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VALORIZATION OF BEETROOT SKIN FOR THE PRODUCTION OF NANOCELLULOSE REINFORCED BIOCOMPOSITE FILMS

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ABSTRACT
The extensive use of petroleum-based packaging significantly contributes to environmental pollution, necessitating sustainable alternatives. This study aimed to valorize beetroot skin, an abundant agricultural by-product, by incorporating it into biocomposite films reinforced with crystalline nanocellulose (CNC) to enhance mechanical and barrier properties. Films were produced by blending beetroot skin with gelatin and glycerol, followed by the addition of 3% and 6% CNC. The films were cast, dried, and analyzed for thickness, opacity, color, water solubility, tensile strength, elongation at break, and water vapor permeability. Statistical analysis identified significant differences among formulations. Incorporating 6% CNC increased thickness from 0.12 mm to 0.26 mm, enhanced opacity by 40%, decreased solubility from 60% to below 40%, improved tensile strength from 10 MPa to 15.6 MPa, and reduced water vapor permeability by over 70% to 0.46 $ng \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$. In conclusion, CNC reinforcement successfully improves beetroot skin-based films, potentially offering a viable sustainable biocomposite film solution in packaging applications.
Keywords: Biocomposite, biodegradable film, beetroot skin, crystalline nanocellulose, biodegradable materials, sustainability

PANCAR KABUĞUNUN NANOSELÜLOZ İLE GÜÇLENDİRİLMİŞ BİYOKOMPOZİT FİLM ÜRETİMİ İÇİN DEĞERLENDİRİLMESİ

ÖZ
Petrol bazlı ambalajların yaygın kullanımı çevre kirliliğine önemli ölçüde katkıda bulunmakta olup, sürdürülebilir alternatifleri zorunlu kılmaktadır. Bu çalışma, bol miktarda tarımsal yan ürün olan pancar kabuğunu değerlendirerek, mekanik ve bariyer özelliklerini artırmak amacıyla kristalin nanoselüloz (CNC) ile güçlendirilmiş biyokompozit filmlerine dahil etmeyi hedeflemiştir. Filmler, pancar kabuğu, jelatin ve gliserolün karıştırılmasıyla üretilmiş, ardından %3 ve %6 CNC eklenmiştir. Filmler dökülmüş, kurutulmuş ve kalınlık, opaklık, renk, suda çözünürlük, çekme mukavemeti, kopma uzaması ve su buharı geçirgenliği açısından analiz edilmiştir. İstatistiksel analiz, formülasyonlar arasında anlamlı farklar belirlemiştir. %6 CNC eklenmesi, kalınlığı 0.12 mm'den 0.26 mm'ye çıkarmış, opaklığı %40 artırmış, çözünürlüğü %60'tan %40'ın altına düşürmüş, çekme mukavemetini 10 MPa'dan 15.6 MPa'ya yükseltmiş ve su buharı geçirgenliğini %70'in üzerinde azaltarak 0.46

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ng·m⁻¹·s⁻¹·Pa⁻¹ seviyesine indirmiştir. Sonuç olarak, CNC takviyesi, pancar kabuğuna dayalı biyokompozit filmlerin mekanik ve bariyer özelliklerini başarıyla geliştirmiş olup, uygulanabilir sürdürülebilir bir ambalaj çözümü sunmaktadır.
Anahtar kelimeler: Biyokompozit, biyobozunur ambalaj, pancar kabuğu, kristalin nanoselüloz, biyobozunur malzemeler, sürdürülebilirlik

INTRODUCTION

Packaging is essential for product protection, ensuring quality and safety during storage and transport. However, the extensive use of petroleum-based materials has created a severe environmental crisis. Globally, packaging accounts for 40% of the 353 million tons of plastic waste generated annually, much of which persists for centuries, contributing significantly to pollution and greenhouse gas emissions (Caleb and Belay, 2023; Ghosh et al, 2024). The packaging sector alone is responsible for 19% of the global carbon budget needed to limit warming to 1.5°C, highlighting the urgent need for sustainable alternatives (Verma et al., 2024).
Biodegradable films have emerged as promising solutions, offering sustainability by utilizing natural polymers and waste materials. Agricultural residues like rice husks and sugarcane bagasse are being converted into biopolymers, reducing waste and production costs. These materials align with circular economy principles, transforming waste into resources (Terán Hilares et al., 2018; Vigneswari et al., 2024). With biodegradable films holding a 53% share of the global bioplastics market and an annual production of 1.14 million tons, their adoption in food packaging reflects the dual benefits of environmental protection and economic viability (Fatima et al., 2024).

Despite their potential, the development of biodegradable films faces significant challenges, particularly in mechanical and barrier properties. Biopolymer films often lack the strength, elasticity, and durability required for robust packaging, limiting their performance under stress or prolonged use. Additionally, these films exhibit higher permeability to water vapor and gases compared to synthetic plastics, which can compromise their effectiveness in preserving food (Karimi Sani et al., 2023). Addressing these limitations is crucial, as biodegradable films must match or exceed the performance of conventional materials to gain broader commercial adoption. Studies have shown that integrating reinforcing agents such as nanocellulose or modifying polymer blends can improve tensile strength and reduce permeability, paving the way for more functional biopolymer films (Ghosh et al., 2024; Ren et al., 2024).

