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ORIGINAL PAPER

Production and Characterization of Bacterial Cellulose from Citrus Peels

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Abstract

Cellulose is the most common polymer in the world, formed by β-1,4 linked glucopyranose units. In this study, citrus peels (lemon, mandarin, orange and grapefruit) were used for the production of bacterial cellulose (BC). The peels were hydrolyzed with dilute acid and hydrolysates were used for BC production. The production of BC was carried out at 28–32 °C for 21 days under static conditions with *Komagataeibacter hansenii* GA2016. BC yields were found to be between 2.06 and 3.92%. It was found that the FTIR spectra of the BCs produced in citrus peel hydrolysates were similar to BC produced in the commercially available nutrients. The result of this study showed that all the BCs produced from citrus peels were characterized to have high water holding capacity, thin fiber diameter, high the thermal stability and high crystallinity.

Keywords Bacterial cellulose · Citrus peels · *Komagataeibacter hansenii* · Waste valorization

Introduction

Cellulose is the most common linear polymer in the world and, it is formed by D-glucose units linked together with β-1,4-glycosidic linkages. It is the major component of plant cell wall together with hemicellulose and lignin (40–60% cellulose, 20–40% hemicellulose and 10–25% lignin) [1, 2]. It can be also produced by various bacterial species such as *Komagataeibacter, Gluconacetobacter, Acetobacter, Agrobacterium, Aerobacter, Achromobacter, Alcaligenes, Azotobacter, Pseudomonas, Rhizobium, Sarcina, Salmonella* and *Escherichia* [3, 4], as an extracellular polymer of their metabolism and known as bacterial cellulose (BC) [2, 5]. It protects microorganisms against unfavourable environmental conditions such as low water content, extreme pHs, pathogenic microorganism [6], UV rays [7] etc. It is produced on the surface of growth medium where the oxygen pressure is high [8].

BC is traditionally used in some foods (Nata de Cocco: jelly-like food produced by the fermentation of coconut water) and functional drinks (Kampuchea or Manchurian tea) in South-East Asia and Japan [9, 10]. However, it has increased applications areas such as pharmaceuticals, biotechnology, biomedical, cosmetics, paper and electronic industry [10–13]. It has excellent properties compared to plant cellulose, such as unique nanostructure [14], purity, water retention capacity [15], polymerization degree [16], crystallinity [17, 18], mechanical and tensile strength [19], elasticity, transparency and biodegradability and adaptability to living body [8, 17, 20]. Since it cannot be digested in the human digestive tract like cellulose [21], it acts as a dietary fiber [22].

In spite of the many advantages, the high cost of BC production limits the industrial implementation and the market share of the polymer. A standard medium used for the production of BC is the Hestrin–Schrammian that is expensive medium, composed of glucose, yeast, peptone, citric acid and potassium. Since the widespread use of BC depends on the reduction of its production cost, it is necessary to find the cheap and sustainable carbon sources for the production of BC that does not compete with the food production. Recently, there have been various studies using agricultural, forestry and industrial wastes as a carbon source in order to reduce production costs of BC [23–25]. In these studies, food processing waste [26], hemicellulose [27], beet and sugar cane molasses [28], various fruit wastes [29], konjac powder [30], rice husk [31], wheat straw [14], cotton-based waste textiles [32], maple syrup [33], coffee cherry bark

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[34], dried olive mill residue [35], waste beer yeast [25], date syrup [36] and wood extract [37] were used for BC production. Among the wastes, fruit peels represent important source of carbohydrates that make them attractive biomass for the production of value-added products such as BC.

Citrus peels, not consumed with fruits due to their bitter taste, represent approximately 30–60 g/100 g of the citrus fruit weights [38]. *Citrus limon* (lemon), *Citrus sinensis* (orange), *Citrus reticulata* (mandarin) and *Citrus paradisi* (grapefruit) are the most cultivated citrus species in Turkey [39]. Based on Turkish Statistical Institute data the production amount of lemon, mandarin, orange and grapefruit were 750.550 tons, 1156.365, 1816.798 tons and 250.025 tons, respectively in 2015 [40] and Turkey ranks 7th and 6th in the world for lemon and grapefruit production, respectively [41]. Although the most common use of the citrus peels is the production of pectin due to their rich content of it [42], they have been used for the preparation of different food products such as marmalade, beverages (limoncello) [43], some traditional foods (chenpi: traditional Chinese food) [44], or for production of essential oils [45]. Alternatively, this low-cost and widely available biomass can be used for the production of BC.

The driving force of this study is to lower the production cost of BC by using fruit peels. The present study aimed to produce BC using different citrus peels (lemon, mandarin, orange and grapefruit peels), as sole nutrients sources. The citrus peels were converted to soluble sugar by dilute hydrolysis, and the hydrolysates were fermented to BC with *Komagataeibacter hansenii* GA2016. In addition, physical, chemical, structural and thermal properties of the produced BC from different citrus peels were determined and compared with each other.

Materials and Methods

Material

The citrus fruits used in the study were freshly supplied at local markets at different times. Their peels (lemon, mandarin, orange and grapefruit) were dried for 48 h at 60 °C and ground by the grinder (Bosch MKM 600, Germany) for 5 min. Samples were stored at + 4 °C until use.

Gallic acid (3,4,5-trihydroxybenzoic acid) and 3,5-dinitrosalicylic acid (DNS) were from Alfa Aesar GmbH & Co KG (Germany), arabinose was supplied from Sigma-Aldrich (St. Louis, MO, USA). Bovine serum albumin (BSA), Folin–Ciocalteu phenol reagent (2 N), Comassie Brillant-blue G 250, xylose, galactose, glucose yeast extract and peptone were purchased from Merck KGaA (Germany). All the chemicals were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, USA) and Merck KGaA (Germany).

Acid Hydrolysis of Citrus Peels and Analysis of the Hydrolysates

Hydrolysis of citrus peel was performed at 100 °C with 0.6 M H_2SO_4 for 2 h using liquid/solid ratio 10 mL/g. After the reaction was completed, the solid material was separated by filtration using a coarse filter paper and the pH of the filtrate was adjusted with $CaCO_3$ powder to 4.50. To collect enough hydrolysate for later experiments, the hydrolytic process was carried out several times. The neutralized hydrolysates of lemon (LPH), mandarin (MPH), orange (OPH) and grapefruit (GPH) peels were filtered on coarse filter paper, autoclaved (121 °C for 15 min) and used for BC production. Reducing sugar and protein contents of the hydrolysates were determined by DNS method [46] using glucose as a standard and Bradford method using the BSA (bovine serum albumin) standard [47], respectively. The results were presented as mean ± standard deviation of three replicates. The sugar compositions of the hydrolysates were quantified with high performance liquid chromatography system 200 (Perkin Elmer), using refractive index detector (Perkin Elmer Series 200) and column Aminex HPX 87H (300 × 7.8 mm), which was preceded by its complimentary cation H cartridge. Sugars and organic acids were eluted with 5 mM H_2SO_4 in their mobile phase from the column at 45 °C and a flow rate of 0.5 mL/min in 45 min [48]. Their concentrations were quantified using the average peak areas and compared with the mixture of standards (xylose, glucose, galactose, arabinose, citric acid and acetic acid). The results were presented as mean ± standard deviation of three replicates.

