

RESEARCH ARTICLE

THE IMPACT OF OPERATIONAL CONDITIONS ON THE STRUCTURAL AND CHEMICAL PROPERTIES OF BIOLOGICALLY PRODUCED NANOCELLULOSE FROM SUBSTRATES CONTAINING DATE PROCESSING WASTES

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ABSTRACT

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This study explores the use of date pomace (DP), a byproduct from the date processing industry, as a sustainable nutrient source for producing bacterial nanocellulose (BNC). By adjusting the Hestrin-Schramm (HS) culture medium with DP juice, we evaluated how key operational factors—such as incubation temperature, initial medium pH, and the ratio of DP juice—affect the structural and chemical properties of BNC. The best conditions for BNC production were found to be an initial pH of 6, an incubation temperature of 30°C, and a DP juice ratio of 20%. We used various analytical techniques, including Field Emission Scanning Electron Microscopy (FESEM), Fourier Transform Infrared Spectroscopy (FTIR), Energy-Dispersive X-ray Spectroscopy (EDX), and Ultraviolet-Visible Spectroscopy (UVS), to characterize the produced BNC. The results showed that increasing the DP juice ratio had a positive effect on the morphology and structure of BNC, while the chemical properties remained stable across different conditions, underscoring the practical potential of using DP for BNC production.

KEYWORDS

Nanocellulose; mother of vinegar; date pomace; incubation; medium

1. INTRODUCTION

Recently, many types of research have been directed toward material recovery in solid waste (SW) through their reuse as secondary industrial raw materials. Replacement of raw materials in the ceramic industry by marble and granite sludge was achieved by (Al-Hamaiedh 2010). Fine aggregate replacement by granite and marble powder in concrete industry has been investigated and proved by (Al-Jrajreh et al. 2023; Al-Hamaiedh and. Khushefati, 2013). The results of a study revealed the possibility of partial replacement of fine aggregate by glass waste (Al-Awabdeh et al. 2022). The feasibility of incorporating face masks in concrete mixes was proved by (Al Swalqah et al. 2024). The organic fraction of municipal SW was successfully reused in compost production (Al-Nawaiseh et al. 2021). Biogas production from co-digestion of sewage sludge and municipal SW was studied by (Aljbour et al. 2021a; Aljbour, El-Hasan et al. 2021b). Also, the generation of methane gas via anaerobic digestion of the organic fraction of municipal SW was studied by (Al-Hajaya et al., 2021). Organic SW can be utilized as a nutrient source for the biological production of nanocellulose which can be produced biologically mainly as bacterial nanocellulose (BNC) or from plants after passing through several chemical and mechanical stages (Figure 1). The analysis highlighted that the properties of BNC are different from the properties of cellulose produced

by other organisms (Carpenter, de Lannoy et al. 2015). On the other hand, stated that due to its supramolecular structure, BNC is characterized by high water retention and high tensile resistance, and it is also of high purity compared to plant cellulose, as it is formed from a very fine and organized network of nanofibers (Carpenter, de Lannoy et al., 2015). Cellulose nanofibers have diameters ranging from 5 to 60 nanometers and varying lengths on the micrometer scale (Delmer, 1999). These materials have excellent properties (mechanical, structural, and optical); therefore, they have been widely used in the paper industry, packaging, automobiles, personal care, construction, and textiles (Delmer, 1999). The study BNC has gained significant attention due to its exceptional physicochemical properties, high tensile strength, porosity, crystallinity, and biodegradability to (Yamanaka et al. 1989; Ullah et al. 2016; Picheth et al. 2017; Ayu, Khalina et al., 2020; Sari, Pruncu et al. 2020; Aisyah et al, 2021, Alsubari, et al., 2021; Azman et al., 2021; Norizan et al., 2021; Norrrahim, et al., 2021; Nurazzi, et al., 2021). Moreover, BNC is an excellent green biopolymer with more applications in bioprocessing, biomedicine, pharmacology, and the food industry (Çakar, et al., 2014; Moradi, et al., 2021). However, the high cost is an important limitation in the production of BNC. To produce nanocellulose cost-effectively, the reuse of different industrial wastes to serve as a nutrient source in the substrate in the fermentation process was investigated earlier.