Beetroot (*Beta vulgaris L.)* is a nutrient-rich root vegetable recognized for its high content of bioactive compounds, including betalains, phenolics, dietary fiber, and substantial amounts of cellulose, hemicellulose, and pectin (Chaari et al., 2022; Pedreño and Escribano, 2000). The skin, an abundant byproduct of beetroot processing, is notably rich in betalain pigments (betacyanins and betaxanthins), which not only impart vibrant coloration but also provide antioxidant activity, enhancing UV protection and oxidation resistance in derived films (Pedreño and Escribano, 2000). Due to its low cost, ready availability, and alignment with waste-reduction principles, beetroot skin serves as a promising source of natural polymers for biodegradable films (Zin et al., 2022). Studies have demonstrated that incorporating beetroot skin into film formulations results in bioactive materials with improved mechanical and antioxidant properties, making them suitable for food packaging and preservation (Chaari et al., 2022; Zin et al., 2022). However, to best of our knowledge, there are no studies in literature that uses beetroot skin as the main ingredient to form biocomposite films.

Gelatin has emerged as a widely used film-forming agent due to its biodegradability, edibility, and excellent film-forming capabilities. Derived from collagen, it has been successfully applied in the production of biodegradable films for applications such as food preservation and pharmaceutical coatings. To enhance the mechanical properties of gelatin-based films, glycerol is commonly employed as a plasticizer (Andiati et al., 2023). By reducing brittleness and increasing flexibility, glycerol improves film usability without compromising structural integrity (Bergo and Sobral, 2007; Jamali et al., 2024). Its compatibility with gelatin and other biopolymers has made it a preferred additive in the formulation of eco-friendly packaging materials (Park et al., 2008).

To overcome the mechanical and barrier limitations of biodegradable films, reinforcing agents like crystalline nanocellulose (CNC) are incorporated to enhance their properties. CNC, derived from cellulose, offers high tensile strength and a large surface area, making it an effective nanofiller in biopolymer matrices. Incorporating CNC can improve tensile strength and reduce permeability, thus enhancing the functionality of biopolymer films (Ren et al., 2024). Chaichi et al. (2017) reported that adding 5% CNC to pectin films increased tensile strength by 84% and reduced water vapor permeability by 40%, demonstrating significant improvements in film performance (Chaichi et al., 2017). Similarly, Reddy and Rhim (2014) found that blending 5% CNC into agar films increased tensile strength by 25% and decreased water vapor permeability by 25%, highlighting CNC's role as a robust reinforcing agent (Reddy and Rhim, 2014) .

This study aims to develop sustainable, biocomposite films utilizing beetroot skin, an abundant industrial waste, in combination with gelatin and glycerol. By incorporating CNC at varying concentrations, we seek to enhance the physical and mechanical properties of the films. This innovative approach not only valorizes beetroot skin, contributing to waste reduction and circular economy principles, but also addresses the critical challenge of improving the performance of these films for practical packaging applications. The novelty of this research lies in the synergistic combination of beetroot skin, gelatin, glycerol, and CNC to produce films with superior properties potentially suitable for food packaging. This advancement has the potential to extend shelf life while mitigating environmental pollution caused by conventional plastics, thereby offering a promising solution for eco-friendly packaging materials.

MATERIAL AND METHODS

Materials

Beetroot skins were purchased in bulk (50 kg) from a local grocery store in Gebze, Turkey. Small beetroots were specifically chosen to obtain a higher skin-to-pulp ratio. This single large purchase was made to ensure consistency and minimize variations that could arise from different suppliers or harvest times throughout the study. Gelatin (Type A, from porcine skin) and glycerol (≥99% purity) were obtained from Sigma-Aldrich Chemie GmbH (Darmstadt, Germany). Crystalline nanocellulose (CNC) powder was also sourced from Sigma-Aldrich Chemie GmbH. Magnesium nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6H_2O$, ≥98% purity) used for desiccator humidity control was purchased from Merck KGaA (Darmstadt, Germany). All chemicals were of analytical grade and used without further purification. Distilled water was utilized throughout all experiments.

Methods

Film formation

Films were produced by wet casting method as described by Aydogdu et al, (2018). Beetroot skin powder was prepared by peeling beetroot skins to an approximate thickness of 0.2 mm. The skins were dried at 60 °C for 24 hours, a condition determined through preliminary trials to ensure complete drying. The dried skins were then ground into a fine powder using a coffee grinder (KSPG-4820, Kiwi, Istanbul, Turkey) until a visually uniform powder was obtained.