The phenolic content was measured by the Folin–Ciocalteu method [49] with slight modifications and expressed as gallic acid equivalents. The samples, 0.1 and 2.3 mL of distilled water were mixed with 0.1 mL of Folin–Ciocalteu reagent and incubated for 8 min, followed by the addition of 1 mL of 70 g/L sodium carbonate solution with 2 mL of water. The mixture was allowed to stand for 2 h at room temperature before reading the absorbance at 750 nm. The results were presented as mean ± standard deviation of three replicates.

Bacterial Cellulose Production

Komagataeibacter hansenii GA2016 that was previously isolated, identified using 16s rRNA analyses [50] and was used for the present investigation. The pre-culture was performed on HS medium (20 g/L glucose, 5 g/L yeast extract, 5 g/L peptone, 2.7 g/L Na_2PO_4 and 1.15 g/L citric acid). All autoclaved citrus peels hydrolysates (500 mL) and HS medium (500 mL) were inoculated with *K. hansenii* GA2016 at a ratio

of 1/1000 (v/v) and incubated at 28–32 °C for 21 days under static conditions. The synthesized celluloses were separated from the medium by filtration using coarse filter paper, centrifuged (Boeco, U-32/32R, Germany) at 4000x*g* for 10 min at room temperature and boiled in 500 mL of 4% NaOH solution for 30 min to inactivate bacterial cells and remove proteins. The celluloses were rinsed five times with deionized water and allowed to stand in water for removal of NaOH and neutralization [51]. The BC samples were dried at 50 °C for 48 h and the yields were calculated according to the following formula. The results were presented as mean ± standard deviation of three replicates.

$$\% \text{ Yield} : \text{A/B} \times 100$$

where A is the amount of dried BC (g), B is the amount of dried peel (g).

Determination of Moisture, Ash and Liquid Holding Capacity of BC

Moisture and ash contents of dried lemon peel (LBC), mandarin peel (MBC); orange peel (OBC); grapefruit peel (GBC) and Hestrin–Schramm (HSBC) BCs were determined gravimetrically [52]. To determine the liquid holding capacity (LHC), dry BC (1 g) was immersed in different liquids (40 g of water, acetone, dimethyl sulphoxide or acetic acid). The suspension was allowed to stand for 2 h and then centrifuged (Hettich EBA 20, Germany) at 1178x*g* for 30 min at 25 °C and the wet sample was weighed. The results were expressed as % LHC that was calculated by the following formula [53]. The results were presented as mean ± standard deviation of three replicates.

$$\text{Liquid Holding Capacity } (\%) = (\text{B} - \text{A})/\text{A} \times 100$$

where A is the dry weight of BC (g), B is the wet weight of BC (g).

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis of dried and ground BCs were performed on Jasco FT/IR-430 spectrophotometer (Japan), using samples prepared as KBr pellets. The spectra of the samples were collected over the range of 400–4000 cm^{-1} with an accumulation of 24 scans, resolution of 4 cm^{-1}.

Thermal Analysis (TG–DTA)

The thermal properties of the BCs were determined by PRIS Diamond TG/DTA thermal analyzer (USA). The samples, ~10 mg, were heated in platinum crucibles from room temperature up to 650 °C with a rate of 10°C/min in nitrogen atmosphere.

Scanning Electron Microscope (SEM) Analysis

The structural and morphological characteristics of BCs were examined by SEM. SEM analyses were performed with QUANTA 450 Field Emission Gun (FEG) SEM High Resolution Scanning Electron Microscope (USA). Images were recorded at different magnifications (×50,000 and ×30,000) and the average fiber diameters (nm) were calculated by determining the diameters of at least 10 BC fibers.

X-Ray Diffraction (XRD) Analysis

Determination of the crystal index values of the BCs and XRD analyses were performed in the Panalytical Empyrean High Performance Diffractometer (Netherlands). The samples were analyzed in Cu X-ray tube device with Ni filter between 2θ = 10°–50°. The determination of the degree of crystallinity was calculated using the following formula by the Curve Fitting Method [54, 55].

$$Crl(\%)(\text{Curve Fitting Method}) = A_{cryst}/A_{total} \times 100$$

where A_{cryst} is the area of the crystalline region, A_{total} is the the total area.

Results and Discussion

Production of BC

The citrus peels were subjected to dilute acid hydrolysis to convert their insoluble polysaccharides to fermentable sugars. The highest reducing sugar yield (30.23 g/L) was obtained in OPH. The resulting hydrolysates were found as composed of glucose (5.29–8.15 g/L), galactose (3.21–6.11 g/L), arabinose (1.41–2.33 g/L), acetic acid (0.25–0.30 g/L), citric acid (0.09–1.74 g/L), phenolics (5.42–12.95 g/L) and protein (39.88–306.63 mg/L) (Table 1). Among them, OPH has the highest glucose and acetic acid content, and LPH has the highest citric acid content. LPH and GPH were found to have higher phenolic and protein content, respectively than the other hydrolysates.

BC production with the bacterial strain *K. hansenii* GA2016 was initially carried out in HS medium leading to BC concentration of 7.44 g/L. LPH, MPH, OPH and GPH without adding any extra nutrient were directly inoculated with *K. hansenii* GA2016 to produce BC and the production yield, moisture and ash contents of all the produced BCs were presented in Table 2. It was determined that the BC yield was the highest (3.92% (w/w)) when MPH was used as the sole cultivation medium. Previous studies showed that the yield was affected by the carbon source depending on the microorganism and the citric acid in the fermentation medium could promote bacteria to produce BC and

Table 1 Chemical properties and phenolic contents of hydrolysates from citrus peels

	LPH	MPH	OPH	GPH
Protein (mg/L)	181.75 ± 2.65^{b}	39.88 ± 0^{d}	106.53 ± 5.51^{c}	306.63 ± 13.83^{a}
Total red. sugar (g/L)	17.75 ± 0.05^{d}	26.03 ± 0.41^{b}	30.23 ± 0.91^{a}	19.74 ± 0.44^{c}
Glucose (g/L)	5.73 ± 0.028^{c}	7.21 ± 0.024^{b}	8.15 ± 0.11^{a}	5.29 ± 0.16^{c}
Galactose^{+} (g/L)	3.21 ± 0.20^{b}	6.11 ± 0.35^{a}	5.55 ± 0.16^{a}	3.32 ± 0.20^{b}
Arabinose (g/L)	2.20 ± 0.09^{a}	2.33 ± 0.10^{a}	2.30 ± 0.12^{a}	1.41 ± 0.12^{b}
Citric acid (g/L)	1.74 ± 0.04^{a}	0.65 ± 0.01^{b}	0.09 ± 0.01^{d}	0.47 ± 0.01^{c}
Acetic acid (g/L)	0.28 ± 0.01^{b}	0.25 ± 0^{c}	0.30 ± 0.01^{a}	0.26 ± 0^{bc}
Total phenolic (g GAE/L)	12.95 ± 0.06^{a}	5.42 ± 0.18^{d}	9.82 ± 0.32^{b}	9.50 ± 0.42^{c}

Peel hydrolysate from lemon (LPH); mandarin (MPH); orange (OPH); and grapefruit (GPH)

^{a,b,c,d}Means followed by different letters within the same line represent significant differences ($p < 0.05$). Data are the average of triplicates

$^{+}$Values for galactose also include xylose since galactose was coeluted with xylose

