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Article

The potential of DNA from industrial vegetables byproducts for the preparation of sustainable materials

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Abstract: Vegetable byproducts from the food and agroforestry industries are a source of several molecules and macromolecules that can find application in the development of high-value materials because of their intrinsic properties. Deoxyribonucleic acid (DNA) is found in all living systems and is widely available in nature. It is a macromolecule well known for its biological function related to carrying and transmitting genetic information. The chemical composition and arrangement of this macromolecule can generate new materials with noble properties that are still being explored for applications apart from their biological function. The purpose of this work was to study the film formation and its properties using the DNA extracted from the food industry byproducts, namely orange and banana, in order to evaluate their properties. The material was capable of forming large films with green, mild, and easy processing techniques. The films were characterized by mechanical tensile tests, Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA), indicating their potential as an alternative natural material for developments in composite and biomedical fields.

Keywords: DNA; biomass; film; sustainable materials

1. Introduction

The interest in using materials from renewable resources and biodegradable materials has been increasing in an attempt to explore their potential new properties. Its use can reduce waste disposal in the environment and avoid accumulation problems [1]. The agroforestry industry generates large amounts of biomass composed of by-products, residues, and derivatives of vegetables from food industries, papermaking, and others [2]. The vegetable biomass from the food industry has been extensively studied, and the components found in small amounts can represent the source of raw material with superior properties; however, they are few studied because of their low yield. These waste materials with superior properties can be useful to generate products with low production costs and high value-added applications [2,3].

Deoxyribonucleic acid (DNA) is found in any living organism, and thus it is available in fresh waste vegetables, an abundant source of raw materials from the agroforestry industry. The DNA molecule displays a double helical structure composed of nitrogenous base pairs (adenine, thymine, cytokine, and guanine) that provides stability to the molecule for the storage of the genetic code [4]. Apart from its biological function as the genetic material in living systems, the most promising DNA application is in the development of tools for diagnosis and disease treatment

[5], which has created possibilities to revolutionize clinical practice [6]. In cell-related applications and biomedical applications, it can be used for the immuno-modeling process, for protein production [7] and to manufacture DNA-based nanostructures such as aptamers, tetrahedrons, molecular beacons, nanoflow, nanotubes, dendrimers, scaffolds [8], hybrid devices [9–11], composites [12] and structured DNA nanoparticles [5]. It was also used for the preparation of thin films [13,14] for use in electronic and optoelectronic devices; however, to the best of our knowledge, much remains to be explored with respect to its structure, morphology, and properties, not deeply studied for application in material development. Thus, a strategy to increase the competitiveness of this unique material would be the study of its intrinsic features, such as its capability of forming films and its mechanical properties, aiming to widen its range of applications and generate high-value products.

Residues from the fruit processing industry could be effectively used in this context. For instance, oranges (*Citrus sinensis* L.) and bananas (*Musa sapientum* L.) are among the most consumed fruits worldwide and generate high amounts of residues, including whole fruits. Orange worldwide production in 2024 is 48.8 million tons [15], and about 115 million tons in 2017–2019 [16].

In this vein, the objective of this work is to evaluate the properties of the DNA from two abundant fresh vegetable wastes, the orange and banana, as the raw material for the preparation of films and to characterize the films, aiming to show their physic-chemical properties. The reuse of these raw materials represents an upgrade of these residues, contributing to the gradual implementation of the biorefinery concept in the food industry. In this perspective, after separating the DNA, their yield and capability of forming films were tested, and then the film properties were characterized by FTIR, tensile tests, and thermal analysis.

2. Materials and methods

2.1. DNA extraction

The whole waste oranges (1 kg) were peeled, cut, and ground using a high-speed kitchen blender (Arno, Power Max LN50, Brazil) for 5 min. A lysis solution was prepared by mixing 100 mL of sodium lauryl sulfate solution (5 wt%), 40 g of sodium chloride (NaCl), and distilled water to complete 600 mL. The lysis solution (600 mL) was added to 1000 g of the milled orange and kept resting for 3 min. The suspension was filtrated into a Becker cup using a sieve, and the filtrate was used for DNA precipitation. Cold ethanol (150 mL) was slowly added (with a pipette) to the edges of the Becker cup. The precipitated DNA was then visualized at the top of the mixture and separated using a glass rod. The precipitated DNA was washed with deionized water (100 mL), followed by cold ethanol (100 mL), and the washing process with water followed by ethanol was repeated twice. DNA from a waste peeled banana (1 kg) was extracted using exactly the same process; however, on the first step, the peeled fruit pulp was handily crushed within a plastic bag, handily homogenized for 5 min and stored at 8 °C.