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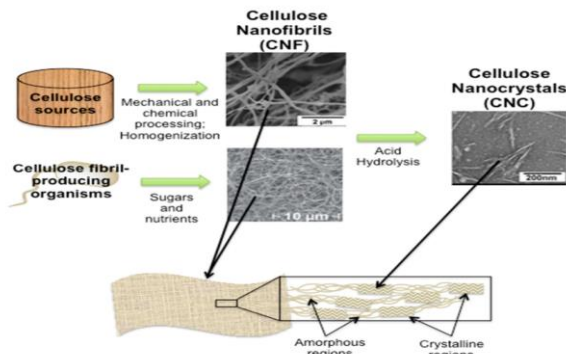


Figure 1: Mechanical and chemical production of cellulose as well as biological production of nanocellulose Carpenter et al, (2015).

This study Highlighted that the variation between these mediums depends mainly on carbon and nitrogen sources, which can be found in many types of industrial wastes or by-products and in inexpensive local sources (Ul-Islam et al, 2020). Date processing wastes are characterized by high sugar content, making them suitable nutrient sources for BNC production. To the researchers investigated the influence of the operational conditions namely the initial pH, incubation temperature, and date pomace (DP) juice ratio in the medium on the yield, water holding capacity, moisture content and morphology of the produced BNC (Al-Hamaiedh et al, 2024). The Maximum BNC wet and dry yields of 31.6 g/L and 0.62 g/L were achieved at an initial pH of 6, an incubation temperature of 30 C, and a juice ratio in the medium of 10%, respectively. Iraq stands at the forefront of date-producing countries globally. Over the past decade, there has been significant growth and revitalization in palm cultivation and date production. According to the 2020 statistical department report, Iraq produced approximately 735.4 tons of dates that year. This growth has spurred the expansion and enhancement of various date-related industries, including packaging, date molasses, and vinegar production. However, these agricultural and industrial activities generate a huge amount of SW, which poses a burden on factories and causes environmental pollution. The main constituent of date waste is the date pomace (DP) or the so-called "press cake," the residual from the process of extracting date juice in some industries such as date molasses and date vinegar.

Table 1: Chemical composition (dry basis) and mineral content of date pomace			
Composition, %		Minerals (mg /kg)	
Moisture	13.37 ± 0.34	Fe	80.75 ± 0.38
Fat	4.92 ± 0.25	Zn	19.72 ± 0.21
Protein	6.35 ± 0.75	Cu	9.06 ± 0.21
Crude fiber	11.74 ± 0.13	Mn	11.03 ± 0.18
Total carbohydrate content	79.06 ± 0.69	Ca	460.46 ± 1.65
Total Sugar	16.25 ± 0.11	Mg	959.50 ± 6.27
Glucose	4.84 ± 0.1	P	853.37 ± 8.28
Fructose	11.05 ± 0.22	Na	2.05 ± 0.18
Sucrose	0.41 ± 0.35	K	29.93 ± 0.75
Ash	2.56 ± 0.09		

DP contains some sugar (about 30% of the weight of processed dates) with or without date kernels (Barreveld 1993). The composition of DP reveals the possibility of its reuse as a nutrient source in the substrate for BNC production. The data presented in Table 1 are the chemical composition (dry basis) and mineral content of the DP (Majzoobi et al. 2019).

This study is considered as the second part of the previous, it study aimed to explore the effect that incubation temperature, initial PH, and DP juice ratio have on the chemical and structural properties BNC. To achieve the stated objective, samples of BNC produced in mediums containing DP juice were tested using Fourier Transform-Infrared Spectroscopy (FTIR), Field Emission Scanning Electron Microscope (FESEM), Energy-dispersive X-ray spectroscopy (EDX), and Ultraviolet Visible Spectroscopy (UVS).