Table 2 Physicochemical properties of BCs

	Production yield (g BC/100 g peel)	Moisture (% w/w)	Ash (% w/w)
LBC	2.06 ± 0.06^{d}	7.25 ± 0.94^{ab}	7.23 ± 0.09^{b}
MBC	3.92 ± 0.07^{a}	6.49 ± 0.46^{b}	3.31 ± 0.02^{d}
OBC	2.33 ± 0.05^{c}	7.73 ± 0.53^{ab}	9.01 ± 0.05^{a}
GBC	2.68 ± 0.08^{b}	8.06 ± 0.01^{a}	4.82 ± 0.03^{c}

Bacterial cellulose from lemon peels (LBC); mandarin peels (MBC); orange peels (OBC); and grapefruit peels (GBC)

^{a,b,c,d}Means followed by different letters within the same column represent significant differences ($p < 0.05$). Data are the average of triplicates

improve its production [56, 57]. However, in this study, it was determined that the phenolic contents of the hydrolysates affected the yield of BC production more than the carbon source and citric acid contents. It was observed that there were negative correlation between phenolic content of hydrolysates and BC yields. Due to the antimicrobial activity of phenolics, the presence of phenolic compounds in the cultivation medium influences the development of microorganisms and affect the production of BC, adversely. Although LPH and OPH had the highest citric acid and reducing sugar content, respectively, their high phenolic contents suppressed the BCs production (Table 1). The low phenolic and high citric acid content of MPH resulted in the higher BC yield. Besides phenolic compounds, the protein content of the hydrolysates also affected the yield (Table 3). It was determined that high protein content of GPH supported the BC production of the *K. hansenii* GA2016 more than LPH and OPH, and resulted in higher BC yield.

Except composition of cultivation medium, BC production can be affected by many other conditions such as cultivation method, microorganism, agitation speed of culture medium, temperature, time, pH and amount of oxygen [31, 36, 58]. Therefore, it is not easy to compare the results obtained in this study with those of previous studies. Castro et al. [19] found that the yield of BC produced from HS broth and pineapple juice with *Gluconacetobacter swingsii* were 2.1 and 2.8 g/L, respectively, Gomes et al. [35] reported 0.81 g/L of BC with *Gluconacetobacter sacchari* in olive mill residue, Mohammadkazemi et al. [36] reported 0.70–1.90 g/L of BC with *Gluconacetobacter xylinus* in different nutrients and various carbon sources and Lin et al. [59] reported 1.2 g/L of BC with *Komagataeibacter intermedius* in fruit juice. This study showed that the citrus peels supported the growth of *K. hansenii* GA2016 very well to produce BC with high yield.

Table 3 Liquid holding capacities of BCs

LHC (%(w/w))	Water	Acetone	Dimethyl sulfoxide	Acetic acid
LBC	886.00 ± 19.80^{a}	414.40 ± 20.36^{a}	904.40 ± 6.22^{a}	488.46 ± 2.18^{c}
MBC	791.45 ± 16.19^{b}	307.80 ± 11.03^{bc}	889.02 ± 1.39^{a}	611.01 ± 15.57^{a}
OBC	595.76 ± 6.00^{d}	306.97 ± 9.85^{bc}	574.18 ± 5.92^{d}	516.75 ± 9.55^{b}
GBC	705.17 ± 7.31^{c}	332.36 ± 3.74^{b}	792.84 ± 11.09^{b}	513.71 ± 5.25^{b}
HSBC	609.30 ± 0.9^{d}	294.12 ± 8.31^{c}	637.31 ± 10.87^{c}	543.73 ± 19.41^{b}

LHC liquid holding capacity; Bacterial cellulose from lemon peels (LBC); mandarin peels (MBC); orange peels (OBC); and grapefruit peels (GBC)

^{a,b,c,d}Means followed by different letters within the same column represent significant differences ($p < 0.05$). Data are the average of triplicates

Liquid Holding Capacity of BCs

LHC is a property of a polymer of adsorbing and retaining liquid to form a viscous solution. Since it affects the texture and viscosity of food, it is an important feature both physiologically and technologically. A polymer with high LHC has the ability to increase the volume and decrease the calorie of the food [60]. Although cellulose does not dissolve in most of the organic solvents due to its crystal structure [61], it can swell in certain solvents [62]. The LHCs of the BCs were presented in Table 3. It was found that their water, dimethyl sulfoxide (DMSO) holding capacities were higher than the acetone and acetic acid holding capacities. These results coincided with other studies [61, 62]. Due to high hydrogen bonding capacity of DMSO [61, 63], DMSO holding capacities of all BCs were relatively higher than those of other liquids. However, low hydrogen bonding capacity of acetone [61] resulted in low acetone holding values of BCs. Among them, LBC was determined to have the highest water holding capacity. The previous study reported that liquid (water, acetone, DMSO and acetic acid) holding capacities of BC (formed during Kampuchea tea fermentation) and commercial crystalline cellulose were 10–160 and 5–70%, respectively [61]. The BCs obtained in this study had higher liquid holding capacities than the previous study and commercial microcrystalline cellulose.

