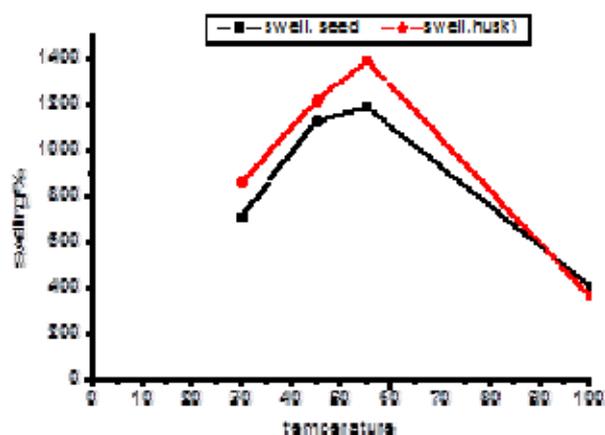

Fig. 6 Effect of temperature on the swelling capacity

The pH-reversibility of hydrogels has been studied by using two buffers with pH 3 (H$_3$PO$_4$/NaOH, 0.1 mol/L of H$_3$PO$_4$ was titrated with 0.1 M of NaOH solution) and 10 NaHCO$_3$/NaOH, 0.1 mol/L of NaHCO$_3$ was titrated with 0.1 M of NaOH solution). The pH values were precisely checked with a pH-meter.

6. Effect of Temperature on Swelling

The effect of temperature on the water absorbency of a hydrogel (swilling) was carried out at various temperatures (30 to 100°C as shown in Fig. 6). Hydrogel reached its maximum swelling capacity at 55°C. Temperatures higher than this resulted in the reduction of swelling. This may be attributed to increased cross-link formation and degradation of the polysaccharide chains of the hydrogel at higher temperatures (T > 55 °C). A similar observation was reported by Lim et al. when working on the hydrogels of sulfate-g-polyacrylonitrile [39].

7. Antimicrobial Assay

The antimicrobial effects of silver have been noticed since ancient times [40] but with the advent of nanotechnology, the use of silver in nanoparticle form has opened new treatment avenues. The mechanism of the growth-inhibitory effects of AgNPs on microorganisms has not been clearly understood. The inhibition may be possibly related to the formation of free radicals from the surface of Ag. Uncontrolled generation of free radicals can attack membrane lipids and then lead to a breakdown of membrane function [41]. The antibacterial activities of hydrogels loaded with AgNPs were investigated against Gram-positive *Staphylococcus aureus*, Gram-negative *Pseudomonas aeruginosa* and the yeast *Candida albicans* by the agar disc diffusion method. The abilities of the hydrogels to inhibit the growth of the test bacteria are shown in Fig. 7.

D. Analysis

1. Infrared Spectroscopy Analysis

Fig. 8 shows FTIR spectra of (a) cellulose seed, husk (b) CMC seed, husk and (c) grafted CMC seed, husk. The FTIR spectra of cellulose (a) show the appearance of a very broad peak at around 3450 cm^{-1} region. This corresponds to O-H stretching vibrations of the hydroxyl groups [42]. By comparing the spectra of cellulose (a), CMC (b) and grafted (c), it can be seen that the broad peak observed in (a), (b) is shifted to lower wavelengths 3430 cm^{-1} and its intensity is decreased this may be as a result of the reaction of acrylamide with OH of CMC and replacement of O-H by N- H group.

I-Staphylococcus aureus   *II- Pseudomonas aeruginosa*
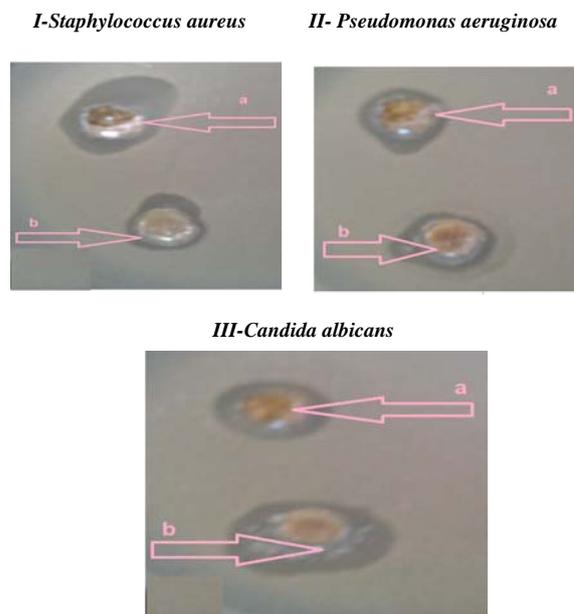

III-Candida albicans

Fig. 7 Antibacterial activities of hydrogels loaded with AgNPs (a) CMC gel Ag seed and (b) CMC gel –Ag husk

The characteristic peak observed around 1655 cm^{-1}

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corresponds to a C=O group of the acrylamide moiety, the relative absorbance of C-O-C at 1115 cm^{-1} decreased in CMC and disappear by grafting. This means that degradation of cellulose occurred during carboxymethylation grafting. The band at 2922 and 2845 cm^{-1} corresponds to C-H stretching vibrations in CH and CH$_2$.

