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# Production and Characterization of Bacterial Cellulose with Different Nutrient Source and Surface–Volume Ratios

## Proizvodnja i karakterizacija bakterijske celuloze pri različitim izvorima hranjivih tvari i različitim omjerima površine i volumena

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**ABSTRACT** • *In this research, commercially available, carrot juice was explored as alternative feedstock for production of bacterial cellulose (BC) by *Gluconacetobacter hansenii* (ATCC® 23769™). Two types of culture media were used: Hestrin–Schramm (HS) and the carrot juice medium and these culture media were incubated statically for 10 days. The effect of different volumes of media on the microbial process and the utilization of substrates by the bacteria, were also examined. The produced BC was analyzed using X-ray diffraction (XRD), scanning electron microscopy (SEM), thermogravimetric analysis (TGA), and Fourier transform infrared spectroscopy (FT-IR). The water holding capacity (WHC) did not vary greatly with 210 mL (38.6 %), 310 mL (35.4 %), 360 mL (36.4 %) and 410 mL (37.3 %) of carrot juice media, however the WHC of 310 mL HS media (77.1 %), actually achieved a greater WHC, compared to 410 mL of HS media (55.8 %). BC produced in the carrot juice media showed higher yields than cellulose produced in HS media, with values of 1.19 g, 1.35 g, 1.33 g and 1.21 g for media with 210 mL, 310 mL, 360 mL and 410 mL, respectively. According to XRD and TGA results, there were no significant differences in the crystallinity and thermal stability of cellulose produced between HS and the carrot juice medium. FT-IR of BC from HS and carrot juice medium also demonstrated a similar spectrum to alpha cellulose and microcrystalline cellulose.*

**Keywords:** *Bacterial cellulose, crystallinity, morphology, carrot, production*

**SAŽETAK** • *U radu je predstavljeno istraživanje komercijalno dostupnog soka mrkve kao alternativne sirovine za proizvodnju bakterijske celuloze (BC) uz pomoć bakterije *Gluconacetobacter hansenii* (ATCC 23769™). Primi-jene su dvije vrste medija za kulturu: Histrin–Schramm (HS) i medij od mrkvina soka te su ti mediji statički inkubirani deset dana. Istraživani su utjecaji različitih obujama medija na mikrobn proces i iskorištenje sup-strata od bakterija. Dobiveni je BC analiziran s pomoću rendgenske difrakcije (XRD), skenirajuće elektronske mikroskopije (SEM), termogravimetrijske analize (TGA) i Fourierove transformirane infracrvene spektroskopije*

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(FT-IR). Kapacitet zadržavanja vode (WHC) nije se znatnije mijenjao pri različitom obujmu medija od mrkvina soka: pri 210 mL (38,6 %), 310 mL (35,4 %), 360 mL (36,4 %) i 410 mL (37,3 %). Međutim, medij HS pri obujmu 310 mL (77,1 %) ostvario je veći WHC u usporedbi s obujmom od 410 mL (55,8 %). Proizvodnja BC-a u mediju od mrkvina soka pokazala je veće prinose od proizvodnje celuloze u mediju HS, s vrijednostima 1,19 g, 1,35 g, 1,33 g i 1,21 g za obujam medija 210 mL, 310 mL, 360 mL i 410 mL. Prema rezultatima XRD i TGA, nije bilo značajnih razlika u kristaliničnosti i toplinskoj stabilnosti proizvedene celuloze između medija HS i mrkvina soka. FT-IR analiza BC-a proizvedenoga u mediju HS i mediju od mrkvina soka također je pokazala sličan spektar alfa-celuloze i mikrokristalne celuloze.

**Ključne riječi:** bakterijska celuloza, kristaliničnost, morfologija, mrkva, proizvodnja

## 1 INTRODUCTION

### 1. UVOD

Bacterial cellulose (BC) is a bio-nanomaterial with unique properties. This material is produced by several species of bacteria. The most notable of this group is *Acetobacter xylinum*, renamed nowadays as *Gluconacetobacter xylinus* and it is found wherever the fermentation of sugars and plant carbohydrates occur (Gama *et al.*, 2013). BC is similar to plant cellulose. However, it is purer and does not contain hemicelluloses and lignin. On the other hand, BC has higher crystallinity, degree of polymerization, water absorbing and holding capacity, mechanical strength in the wet state and stronger biological adaptability (Castro *et al.*, 2011; Wan *et al.*, 2007). BC has a wide application in medicine (artificial blood vessels, skin tissue repair), cosmetics, food industry (Nata de Coco) and in the production of magnetic aerogels and magnetic nano papers (Klemm *et al.*, 2001; Olsson *et al.*, 2010; Halib *et al.*, 2012; Fu and Yang, 2013). Many studies were carried out to decrease the high production costs of bacterial cellulose, which is the main problem for industrial scale production. Alternative carbon sources, such as olive oil residues, molass, corn steep liquor and fruits, were used and evaluated (El-Saied *et al.*, 2008; Gomes *et al.*, 2013; Castro *et al.*, 2011).

