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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Optimization of Cellulose Extraction Parameters and Production of Nanocellulose from Black Carrot Juice Wastes*

Siyah Havuç Suyu Atıklarından Selüloz Ekstraksiyon Parametrelerinin Optimizasyonu ve Nanoselüloz Üretimi

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Abstract

Agricultural wastes are abundant worldwide with increased production and consumption activities as a result of human population growth. Waste recycling processes, which are important to support sustainable production, remain popular due to the increasing amount of agricultural waste. In particular, there are various studies on the recovery of valuable components from waste. In this context, the recovery of cellulose and nanocellulose from waste, which has the potential to be used and applied in many sectors, especially in food, draws attention. Although black carrot juice waste, which is one of the important agricultural wastes, is frequently used in the production of natural colorants, it was used for the production of cellulose and nanocellulose in this study due to its high cellulose content. Response Surface Method-Central Composite Design was used to improve the alkaline extraction conditions of cellulose for the optimum yield and whiteness index by using process parameters of NaOH concentration (2–12%), process temperature (25–110 °C), and time (60–240 min). The optimum process parameters were determined as the NaOH concentration (7.06%), process temperature (44.83°C), and time (114.21 min) for alkaline extraction of cellulose from black carrot juice waste where the yield of cellulose was 22.90±2.48%, and whiteness index was 60.32±0.07%. Nanocellulose was produced from cellulose obtained from black carrot juice waste by acidic hydrolysis using 25% H_2SO_4. Nanocellulose yield and whiteness index were found as 15.76±0.16% and 58.77±0.26% respectively. The average diameter (61±2.89 nm) and length (281±18.50 nm) of the nanocellulose were determined by Atomic Force Microscopy (AFM). As a result of the Fourier Transform Infrared (FTIR) spectroscopy, it was determined that non-cellulosic components were removed.

Keywords: Black carrot juice waste, Cellulose, Optimization, Acid hydrolysis, Nanocellulose

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Öz

Dünya nüfusunun sürekli artış göstermesiyle birlikte, üretim ve tüketim faaliyetleri de hızla artmakta ve sonucunda tarımsal atıklar dünya genelinde büyük miktarlarda birikmektedir. Bu durum, sürdürülebilir üretimi desteklemek amacıyla atık geri dönüşüm süreçlerine olan ihtiyacı da artırmaktadır. Özellikle tarımsal atık miktarının artmasıyla birlikte, atıklardan değerli bileşenlerin geri kazanımı konusunda çeşitli çalışmalar yoğunlaşmıştır. Bu bağlamda, selüloz ve nanoselüloz gibi maddelerin atıklardan geri kazanımı, özellikle gıda sektörü başta olmak üzere birçok sektörde kullanım ve uygulama potansiyeline sahip olduğu için önem taşımaktadır. Siyah havuç suyu atığı da önemli bir tarımsal atık olarak kabul edilmekte ve genellikle doğal renklendirici üretiminde sıklıkla kullanılmaktadır. Renk maddesi üretiminin yanı sıra yüksek selüloz içeriği sebebiyle bu çalışmada siyah havuç suyu atığı, selüloz ve nanoselüloz üretiminde kullanılmıştır. Yanıt Yüzey Yöntemi (Response Surface Method)-Merkezi Tümleşik Tasarım (CCD) yöntemi kullanılarak, selülozun alkali ekstraksiyon koşulları optimize edilmiştir. Bu optimizasyon sürecinde, NaOH konsantrasyonu (%2–12), proses sıcaklığı (25–110 $^{\circ}$C) ve proses süresi (60–240 dakika) gibi parametreler dikkate alınarak yanıt olarak selüloz verimi ve beyazlık indeksi seçilmiştir. Bu sayede, farklı parametre kombinasyonlarına karşılık gelen selüloz verimi ve beyazlık indeksi değerleri ile birlikte optimum ekstraksiyon koşulları belirlenmiştir. Selüloz veriminin %22,90±2,48 ve beyazlık indeksinin %60,32±0,07 olduğu siyah havuç suyu atıklarından selülozun alkali ekstraksiyonu için optimum proses parametreleri NaOH konsantrasyonu %7,06; proses sıcaklığı 44,83 $^{\circ}$C ve proses süresi 114,21 dk olarak belirlenmiştir. Siyah havuç suyu atıklarından elde edilen selülozdan %25 konsantrasyonda H_2SO_4 kullanılarak asidik hidroliz ile nanoselüloz üretilmiştir. Nanoselüloz verimi ve beyazlık indeksi sırasıyla %15,76±0,16 ve %58,77±0,26 olarak bulunmuştur. Nanoselülozun ortalama çapı (61±2,89 nm) ve uzunluğu (281±18,50 nm) Atomik Kuvvet Mikroskobu (AFM) ile belirlenmiştir. Fourier Transform Infrared (FTIR) spektroskopisi sonucunda ise selülozik olmayan bileşenlerin uzaklaştırıldığı belirlenmiştir.