Beetroot skin powder (8 g) was dispersed in 100 ml distilled water, then gelatin and glycerol were added at either 4% or 8% (w/v), respectively, as per formulations in Table 1. Each mixture was stirred at 60°C and 400 rpm for 10 min (MR 3001 K Hot Plate with Magnetic Stirrer, Heidolph Instruments, Germany) until complete dissolution. The sequence involved adding beetroot skin first, followed by gelatin, then glycerol. After filtration through cheesecloth, 17 ml of each solution was cast into glass petri dishes (10 cm diameter) and dried at 60°C for 17 h (ED 56 Oven, Binder GmbH, Germany). Dried films were stored in a desiccator with $Mg(NO_3)_2$.

Table 1. Film Solution Formulations

Sample	Beetroot Skin (% w/v)	Gelatin (% w/v)	Glycerol (% w/v)	CNC (% w/v)
G8Gl8	8	8	8	0
G8Gl4	8	8	4	0
G4Gl8	8	4	8	0
G4Gl4	8	4	4	0
G8Gl8-C3	8	8	8	3
G8Gl4-C3	8	8	4	3
G8Gl8-C6	8	8	8	6
G8Gl4-C6	8	8	4	6

For CNC-containing films, CNC was incorporated at 3% or 6% (w/v). CNC was incorporated into films containing 8% w/v gelatin in the film solution. Preliminary mechanical experiments, on the initial trials showed that G8 samples exhibited slightly better mechanical properties (i.e. higher tensile strength) compared to G4 samples. Concentrations were chosen after preliminary trials ensuring stable dispersion. CNC was ultrasonically treated for 4 min at 60% power and 5 cycles (HD2200.2 Ultrasonic Homogenizer, Bandelin Elektronik GmbH and Co. KG, Germany) before adding beetroot skin and proceeding as above. Films were peeled from the glass plates and analyzed within one hour of removal.

Thickness

Film thickness was measured using a digital micrometer (Digimatic Micrometer 293-821-30, Mitutoyo Corp., Japan; accuracy 0.001 mm) at five points per sample (center and four perimeter locations). At least five samples per formulation were measured, and average values with standard deviations were reported (Aydogdu et al, 2018).

Opacity

Opacity was determined with a UV-Vis spectrophotometer (Lambda 35, PerkinElmer Inc., USA) at 600 nm. Rectangular film strips were placed directly in the light path. Absorbance (A600) was recorded, and opacity was calculated as A600 divided by film thickness (in mm). Care was taken to avoid fingerprints, wrinkles, or bending (Aydogdu et al, 2018).

Color

Color parameters (*L*, a*, b**) were measured using a Chroma Meter CR-400 (Konica Minolta, Japan) calibrated with a white reference. Films were placed on a white background, and readings were taken at five distinct points per sample. The color parameters were recorded in the CIE *Lab** color space system, where:

- *L** represents the lightness index (ranging from 0 [black] to 100 [white]),
- *a** indicates the red-green coordinate (positive values for red, negative values for green),
- *b** indicates the yellow-blue coordinate (positive values for yellow, negative values for blue).

Bulk density

Samples were prepared by cutting 20 × 20 mm² square pieces from the films, and an average thickness (x) was determined from three random measurements on these samples. The samples were then dried in a vacuum oven at 70°C for 48 h and weighed. The density was calculated as the ratio of the weight to the volume (x × Area) of the film (Aydogdu et al, 2018).

Water solubility

Square film samples (2 cm × 2 cm) were dried at 60°C for 24 h, weighed (W_i), then immersed in distilled water at ~25°C for 24 h. After immersion, undissolved material was dried again at 60°C for 24 h and weighed (W_f) (Aydogdu et al, 2018).

The solubility percentage (S) of the films was calculated using Equation 1:

$$S(\%) = \frac{W_i - W_f}{W_i} \times 100 \qquad [1]$$

Mechanical properties
Mechanical tests were conducted using a universal testing machine (Z250, Zwick/Roell, Germany) equipped with a flat-faced tensile grip. Film strips (5 mm × 50 mm) conditioned at 50% RH and 23±2°C for 48 h were tested at 25 mm/min crosshead speed. Tensile strength and elongation at break were calculated from the stress-strain data (Aydogdu Emir et al, 2023).

Water vapor permeability (WVP)
Water vapor permeability (WVP) was measured using a modified gravimetric cup method (ASTM E96/E96M-16). The method was used in the author's previous studies (Kirtil et al, 2021). Custom permeability cups (40 mm inner diameter) were filled with 30 ml distilled water to maintain 100% RH inside. Films were sealed onto the cup mouths with rubber gaskets and screw-on lids, ensuring water vapor diffused only through the film surface. Cups were placed in desiccators with silica gel to create a low-RH environment outside; actual outside RH (~15%) was monitored with a digital hygrometer. Each cup was weighed initially and at 1 h intervals for 10 h using an analytical balance (As 220.R2 Plus, Radwag, Radom, Poland; accuracy 0.0001 g). Weight loss over time was plotted, and the linear slope normalized by exposed film area (A) provided the water vapor transmission rate (WVTR).

WVP was calculated by Equation 2:

$$WVP = \frac{WVTR \times \Delta x}{\Delta P} \qquad [2]$$

where WVTR is the water vapor transmission rate ($g\ m^{-2}\ s^{-1}$), Δx is the average film thickness (m), ΔP is the water vapor partial pressure difference across the film (Pa).