The aim of this study was to determine the ability of carrot juice media as a nutrient source in different surface-volume ratios; 15 x 20 cm - 210 ml (A), 15 x 20 cm - 310 ml (B), 15 x 20 cm - 360 ml (C) and 15 x 20 cm - 410 (D) ml compared to standard Hestrin-Schramm (HS) media (KA), (KB), (KC) and (KD), respectively.

## 2 MATERIALS AND METHODS

### 2. MATERIJALI I METODE

#### 2.1 Materials

##### 2.1. Materijali

*Gluconacetobacter hansenii* strain used in this research was obtained from the American Type Culture Collection (ATCC® 23769™). The seed culture was prepared according to the ATCC procedure. The main culture was started by inoculating 10 % (v/v) of the seed culture with the standard Hestrin-Schramm culture medium (Hestrin and Schramm, 1954). It was incubated at 30 °C for 7 days under stable conditions in 250 ml Erlenmeyer flasks. Two types of culture media were used for the experiments: Carrot juice (Carrot

media; squeezed juice of 1.5 kg planed fresh carrots in 1/1.5 ratio with deionized water and Hestrin-Schramm (HS) media for control.

#### 2.2 Methods

##### 2.2. Metode

The culture media used were sterilized at 121 °C in an autoclave for 20 min and poured into Erlenmeyer flasks. Experiments were prepared by adding 10 % (v/v) inoculums to the Carrot and HS media in different surface-volume ratios, namely 17.5 x 11.5 x 1.5 cm = 210 ml (A), 17.5 x 11.5 x 2.0 cm = 310 ml (B), 17.5 x 11.5 x 2.2 cm = 360 ml (C), 17,5 x 11,5 x 2,5 cm = 410 ml (D) and (KA), (KB), (KC) and (KD), respectively, and they were statically incubated at 30 °C for 14 days. The collected pellicles were boiled in water for 1h and treated for 12 h in a 0.5 M NaOH solution, rinsed overnight with tap water and followed by washing with deionized water to neutral pH and weighed (wet weight). Freeze dried samples were prepared after a pre-treatment for 15 °C (48 h) under 0.454 mBar and -55 °C conditions and weighted (dry weight).

The water holding capacity (WHC) and yield (Y) were calculated as follows (Shezad *et al.* 2010);

$$WHC = \frac{\text{Mass of water removed during drying (g)}}{\text{Dry weight of BC sample (g)}} \times 100 \quad (1)$$

$$Y = \frac{\text{Dry weight of BC sample (g)}}{\text{Volume of each nutrient source (ml)}} \times 100 \quad (2)$$

##### 2.2.1 Scanning electron microscopy (SEM)

###### 2.2.1. Skenirajuća elektronska mikroskopija (SEM)

The freeze-dried samples were coated with gold (Quorum, UK). Analysis of the BC structure was performed by using a SEM (Quanta FEG 450, Netherlands) at 5 kV. Images were taken with 50000 x SEM micrograph magnifications.

##### 2.2.2 X-Ray diffraction (XRD)

###### 2.2.2. Rendgenska difrakcija (XRD)

XRD was performed with a high resolution X-ray diffractometer (Model Rigaku Smartlab, Made in Japan) with a Ni-filtered Cu K $\alpha$  (2 kW,  $\lambda$ : 1.54 Å) radiation source operated at voltage of 40 kV and 30 mA. The samples were scanned from 10°-40° 2 $\theta$  range with

a step of 10 °/min. Crystallinity index (C.I.) of BC samples were calculated from the reflected intensity data using Segal method (Keshk, 2014; Terinte *et al.*, 2011):

$$CI = 100 \cdot \frac{I_{020} - I_{\text{non-cr}}}{I_{020}} (\%) \quad (3)$$

Where;  $I_{020}$  is the maximum intensity of lattice diffraction ( $2\theta$  of 16° to 17°) and  $I_{\text{non-cr}}$  is that of the amorphous material between  $2\theta$  of 14° to 15° where the intensity is minimum.

### 2.2.3 FT-IR spectroscopy

#### 2.2.3. FT-IR spektroskopija

Fourier-Transform InfraRed (ATR-FTIR) spectroscopy analysis of the BC sample was carried out on a Shimadzu IRAffinity- One FTIR spectrometer (Japan), equipped with a Universal ATR accessory, using 200 scans and a resolution of 4  $\text{cm}^{-1}$ , over the range 4000–800  $\text{cm}^{-1}$ .

### 2.2.4 Thermogravimetric analysis (TGA)

#### 2.2.4. Termogravimetrijska analiza (TGA)

TGA analysis was evaluated with an SII Model TG/DTA 7200 EXSTAR (Made in Japan) analyser. Each sample (5 mg) was scanned from 30° to 450 °C at a heating rate of 10 °C/min in the presence of nitrogen with a flow rate of 20 ml/min to avoid sample oxidation.