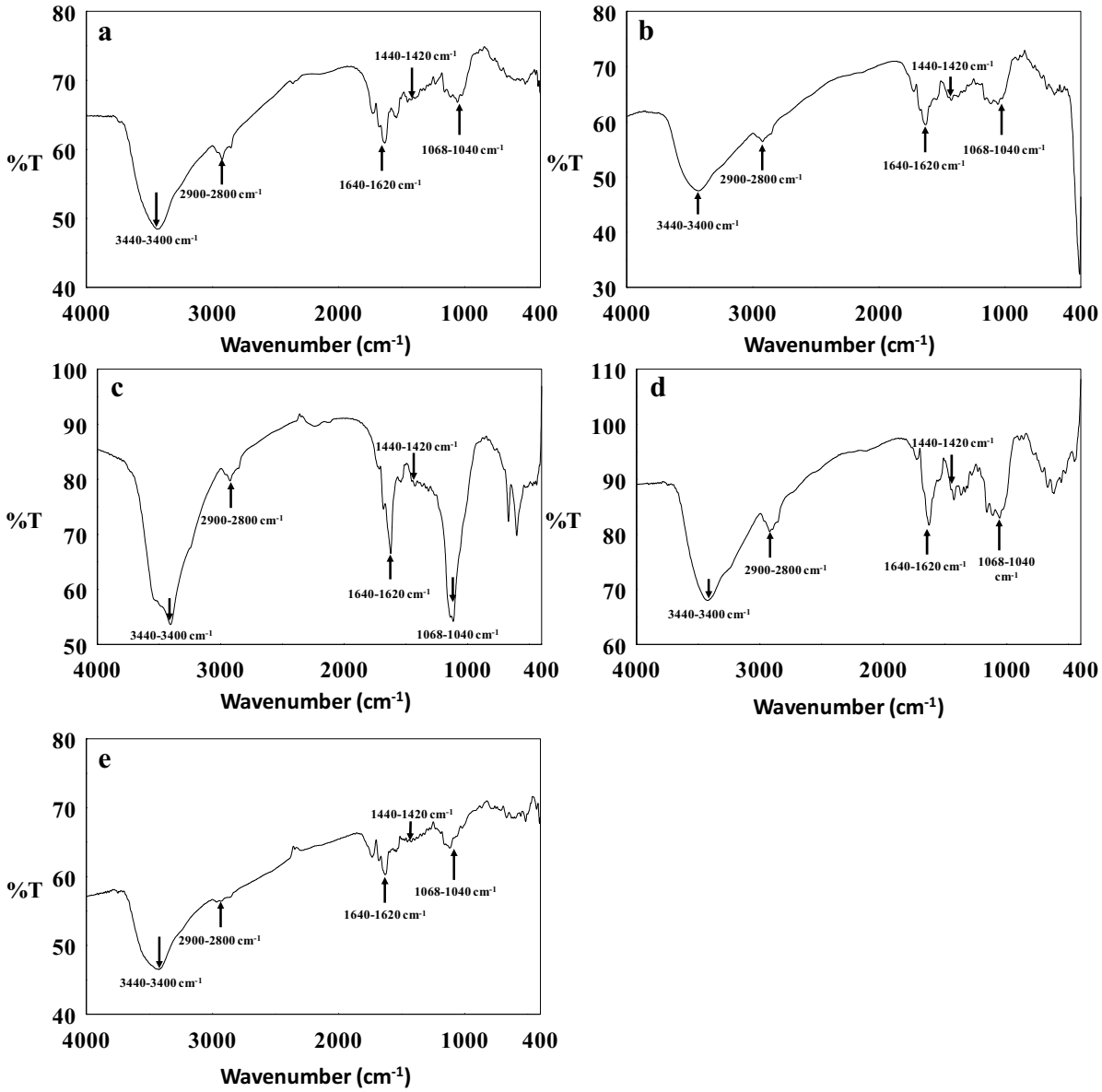

Fig. 1 FTIR spectra of BCs. BC from lemon peel (**a**), mandarin peel (**b**); orange peel (**c**); grapefruit peel (**d**) and Hestrin–Schramm medium (**e**)

FTIR Analysis of BCs

The FTIR spectra of all the BCs, presented in Fig. 1, give the information about their functional groups and the state of bonds in their structure. Absorption bands at 800 and 1200 cm^{-1} are considered fingerprint regions for carbohydrates, and the location and density of these bands are specific for each polysaccharide and allow the identification of important chemical groups in polysaccharides [64–66]. Studies have indicated that the characteristic peaks of the typical BC spectrum are hydroxyl groups (–OH) at 3400–3440 cm^{-1}, methylene stretching vibration (–CH2–) at 2800–2900 cm^{-1}, carboxyl groups (COOH) at 1620–1640 cm^{-1}, carbonyl groups (C=O) at 1420–1440 cm^{-1} and C–O–C and C–O–H stretching vibrations of the sugar ring at 1040–1068 cm^{-1} [67, 68]. The FTIR spectra of BCs produced from citrus peel hydrolysates and HSBC were similar to each other and the results are consistent with the literature [69–71].

Thermogravimetric Analysis of BCs

The decreases in their mass against temperature change of BCs were measured and the thermogravimetric differential thermal analysis curves of them were shown in Fig. 2. As seen from the figure, the thermal degradation curves of BCs from citrus peels are similar to that of HSBC and composed of three regions. The slight weight loss in the first region is due to the evaporation of the water in the sample with the increase in temperature. A second weight loss between 200 and 360 °C, is due to the removal of small molecular weight fractions such as hydroxyl groups of celluloses [36]. Third weight loss occurring between 360 and 600 °C is due to degradation of polymeric chains and pyran structures [72, 73]. The maximum decomposition temperature (DTG_{max}) is a critical point for the thermal stability and it shows the sharpest weight loss slope (%/°C) during decomposition [74]. It was determined that these values varied between 228 and 359 °C for all the BCs and GBC had the highest while LBC had lowest DTG_{max} (Table 4). In the previous studies, DTG_{max} was found as 330–350 °C for pure cellulose and whatman paper, 330–370 °C for Nata de Coco [75] and 333 °C for the plant cellulose [76]. Except LBC, the DTG_{max} of BCs of this study was found to be similar to literature values.

SEM Analysis of BCs

The visual surface morphology characteristics of the BCs were examined by SEM with different magnifications and images are shown in Fig. 3. It was found that the morphological structures of the BCs from the citrus peels were similar to the HSBC and the results were consistent with the SEM pictures of BCs presented in the literature [36, 72, 77]. Average fiber diameters of the BCs from the citrus peels were found to be in between 47.92 and 66.32 nm that were thinner than HSBC (74.29 nm) (Table 5). Thin fibers

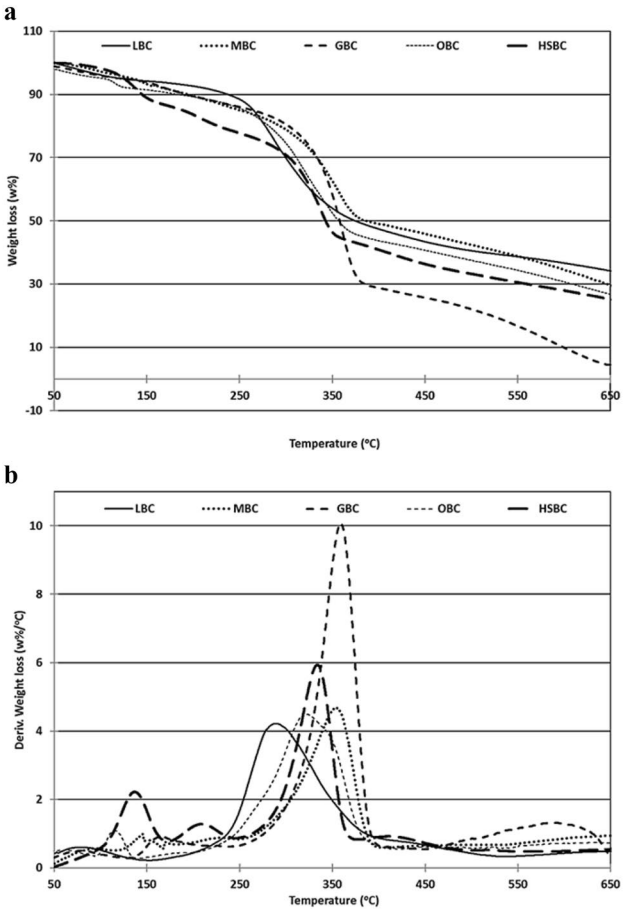

Fig. 2 TGA (**a**) and DTA (**b**) curves of BCs. BC from lemon peel (LBC); mandarin peel (MBC); orange peel (OBC); grapefruit peel (GBC) and Hestrin–Schramm medium (HSBC)

Table 4 Thermal degradation values of BCs

	$T_{\%50}$ (°C)	DTG_{max} (°C)	Mass loss (650 °C) (%)
LBC	374	228	66
MBC	378	354	70
OBC	354	322	73
GBC	357	359	95
HSBC	342	333	75

Bacterial cellulose from lemon peel (LBC), mandarin peel (MBC); orange peel (OBC); grapefruit peel (GBC); Hestrin–Schramm medium (HSBC)