2. MATERIAL AND METHODS

2.1 Experimental setup

The methodology employed in this study aligns with the approach detailed in as this research serves as a continuation of the work presented in (Al-Hamaiedh et al. 2024; Al-Hamaiedh et al. 2024). Samples of date pomace (DP) from Zahdi-type dates were sourced from a factory in Hit, Iraq, specializing in the production of date molasses and vinegar. Juice extraction from the DP followed the method outlined by (Jozala, Pértile et al. 2015). Specifically, 500 g of DP was soaked in 800 ml of distilled water for 30 minutes, then blended and filtered using a fine cotton-textured medium. The filtrate was centrifuged at 4000 rpm for 20 minutes to extract the juice. The supernatant (juice) was collected, sterilized in an autoclave at 121°C for 30 minutes, and stored at 4°C. Figure 2 illustrates the DP before and after juice extraction, alongside the extracted juice. For bacterial nanocellulose (BNC) production, a modified Hestrin-Schramm (HS) culture medium was utilized. The standard HS medium consists of glucose (20 g/L), peptone (5 g/L), yeast extract (5 g/L), sodium dihydrogen phosphate (2.7 g/L), and citric acid (1.15 g/L). The modified HS medium, prepared following the method described by Abol-Fotouh et al (2020), substituted glucose with DP juice.

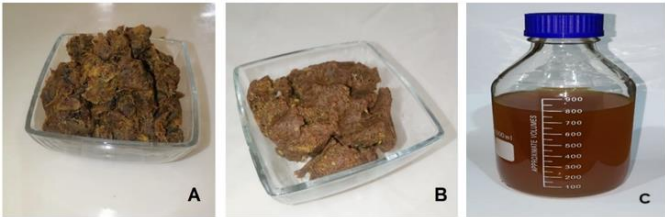


Figure 2: Date pomace A- before extraction, B-after extraction, and C- the extract of DP "juice"

The studied operational parameters encompassed incubation temperature, DP juice ratio in the medium and initial pH. The experiment was carried out in a 1-liter beaker containing 500 ml of substrate, which included 3% mother of vinegar (inoculum culture), date juice extract, and the modified Hestrin-Schramm (HS) culture medium (Goh et al., 2012). The medium components were accurately weighed using an analytical balance with a precision of 0.001 g, and distilled water was measured using a graduated cylinder. The initial pH of the medium was adjusted to the target value using HCl and NaOH prior to inoculum addition. Following pH adjustment, the samples were sterilized in an autoclave at 121°C for 20 minutes to ensure a sterile environment. Three replicates per experimental set were incubated for 168 hours in a dark, open environment at the specified temperature. Upon completion of the incubation period, the cellulose layer formed on the medium's surface was collected. The harvested BNC was tested using Field Emission Scanning Electron Microscope (FESEM), Fourier Transform-Infrared Spectroscopy (FTIR), Energy-dispersive X-ray spectroscopy (EDX), and Ultraviolet Visible Spectroscopy (UVS). FESEM was conducted to visualize the extremely fine surface details (nanostructures), as it operates with electrons rather than light. FTIR is an authoritative and inexpensive analytical tool for the identification of polymers and the assessment of their quality. When a polymer material absorbs mid-infrared light, the resulting spectrum (absorbance or transmittance) gives a unique imprint that distinguishes this polymer and can be used to easily screen and test samples. FTIR can be defined as an effective method used to measure the emission, absorption, and photoconductivity of liquid, solid, and gas matter for each wavelength. It is employed to detect various functional groups at a spectrum recorded between 4000 and 400 cm⁻¹ (Pandey et al, 2015; Veerasingam et al., 2021). Energy-dispersive X-ray spectroscopy (EDX) was also conducted, along with Ultraviolet Visible Spectroscopy (UVS), which measures the fade away (scatter + absorption) of passing light through samples. Nanomaterials have unique optical properties that are sensitive to the size, shape, concentration, and refractive index on the nanoparticle surface, making UV-Vis an effective tool for identifying, characterizing, and studying nanomaterials. A 500 ml medium was prepared with modified HS ratios of 90% and 80%, corresponding to DP ratios of 10% and 20%, respectively and inoculator weight of 15 grams. Samples P1, P3, P6, and P7, with a fixed pH of 6 and a DP ratio of 10%, were incubated at different temperatures (24°C, 26°C, 30°C, and 34°C) to determine the optimal incubation temperature. Similarly, samples with a DP ratio of 10% and pH values of 5, 6, and 7 were incubated at 30°C to identify the optimal pH level. Furthermore, samples P3 and P5, with DP ratios of 10% and 20%, were incubated at a constant pH of 6 and a temperature of 30°C to evaluate the ideal juice ratio in the medium.