## 3 RESULTS AND DISCUSSION

### 3. REZULTATI I RASPRAVA

Some data such as the yield, wet-dry weight values, and water holding capacity (WHC) of BC are given in Table 1, 2 and 3.

The composition of the nutrient medium, pH, temperature and the interaction of the surface area to the volume of substrate, as well as strain activity, are the fundamental factors affecting BC production and the profitability of the biotechnological process (Krystynowicz *et al.*, 2002; Poyrazoglu and Biyik, 2011; Ruka *et al.*, 2012). The yield increased in Carrot-BC (1.27 g/L) compared to HS-BC (0.60 g/l). The water holding capacity (WHC) is considered one of the most important physical characteristics of BC, which is directly involved in the biomedical applications of BC as wound dressing material (UI-Islam *et al.*, 2012; Tsouko *et al.*, 2015). The variations between the WHC are related to the porosity and surface area of each BC and it is also known that the greater the surface area and the larger the pore size, the greater will be the WHC of the BC sample (Tsouko *et al.*, 2015). The results showed that the HS-BC absorbed 68 times its dry weight of water. The WHC decreased to 37 in Carrot-BC medium compared to HS-BC.

Scanning electron micrographs (SEM) of freeze-dried Carrot-BC pellicle and of the reference medium HS-BC were evaluated. The fracture surface morphology of the Carrot-BC pellicles exhibits a slightly smaller and narrower diameter distribution in comparison with HS-BC. The SEM micrographs indicate that most of the fibers are in the range of 60 to 70 nm for Carrot-

**Table 1** Bacterial cellulose yield (dry-basis)

**Tablica 1.** Prinos bakterijske celuloze (suha tvar)

Volume Obujam mL	Bacterial cellulose yield, g/l <i>Prinos bakterijske celuloze, g/l</i>		Difference Razlika %
	Carrot-BC <i>BC - medij od mrkvina soka</i>	HS-BC <i>BC - medij HS</i>	
210	1.19	0.61	95
310	1.35	0.51	164
360	1.33	0.63	111
410	1.21	0.65	86
Average <i>Prosječna vrijednost</i>	1.27	0.60	112

**Table 2** BC wet - dry weight values

**Tablica 2.** Vrijednosti mase suhe i vlažne bakterijske celuloze

Volume Obujam mL	Carrot-BC <i>BC - medij od mrkvina soka</i>		HS-BC <i>BC - medij HS</i>	
	Wet <i>Vlažna</i> g	Dry <i>Suha</i> g	Wet <i>Vlažna</i> g	Dry <i>Suha</i> g
210	9.90	0.25	9.11	0.13
310	15.28	0.42	12.56	0.16
360	17.93	0.48	16.06	0.23
410	19.14	0.50	15.33	0.27

**Table 3** BC water holding capacity

**Tablica 3.** Kapacitet zadržavanja vode BC-a

Volume Obujam mL	Water holding capacity <i>Kapacitet zadržavanja vode, %</i>	
	Carrot-BC <i>BC - medij od mrkvina soka</i>	HS-BC <i>BC - medij HS</i>
210	39.60	69.07
310	35.38	77.05
360	36.35	71.17
410	37.28	55.77
Average <i>Prosječna vrijednost</i>	37.20	68.27

BC and 80-100 nm for HS-BC pellicles. The Carrot-BC pellicles showed much thinner and better network structure than the HS-BC pellicles (Figure 1).

Thermal stabilities of BCs obtained from different nutrition resources and surface–volume ratios were investigated by thermogravimetric analysis (TGA). According to TGA curves in Figure 2, it was observed that Carrot-BC showed lower thermal stability compared to HS-BC.

For TGA curves of the carrot-BC and HS-BC, low weight loss was detected over the temperature range of 50-200 °C because of evaporation of water bounded in the BCs, and the  $T_{\text{onsets}}$  were generally found to be 265-290 °C for all BCs. According to TGA curves, all TGA results are summarized in Table 4.

As seen in Table 4, the best stability of the BCs was determined as KA.  $T_{10\%}$  and  $T_{50\%}$  of KA were found