The partial pressure difference (ΔP) was calculated using Equation 3:

$$\Delta P = P_{sat}(RH_i - RH_o) \qquad [3]$$

where Psat is the saturation vapor pressure of water at the experimental temperature, obtained from standard reference tables, RH_i is the relative humidity inside the cup (100%), RH_o is the measured relative humidity outside the cup. For each measurement interval, the RH_o was recorded, and the corresponding ΔP was calculated accordingly.

Statistical analysis
All experiments were conducted using three independent film samples, with each measurement repeated three times for replicates. Data are reported as mean values±standard deviation (SD). Statistical analysis was performed using JMP Pro 18 Student Version (SAS Institute Inc., Cary, NC, USA). One-way analysis of variance (ANOVA) was applied to evaluate significant differences among film solution formulations at a significance level of $P<0.05$. Tukey's Honestly Significant Difference (HSD) post hoc test was used for pairwise comparisons when significant differences were detected.

RESULTS AND DISCUSSION

Thickness
The thickness of films directly affects their mechanical properties, water vapor permeability, and integrity (Park et al., 2008). In this study, thickness values ranged from 0.118±0.003 mm (G4Gl4) to 0.258±0.003 mm (G8Gl8-C6) (Table 2), reflecting the influence of varying glycerol, gelatin, and CNC content, as well as the incorporation of beetroot skin. Higher glycerol concentrations significantly increased thickness compared to lower-glycerol formulations, attributed to the plasticizing effect of glycerol, which enhances molecular mobility during drying (Bergo and Sobral, 2007). In line with the study's goal of improving film properties through natural wastes like beetroot skin and CNC reinforcement, the addition of CNC also led to marked increases in thickness. For example, G8Gl8-C6, showed the highest thickness, consistent with findings by Chaichi et al. (2017), who reported increased thickness in pectin films containing CNC. This reinforcement occurs through CNC's network formation and hydrogen bonding with the gelatin and beetroot skin matrix (Reddy and Rhim, 2014).

Increasing CNC from 3% to 6% further boosted thickness, highlighting CNC's ability to create a denser structure. Similarly, higher gelatin and glycerol contents (G8Gl8) increased thickness by elevating the solid fraction and improving material dispersion, including beetroot skin particles (Andiati et al., 2023). The thickness values observed here align with those reported for other gelatin-glycerol films and CNC-based bioplastics, typically 0.100–0.300 mm (Park et al., 2008; Ren et al., 2024).

Table 2. Physical properties of films

Sample Names	Thickness (mm)	Opacity	*L**	*a**	*b**
G4Gl4	0.118±0.003[h]	10.35±0.13[c]	24.4±0.5[c]	20.6±0.6[ab]	7.6±0.6[c]
G4Gl8	0.143±0.003[fg]	10.18±0.24[c]	24.1±0.8[c]	20.9±0.2[a]	7.8±0.4[c]
G8Gl4	0.158±0.002[f]	10.38±0.16[c]	24.2±0.6[c]	20.7±0.6[ab]	7.6±0.3[c]
G8Gl8	0.185±0.001[de]	10.48±0.16[c]	24.4±0.6[c]	20.9±0.4[a]	7.5±0.5[c]
G8Gl4-C3	0.198±0.003[d]	12.62±0.13[b]	29.3±0.6[b]	20.4±0.1[ab]	13.2±0.3[b]
G8Gl8-C3	0.215±0.002[c]	12.73±0.19[b]	29.4±0.6[b]	20.5±0.2[ab]	12.9±0.3[b]
G8Gl4-C6	0.233±0.002[b]	14.85±0.15[a]	34.1±0.5[a]	19.9±0.2[b]	17.6±0.3[a]
G8Gl8-C6	0.258±0.003[a]	14.54±0.11[a]	34.5±0.6[a]	19.8±0.2[b]	17.5±0.4[a]

Sample Names	Bulk Density (g/cm^3)	Water Solubility (%)	Tensile Strength (MPa)	Elongation at Break (%)	WVP ($ng \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$)
G4Gl4	0.692±0.008[d]	64.3±1.5[c]	10.58±0.14[e]	49.5±1.3[cd]	2.051±0.048[b]
G4Gl8	0.673±0.007[d]	75.3±2.1[a]	10.02±0.16[e]	58.4±0.8[a]	2.423±0.074[a]
G8Gl4	0.753±0.005[c]	59.7±1.5[cd]	12.89±0.25[c]	47.5±1.4[de]	1.554±0.092[d]
G8Gl8	0.735±0.004[c]	70±2.1[b]	11.89±0.16[d]	58.3±0.8[a]	1.857±0.044[c]
G8Gl4-C3	0.865±0.011[a]	49.7±1.8[e]	14.42±0.25[b]	46.3±0.9[ef]	0.850±0.035[f]
G8Gl8-C3	0.780±0.005[b]	55±2.0[d]	13.42±0.25[c]	53.2±1.3[b]	1.053±0.076[e]
G8Gl4-C6	0.872±0.012[a]	39.7±1.7[g]	15.62±0.15[a]	44.4±1.0[f]	0.458±0.059[g]
G8Gl8-C6	0.858±0.007[ab]	44.7±1.6[f]	14.96±0.20[b]	52.2±1.0[bc]	0.651±0.031[fg]

The increase in thickness observed, particularly in CNC-rich formulations, may also contribute to enhanced opacity and improved moisture barrier properties. Thicker films generally present more tortuous paths for light transmission and water diffusion, potentially augmenting the optical density and reducing WVP. This relationship aligns with the denser, CNC-rich structures previously observed, highlighting the interconnectedness of thickness, opacity, and barrier functionality.