3. RESULTS AND DISCUSSIONS

3.1 FESEM

A nanoporous or sponge-like structure characterized by different nanoporous sizes can be seen in the structure of the produced BNC (Figures 3 and 4). Comparing BNC produced in samples P3, P4 with lower initial pH values. The structure of BNC formed in sample P2 with high pH

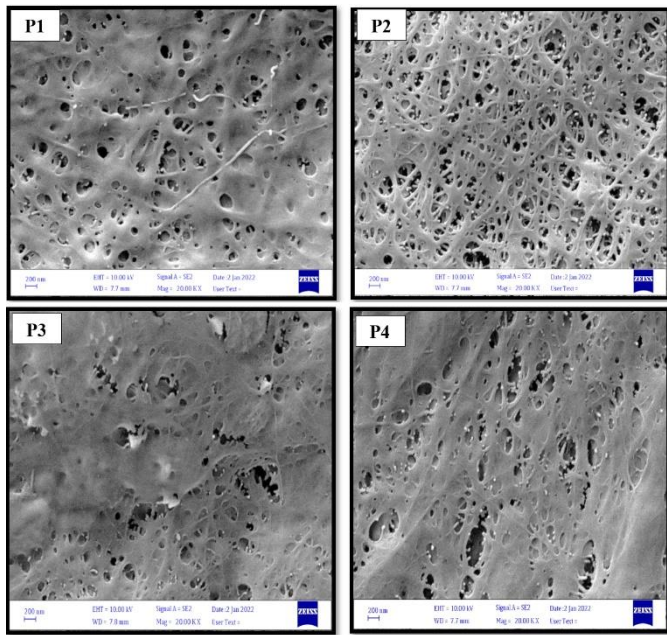


Figure 3: Morphological images of samples from sets P1-P4

In comparison to samples P1 and P3, which have the same pH and DP juice ratio, sample P6 had a positive effect on the alignment of nanostructure and porosity by raising the incubation temperature. Using FESEM, the results revealed that the change in control parameters and conditions resulted in porous nanostructures that had different nanoscale diameters of less than 100 nm and different porosities. Due to an increase in the ratio of DP juice in the culture medium, the porous structure of produced BNC for sample P5 increased in porosity and regularity when compared to sample P3, which had the same pH and incubation temperature and half the DP juice ratio. The morphology and structure of BNC were improved by increasing the DP juice ratio in the samples.