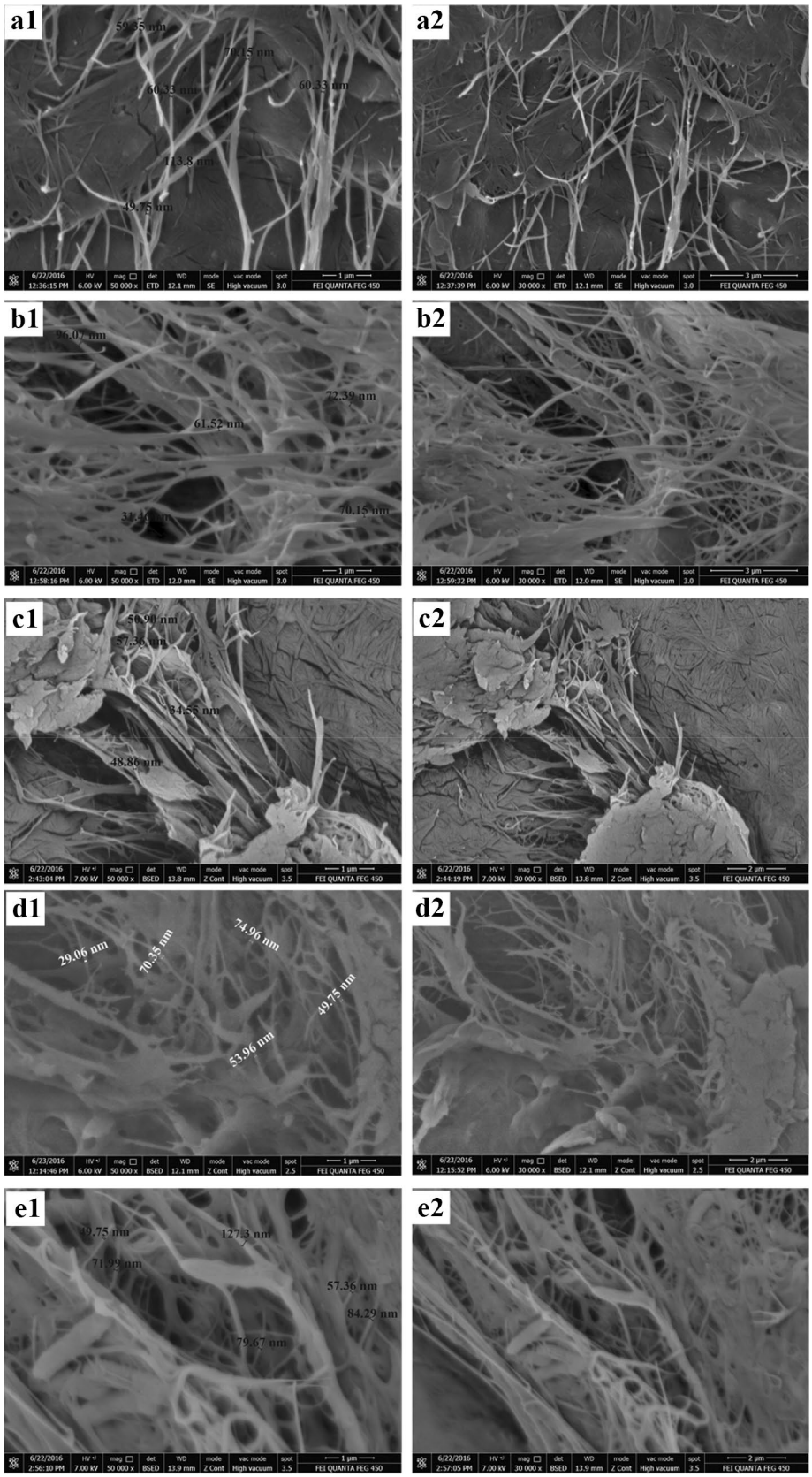

Fig. 3 SEM of BCs. BC from lemon peel (**a**), mandarin peel (**b**); orange peel (**c**); grapefruit peel (**d**) and Hestrin–Schramm medium (**e**), ×50,000 (1) and ×30,000 (2) magnification

Table 5 The average fiber diameter and degree of crystallinity of celluloses

	Average fiber diameter (nm)	Crystallinity (%)
LBC	59.98	88.55
MBC	66.32	79.48
OBC	47.92	91.54
GBC	55.45	91.96
HSBC	74.29	87.47

Bacterial cellulose from lemon peel (LBC), mandarin peel (MBC); orange peel (OBC); grapefruit peel (GBC); Hestrin–Schramm medium (HSBC)

increase the tensile strength and elongation properties of the polymers, reduce the water vapour permeability and forms a smoother structure [78]. Due to the thinner diameter of the polymer fiber, the surface area of the polymer is increased, thus providing a larger and porous hydrogel layer [79–81]. Among them OBC has the thinnest fiber diameter, thus it has the advantages mentioned above, compared to other BCs.

XRD of BCs

Although cellulose has a highly ordered structure, it also contains less ordered regions called amorphous regions [82]. The interruption crystalline region by amorphous region has been described as a degree of crystallinity and is determined by XRD technique. The crystal part of the sample forms a sharp diffraction peak while, the amorphous part forms scattered peaks in the X-ray diffractograms [82]. X-ray diffractograms of all the BCs are presented in Fig. 4 and all them exhibit three characteristic 2θ angles, 14.05°–16.77° and 22.68° [83, 84] which correspond to 101, $10\bar{1}$ and 002 planes of Cellulose I crystal structure. The largest 2θ angles of 14.05°–16.77° and 22.68° were used to calculate the crystallinity and other linear regions were excluded from the calculation. The degrees of crystallinity of the BCs were determined in between 79.48% and 91.96%. GBC and OBC were found to have the highest degree of crystallinity (Table 5). Studies showed that there were many factors affecting crystallinity of BC such as cultivation method [31], carbon sources [31, 36, 58], pH [16], agitation speed [85], temperature [86], fermentation time [16] and drying methods [87]. The previous studies reported that the crystallinity of BCs produced with different microorganisms and different carbon sources were between 46.7° and 89° [36, 85] and the crystallinity of commercial microcrystalline cellulose was between 65 and 83% [88–90]. The crystallinities of the BCs found in this study were consistent with the literature.

The present study showed that citrus peels hydrolysates provided all nutrients required for bacterial growth (*K. hansenii GA 2016*), supported the production of BCs with high yield and produced BCs with similar properties as those