Fig. 9 shows the FT-IR spectra of lignin from seed and husks. FT-IR spectra showed characteristic lignin peaks of husks and seed as 3430 cm^{-1} for O-H stretching vibration phenolic compound. The band at 2925 cm^{-1} for C-H stretching vibration. The bands in the range 2832–2815 cm^{-1}, which are very characteristic for -OCH$_3$ groups. At 1710 cm^{-1} C=O aryl conjugated acid or ester. The peak at 1665 cm^{-1} for C=C Aromatic skeletal vibrations. 1271-1281 cm^{-1} C-O stretching in guguciyl ring in husk only where at 1325 cm^{-1} for C-O stretching in syringyl ring in husk and seed. At 722 -637 cm^{-1} C-H in plan and out of plan syringyl ring.

TABLE IV
CLEAR ZONE *STAPHYLOCOCCUS AUREUS*, *PSEUDOMONAS AERUGINOSA*, AND *CANDIDA ALBICANS*

Sample	Clear zone (φmm)		
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Candida albicans*
(a) Seed CMCgel –Ag	14	12	14
(b) Husk CMCgel -Ag	10	13	18

2. Scanning Electron Microscope (SEM)

The morphology of cellulose in the residues of olive oil fibers, CMC and hydrogel was observed using SEM. Fig. 10 shows the SEM images of cellulose, CMC, and hydrogel, which indict that crystallinity of cellulose is decreased after carboxymetheylation in the image (Figs. 10 (a), (c)) CMC (seed and husk) also there are pores on the surface of gel and more cross-linking in the image (Figs. 10 (e), (f)) this proof the conversion of cellulose to CMC and then to hydrogel.
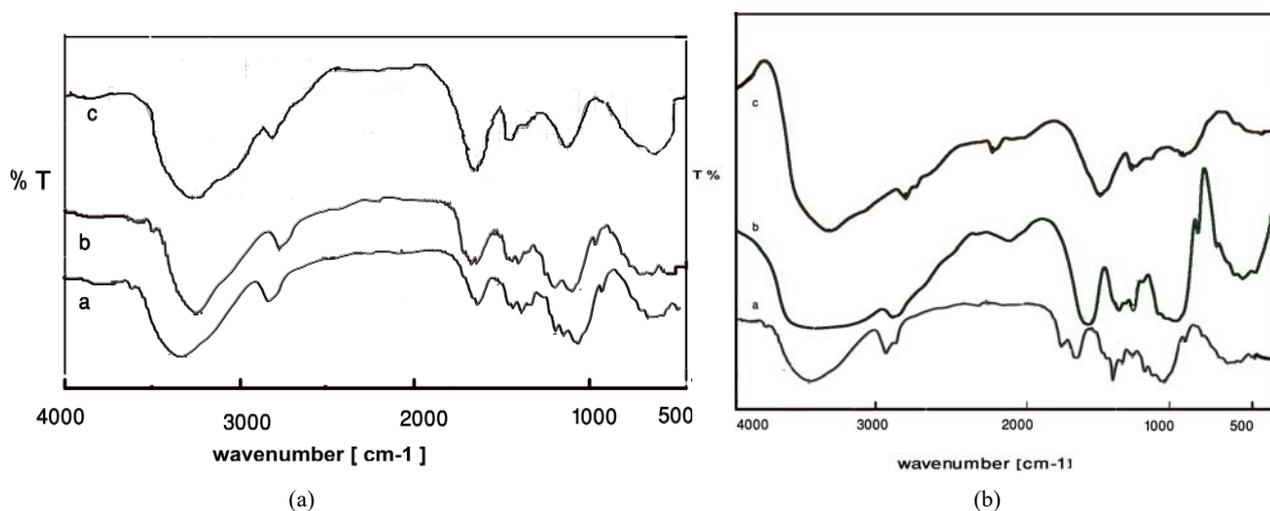

(a)

(b)