Anahtar Kelimeler: Siyah havuç suyu atığı, Selüloz, Optimizasyon, Asit hidrolizi, Nanoselüloz

1. Introduction

The black carrot (*Daucus carota* L. *ssp. sativus* var. *atrorubens* Alef.) is a type of carrot originated from Middle Asia and has been cultivated for thousands of years (Sucheta et al., 2020; Ağçam and Akyıldız, 2015). Nowadays, mostly in eastern countries such as Turkey, India, and the Far East black carrots are grown and consumed widely. Particularly in Turkey, they are raw materials of a fermented beverage called *şalgam* (Ünal and Bellur, 2009). The consumption of black carrots has been increasing in recent years due to their rich content of phytochemicals like flavonoids that play a role in preventing diseases (Akhtar et al., 2017; Sucheta et al., 2020). Furthermore, previous studies have reported that they are rich sources of many bioactive components such as carotenoids, and anthocyanins (Arscott and Tanumihardjo, 2010; Akhtar et al., 2017) which can be used as natural colorants in processed food products such as fruit juices, jam, and marmalade. In addition, it is known that the pulp that emerged during the processing of black carrot is an important waste in terms of vegetable fiber content (Ünal and Bellur, 2009; Singh et al., 2016).

Cellulose, a vegetable fiber, is the most abundant raw material in nature (Perez and Mazeau, 2005). Cellulose forms the structure called lignocellulose together with hemicellulose, lignin, ash, and extractives in the plant cell wall (Aksoy et al., 2023; Ghaemi et al., 2019; Smyth et al., 2017). The composition of lignocellulose found in plants and their wastes varies according to its source (Ghaemi et al., 2019). Previous studies showed that the plants and their wastes such as cotton, flax, hemp, sugar cane (Dorez et al., 2014), corn stover (Wartelle and Marshall, 2006; Liu et al., 2016), corn husk (Smyth et al., 2017) and rice straw (Wartelle and Marshall, 2006; Krishania et al., 2018) has high cellulose content in their structure. Cellulose is used in the production of many materials such as paper, coatings, and films, as well as cosmetics and pharmaceutical products (Perez and Mazeau, 2005).

Cellulosic materials with particle sizes generally below 100 nm are called nanocellulose. Nanocelluloses, each of which has different sizes and functions, are divided into three groups according to the cellulose source and process conditions. With the help of mechanical methods, acid hydrolysis or bacterial synthesis, microfibrillated cellulose (MFC), nanocrystalline cellulose (NCC), and bacterial nanocellulose (BC) can be obtained (Klemm et al., 2011). In previous studies, MFC was obtained from hemp (Pacaphol and Aht-Ong, 2017), kenaf (Davoudpour et al., 2015), bamboo (Wang et al., 2015), and lemon (Impoolsup et al., 2020) where the NCC was produced from apple pomace (Melikoğlu et al., 2019), rice peel (Johar et al., 2012), coffee peel (Collazo-Bigliardi et al., 2018) and mango seed (Henrique et al., 2013).

In this study, the lignocellulosic composition and physicochemical properties of black carrot juice waste were determined, and it was used as a source for the production of cellulose and nanocellulose. Extraction conditions were optimized to obtain a high-yield cellulose and whiteness index according to the properties of the black carrot juice waste.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Materials

Black carrot (Daucus carota L. ssp. sativus var. atrorubens Alef.) juice waste (BCW) was obtained from Döhler Gıda San. A.Ş. BCW was dried in a vacuum oven (Nüve EV 018, Turkey) at 65 ˚C until reaching to 10-11% moisture content and grounded to ≈500 microns in a laboratory-type grinder (Brook Crompton Series 2000, England). The dry powder product was stored at 4 ˚C.

2.1.2. Chemicals

Merck (Darmstadt, Germany) branded benzene (C_6H_6), ethanol (C_2H_5OH), hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium hypochlorite (NaClO), and sulfuric acid (H_2SO_4) were used for extractions. All chemicals were used in analytical purity.

2.2. Methods

2.2.1. Raw Material Analysis

The total dry matter content (TDM) of BCW was determined in triplicates (AOAC, 1990). Known amount weighed samples were placed in aluminum weighing dishes and then dried in a vacuum oven (Nüve EV 018, Turkey) (65 °C, 515-775 mmHg) until reaching a constant weight. The TDM content was calculated according to Eq. 1.

$$\boldsymbol{TDM}\ (\%) = \frac{\boldsymbol{Weight_{dish+sample} - Weight_{dish}}}{\boldsymbol{Weight_{initial\ sample}}} \times \mathbf{100} \quad \text{(Eq. 1)}$$

The water activity of BCW was measured by a Testo 645 water activity analyzer (Cemeroğlu, 2013). The pH value was measured potentiometrically by a pH meter (Inolab, WTW pH 720) (Albaş et al., 2022; Cemeroğlu, 2013). L^*, a^*, and b^* values (CIELAB color values) of BCW were measured by Hunter-Lab colorimeter Colorflex model color measurement device (Management Company, USA) (Toews and Wang, 2013; Tekin et al., 2023).