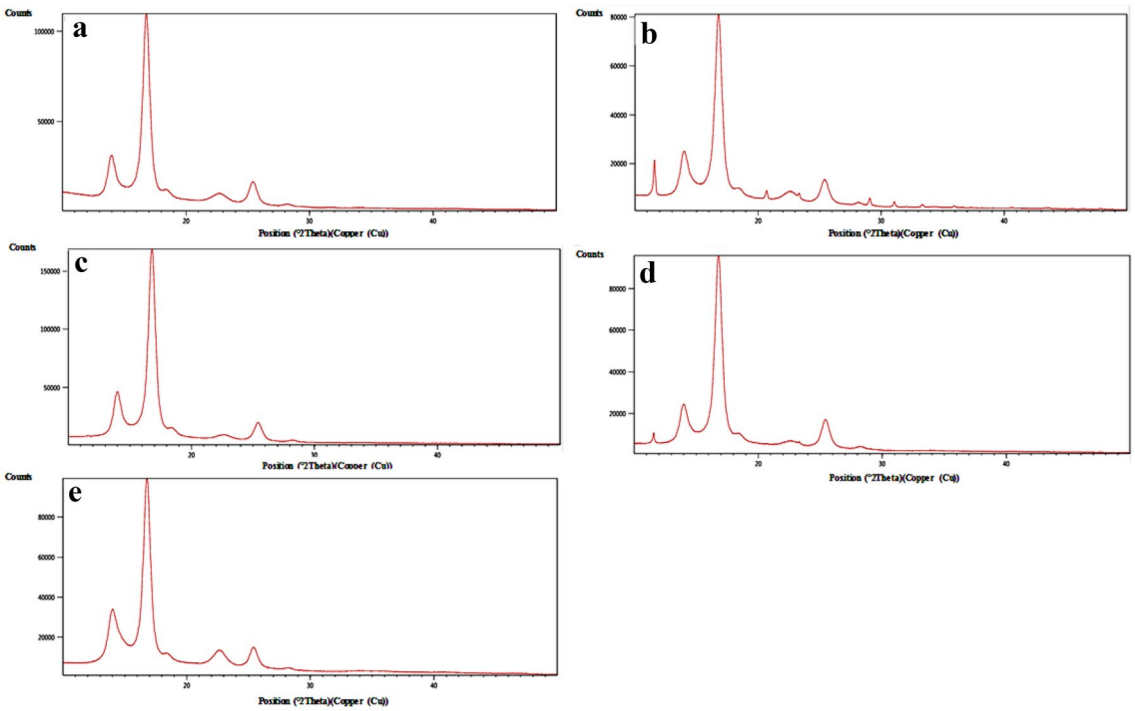

Fig. 4 X-ray diffractograms of BC. BC from lemon peel (**a**), mandarin peel (**b**); orange peel (**c**); grapefruit peel (**d**); Hestrin–Schramm medium (**e**)

produced with commercially available nutrients. All the produced BCs have superior features such as high crystallinity, thermal stability, LHC and thin fibers. Among them, LBC was found to have the highest water holding capacity and thermal stability, while OBC and GBC had the highest crystallinity degrees and OBC had the thinnest fiber diameter. Utilization of citrus peels for BC production could decrease its production cost, increase its market share among the polysaccharides and increase its potential use in food and other industrial applications.

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References

1. Lynd, L.R., Weimer, P.J., Van Zyl, W.H.: Pretorius IS: Microbial cellulose utilization: fundamentals and biotechnology. Microbiol. Mol. Biol. Rev. **66**(3), 506–577 (2002)
2. Klemm, D., Heublein, B., Fink, H.P., Bohn, A.: Cellulose: fascinating biopolymer and sustainable raw material. Angew. Chem. Int. Ed. Engl. **44**(22), 3358–3393 (2005)
3. Brown, R.M.: Cellulose structure and biosynthesis: what is in store for the 21th century. J. Polym. Sci. A **42**, 487–495 (1991)
4. Huang, Y., Zhu, C., Yang, J., Nie, Y., Chen, C., Sun, D.: Recent advances in bacterial cellulose. Cellulose **21**(1), 1–30 (1991)
5. Perez, S., Samain, D.: Structure and engineering of celluloses. Adv. Carbohydr. Chem. Biochem. **64**, 25–116 (2010)
6. Bielecki, S., Krystynowicz, A., Turkiewicz, M., Kalinowska, H.: Bacterial cellulose. In: Steinbuchel, A. (ed.), Biopolymers: Polysaccharides I., vol. 7, pp. 37–90. Wiley, Munster (2000)
7. Ross, P., Mayer, R., Benziman, M.: Cellulose biosynthesis and function in bacteria. Microbiol. Rev. **55**(1), 35–58 (1991)
8. Iguchi, M., Yamanaka, S., Budhiono, A.: Bacterial cellulose—a masterpiece of nature's arts. J. Mater. Sci. **35**, 261–270 (1991)
9. Ng, C., Sheu, F., Wang, C., Shyu, Y.: Fermentation of Monascus purpureus on agri-by-products to make colorful and functional bacterial cellulose (NATA). Microbiol. Indones. **4**(1), 6–10 (2004)
10. Shi, Z., Zhang, Y., Phillips, G.O., Yang, G.: Utilization of bacterial cellulose in food. Food Hydrocoll. **35**, 539–545 (2014)
11. Zhu, H., Jia, S., Yang, H., Tang, W., Jia, Y., Tan, Z.: Characterization of bacteriostatic sausage casing: a composite of bacterial cellulose embedded with polylysine. Food Sci. Biotechnol. **19**, 1479–1484 (2014)
12. Shah, J., Brown, R.M. Jr.: Towards electronic displays made from microbial cellulose. Appl. Microbiol. Biotechnol. **66**(4), 352–355 (2005)
13. Çakar, F., Özer, İ, Aytekin, A.O., Şahin, F.: Improvement production of bacterial cellulose by semi-continuous process in molasses medium. Carbohydr. Polym. **106**, 7–13 (2014)
14. Chen, P., Cho, S.Y., Jin, H.J.: Modification and applications of bacterial celluloses in polymer science. Macromol. Res. **18**, 309–320 (2010)
15. Saibuatong, O.A., Phisalaphong, M.: Novo aloe vera-bacterial cellulose composite film from biosynthesis. Carbohydr. Polym. **79**(2), 455–460 (2010)
16. Dahman, Y.: Nanostructured biomaterials and biocomposites from bacterial cellulose nanofibers. J. Nanosci. Nanotechnol. **9**, 5105–5122 (2009)
17. Yamanaka, S., Sugiyama, J.: Structural modification of bacterial cellulose. Cellulose **7**(3), 213–225 (2000)
18. Keshk, S.M.A.S.: Vitamin C enhances bacterial cellulose production in *Gluconacetobacter xylinus*. Carbohydr. Polym. **99**, 98–100 (2014)
19. Castro, C., Zuluaga, R., Putaux, J.L., Caroa, G., Mondragon, I., Ganán, P.: Structural characterization of bacterial cellulose produced by *Gluconacetobacter swingsii* sp. from Colombian agroindustrial wastes. Carbohydr. Polym. **84**(1), 96–102 (2011)
20. Watanabe, K., Tabuchi, M., Morinaga, Y., Yoshinaga, F.: Structural features and properties of bacterial cellulose produced in agitated culture. Cellulose **5**(3), 187–200 (1998)
21. Lin, K.W., Lin, H.Y.: Quality characteristics of Chinesestyle meatball containing bacterial cellulose (Nata). J. Food Sci. **69**, 107–111 (2004)
22. Stephens, S.R., Westland, J.A., Neogi, A.N.: Method of using bacterial cellulose as a dietary fiber component. US patent 4960763 (1990)
23. Guo, X., Cavka, A., Jönsson, L.J., Hong, F.: Comparison of methods for detoxification of spruce hydrolysate for bacterial cellulose production. Microb. Cell Fact. **12**, 93 (2013)
24. Charreau, H., Foresti, M.L., Vazquez, A.: Nanocellulose patents trends: a comprehensive review on patents on cellulose nanocrystals, microfibrillated and bacterial cellulose. Recent Pat. Nanotechnol. **7**, 56–80 (2013)
25. Lin, D., Sanchez, P.L., Li, R., Li, Z.: Production of bacterial cellulose by *Gluconacetobacter hansenii* CGMCC 3917 using only waste beer yeast as nutrient source. Bioresour. Technol. **151**, 113–119 (2014)
26. Carreira, P., Mendes, J.A., Trovatti, E., Serafim, L.S., Freire, C.S., Silvestre, A.J., Neto, C.P.: Utilization of residues from agro-forest industries in the production of high value bacterial cellulose. Bioresour. Technol. **102**, 7354–7360 (2011)
27. Uraki, Y., Morito, M., Kishimoto, T., Sano, Y.: Bacterial cellulose production using monosaccharides derived from hemicelluloses in water-soluble fraction of waste liquor from atmospheric acetic acid pulping. Holzforschung **56**, 341–347 (2002)
28. Bae, S., Shoda, M.: Production of bacterial cellulose by *Acetobacter xylinum* BPR2001 using molasses medium in a jar fermentor. Appl. Microbiol. Biotechnol. **67**, 45–51 (2005)
29. Hungund, B., Prabhu, S., Shetty, C., Acharya, S., Prabhu, V.: Production of bacterial cellulose from *Gluconacetobacter persimmonis* GH-2 using dual and cheaper carbon sources. J. Microb. Biochem. Technol. **5**, 31–33 (2013)
30. Hong, F., Qiu, K.: An alternative carbon source from konjac powder for enhancing production of bacterial cellulose in static cultures by a model strain *Acetobacter aceti* subsp. xylinus ATCC 23770. Carbohydr. Polym. **72**, 545–549 (2008)
31. Goelzer, F., Faria-Tischer, P., Vitorino, J., Sierakowski, M.R., Tischer, C.: Production and characterization of nanospheres of bacterial cellulose from *Acetobacter xylinum* from processed rice bark. Mater. Sci. Eng. C. **29**, 546–551 (2009)
32. Hong, F., Guo, X., Zhang, S., Han, S.F., Yang, G., Jönsson, L.J.: Bacterial cellulose production from cotton-based waste textiles: enzymatic saccharification enhanced by ionic liquid pretreatment. Bioresour. Technol. **104**, 503–508 (2012)
33. Zeng, X., Liu, J., Chen, J., Wang, Q., Li, Z., Wang, H.: Screening of the common culture conditions affecting crystallinity of bacterial cellulose. J. Ind. Microbiol. Biotechnol. **38**, 1993–1999 (2011)
34. Usha, R.M., Appaiah, K.A.: Statistical optimization of medium composition for bacterial cellulose production by *Gluconacetobacter hansenii* UAC09 using coffee cherry husk extract—an agro-industry waste. J. Microbiol. Biotechnol. **21**, 739–745 (2011)
35. Gomes, F.P., Silva, N.H.C.S., Trovatti, E., Serafim, L.S., Duarte, M.F., Silvestre, A.J.D., Neto, C.P., Freire, C.S.R.: Production of bacterial cellulose by *Gluconacetobacter sacchari* using dry olive mill residue. Biomass Bioenergy **55**, 205–211 (2013)