Fig. 8 (a) FTIR spectroscopy of seed a) Cellulose, b) CMC, c) CMC –gel, (b) FTIR spectroscopy of husk a) Cellulose, b) CMC, c) CMC –gel
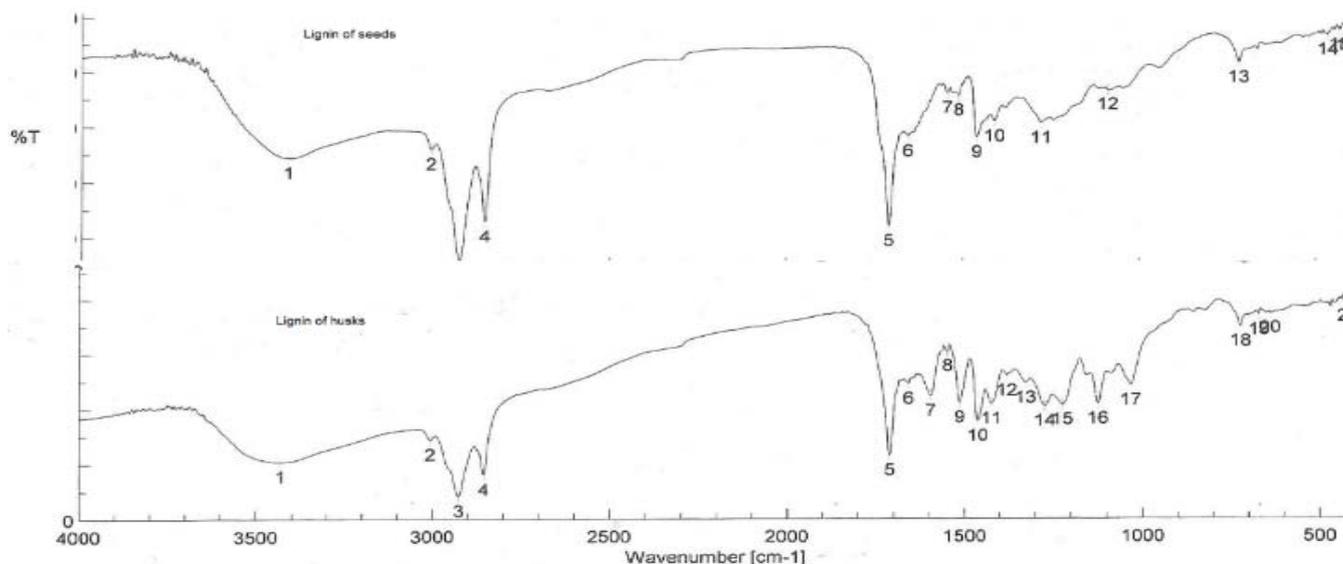

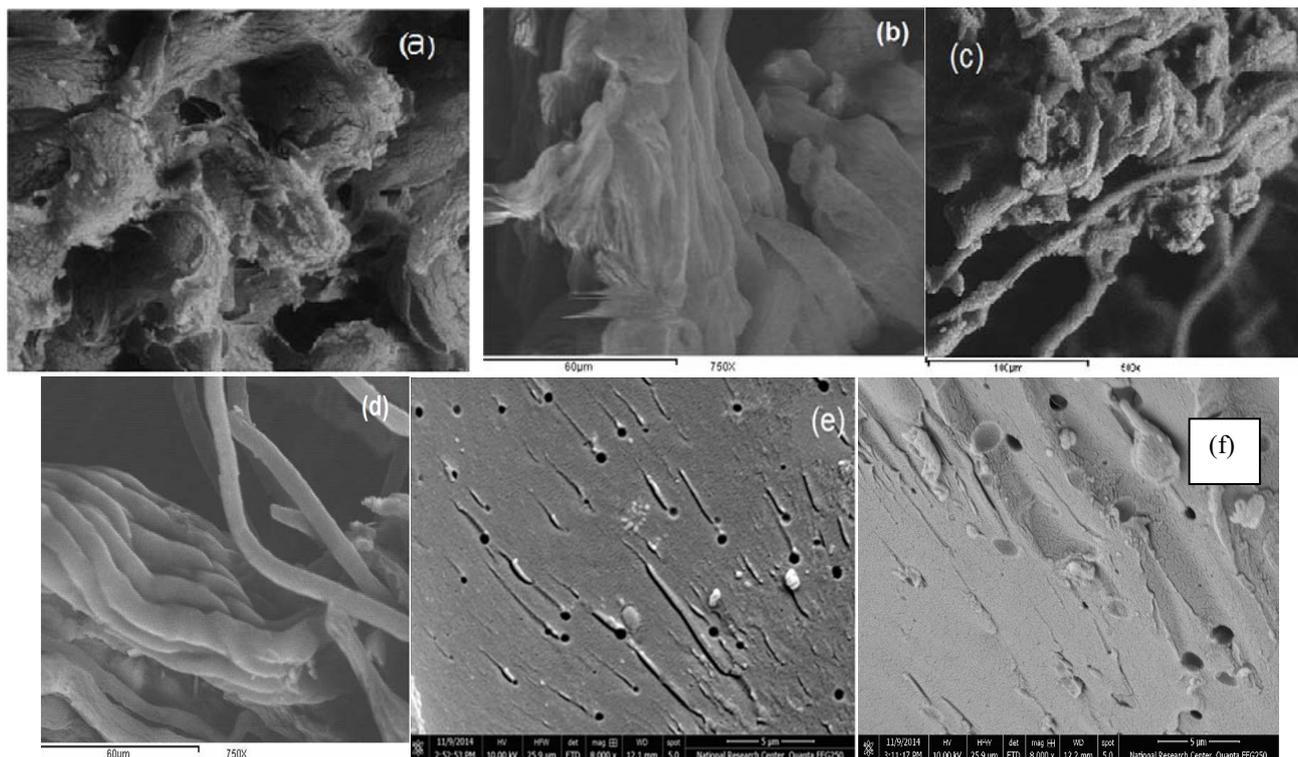Fig. 9 FTIR spectroscopy of lignin (seed, husk)