2.2.2. Compositional Analysis

For ash content, the BCW samples were weighed into the tared crucible and then burned at 550 °C for 5 hours (Rannou et al., 2015) and calculated according to (Eq. 2).

$$\boldsymbol{Ash}\ (\%) = \frac{\boldsymbol{Weight_{crucible+sample} - Weight_{pot}}}{\boldsymbol{Weight_{initial\ sample}}} \times \mathbf{100} \quad \text{(Eq. 2)}$$

The extractive content of 10 g of BCW (W_0) was measured by treated with a solution of 2:1 benzene:ethanol (1:10 v/w) for 2 hours at 20 °C. After that sample was filtered and washed with distilled water and dried in a vacuum oven at 105 °C until reaching a constant weight (W_1) (Eq. 3) (Değermenci et al., 2019).

$$\boldsymbol{Extractives}\ (\%) = \left(\frac{\boldsymbol{W_0 - W_1}}{\boldsymbol{W_0}}\right) \times \mathbf{100} \quad \text{(Eq. 3)}$$

Holocellulose content was determined as the remaining part of the sample was treated with 100 mL sodium hypochlorite (NaClO - 5%) solution containing 2 mL acetic acid (CH_3COOH). The sample was kept in a water bath at 70 °C for 4 hours, then filtered and washed with distilled water. The sample was dried at 105 °C and then weighed (W_2) (Eq. 4) (Değermenci et al., 2019).

$$\boldsymbol{Holocellulose}\ (\%) = \frac{\boldsymbol{W_2}}{\boldsymbol{W_0}} \times \mathbf{100} \quad \text{(Eq. 4)}$$

For cellulose content of BCW determination, the remaining sample was treated with 50 mL of 17.5% sodium hydroxide (NaOH) at 20 °C for 2 hours. The sample was filtered and washed with distilled water and weighed after drying at 105 °C (W_3) (Eq. 5) (Değermenci et al., 2019).

$$\boldsymbol{Cellulose}\ (\%) = \frac{\boldsymbol{W_3}}{\boldsymbol{W_0}} \times \mathbf{100} \quad \text{(Eq. 5)}$$

The amount of hemicellulose was calculated by subtracting the amount of cellulose from the amount of holocellulose determined experimentally.

To determine the lignin content, the extractive-free sample was treated with 30 mL of 72% sulfuric acid (H_2SO_4) solution at 20 °C for 2 hours. Next, 300 mL of distilled water was added to the sample and boiled for 4 hours. The sample filtered and washed with distilled water was weighed after drying at 105 °C (W_4) (Eq. 6) (Değermenci et al., 2019).

$$\boldsymbol{Lignin}\ (\%) = \frac{\boldsymbol{W_4}}{\boldsymbol{W_0}} \times \mathbf{100} \quad \text{(Eq. 6)}$$

2.2.3. Preparation of Cellulose from Black Carrot Juice Waste

The sequential extraction method was used to obtain cellulose from BCW (Szymanka-Chargot et al., 2017; Melikoğlu et al., 2023). The sample was treated with a 2:1 benzene:ethanol (1:10 w/v) solution at 10 °C for 16 h and then boiled with 1 L distilled water at 80±5 °C for 30 min to remove extractives and water-soluble compounds. After these pre-treatments, the sample was boiled with 1 L of 1 M HCl solution at 80±5 °C for 30 min for acid treatment. In order to remove hemicellulose from BCW, the alkaline process parameters NaOH concentration (2-12%), temperature (25-110 °C), and time (60-240 min) were optimized (Chirayil et al., 2014; Collazo-Bigliardi et al., 2018; Dinand et al., 1999). Then, bleaching treatment was carried out using 5% NaClO solution at 90 °C for 30 min. The sample was filtered, washed with distilled water, and dried in a vacuum oven at 65 °C for 24 h.

2.2.4. Optimization of Alkaline Treatment Conditions

For the optimization of alkaline treatment parameters based on a previous study by Melikoğlu et al. (2023), Central Composite Design (CCD) of the response surface method (RSM) in the Design Expert program version 7.0.0 was used (State-Ease, Inc., Minneapolis, MN, USA). In this context, NaOH concentration (2-12%) (A), process temperature (25-110 °C) (B), and time (60-240 min) (C) were chosen as independent variables. Cellulose yield (%) and whiteness index (%) were chosen as responses. A total of 20 experiments were carried out under different conditions, with 6 repetitions at the central point. According to the experiment results, a mathematical model was created for dependent variables using the multiple regression analysis method. The significant terms in the model were determined with the one-way analysis of variance (ANOVA), and the accuracy of the model was evaluated with the lack of fit and Fischer's test (F-value).