2.2. DNA film preparation

The extracted DNA dispersed in ethanol was poured into Petri dishes and dried at room temperature (25 ℃) until the mass was stable, giving rise to the film.

2.3. DNA yield

The wet DNA (after washing with ethanol) was weighed, dried as described in item 2.2, and weighed on an analytical balance (final mass). The initial mass corresponds to the whole peeled vegetables before the extraction described in item 2.1. The mass values were used to calculate the yield, as shown in Equation (1):

$$\eta = (M_F \times 100)/M_i \quad (1)$$

where: η = yield (%), M_F = Final mass (g), M_i = Initial mass (g).

2.4. DNA film characterization

The chemical structure of DNA was analyzed using FTIR, their thermal and mechanical properties were determined using Differential Scanning Calorimetry (DSC), Thermogravimetric analysis (TGA) and tensile tests, respectively.

2.4.1. Fourier transform infrared (FTIR)

The dried films were used for the analyses using a Perkin-Elmer Spectrum 100 FT-IR Spectrometer equipped with an attenuated total reflectance (ATR) device of diamond coated with zinc selenide crystal. The spectra were in the range of 650 to 4000 cm^{-1}, with 4 cm^{-1} resolution after 16 spectrum scans, and the spectral outputs were recorded in transmittance.

2.4.2. Differential scanning calorimetry (DSC)

The samples were cut in a circular shape with approximately 8 mg and analyzed using a Perkin-Elmer DSC 8000 calorimeter calibrated with indium. The analyses were carried out in a nitrogen atmosphere at a 50 $mL \cdot min^{-1}$ flow rate. The heating and cooling cycles ranged from −25 to 200 ℃ at a scan rate of 10 ℃ min^{-1} using 40 μL aluminum standard pans.

2.4.3. Thermogravimetric analysis (TGA)

Analyzes were performed using a Perkin Elmer Thermogravimetric Analyzer Pyris 1 TGA at 20 mL/min nitrogen flow atmosphere. The samples (3–8 mg) were heated at 10 ℃ min^{-1} in the temperature range from 25 to 600 ℃, using a platinum crucible as a sample holder.

2.4.4. Mechanical tests (tensile tension)

Mechanical tests were performed in an Instron Universal Testing Machine, model 5969, with a 0.5 kN load cell and a deformation rate of 50 $mm \cdot min^{-1}$. Six specimens (9 × 1 × 0.1 cm) of each sample were tested. The samples were kept at 25 ℃ and 50% RH for 72 h before the tests. Young's modulus, stress at break, and elongation at break were calculated using the resulting curves from the mechanical tests.

3. Results and discussion

3.1. DNA extraction, film preparation and yield

DNA extraction followed the conventional approach reported for extraction, in which the surfactant (sodium laurel sulfate) was used to disrupt the lipid bilayer cell membrane, releasing the DNA; NaCl was used to remove the proteins bound to the DNA and keep them soluble in the aqueous medium; and ethanol was used for precipitation and separation of the DNA. **Figure 1** shows the extraction (A and B) and double washing with ethanol, showing the cleaner appearance of the DNA after treatment.

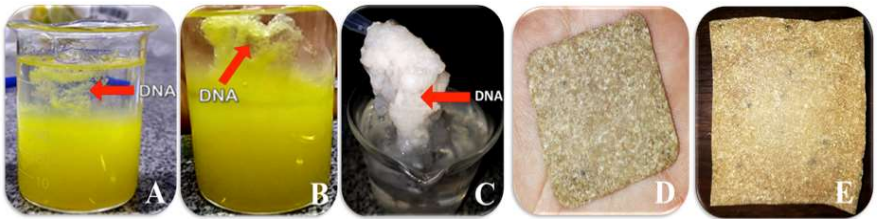

Figure 1. DNA extraction. **(A**, **B)** DNA extraction; **(C)** washed DNA in cold ethanol; **(D)** orange DNA film; **(E)** banana DNA film.

After extraction and washing, the films were prepared for further characterization. The films from banana and orange, after drying, were similar in visual appearance, showing a white, opaque color, a rough surface, and about 0.5 mm of thickness, as shown in **Figures 1D and 1E**. The solvent evaporation from the suspension of the precipitated DNA strands gave rise to the films. The ethanol evaporation led the DNA macromolecules to approximate each other; the molecules bind each other by intermolecular forces, including hydrogen bonds and van der Waals. These weak bonds are responsible for the intrinsic properties of the DNA, such as the mechanical strength and melting point [17].