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Fig. 10 SEM of (a) CMC of seed, (b) α-cellulose of seed, (c) CMC of Husk, (d) α-cellulose of Husk, (e) CMC gel seed and (f) CMC gel husk

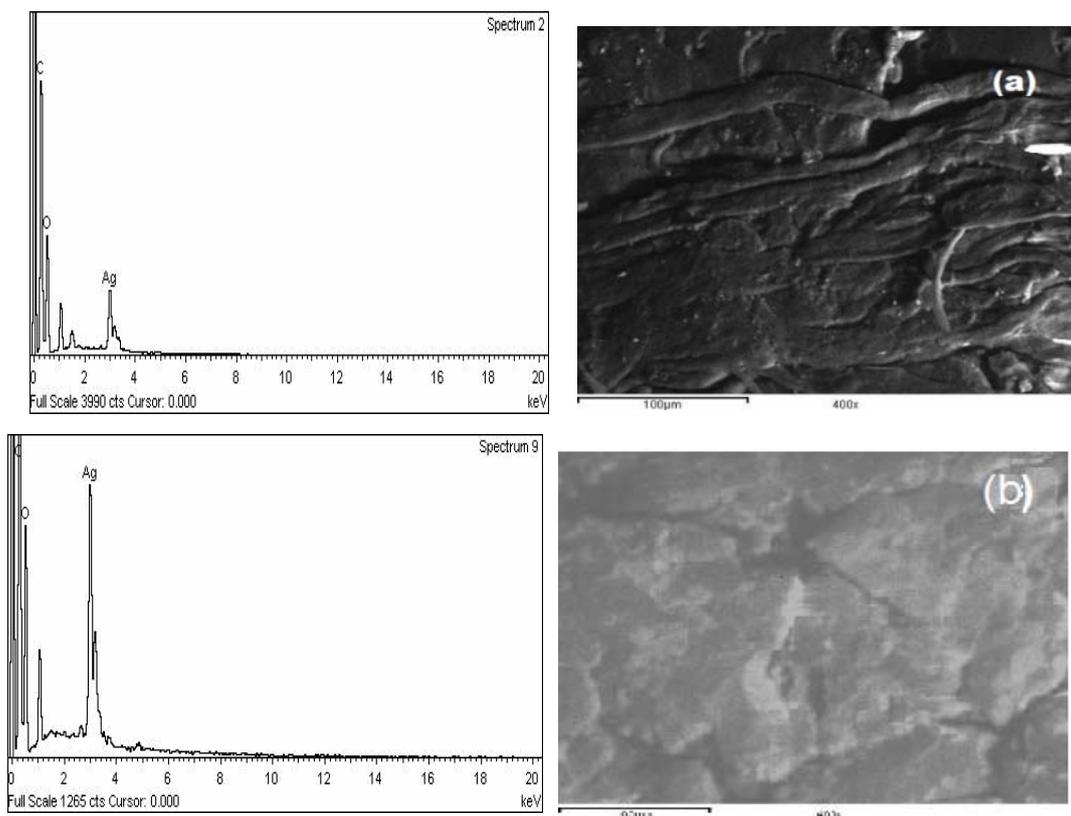Fig. 11 SEM of CMC-gel with Ag (a) Seed CMC gel –Ag (b) Husk CMC gel-Ag

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