2.2.5. Nanocellulose Preparation from Black Carrot Juice Waste Cellulose

The acid hydrolysis process was applied to obtain nanocellulose from black carrot juice waste cellulose (BCW-C). Firstly, 10 mL of distilled water was added to the 5 g sample. In order to prevent overheating of the solution, 25 mL of H_2SO_4 in the burette was slowly dropped on the sample, which was also kept in an ice bath, and the sample's volume was made up to 100 mL by adding distilled water. Then, the sample was treated with 25% H_2SO_4 solution and mixed in a magnetic stirrer (MTOPS MS300, South Korea) at 40 °C for 40 min. After that, centrifugation and dialysis processes were carried out to remove the acid residue in the samples. Centrifugation was performed at 4000 rpm for 15 min (Nüve NF 800, Turkey) and the supernatant was removed. Centrifugation was repeated 3 times by adding distilled water, the collected suspension was diluted with distilled water and kept in a dialysis bag (width of 33 mm, cut-off value: 14000). The dialysis process was continued for 4-7 days at 4 °C until the sample reached to neutral pH level. Then, the sample was homogenized with Ultra-Turax T25 (France) at 9000 rpm for 2 min. The next stage involved redistributing the nanocellulose by homogenizing the sample that had been held in an ice bath for 15 minutes (probe: 3 mm, power: 70 W) using an ultrasonic homogenizer (Bandelin Sonopuls 2450, Germany). The nanocellulose sample was dried in a freeze dryer at -48 °C for 24 h (Kumar et al., 2014; Melikoğlu et al., 2023).

2.2.6. Yield (%) Determination

The yield of cellulose and nanocellulose obtained from BCW was calculated with Eq. 7 (Melikoğlu et al., 2019).

$$\boldsymbol{Yield}\ (\%) = \frac{\boldsymbol{Obtained\ cellulose\ or\ nanocellulose\ (g)}}{\boldsymbol{BCW\ sample\ (g)}} \times \mathbf{100} \qquad \text{(Eq. 7)}$$

2.2.7. Whiteness Index (%) Determination

The whiteness index of the cellulose and nanocellulose was calculated using Eq. 8 (Rodsamran and Sothornvit, 2015).

$$\boldsymbol{Whiteness\ index}\ (\%) = \sqrt{(\mathbf{100} - \boldsymbol{L^*})^{\mathbf{2}} + \boldsymbol{a^{*2}} + \boldsymbol{b^{*2}}} \qquad \text{(Eq. 8)}$$

2.2.8. Fourier Transform-Infrared (FTIR) Analysis

Changes in the chemical composition of cellulose and nanocellulose obtained from BCW were determined with a Perkin Elmer FTIR spectrophotometer (Model System 2000, Perkin Elmer, USA). The spectra were recorded at the wavenumber range of 4000-500 cm^{-1} (Kumar et al., 2014; Melikoğlu et al., 2023).

2.2.9. Atomic Force Microscopy (AFM) Analysis

The AFM analysis was performed using a Dimension Edge with ScanAsyst-SPM (Bruker, US) microscopy in a non-contact mode. One drop of a diluted suspension (1:100, v/v) of the dried BCW-C was placed on a mica substrate and allowed to air dry. The morphology and rough estimation of the size of the BCW-C were examined by the DME SPM software version 14.06 (Melikoğlu et al., 2019).

2.2.10. Statistical Analysis

The evaluation of the results of the analysis was made with the IBM SPSS 22 package program (SPSS Inc., Chicago, IL, USA). The effect of the parameters on the results was determined by performing variance analysis and the differences between the means ($P < 0.05$) were determined by Duncan multiple comparison test.

3. Results and Discussion

3.1. Raw Material Analysis

Total dry matter, water activity, pH value, and color value analysis results of BCW are given in *Table 1*. The total dry matter content of BCW was 10.90%. The total dry matter content for black carrots was found as 13.15% (Ersus and Yurdagel, 2007), 11.62% (Demir, 2010), and 11.54% (Uçan Türkmen et al., 2018) in previous research. The samples were found very acidic (pH 2.96) and the water activity was 0.98.