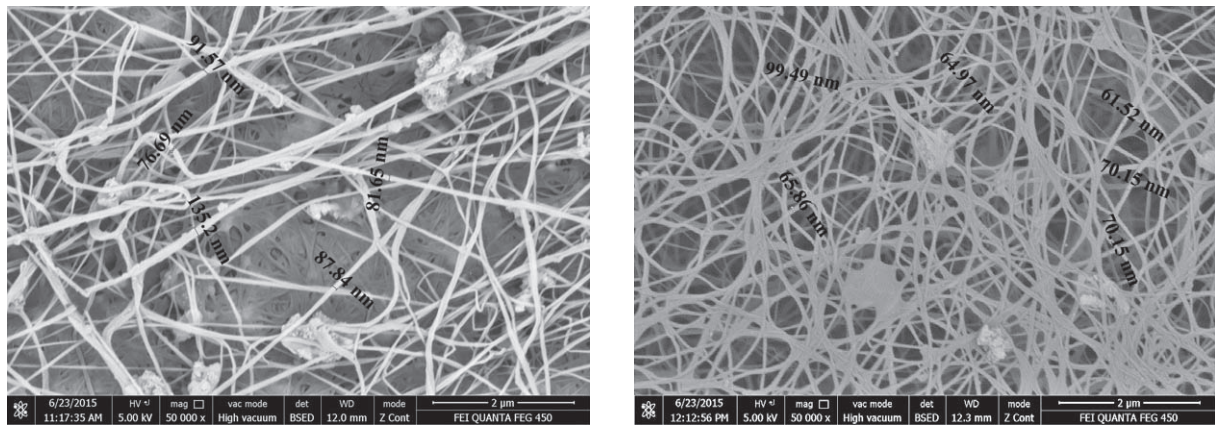


Figure 1 SEM micrographs of Carrot-BC (left) and HS-BC pellicles (right)

Slika 1. SEM mikrografije bakterijske celuloze proizvedene u mediju od mrkvina soka (lijevo) i u mediju HS (desno)

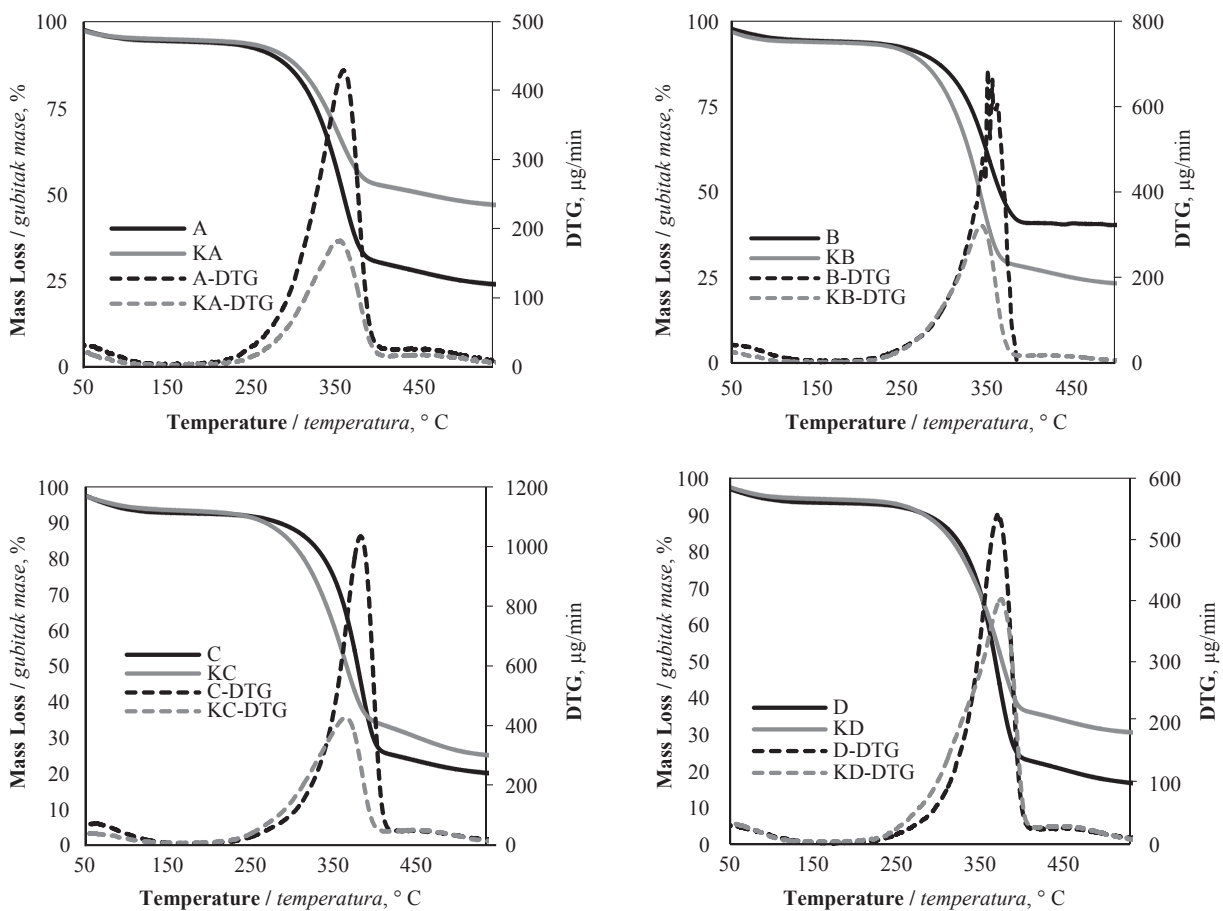


Figure 2 TGA and derivate TGA (DTG) of cellulose produced by *G. hansenii* from Carrot-BC (A, B, C and D) and HS-BC (KA, KB, KC and KD).