Opacity

Opacity is a critical optical property of films, influencing both visual appeal and protection against light-induced quality loss in packaged foods (Chaari et al., 2022). In line with the study's goal of utilizing beetroot skin and CNC to enhance film functionality, our results showed that opacity ranged from 10.18±0.24 to 14.85±0.15 (Table 2), with formulations containing CNC consistently more opaque. Films without CNC maintained relatively low opacity values (around 10.18–10.48), attributed to a more homogenous distribution of gelatin and glycerol that reduces light scattering (Bergo and Sobral, 2007). The inclusion of beetroot skin, rich in natural pigments, likely contributed to the base-level opacity, providing a foundation upon which CNC further influenced optical properties.

In contrast, adding CNC significantly heightened opacity, as seen in G8Gl4-C3 (12.62±0.13) and G8Gl4-C6 (14.85±0.15). This effect arises from CNC's high refractive index and nanoscale dimensions, which increase light scattering within the dense biopolymer matrix (Chaichi et al., 2017). Increasing CNC content from 3% to 6% consistently amplified this effect, indicating that CNC concentration outweighs slight compositional shifts in gelatin or glycerol.

Although higher gelatin content (e.g., G8Gl8) marginally increased opacity (10.48±0.16) compared to lower-gelatin films (e.g., G4Gl8), these differences were not statistically significant. Similar observations have been reported for gelatin-glycerol systems (Park et al., 2008). Literature comparisons further support these findings: CNC additions have been shown to raise opacity by 25–30% in pectin-based films (Chaichi et al., 2017), and analogous results were reported in agar films (Reddy and Rhim, 2014).

Interestingly, the same CNC-induced structural densification that increased opacity and influenced color parameters may also reinforce the polymer network. This structural reinforcement appears to improve mechanical strength while concurrently reducing WVP, illustrating how modifications that affect optical characteristics can cascade into improvements in mechanical robustness and barrier efficiency. These results confirm that CNC plays a dominant role in modulating opacity, building on the inherent contributions of beetroot skin and providing a strategic pathway to tailor optical properties in sustainable, bio-based packaging materials.

Color and appearance

The color and appearance of the films are pivotal for both their visual appeal and potential functional roles, such as providing light protection. Evaluations using the CIE *Lab* system (L^*, a^*, b^*) and examining surface characteristics allowed for an objective analysis of these properties (Pedreño and Escribano, 2000). In line with the study's goal of creating functional films from beetroot skin and CNC, the variations in color metrics presented in Table 2 and Figure 1 offer valuable insights into how composition influences film properties.

L^* values ranged from 24.1±0.8 (G4Gl8) to 34.5±0.6 (G8Gl8-C6). Films without CNC (top 2 rows, Figure 1) appeared darker, while those incorporating CNC (bottom 2 rows, Figure 1) were significantly lighter ($P<0.05$), reflecting CNC's light-scattering properties (Chaichi et al., 2017). Increasing CNC content further brightened the films. Regarding a^*, values were relatively stable (19.8±0.2 to 20.9±0.2), but non-CNC films showed slightly more pronounced redness. Beetroot skin's betalains likely contributed to this hue, while CNC addition diluted these pigments, reducing redness in samples such as G8Gl8-C6 (Pedreño and Escribano, 2000).

In contrast, b^* values increased substantially with CNC, rising from about 7.5–7.8 in non-CNC films to 17.6±0.3 in G8Gl4-C6. This enhancement in yellowness may result from the interaction of CNC with the polymer-beetroot matrix, increasing light scattering and altering optical perception (Reddy and Rhim, 2014). Surface characteristics, shown in Figure 1, also changed markedly. Films without CNC (top rows) were smooth and uniform, while those with CNC (bottom rows) exhibited greater heterogeneity. At higher CNC concentrations, structures like G8Gl8-C6 showed pronounced bumps, likely due to CNC aggregation—a phenomenon also reported in agar films (Reddy and Rhim, 2014). Overall, CNC integration influenced the optical and surface properties by brightening the films, shifting their color metrics, and increasing surface irregularities, while the beetroot skin contributed base-level pigmentation.