Table 1. Total dry matter, water activity, pH value, and color value analysis results of BCW

Sample	Total dry matter (%)	Water activity	pH Value	Color Values		
				L^*	a^*	b^*
BCW	10.90±0.46	0.98±0.01	2.96±0.01	45.09±0.05	13.95±0.02	9.50±0.02

3.2. Compositional Analysis

Compositional analysis results of BCW are given in *Table 2*. It has been determined that the most abundant lignocellulosic compound in BCW is extractives. Extractives in the plant cell wall, consist of low molecular weight carbohydrates, salts, waxes, oils, resins, phenolic compounds, and flavonoids (Ghaemi et al., 2019). In previous studies, BCW has been reported to be a good source of carbohydrates, terpenes (terpinolene, terpinene, caryophyllene), and phenolic compounds (Smeriglio et al., 2018; Sharma et al., 2012). Also, in previous studies, the hemicellulose, lignin, and cellulose contents of carrot, which is a different species from black carrot, were reported to vary between 1.80-17.50%, 0.49-6.70% and 10.01-28.00%, respectively (Rani and Kawatra, 1994; Nawirska and Kwasniewska, 2005; Szymanska-Chargot et al., 2017). It was found that the lignin and cellulose concentration of BCW was comparable to that of previous research on carrots. However, it was determined that the hemicellulose content of BCW was higher than the literature where the type of raw material, the time of planting and harvesting, the cultivation method and extraction conditions could be the reasons of these differences (Ghaemi et al., 2019).

Table 2. Compositional analysis results of BCW

Analysis	Result
Ash (%)	3.10±0.30
Extractives (%)	44.27±2.06
Holocellulose (%)	38.58±5.10
Hemicellulose (%)	17.92±5.24
Lignin (%)	2.03±0.32
Cellulose (%)	20.67±1.35

3.3. Preparation of Cellulose from Black Carrot Juices Waste

3.3.1. Optimization of the Alkaline Treatment

The effects of alkaline treatment parameters on the yield (%) and whiteness index (%) of BCW-C are given in *Table 3*. In the RSM-CCD experimental design of BCW-C, it was found that the cellulose yield varied between 17.45 and 25.63% and the whiteness index value ranged from 60.91 to 67.66% (*Table 3*).

According to ANOVA results, with a 95% confidence level ($P < 0.05$), it was determined that the model is significant for cellulose yield ($P < 0.0001$) and whiteness index values ($P < 0.0001$). Also, the lack of fit values for both responses ($P = 0.2865$ and $P = 0.1187$, respectively) was found to be insignificant. The significance of the model and the insignificance of the lack of fit shows the suitability of the mathematical model in terms of cellulose yield and whiteness index of BCW (Behera et al., 2018). Also, the R^2 value, which is a value that shows how much of the experimental data change is met in the model, is above 0.75 proves that the model is successful (Le Man et al., 2010; Owolabi et al., 2018). However, for a good model and a correct optimization, the adj-R^2 value, which is one of the other criteria, should also be higher than 0.80 (Myers et al., 2009; Rheem et al., 2017). The R^2 value was 0.95 for the cellulose yield and 0.94 for the whiteness index, and the adj-R^2 values were 0.91 for both responses. Therefore, considering these results, it has been seen that the model is suitable for optimization. ANOVA results also stated that all three alkali process parameters alone did not have significant effects on the cellulose yield ($P > 0.05$) and have significant effects on the whiteness. index ($P < 0.05$). For cellulose yield, the effects of temperature x temperature, time x time, NaOH concentration x NaOH concentration x temperature, and NaOH concentration x temperature x temperature were found to be statistically significant (P <0.05). The effects of NaOH concentration x NaOH concentration, and NaOH concentration x NaOH concentration x time were found to have significant effects on the whiteness index (P <0.05).

Table 3. RSM-CCD experimental design prepared for BCW-C

Run	NaOH concentration (%) (A)	Temperature (°C) (B)	Time (min) (C)	Yield (%)	Whiteness index (%)
1	7.00	25.00	150.00	21.67	63.79
2	4.03	42.23	96.49	25.17	62.05
3	4.03	42.23	203.51	25.63	62.46
4	9.97	42.23	96.49	23.18	63.92
5	9.97	42.23	203.51	23.35	64.25
6	2.00	67.50	150.00	22.17	61.53
7	7.00	67.50	60.00	21.69	60.91
8	7.00	67.50	150.00	22.36	65.30
9	7.00	67.50	150.00	23.13	64.82
10	7.00	67.50	150.00	23.64	65.87
11	7.00	67.50	150.00	23.67	65.02
12	7.00	67.50	150.00	23.86	65.40
13	7.00	67.50	150.00	23.08	65.04
14	7.00	67.50	240.00	19.87	67.66
15	12.00	67.50	150.00	21.73	66.20
16	4.03	92.77	96.49	21.01	64.75
17	4.03	92.77	203.51	20.76	65.20
18	9.97	92.77	96.49	17.45	67.51
19	9.97	92.77	203.51	17.86	66.50
20	7.00	110.00	150.00	20.89	66.17

Model equations for cellulose yield and whiteness index of BCW are given in Eq. 9 and 10.