Slika 2. TGA i DTG krivulje celuloze proizvedene bakterijom *G. hansenii* u mediju od mrkvina soka (A, B, C i D) i u kontrolnom HS mediju (KA, KB, KC i KD)

as 273.2 °C and 427.9 °C, respectively.  $T_{10\%}$  and  $T_{50\%}$  of the others were found to be lower than KA except for D in  $T_{10\%}$ . According to the weight loss, the maximum degradation was found as 83.4 % for H, and the minimum degradation was measured as 52.6 % for KA. DTG curves showed maximum degradation at 354.2 °C for F. As seen in TGA-DTG curves of the BCs, they exhibited three different degradation stages: (1) in the range of 50–200 °C with weight loss of small percentage (%). This mass loss may be attributed to vaporiza-

tion of water; the free water is evaporated below 100 °C, while linked water that forms physical bounds with polymers is only evaporated above 100 °C. (2) in the range of 200–370 °C as a result of thermal degradation of cellulose main chains, and (3) at 370–500 °C due to thermal degradation of BCs.

The FT-IR spectra demonstrated a similar spectrum to cellulose (alpha cellulose and microcrystalline cellulose), which proved that the material produced by *G. hansenii* was cellulose (HS-BC). The band at 1045

**Table 4** Summarized results of TGA curves of BCs

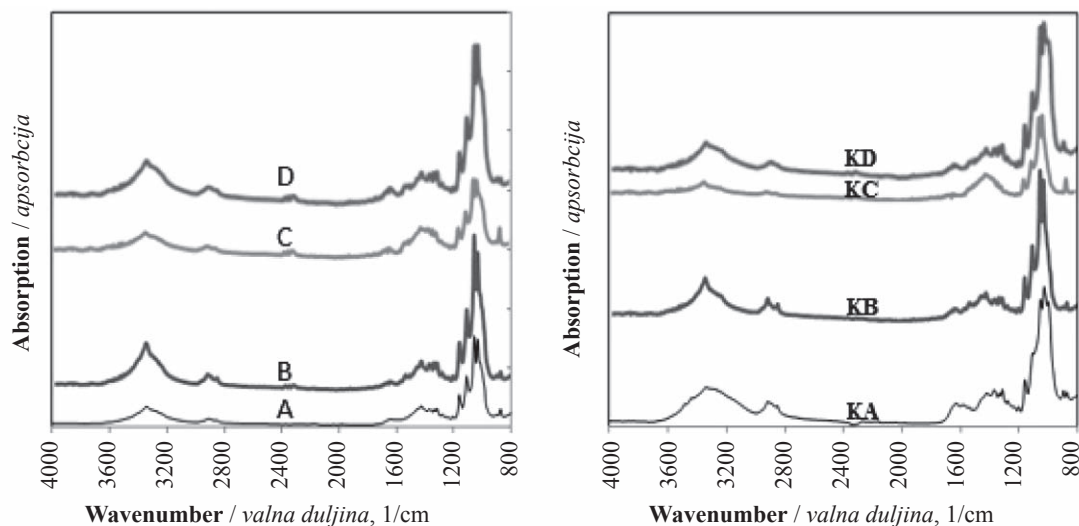
**Tablica 4.** Sažeti prikaz rezultata dobivenih TGA i DTG analizom BC-a

BC Source <i>Izvor BC-a</i>	$T_{\%10}$ °C	$T_{\%50}$ °C	$DTG_{max}$ °C	Weight loss / <i>Gubitak mase</i> %
A	259.5	335.7	335.3	75.9
KA	273.2	427.9	328.3	52.6
D	278.6	366.2	348.2	64
KE	260.8	330.3	336.2	77.2
F	267.2	355.7	354.2	80.2
KG	250.5	341.8	336.5	75.5
H	271.5	348.6	344.3	83.4
KH	266.9	353.9	347.8	69.8

to 1065  $cm^{-1}$  is related to C-O-C and C-O-H stretching vibration and at 1430 and 1660  $cm^{-1}$  for carboxylate groups and carboxylic acid (Moosavi-Nasab and Yousefi, 2010). The bands at 2900 and 3300 to 3400  $cm^{-1}$  are attributed to the  $CH_2$  stretching and the intramolecular hydrogen bonding, respectively, and the band at 3300 to 3400  $cm^{-1}$  is important for elucidating hydrogen-bonding patterns (Sturcova *et al.*, 2004). There is no significant difference between HS-BC and

Carrot-BC and the results confirmed that both BC samples exhibited similar chemical binding (Table 5, 6 and Figure 3).