36. Mohammadkazemi, F., Azin, M., Ashori, A.: Production of bacterial cellulose using different carbon sources and culture media. Carbohydr. Polym. **117**, 518–523 (2015)
37. Kızıltaş, E.E., Kızıltaş, A., Gardner, D.J.: Synthesis of bacterial cellulose using hot water extracted wood sugars. Carbohydr. Polym. **124**, 131–138 (2015)
38. Pinzon, K.M., Rodriguez, M.C., Sandova, E.R.: Effect of drying conditions on the physical properties of impregnated orange peel. Braz. J. Chem. Eng. **30**(3), 667–676 (2013)
39. Agriculture Production Data. http://faostat.fao.org (2016). Accessed 16 Aug 2016
40. TUIK: Crop Production Statistics. http://www.tuik.gov.tr/PreHaberBultenleri.do?id=18706 (2015). Accessed 16 Aug 2016
41. Bitkisel Üretim İstatistikleri. http://www.tuik.gov.tr/PreHaberBultenleri.do?id=18706 (2015). Accessed 16 Aug 2016
42. Marin, F.R., Soler-Rivas, C., Benavente-Garcia, O., Castillo, J., Perez-Alvarez, J.A.: By-products from different citrus processes as a source of customized functional fibres. Food Chem. **100**(2), 736–741 (2007)
43. Crupi, M.L., Costa, R., Dugo, P., Dugo, G., Mondello, L.: A comprehensive study on the chemical composition and aromatic characteristics of lemon liquor. Food Chem. **105**(2), 771–783 (2007)
44. Peel: (fruit). http://en.wikipedia.org/wiki/Peel (2014). Accessed 16 Aug 2016
45. Başer, H.C.: Tıbbi ve aromatik bitkilerin ilaç ve alkollü içki sanayilerinde kullanımı, Istanbul Technical University Publications, Publication no: 1997-39, İstanbul (1997)
46. Miller, G.L.: Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. **31**, 426–428 (1959)
47. Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. **72**, 248–254 (1976)
48. Canettieri, E.V., Moraes Rocho, G.J., Carvalho, K.A. Jr., Almeida de Silva, J.B.: Optimization of acid hydrolysis from the hemicellulosic fraction of Eucalyptus grandis residue using response surface methodology. Bioresour. Technol. **98**(2), 422–428 (2007)
49. Singleton, V.L., Rossi, J.A.: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. **16**, 144–158 (1965)
50. Güzel, M., Akpinar, O.: *Komagataeibacter hansenii* GA2016 ile bakteriyel selüloz üretimi ve karakterizasyonu. J. Food **42**(5), 620–633 (2017)
51. Son, C., Chung, S., Lee, J., Kim, S.: Isolation and cultivation characteristics of *Acetobacter xylinum* KJ-1 producing bacterial cellulose in shaking cultures. J. Microbiol. Biotechnol. **12**(5), 722–728 (2002)
52. AOAC: Official Methods of Analysis, 15th edn. Association of Analytical Chemists, Arlington, Virginia, (1989)
53. Tappi: Tappi Useful Method UM256. Water Retention Value (WRV), Tappi Useful Methods. Tappi Press, Atlanta (1991)
54. Hermans, P.H., Weidinger, A.: Quantitative X-ray investigations on the crystallinity of cellulose fibers. A background analysis. J. Appl. Phys. **19**(5), 491 (1948)
55. Fang, L., Catchmark, J.M.: Characterization of water-soluble exopolysaccharide from *Gluconacetobacter xylinus* and their impacts on bacterial cellulose crystallization and ribbon assembly. Cellulose **21**, 3965–3978 (2014)
56. Mikkelsen, D., Flanagan, B., Dykes, G., Gidley, M.: Influence of different carbon sources on bacterial cellulose production by *Gluconacetobacter xylinus* strain ATCC 53524. J. Appl. Microbiol. **107**, 576–583 (2009)
57. Santos, S.M., CArbajo, J.M., Villar, J.C.: The effect of carbon and nitrogen sources on bacterial cellulose production and properties from *Gloconacetobacter sucrofermentans* CECT 7291 focused on its use in degraded paper restoration. Bioresources **8**(3), 3630–3645 (2013)
58. Miyamoto, H., Tsuduki, M., Ago, M., Yamane, C., Yamane, C., Ueda, M., Okajima, K.: Influence of dyestuffs on the crystallinity of a bacterial cellulose and a regenerated cellulose. Text. Res. J. **84**(11), 1147–1158 (2014)
59. Lin, S.P., Huang, Y.H., Hsu, K.D., Lai, Y.J., Chen, Y.K., Cheng, K.C.: Isolation and identification of cellulose-producing strain *Komagataeibacter intermedius* from fermented fruit juice. Carbohydr. Polym. **151**, 827–833 (2016)
60. Rodriguez, R., Jiménez, R., Fernández-Bolaños, J., Guillén, R., Heredia, A.: Dietary fibre from vegetable products as source of functional ingredients. Trends Food Sci. Technol. **17**(1), 3–15 (2006)
61. Goh, W.N., Rosma, A., Kaur, B., Fazilah, A., Karim, A.A., Bhat, R.: Microstructure and physical properties of microbial cellulose produced during fermentation of black tea broth (Kombucha). II. Int. Food Res. J. **19**(1), 153–158 (2012)
62. Mantanis, G.I., Young, R.A., Rowell, R.M.: Swelling of compressed cellulose fiber webs in organic liquids. Cellulose **2**, 1–22 (1995)
63. Robertson, A.A.: Cellulose-liquid interactions. Pulp Pap. Mag. Can. **65**, 171–178 (1964)
64. Fabio, P.G., Nuno, H.C.S., Trovatti, E., Serafim, L.S., Duarte, M.F., Silvestre, A.J.D., Neto, C.P., Carmen, S.R.F.: Production of bacterial cellulose by *Gluconacetobacter sacchari* using dry olive mill residue. Biomass Bioenergy **55**, 205–211 (2013)
65. Nesic, A.R., Trifunovic, S.S., Grujic, A.S., Velickovic, S.J., Antonovic, D.G.: Complexation of amidated pectin with poly(itaconic acid) as a polycarboxylic polymer model compound. Carbohydr. Res. **346**(15), 2463–2468 (2011)
66. Sivam, A.S., Sun-Waterhouse, D., Perera, C.O., Waterhouse, G.I.N.: Exploring the interactions between blackcurrant polyphenols, pectin and wheat biopolymers in model breads; a FTIR and HPLC investigation. Food Chem. **131**(3), 802–810 (2012)
67. Park, J.K., Park, Y.H., Jung, J.Y.: Production of bacterial cellulose by *Gluconacetobacter hansenii* PJK isolated from rotten apple. Biotechnol. Bioprocess. Eng. **8**(2), 83–88 (2003)
68. Gao, C., Yan, T., Du, J., He, F., Luo, H., Wan, Y.: Introduction of broad spectrum antibacterial properties to bacterial cellulose nanofibers via ımmobilising ε-polylysine nanocoatings. Food Hydrocoll. **36**, 204–211 (2014)
69. Klemm, D., Schumann, D., Udhardt, U., Marsch, S.: Bacterial synthesized celluloseartificial blood vessels for microsurgery. Prog. Polym. Sci. **26**, 1561–1603 (2001)
70. Wan, Y.Z., Hong, L., Jia, S.R., Huang, Y., Zhu, Y., Wang, Y.L., Jiang, H.J.: Synthesis and characterization of hydroxyapatite–bacterial cellulose nanocomposites. Compos. Sci. Technol. **66**, 1825–1832 (2006)
71. El-Saied, H., El-Diwany, A., Basta, A.H., Atwa, N.A., El-Ghawas, E.: Economical bacterial cellulose. BioResources **3**(4), 1196–1217 (2008)
72. De Souza, C.F., Lucyszyn, N., Woehl, M.A., Riegel-Vidotti, I.C., Borsali, R., Sierakowski, M.R.: Property evaluations of dry-cast reconstituted bacterial cellulose/tamarind xyloglucan biocomposites. Carbohydr. Polym. **93**, 144–153 (2013)
73. Vazquez, A., Foresti, M.L., Cerrutti, P., Galvagno, M.: Bacterial Cellulose fromsimple and low cost production media by *Gluconacetobacter xylinus*. J. Polym. Environ. **21**(2), 545–554 (2013)
74. Ang, A., Ashaari, Z., Bakar, E.S., Ibrahim, N.A.: Characterization and optimization of the glyoxalation of a methanol-fractioned alkali lignin using response surface methodology. Bioresources **10**(3), 4795–4810 (2015)
75. Halib, N., Iqbal, M.C., Ahmad, A.M.: I.: Physicochemical properties and characterization of Nata de Coco from local food ındustries as a source of cellulose. Sains Malays. **41**(2), 205–211 (2012)