3.2 UVS

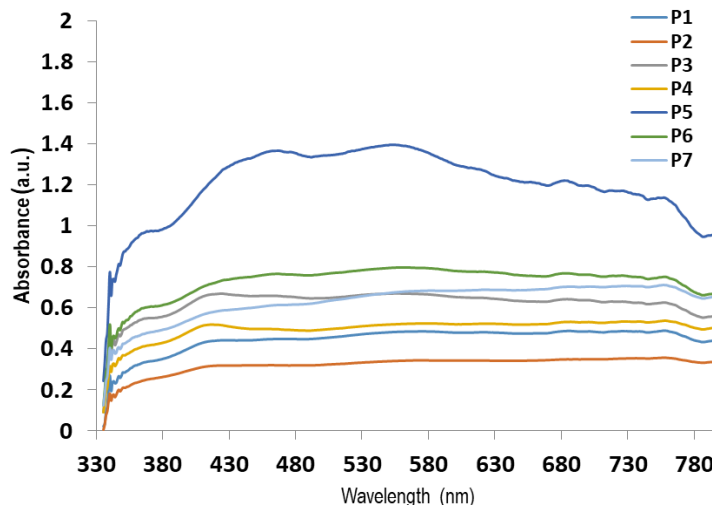


Figure 5: Uv-Visible spectrum of as prepared samples.

3.3 EDX

Figures 6 and 7 display the EDS spectrum of the as-prepared samples under different conditions. The results confirm the presence of a high

value shows great compactness and porosity. A BNC produced in samples containing a higher DP juice ratio P5 is distinguished by nanostructures that resemble nanofibers but have a very small diameter, as shown in images P5 and P5-1. There are two types of nanoporous structures: membranes and bulk materials, and they can consist of organic and inorganic frameworks that support a regular, porous structure. A typical pores size is 100 micrometers or smaller. By increasing the ratio of juice to the medium, the surface morphology of the BNC is altered.

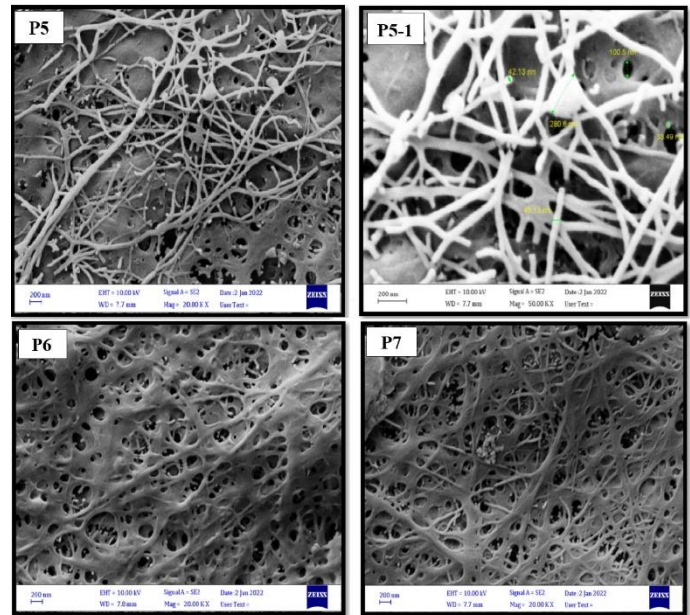


Figure 4: Morphological images of samples from sets (P5-P7)

UV-Vis is the Ultraviolet-Visible spectroscopy, which measures the fade away (scatter + absorption) of passing light through samples. Nanomaterials have exclusive optical properties that are sensitive to the size, shape, concentration, and refractive index on the nanoparticle surface, so UV-Vis is an effective tool for identifying, characterizing, and studying nanomaterials. Figure 5 illustrates the relationship between the absorbance spectrum and the wavelength of the prepared samples. From the figure, the results indicate that sample P5 has the highest absorption value in the UV-Vis curve. This can be attributed to the decreased percentage of Modified HS and the increased percentage of DP juice in the sample compared to the other samples. In the same context, the results confirm a decrease in absorbance at the near edge of the ultraviolet region for all samples. This behavior can be attributed to the formation of nanocellulose (Hassan et al., 2012; Morawski et al., 2013; Mun et al., 2017).

percentage of carbon and oxygen combined with small percentages of sodium, calcium, phosphorus, and nitrogen in samples P1, P4, and P6. In the same context, lignocellulose biomass is characterized by having more than half of its compounds in the form of carbon elements. The results

suggest the formation of nanocellulose supported by the presence of large amounts of carbon and oxygen. These findings are compatible with the

results of previous studies (Kim et al., 2019; Shaikh et al., 2021).