XRD analysis of BC from Carrot-BC and HS-BC medium showed three major characteristic peaks around  $2\theta = 14^\circ, 16^\circ$  and  $25^\circ$ , indicating the typical cellulose I structure. The only difference between the samples is a slight intensity change in the peaks. The crystalline indices (CI) of Carrot-BC (83 %) were also



**Figure 3** FT-IR spectra of cellulose produced by *G. hansenii* from Carrot-BC (A, B, C and D) and HS-BC (KA, KB, KC and KD)  
**Slika 3.** FT-IR spektar celuloze proizvedene bakterijom *G. hansenii* u mediju od mrkvina soka (A, B, C i D) i u kontrolnom HS mediju (KA, KB, KC i KD)

**Table 5** FT-IR Spectra of Carrot-BC ( $cm^{-1}$ )

**Tablica 5.** FT-IR spektar bakterijske celuloze proizvedene u mediju od mrkvina soka

Analysis / <i>Analiza</i>	Wave Number, $cm^{-1}$ / <i>Broj valova, <math>cm^{-1}</math></i>				
A (210 ml)	1031, 1056	1107, 1163	1315, 1336, 1369, 1425	2919	3342
B (310 ml)	1056, 1033	1107, 1163	1427, 1336, 1315, 1371	2916	3346
C (360 ml)	1053, 1029	1109, 1161	1315, 1342, 1375, 1421	2914	3346
D (410 ml)	1056, 1031	1109, 1161	1315, 1338, 1369, 1427	2918	3340

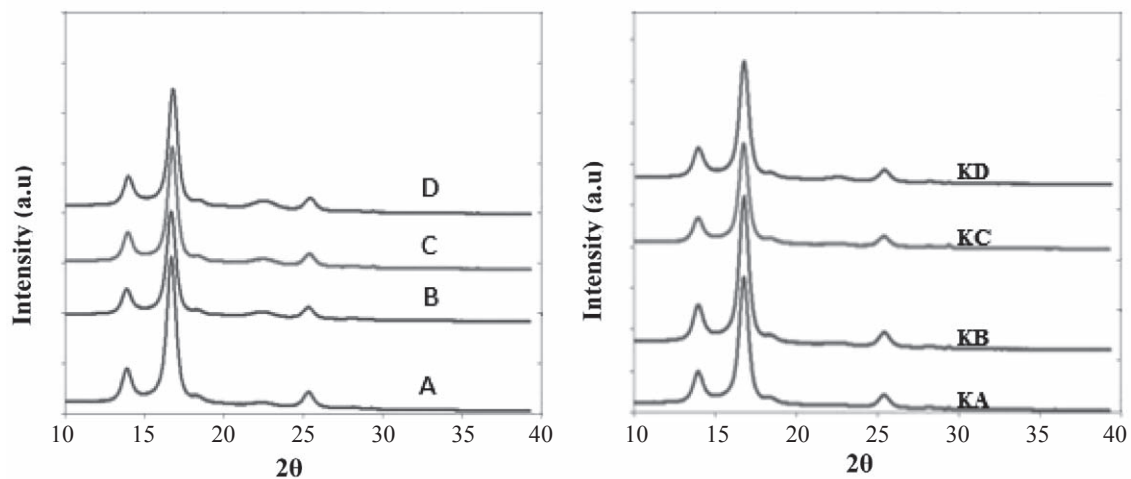
**Table 6** FT-IR Spectra of HS-BC ( $cm^{-1}$ )

**Tablica 6.** FT-IR spektar bakterijske celuloze proizvedene u mediju HS

Analysis / <i>Analiza</i>	Wave Number, $cm^{-1}$ / <i>Broj valova, <math>cm^{-1}</math></i>				
KA (210 ml)	1028, 1055	1107, 1159	1315, 1336, 1371, 1421	2916	3336
KB (310 ml)	1056, 1033	1110, 1161	1425, 1336, 1317, 1361	2918	3350
KC (360 ml)	1056, 1033	1109, 1163	1317, 1340, 1369, 1423	2920	3336
KD (410 ml)	1055, 1031	1109, 1161	1315, 1336, 1369, 1425	2895	3334

**Table 7** Crystalline indices values (%) of different cellulose sources**Tablica 7.** Vrijednosti indeksa kristaliničnosti (%) BC-a iz različitih izvora

Sample / Uzorak	Crystalline indices, % Indeks kristaliničnosti, %	Reference Izvor literature
Bacterial Cellulose (Carrot_BC)	83	Study Results
Bacterial Cellulose (HS-BC)	84	Study Results
Bacterial Cellulose	82	(Keshk, 2014)
Bacterial Cellulose	75	(Grande <i>et al.</i> 2009)
Bacterial Cellulose	79	(Carreira <i>et al.</i> 2011)
Bacterial Cellulose	78	(Shezad <i>et al.</i> 2010)
Bacterial Cellulose	85-93	(Cheng <i>et al.</i> 2009)
Bacterial Cellulose	84-89	(Czaja <i>et al.</i> 2004)
Bacterial Cellulose	63-81	(Sheykhnazari <i>et al.</i> 2011)
Bacterial Cellulose	74-85	(Amin <i>et al.</i> 2014)
Bacterial Cellulose	80	(Gomes <i>et al.</i> 2013)
Microcrystalline Cellulose	77	(Keshk, 2014)
Microcrystalline Cellulose	79	(Amin <i>et al.</i> 2014)
Cotton	78	(Terinte <i>et al.</i> 2011)

**Figure 4** X-ray pattern of cellulose produced by *G. hansenii* from Carrot-BC (A, B, C and D) and HS-BC (KA, KB, KC and KD).