Bulk density

Bulk density, shown in Table 2, reflects the compactness of the films and their potential effects on mechanical and barrier properties (Bergo and Sobral, 2007). In line with the study's goal of developing sustainable films from beetroot skin and CNC, films without CNC, such as G4Gl4 (0.692±0.008) and G4Gl8 (0.673±0.007), exhibited lower bulk densities, indicating that glycerol variations alone did not significantly enhance compactness. Although glycerol improves flexibility by increasing molecular mobility (Bergo and Sobral, 2007), it contributes minimally to density when reinforcing agents are absent. In contrast, elevating gelatin content yielded denser matrices (G8Gl4: 0.753±0.005; G8Gl8: 0.735±0.004), likely due to greater solid fractions that incorporate beetroot skin polymers more effectively (Andiati et al., 2023).

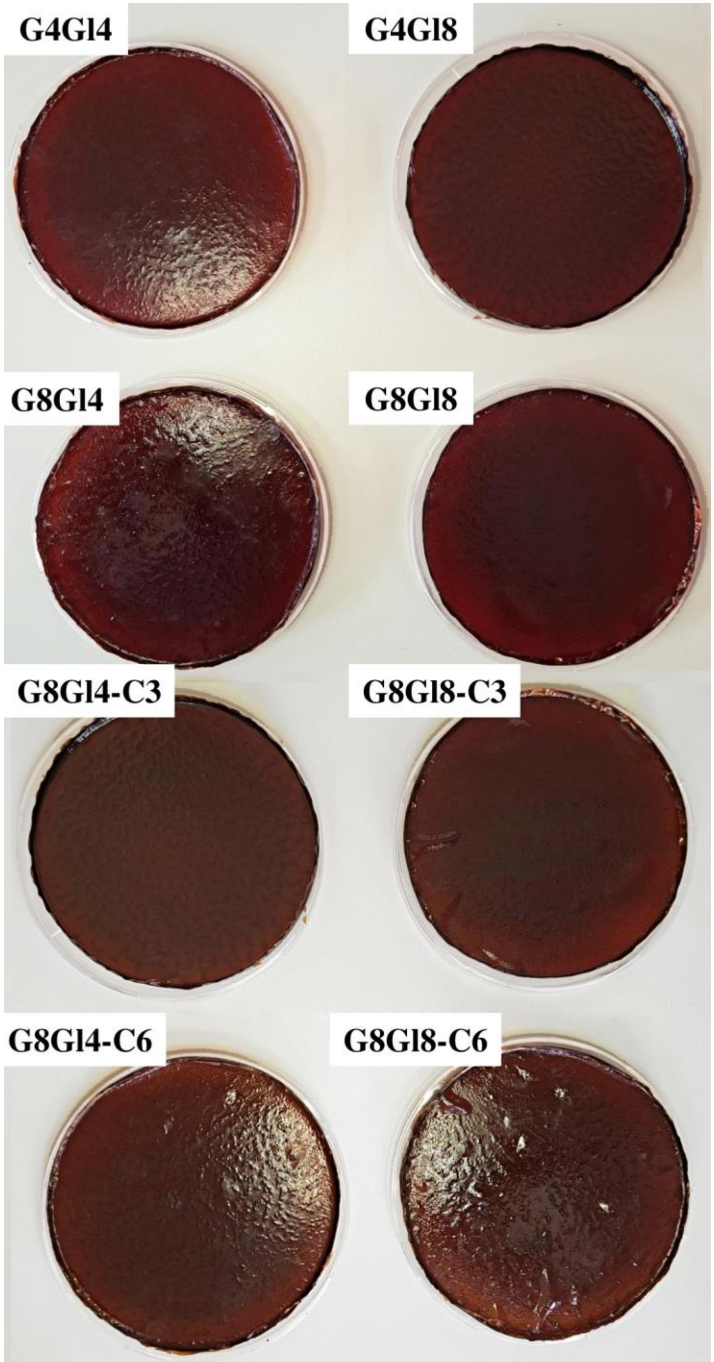

Figure 1. Photos of film samples

Incorporating CNC significantly increased bulk density ($P<0.05$). G8Gl4-C3 (0.865±0.011) and G8Gl4-C6 (0.872±0.012) were markedly denser than G8Gl4 (0.753±0.005), and similarly, G8Gl8-C3 (0.780±0.005) and G8Gl8-C6 (0.858±0.007) surpassed G8Gl8 (0.735±0.004). These findings, supported by Table 2, suggest that CNC establishes hydrogen bonds with gelatin and beetroot skin constituents, creating more compact networks (Chaichi et al., 2017; Reddy and Rhim, 2014). Increasing CNC concentration further reduced void spaces and enhanced matrix packing, in agreement with previous reports on CNC's reinforcing effects in pectin films (Chaichi et al., 2017). Although G8Gl4-C6, G8Gl8-C6 and G8Gl8-C3 showed high densities, their differences were not statistically significant, indicating that once CNC levels are elevated, variations in glycerol content have a diminished influence on density.

As bulk density rises due to the incorporation of CNC, the films not only become mechanically stronger but also less permeable to water vapor, with lower solubility. This compact, tightly bound matrix correlates with the reduced free volume and enhanced intermolecular interactions that govern mechanical integrity, water resistance, and moisture barrier performance.