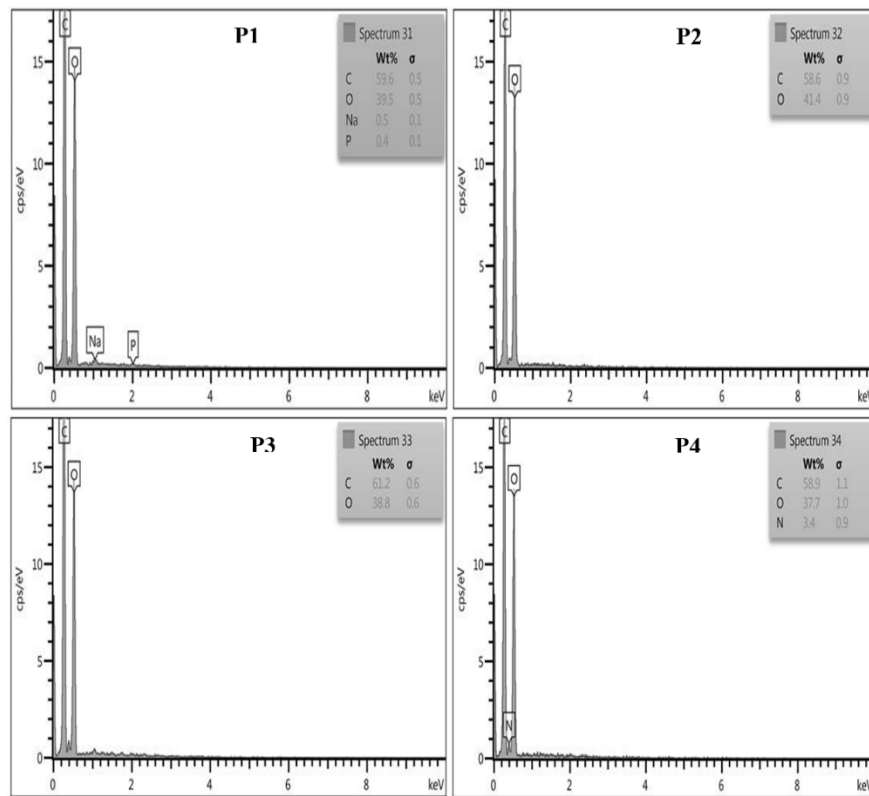


Figure 6: EDS spectrum of samples P1-P4

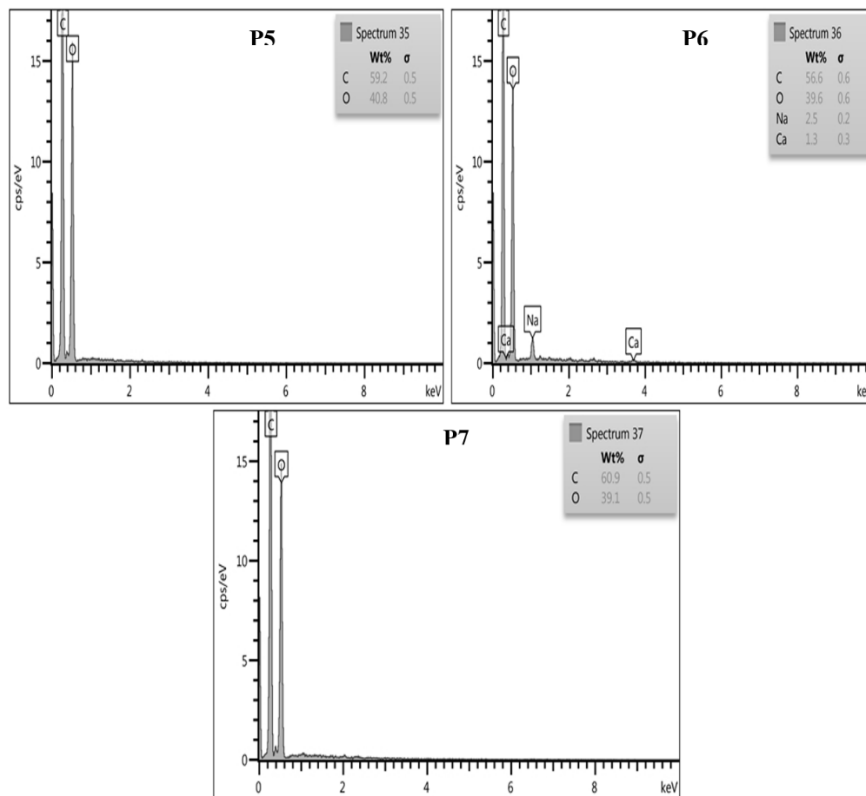


Figure 7: EDS spectrum of samples P5-P7

3.4 FTIR

The functional groups and chemical bonds present in BNC samples (DP juice/HS media) were predicted using FTIR spectroscopy. These bonds were defined by interpreting the infrared transmission spectrum. Figures 8 and 9 display the FTIR transmittance spectrum as a function of wavenumber. The results indicate similar behavior in the spectrum across all samples with very slight differences in the wavenumber. The samples

show peaks in the wavenumber range 3332-3336 cm^{-1} , 1620-1631 cm^{-1} , 1508-1531 cm^{-1} , 1419-1453 cm^{-1} , 1311 cm^{-1} , 1257-1261 cm^{-1} , 1157-1161 cm^{-1} , and 798-794 cm^{-1} , due to the presence of the vibration bonds

of O-H stretching, C=O stretching, N-O stretching, C-H bending deformation vibration, O-H bending vibration, C-O stretching, C=O symmetric stretch, and C=C bending, respectively (Pharmawati and Wrasati 2020; Flores-Hernández et al., 2021; Teixeira et al., 2021).

The peaks in the range 1053-1002 cm^{-1} in original cellulose represented C-O valence vibration (Wang et al. 2015). The wave number ranges from 600 to 540 cm^{-1} may be attributed to the presence of C-Br stretching (halo

compound) or C-I stretching (halo compound). The results were calculated based on the Sigma Aldrich Company table reference standard of FTIR spectrum (Abdelhakim et al., 2021).