$$Yield\ (\%) = 23.27 - 0.13x(A) - 0.23x(B) - 0.17x(C) - 0.27x(A)x(B) - 0.35x(A^2) - 0.59x(B^2) - 0.76x(C^2) - 2.30x(A^2)x(B) - 1.21x(A)x(B^2) \quad \text{(Eq. 9)}$$

$$Whiteness\ Index\ (\%) = 65.20 + 1.14x(A) + 1.12x(B) + 2.01x(C) - 0.19x(A)x(C) - 0.43x(A^2) - 0.28x(C^2) - 1.98x(A^2)x(C) \quad \text{(Eq. 10)}$$

The effect of the interaction between NaOH concentration, temperature, and time on the cellulose yield and whiteness index was represented by the 3D response surface plots (*Figure 1-2*). It was observed that the cellulose yield increased at the experimental points where the NaOH concentration and temperature were low, while it decreased at the points where both parameters increased (*Figure 1a*). Also, in *Figure 1b, c*, where the effects of time x NaOH concentration and time x temperature interactions on cellulose yield are given, it is seen that cellulose with high yield is obtained regardless of time, where NaOH concentration and temperature were low. As can be seen from the results, it can be concluded that the cellulose was damaged at the points where the concentration and the temperature were high, thus decreasing the cellulose yield (Rodsamran and Sothornvit, 2015; Melikoğlu et al., 2019).

When the effects of alkali process parameters such as NaOH concentration, temperature, and time interactions on the whiteness index were examined, it was observed that the whiteness index value increased due to the increase of all three parameters (*Figure 2a, b, c*). In previous studies, it has been reported that the whiteness index value of cellulose increases with the removal of non-cellulosic components that form brown shades with high alkali treatment conditions applied before bleaching (Rodsamran and Sothornvit, 2015).

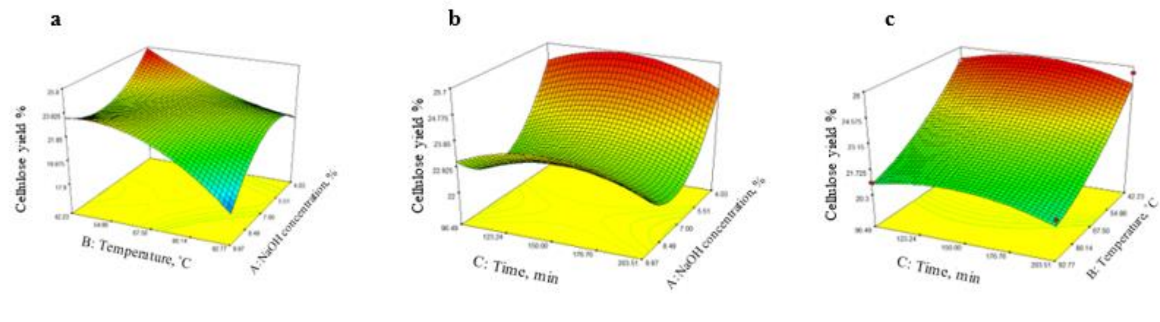

Figure 1. 3D response surface plots of optimization (a: Effect of NaOH concentration and temperature on cellulose yield, b: Effect of NaOH concentration and time on cellulose yield, c: Effect of time and temperature on cellulose yield.)

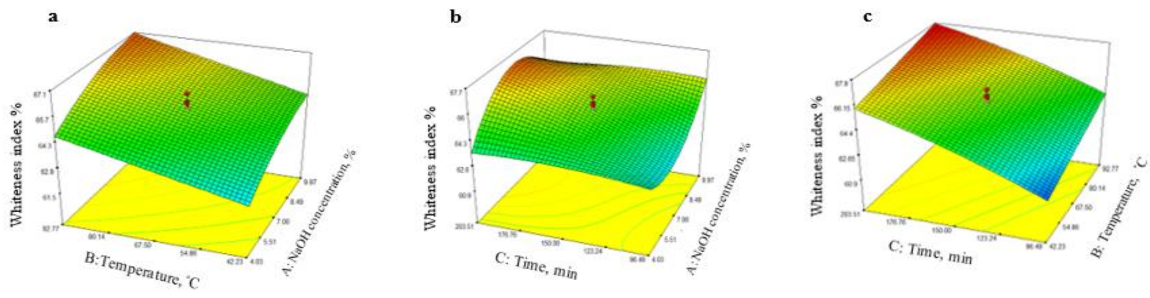

Figure 2. 3D response surface plots of optimization (a: Effect of NaOH concentration and temperature on whiteness index, b: Effect of NaOH concentration and time on whiteness index, c: Effect of time and temperature on whiteness index)

The effects of alkali process parameters on the cellulose yield and whiteness index of the wastes were determined by one-way analysis of variance (ANOVA). In the next step, the point where the alkali process parameters give the optimum cellulose yield and whiteness index values was found using the RSM-CCD in the Design Expert program. According to the RSM-CCD results, a NaOH concentration of 7.06%, a temperature of 44.83 ˚C, and a time of 114.21 min were found as optimum points. Cellulose yield and whiteness index values at the optimum points were predicted by the program as 22.76 and 62.76%, respectively. When the experiments were performed at the optimum points, cellulose with 22.90±2.48% yield and 60.32±0.07% whiteness index was handled from BCW which fits with the theoretical results properly.