These bulk density values align with those observed in CNC-reinforced agar films (Reddy and Rhim, 2014) and fall within a comparable range to other biopolymer matrices. The mechanism involves CNC filling interstitial gaps, thereby reducing porosity and improving compactness (Ren et al., 2024). This synergy between beetroot skin, gelatin, glycerol and CNC contributes to developing denser, high-performance biopolymer films, advancing their potential application in sustainable packaging solutions.

Water solubility

Solubility is a critical parameter in biodegradable films, reflecting their water resistance and suitability for various packaging applications (Cheng et al., 2024). The results, presented in Table 2, indicate how variations in gelatin, glycerol, and CNC content, as well as the inclusion of beetroot skin, influence water solubility. In line with the study's goal of developing sustainable, functional films from beetroot skin and CNC, formulations without CNC (e.g., G4Gl4: 64.3±1.5; G4Gl8: 75.3±2.1) were more soluble. The higher solubility of G4Gl8 compared to G4Gl4 ($P<0.05$) can be attributed to increased glycerol, which enhances hydrophilicity by disrupting polymer-polymer interactions and absorbing water (Bergo and Sobral, 2007; Park et al., 2008). Likewise, G8Gl8 (70±2.1) showed greater solubility than G8Gl4 (59.7±1.5) ($P<0.05$), reinforcing glycerol's role in promoting water uptake.

Incorporating CNC significantly reduced solubility ($P<0.05$), as observed when comparing G8Gl4-C3 (49.7±1.8) and G8Gl4-C6 (39.7±1.7) with G8Gl4 (59.7±1.5). A similar pattern emerged for G8Gl8-C3 (55±2.0) and G8Gl8-C6 (44.7±1.6) relative to G8Gl8 (70±2.1). CNC's hydrophobicity and ability to form dense, interwoven networks with gelatin and beetroot-derived polymers limit water penetration (Chaichi et al., 2017; Ren et al., 2024). Increasing CNC concentration further enhanced water resistance, compacting the matrix and reducing polymer chain mobility. These trends align with earlier studies, where CNC addition in agar films lowered solubility by creating a more crystalline, impermeable structure (Reddy and Rhim, 2014; Xiao et al., 2021).

Higher gelatin content (e.g., G8Gl4 vs. G4Gl4) also contributed to reduced solubility, as stronger intermolecular networks form more water-resistant matrices (Andiati et al., 2023). However, once CNC was incorporated at elevated levels, its impact overshadowed gelatin and glycerol effects. The reduced solubility in CNC-rich formulations reflects a tighter, more crystalline network that simultaneously supports higher tensile strength and greater opacity. By limiting water ingress, CNC helps maintain the structural integrity of the film, thereby influencing parameters like mechanical resilience and even color stability over storage.

Mechanical properties

Mechanical properties such as tensile strength and elongation at break are essential parameters for assessing the durability and performance of films in packaging applications (Fatima et al., 2024). The results in Table 2 show that variations in gelatin, glycerol, and CNC content, as well as the inclusion of beetroot skin, significantly influenced these properties. In line with the study's goal of creating robust, eco-friendly films from beetroot skin and CNC, careful adjustment of these components yielded distinct mechanical behaviors suitable for different end-uses.

Tensile strength values ranged from 10.02±0.16 MPa (G4Gl8) to 15.62±0.15 MPa (G8Gl4-C6). Formulations without CNC, such as G4Gl8 and G8Gl8, exhibited comparatively lower tensile strengths, primarily due to glycerol's plasticizing effect, which diminishes intermolecular interactions within the matrix (Bergo and Sobral, 2007). Increasing gelatin content, as observed in G8Gl4 compared to G4Gl4, enhanced tensile strength (*P<0.05*) by promoting a stronger polymer network that likely interacts favorably with the beetroot skin's inherent biopolymers (Andiati et al., 2023). The substantial improvements in tensile strength upon CNC addition, especially in G8Gl4-C6 and G8Gl8-C6, can be attributed to CNC's ability to form hydrogen bonds with the matrix, reinforcing the structure and integrating with beetroot-derived polymers to produce a denser, more cohesive network (Chaichi et al., 2017). Lower glycerol content further amplified CNC's reinforcing effect, as excess glycerol can disrupt polymer packing (Park et al., 2008).

Elongation at break displayed a contrasting trend. Films with higher glycerol content, such as G4Gl8 (58.4±0.8%) and G8Gl8 (58.3±0.8%), had significantly greater elongation at break (*P<0.05*), reflecting increased flexibility and molecular mobility (Bergo and Sobral, 2007; Reddy and Rhim, 2014). In contrast, CNC incorporation reduced elongation by introducing rigidity and reducing polymer chain mobility (Ren et al., 2024). Nonetheless, intermediate CNC concentrations (3%) offered a compromise, as seen in G8Gl4-C3 and G8Gl8-C3, which maintained moderate elongation while still benefiting from CNC's reinforcing properties (Chaari et al., 2022).