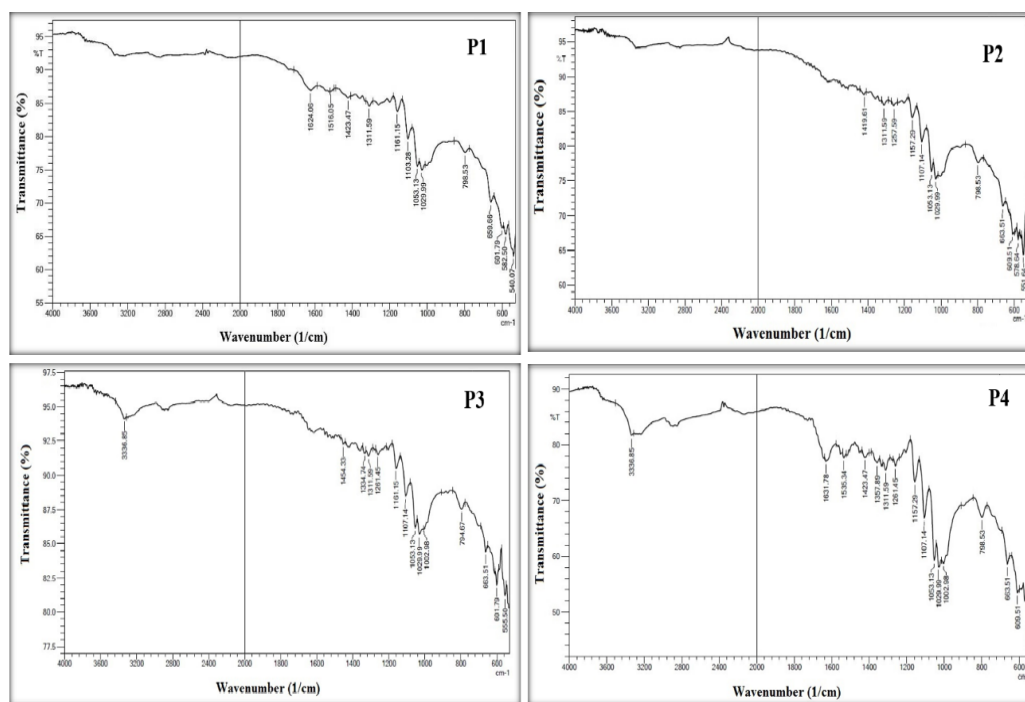


Figure 8: FTIR spectrum of samples P1-P4 at different conditions.

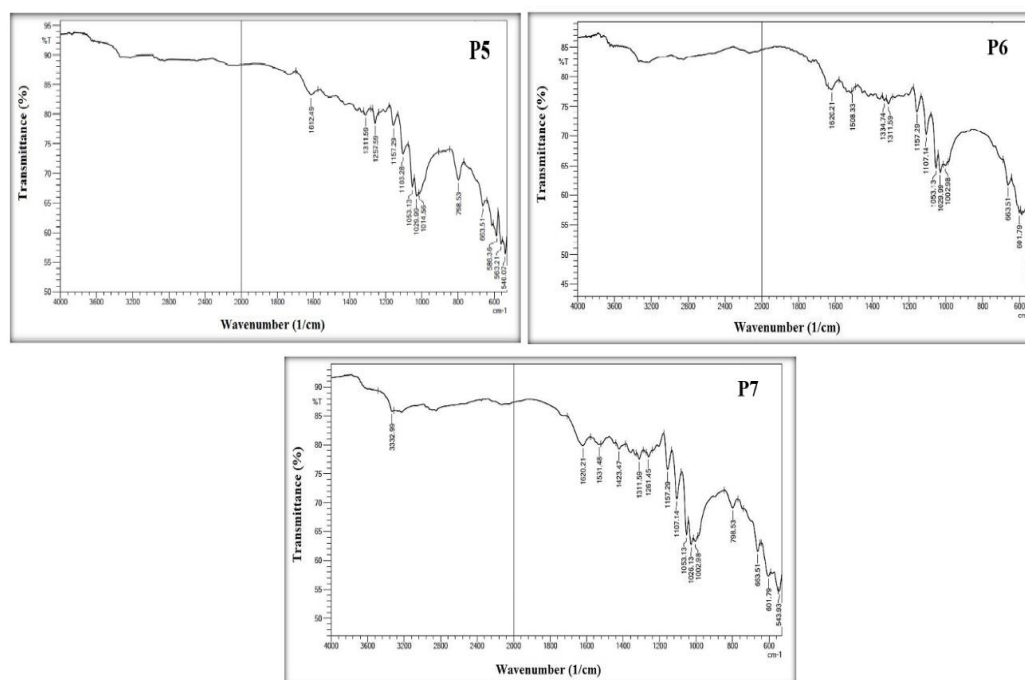


Figure 9: FTIR spectrum of samples P5-P7 at different conditions.

As a result of these measurements, it can be inferred that the production of BNC using DP juice under different conditions did not show any effect on its chemical properties which constitute an important role in its practical applications.

4. CONCLUSIONS

The study shows that waste from date processing, especially date pomace DP, can be reused effectively as a nutrient source for the sustainable production of bacterial nanocellulose (BNC). The best conditions for producing BNC were found to be an initial pH of 6, an incubation temperature of 30°C, and a DP juice ratio of 20%. These conditions led to improved morphology and structure of the BNC. Analytical results indicated that these operational adjustments had a significant impact on the nanostructural properties of BNC, while its chemical properties

remained stable, highlighting the material's versatility and reliability for various uses. This method not only offers a cost-effective way to produce BNC but also provides an eco-friendly solution for handling industrial date processing waste.

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