3.4. Nanocellulose Preparation From Black Carrot Juice Waste Cellulose

Nanocellulose was obtained from BCW-C by acid hydrolysis (*Figure 3*). For this purpose, 25% H_2SO_4 solution was used, and the process was applied at 40 ˚C for 40 min. When the previous studies on the production of nanocellulose were examined, it was found that H_2SO_4 was generally used above 60% concentration for acid hydrolysis (Frone et al., 2017; Collazo-Bigliardi et al., 2018; Smyth et al., 2017). However, in the present study, it has been observed that the obtained nanocellulose burns, and its color turns black when the H_2SO_4 exceeds 25% due to the increase in acid concentration and temperature. Similar results were reported by Vanderfleet et al. (2019) and Yu et al. (2021). As a result of acid hydrolysis, nanocellulose with 15.76±0.13% yield and 58.77±0.26% whiteness index was found for BCW-C. It has been found that the whiteness index value of the nanocellulose obtained from BCW-C is lower than the cellulose. This is due to the conversion of some of the hydroxyl groups into sulfate groups in the crystalline region of cellulose depending on the acid hydrolysis conditions (Börjesson and Westman, 2015).

Figure 3. a: BCW, b: BCW-C, c: Nanocellulose from BCW-C

3.5. Fourier Transform-Infrared (FTIR) Analysis

The FTIR spectrum of the BCW, BCW-C, nanocellulose, and their typical functional groups are shown in *Table 4* and *Figure 4*. BCW, BCW-C, and nanocellulose peaks were observed in the O-H stretching band, which is an indicator of hydrophilic tendency (Chirayil et al., 2014). It has been reported that the band gap of 1720 to 1750 cm^{-1} in the FTIR spectrum corresponds to the C=O stretching vibrations of the xylene, carbonyl, and acetyl groups in hemicellulose and lignin, thus the peaks formed at this point prove the presence of hemicellulose and lignin (Qazanfarzadeh and Kadivar, 2016). The peak which is clearly observed in the spectrum of BCW at 1731 cm^{-1}, but disappears in the cellulose and nanocellulose spectrum, shows that the chemical processes applied to the BCW effectively remove the hemicellulose and lignin in its structure (Qazanfarzadeh and Kadivar, 2016). Furthermore, there is a prominent peak in the BCW spectrum at the 1237 cm^{-1} band, indicative of the presence of lignin (Smyth et al., 2017; Szymanska-Chargot et al., 2018; Borges de Oliveira et al., 2016). The disappearance of this peak in BCW-C and nanocellulose spectra indicates that lignin is removed from their structure. Effective processes for removing hemicellulose and lignin from black carrot waste include alkali treatment, acid hydrolysis, steam explosion, enzymatic hydrolysis, microbial pretreatment, and solvent pretreatment, often employed individually or in combination to optimize lignin and hemicellulose removal while preserving cellulose (Tian et al., 2017). When the FTIR results obtained in this study are evaluated, it can be concluded that the applied alkaline and bleaching process is effective in removing hemicellulose and lignin in the structure of BCW.

Table 4. Spesific band in FTIR spectrum

Absorption band	**Wave numbers (cm^{-1})**	**BCW**	**BCW-C**	**Nanocellulose**
O-H stretching intramolecular hydrogen bond	3250-3400	3360	3360	3360
C-H stretching	≈2900	2917	2917	2917
C=O stretching vibration of the carbonyl and acetyl groups	1720-1750	1731	-	-
O-H bending of water	≈1600	1624	-	-
C-O-C streching	≈1230	1237	-	-
C-O stretching vibration band in the structure of cellulose	≈1000	1031	1031	1031
Glycosidic C-H stretch vibration band of cellulose	850-900	897	897	897

3.6. Atomic Force Microscopy (AFM) Analysis

2D and 3D images of the nanocellulose obtained from BCW-C are given in *Figure 5*. It can be seen that the obtained nanocellulose is interlocked like a network structure. In addition, the average values of the nanocellulose were calculated as 281±18.50 nm in length with a diameter of 61±2.89 nm. It has been determined that the mean diameter of nanocellulose obtained from BCW is in the range of diameter given for the nanocellulose samples obtained by acid hydrolysis in the literature. In previous studies, it was determined that the diameter values of 30 to 70 nm for nanocellulose obtained from corn cob and 50 nm for nanocellulose obtained from rice husk are close to the diameter values of nanocelluloses obtained in the present study (Ditzel et al., 2017; Islam et al., 2018).