76. Soares, S., Camino, G., Levchik, S.: Comparative study of the thermal decomposition of pure cellulose and pulp paper. Polym. Degrad. Stab. **49**, 275–283 (1995)
77. Martins, I.M.G., Magina, S.P., Oliveira, L., Freire, C.S.R., Silvestre, A.J.D.: New biocomposites based on thermoplastic starch and bacterial cellulose. Compos. Sci. Technol. **69**, 2163–2168 (2009)
78. Luddee, M., Pivsa-Art, S., Sirisansaneeyakul, S., Pechyen, C.: Particle size of ground bacterial cellulose affecting mechanical, thermal, and moisture barrier properties of PLA/BC biocomposites. Energy Procedia **56**, 211–218 (2014)
79. Johnson, D.C., Neogi, A.N.: Sheeted products formed from reticulated microbial cellulose. US Patent 4863565 (1989)
80. Jonas, R., Farah, L.F.: Production and application of microbial cellulose. Polym. Degrad. Stab. **59**, 101–106 (1998)
81. Vandamme, E.J., De Baets, S., Vanbaelen, A., Joris, K., De Wulf, P.: Improved production of bacterial cellulose and its application potential. Polym. Degrad. Stab. **59**(1–3), 93–99 (1998)
82. Park, S., Baker, J.O., Himmel, M.E., Parilla, P.A., Johnson, D.K.: Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. Biotechnol. Biofuel **3**, 10 (2010)
83. Kong, F.L., Zhang, M.W., Kuang, R.B., Yu, S.J., Chi, J.W., Wei, Z.C.: Antioxidant activities of different fractions of polysaccharide purified from pulp tissue of litchi (*Litchi chinensis* Sonn.). Carbohydr. Polym. **81**, 612–616 (2010)
84. Sun, J., Jiang, Y., Shi, J., Wei, X., Xue, S.J., Shi, J., Yi, C.: Antioxidant activities and contents of polyphenol oxidase substrates from pericarp tissues of litchi fruit. Food Chem. **119**, 753–757 (2010)
85. Czaja, W., Romanovicz, D., Brown, R.M. Jr.: Structural investigation of microbial cellulose produced in stationary and agitated culture. Cellulose **11**, 403–411 (2004)
86. Hirai, A., Tsuji, M., Horii, F.: Culture conditions producing structure entities composed of cellulose I and II in bacterial cellulose. Cellulose **4**(3), 239–245 (1997)
87. Pa'e, N., Hamid, N.I.A., Khairuddin, N., Zahan, K.A., Seng, K.F., Siddique, B.M., Muhamad, I.I.: Effect of different drying methods on the morphology, crystallinity, swelling ability and tensile properties of Nata de Coco. Sains Malays. **43**(5), 767–773 (2014)
88. Teeäär, R., Serimaa, R., Paakkari, T.: Crystallinity of cellulose, as determined by cp/mas nmr and xrd methods. Polym. Bull. **17**, 231–237 (1987)
89. Leppänen, K., Anderson, S., Torkkeli, M., Knaapila, M., Kotelnikova, N., Serimaa, R.: Structure of cellulose and microcrystalline cellulose from various species, cotton and flax studied by X-ray scattering. Cellulose **16**, 999–1015 (2009)
90. Nada, A.M.A., El-Kady, M.Y., El-Sayed, E.S., Amine, F.M.: Preparation and characterization of microcrystalline cellulose (MCC). BioResources **4**, 1359–1371 (2009)