These mechanical enhancements, stemming from CNC-induced matrix reinforcement, also coincide with reductions in WVP and adjustments in opacity, indicating that a single structural modification can produce widespread improvements in both protective functions and visual characteristics of the film. These findings underscore the delicate interplay among beetroot skin components, gelatin, glycerol, and CNC, enabling the tailoring of mechanical attributes. By balancing rigidity and extensibility, it is possible to produce films with specific mechanical profiles that meet varying packaging requirements. The improved tensile strength from CNC reinforcement and the tunable elongation achieved through glycerol adjustments highlight a versatile strategy for developing sustainable, high-performance biodegradable films.

Water vapor permeability (WVP)

Water vapor permeability (WVP) is a crucial parameter in films particularly the ones designed for food packaging applications, since it reflects their capacity to serve as moisture barriers and maintain food quality (Henning et al., 2022). The results presented in Table 2 show that formulations without CNC, including those containing beetroot skin, exhibited relatively high WVP values. For instance, G4Gl8 recorded 2.423±0.074 $ng \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$, significantly higher than G4Gl4 (2.051±0.048 $ng \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$) (*P<0.05*), a difference primarily linked to the higher glycerol content. Glycerol, as a hydrophilic plasticizer, disrupts polymer networks (including those formed by beetroot-derived polymers) and increases water affinity, thus raising WVP (Bergo and Sobral, 2007; Park et al., 2008). A similar pattern appeared in G8Gl4 (1.554±0.092 $ng \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$) and G8Gl8 (1.857±0.044 $ng \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$), confirming glycerol's influence on permeability.

In contrast, incorporating CNC markedly reduced WVP, reflecting CNC's ability to form dense, crystalline networks that limit water diffusion. In line with the study's objective of enhancing functional properties through CNC addition to beetroot skin-based films, G8Gl4-C3 (0.850±0.035 $ng \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$) and G8Gl4-C6 (0.458±0.059 $ng \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$) displayed substantial improvements compared to G8Gl4 (1.554±0.092 $ng \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$) ($P<0.05$). Similarly, G8Gl8-C3 (1.053±0.076 $ng \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$) and G8Gl8-C6 (0.651±0.031 $ng \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$) surpassed G8Gl8 (1.857±0.044 $ng \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$), demonstrating CNC's efficacy even in formulations with high glycerol levels (Chaichi et al., 2017; Ren et al., 2024).

Higher CNC concentrations (6%) yielded the lowest WVP values, with G8Gl4-C6 achieving the most pronounced reduction. CNC's rigid, hydrogen-bonding structures minimize free volume and create tortuous paths for water molecules, counteracting the permeation-facilitating effect of glycerol (Chaari et al., 2022; Reddy and Rhim, 2014). Notably, even in glycerol-rich matrices, CNC significantly offset glycerol's negative influence, as evidenced by G8Gl8-C6.

The synergy between beetroot skin's intrinsic biopolymers and CNC not only affects thickness and color but also leads to improved mechanical strength and lower WVP. As beetroot-derived polymers integrate into the denser, CNC-reinforced network, they contribute not only to the film's base opacity but also support the overall structural cohesion that underpins improved barrier, mechanical, and optical performance. These findings highlight CNC's dominant role in improving moisture barrier properties, providing a key strategy to enhance WVP in beetroot skin-based biocomposite films. By incorporating CNC, it is possible to produce films with greater resistance to water vapor transmission, potentially extending shelf life and maintaining product quality.

CONCLUSION

This study demonstrates that beetroot skin, an underutilized agro-industrial residue, can serve as a viable base material for producing biocomposite films when combined with gelatin and glycerol. By incorporating crystalline nanocellulose (CNC), the films achieved substantial enhancements in mechanical strength, density, and moisture barrier properties, surpassing the limitations commonly observed in many biopolymer-based materials. In particular, the CNC-reinforced formulations exhibited reduced solubility, improved tensile strength, and significantly lowered water vapor permeability, indicating their potential for maintaining product quality and extending shelf life in food packaging applications.

Beyond improving functional performance, the developed films retained appealing optical characteristics, with beetroot-derived pigments imparting a distinctive coloration. Although introducing CNC altered film opacity and surface texture, the overall aesthetic remained suitable for commercial uses. The synergy between beetroot skin polymers and CNC created a denser, more cohesive network that balanced rigidity and flexibility, enabling customization of the films' mechanical attributes by adjusting glycerol and CNC concentrations.

These findings highlight the promise of valorizing agricultural byproducts to produce advanced, eco-friendly packaging solutions. The approach aligns with circular economy principles, transforming waste streams into value-added products while mitigating reliance on conventional petroleum-based plastics. Future investigations may focus on fine-tuning CNC content, exploring additional biopolymer combinations, and assessing the films' performance under various storage conditions. By refining these strategies, it may be possible to further improve functional, sensory, and environmental attributes, ultimately guiding this emerging technology toward broader industrial adoption.

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AUTHOR CONTRIBUTIONS

Emrah Kirtil: Conceptualization, Methodology, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review Editing, Supervision, Project Administration; Rukiye Nur Üngür: Methodology, Investigation; Gülşah Gürbüz: Methodology, Investigation, Visualization

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could have influenced the conduct, interpretation, or publication of this research.

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