**Slika 4.** Rezultati rendgenske difrakcije celuloze proizvedene bakterijom *G. hansenii* u mediju od mrkvina soka (A, B, C i D) i u kontrolnom HS mediju (KA, KB, KC i KD)

slightly lower than those of HS-BC (84 %) (Figure 4). Similar results were also found for the utilization of dry olive mill residues for the production of BC (Gomes *et al.*, 2013). Comparison of XRD results of different cellulose structures are given in Table 7.

## 4 CONCLUSIONS

### 4. ZAKLJUČAK

It is generally accepted that fruits containing sufficient glucose can be used as a nutrient source for BC production. The results of this research demonstrated the possibility to produce BC in carrot juice instead from Hestrin-Schramm as a nutrient source. The fracture surface morphology of the carrot-BC medium pellicles provided a smaller cellulose fibril diameter and a better network in comparison with the BC pellicle from the Hestrin-Schramm medium (HS-BC). The HS-BC absorbed 68 times its dry weight of water. The WHC decreased to 37 in Carrot-BC medium compared to HS-

BC. The average yield of Carrot-BC was found to be 112 % higher than that of control samples (HS-BC). The yield also increased in both media with volume ratio. The crystalline indices (CI) of Carrot-BC (83 %) were similar to those of HS-BC (84 %). The FT-IR spectra showed no significant difference between Carrot-BC and HS-BC. The results confirmed that BC samples exhibited similar chemical binding. Carrot-BC showed lower thermal stability compared to HS-BC.

## 5 REFERENCES

### 5. LITERATURA

- Amin, M. C. I. M.; Abadi, A. G.; Katas, H., 2014: Purification, characterization and comparative studies of spray-dried bacterial cellulose microparticles. *Carbohydrate Polymers*, 99: 180-189. <https://doi.org/10.1016/j.carbpol.2013.08.041>.
- Carreira, P.; Mendes, J. A. S.; Trovatti, E.; Serafim, L. S.; Freire, C. R. S.; Silvestre, A. J. D.; Neto, C. P., 2008: Utilization of residues from agro-forest industries in the