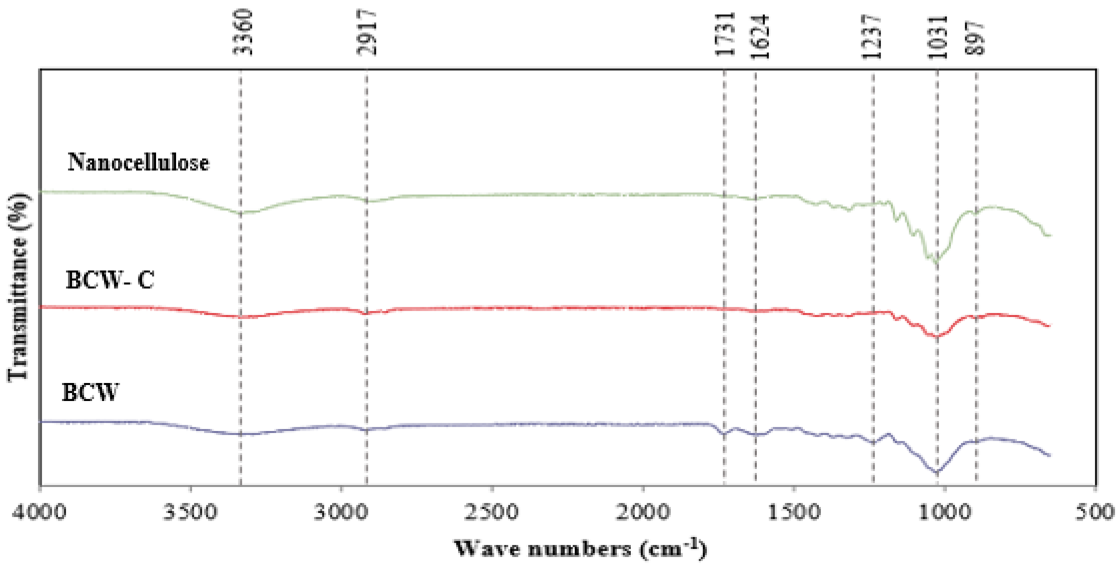

Figure 4. FTIR spectrum of BCW, BCW-C, and nanocellulose.

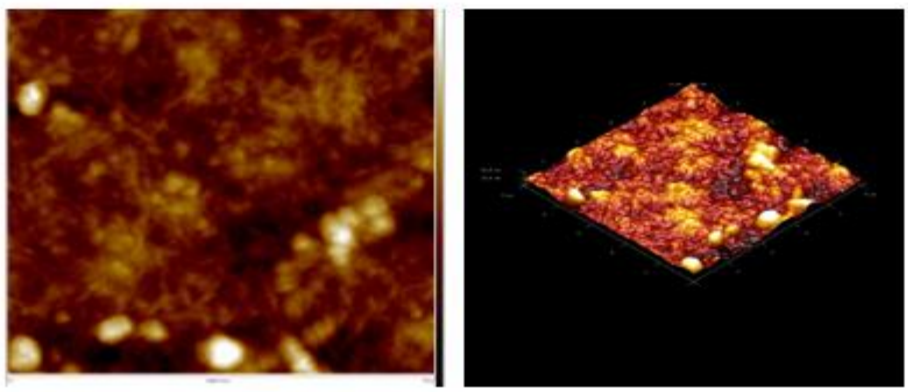

Figure 5. 2D and 3D images of nanocellulose from BCW-C

4. Conclusions

It is known that the production of black carrot juice and concentrate as a natural colorant is quite high, therefore waste assessment is very important in economic and environmental approaches. The waste is a very valuable source for nanocellulose production due to the high cellulose (20.67%) content. Cellulose with a yield of 22.90±2.48% and a whiteness index of 60.32±0.07% was gained at the optimum alkaline extraction conditions which were 44.83 ˚C / 114.21 min with 7.06% NaOH solution. Nanocellulose with 15.76±0.16% yield and 58.77±0.26 whiteness index was obtained from cellulose. Chemical treatments were found effective in the removal of lignin and hemicellulose and did not affect the structure of cellulose negatively. The nanocellulose gained from cellulose had an average diameter of 61 nm and a length of 281 nm.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Seda ERSUS; Design: Seda ERSUS; Data Collection or Processing: Nergiz HAYATİOĞLU, İdil TEKİN; Statistical Analyses: Nergiz HAYATİOĞLU, İdil TEKİN; Literature Search: Nergiz HAYATİOĞLU, İdil TEKİN, Seda ERSUS; Writing, Review and Editing: Nergiz HAYATİOĞLU, İdil TEKİN, Seda ERSUS.

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