- production of high value bacterial cellulose. *Bioresource Technology*, 102: 7354-7360.  
<https://doi.org/10.1016/j.biortech.2011.04.081>.
3. Castro, C.; Zuluaga, R.; Putaux, J. L.; Caroa, G.; Mondragon, I.; Ganán, P., 2011: Structural characterization of bacterial cellulose produced by *Gluconacetobacter swingsii* sp. from Colombian agro industrial wastes. *Carbohydrate Polymers*, 84 (1): 96-102.  
<https://doi.org/10.1016/j.carbpol.2010.10.072>.
  4. Cheng, K. C.; Catchmark, J. M.; Demirci, A., 2009: Enhanced production of bacterial cellulose by using a bio-film reactor and its material property analysis. *Journal of Biological Engineering*, 3 (1): 12.  
<https://doi.org/10.1186/1754-1611-3-12>.
  5. Czaja, W. K.; Young, D. J.; Kawecki, M.; Brown, R. M., 2007: The future prospects of microbial cellulose in biomedical applications. *Biomacromolecules*, 8 (1): 1-12.  
<https://doi.org/10.1021/bm060620d>.
  6. El-Saied, H.; El-Diwany, A.; Basta, A. H.; Atwa, N. A.; El-Ghwas, D. N., 2008: Production and characterization of economical bacterial cellulose. *BioResources*, 3 (4): 1196-1217.
  7. Fu, L.; Zhang, J.; Yang, G., 2013: Present status and applications of bacterial cellulose-based materials for skin tissue repair. *Carbohydrate Polymers*, 92: 1432-1442.  
<https://doi.org/10.1016/j.carbpol.2012.10.071>.
  8. Gama, M.; Gatenholm, P.; Klemm, D., 2013: *Bacterial NanoCellulose A Sophisticated Multifunctional Material*. CRC Press Taylor & Francis Group, 304 pp.
  9. 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., 2013: Production of bacterial cellulose by *Gluconacetobacter sacchari* using dry olive mill residue. *Biomass and Bioenergy*, 55: 205-211.  
<https://doi.org/10.1016/j.biombioe.2013.02.004>.
  10. Grande, C. J.; Torres, F. G.; Gomez, C. M.; Troncoso, O. P.; Ferrer, J. C.; Pastor, J. M., 2009: Development of self-assembled bacterial cellulose-starch nanocomposites. *Materials Science and Engineering C*, 29: 1098-1104.  
<https://doi.org/10.1016/j.msec.2008.09.024>.
  11. Halib, N.; Amin, M. C. I. M.; Ahmad, I., 2012: Physico-chemical Properties and Characterization of Nata de Coco from Local Food Industries as a Source of Cellulose. *Sains Malaysiana*, 41 (2): 205-211.
  12. Hestrin, S.; Schramm, M., 1954: Synthesis of cellulose by *Acetobacter xylinum*. 2. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. *Biochemical Journal*, 58 (2): 345.  
<https://doi.org/10.1042/bj0580345>
  13. Keshk, S., 2014: Vitamin C enhances bacterial cellulose production in *Gluconacetobacter xylinus*. *Carbohydrate Polymers*, 99: 98-100.  
<https://doi.org/10.1016/j.carbpol.2013.08.060>.
  14. Klemm, D.; Schumann, D.; Udhardt, U.; Marsch, S., 2001: Bacterial synthesized cellulose e artificial blood vessels for microsurgery. *Prog Polym Sci.*, 26 (9): 1561-1603. [https://doi.org/10.1016/S0079-6700\(01\)00021-1](https://doi.org/10.1016/S0079-6700(01)00021-1).
  15. Krystynowicz, A.; Czaja, W.; Wiktorowska-Jezierska, A.; Gonçalves-Miśkiewicz, M.; Turkiewicz, M., 2002: Factors affecting the yield and properties of bacterial cellulose. *Journal of Industrial Microbiology and Biotechnology*, 29 (4): 189-195.  
<https://doi.org/10.1038/sj.jim.7000303>.
  16. Moosavi-Nasab, M.; Yousefi, A. R., 2010: Investigation of physicochemical properties of the bacterial cellulose produced by *Gluconacetobacter xylinus* from date syrup. *World Academy of Science, Engineering & Technology*, 44:1258-1263.
  17. Olsson, R. T.; Samir, A.; Salazar-Alvarez, G.; Belova, L.; Ström, V.; Berglund, L. A.; Ikkala, O.; Nogués, J.; Gedde, U. W., 2010: Making Flexible Magnetic Aerogels and Stiff Magnetic Nanopaper Using Cellulose Nanofibrils as Templates. *Nature Nanotechnology*, 5 (8): 584-588. <https://doi.org/10.1038/nnano.2010.155>.
  18. Poyrazoglu Coban, E.; Biyik, H., 2011: Effect of various carbon and nitrogen sources on cellulose synthesis by *Acetobacter lovaniensis* HBB5. *African Journal of Biotechnology*, 10 (27): 5346-5354.
  19. Ruka, D. R.; Simon, G. P.; Dean, K. M., 2012: Altering the growth conditions of *Gluconacetobacter xylinus* to maximize the yield of bacterial cellulose. *Carbohydrate Polymers*, 89: 613-622.  
<https://doi.org/10.1016/j.carbpol.2012.03.059>.
  20. Sheykhnazari, S.; Tabarsa, T.; Ashori, A.; Shakeri, A.; Gholipour, M., 2011: Bacterial synthesized cellulose nanofibers; Effects of growth times and culture mediums on the structural characteristics. *Carbohydrate Polymers*, 86: 1187-1191.  
<https://doi.org/10.1016/j.carbpol.2011.06.011>.
  21. Shezad, O.; Khan, S.; Khan, T.; Park, J. K., 2010: Physicochemical and mechanical characterization of bacterial cellulose produced with an excellent productivity in static conditions using a simple fed-batch cultivation strategy. *Carbohydrate Polymers*, 82: 173-180.  
<https://doi.org/10.1016/j.carbpol.2010.04.052>.
  22. Sturcova, A.; His, I.; Apperley, D. C.; Sugiyama, J.; Jarvis, M. C., 2004: Structural details of crystalline cellulose from higher plants. *Biomacromolecules*, 5: 1333-1339. <https://doi.org/10.1021/bm034517p>.
  23. Terinte, N.; Ibbett, R.; Schuster, K. C., 2011: Overview on native cellulose and microcrystalline cellulose I structure studied by x-ray diffraction (WAXD): comparison between measurement techniques. *Lenzinger Berichte*, 89: 118-131.
  24. Tsouko, E.; Kourmentza, C.; Ladakis, D.; Kopsahelis, N.; Mandala, I.; Papanikolaou, S.; Paloukis, F.; Alves, V.; Koutinas, A., 2015: Bacterial cellulose production from industrial waste and by-product streams. *International Journal of Molecular Sciences*, 16 (7): 14832-14849. <https://doi.org/10.3390/ijms160714832>
  25. Ul-Islam, M.; Khan, T.; Park, J. K., 2012: Water holding and release properties of bacterial cellulose obtained by in situ and ex situ modification. *Carbohydrate Polymers*, 88: 596-603.  
<https://doi.org/10.1016/j.carbpol.2012.01.006>.
  26. Wan, Y. Z.; Huang, Y.; Yuan, C. D.; Raman, S.; Zhu, Y.; Jiang, H. J.; He, F.; Gao, C., 2007: Biomimetic synthesis of hydroxyapatite/bacterial cellulose nanocomposites for biomedical applications. *Materials Science and Engineering C*, 27: 855-864.  
<https://doi.org/10.1016/j.msec.2006.10.002>.

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