The yield of the DNA extraction from the waste fruits was calculated using Equation (1), and the yields were 6 wt% for bananas and 4.5 wt% for oranges. Even with its low yield when compared to other natural products, such as cellulose (about 40 wt%), it can be seen as a potential source of material with noble properties for the search for novel applications.

3.2. Fourier transform infrared (FTIR)

The infrared absorption spectrum in both experiments showed the typical bands of the DNA functional groups, as shown in **Figure 2** for the banana film. The presence of intense bands in the region of 1009 cm^{-1} corresponds to the vibrations of the furan ring breath present in the deoxyribose; in the regions near the wavelength of 1630 cm^{-1} corresponds to the vibrations of the primary amides in nitrogenous bases; at 1735 cm^{-1} correspondent to the C=O groups, 1076, corresponding to the vibrations of the C–O–C; at 764 to the vibrations of the C=C bonds; at 1235 cm^{-1} correspondent to the phosphate ions vibrations; the peaks at 2930 and 2855 cm^{-1} corresponding to the aliphatic CH_2 present in deoxyribose; and at 3276 cm^{-1} corresponding to the free NH_2 of the nitrogenous bases [18]. The band at 3280 cm^{-1} corresponds to the OH groups.

The FTIR spectra of banana and orange DNA films are similar, suggesting the DNA structure dominates the spectrum, and no detectable impurities were revealed by this analysis. Also, both spectra are similar to the pure DNA spectrum from the literature [19,20].

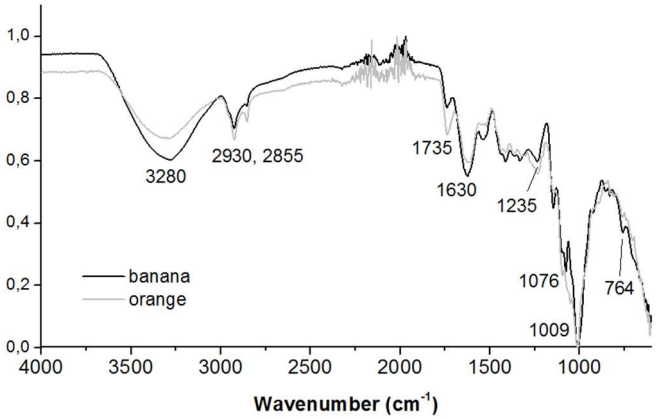

Figure 2. Infrared absorption spectrum (FTIR) of banana DNA.

The cell disruption using surfactant (sodium lauryl sulfate) was useful to separate the DNA from other cell components, including macromolecules such as cellulose and hemicelluloses, which are present in the cell wall and are not solubilized by surfactants. In agreement, the FTIR spectrum revealed no typical peaks for cellulose. The salt NaCl kept the proteins soluble even after the treatment with ethanol; thus, most of the proteins were separated from the DNA at this step, as revealed by the absence of the typical peaks of proteins in the spectrum.

3.3. Differential scanning calorimetry (DSC)

The DSC thermograms of DNA samples are shown in **Figure 3**. The results indicated an endothermic transition at about 80 ℃ for banana DNA and at about 100 ℃ for orange DNA, as the result of their intrinsic sequence of nitrogen bases, which can drive the force between the DNA strands. The DSC measurement determines the heat flow and the temperature associated with the changes in the structural/conformational state of the molecules and, for DNA, especially to study the nucleic acid-folding transitions [21,22]. The DNA molecule is formed by a double strand of nitrogenous bases. The two strands are stabilized by hydrogen bonds, which start to break when the temperature increases and result in their separation. The temperature at which half of the DNA is unwound or separated into single strands is called the melting temperature (T_m) [21,22]. The T_m depends on the composition of the DNA. The DNA double strand is formed by the intermolecular interaction of the base pairs A-T and C-G. The A-T base pairs interact via two hydrogen bonds, and the C-G base pairs interact, forming three hydrogen bonds. Each DNA species has a typical composition in A-T and C-G base pairs and a typical T_m. The higher the content of G-C base pairs, the higher their T_m because C-G base pairs require more energy to dissociate than A-T base pairs [22]. The DSC heating curves of the DNA indicated the endothermic event at the region of DNA typical T_m, indicating the heat treatment partially separated the DNA. The temperature effect was most intense for the banana

DNA. The slight event shown by the orange DNA can be the result of a molecule rich in C-G nitrogenous base pairs, indicating the heating DSC treatment separated just small parts of the DNA strands. The DSC thermogram profiles and the temperature revealed here for banana and orange DNA are in agreement with the DSC data range for DNA from other sources described in the literature [14].

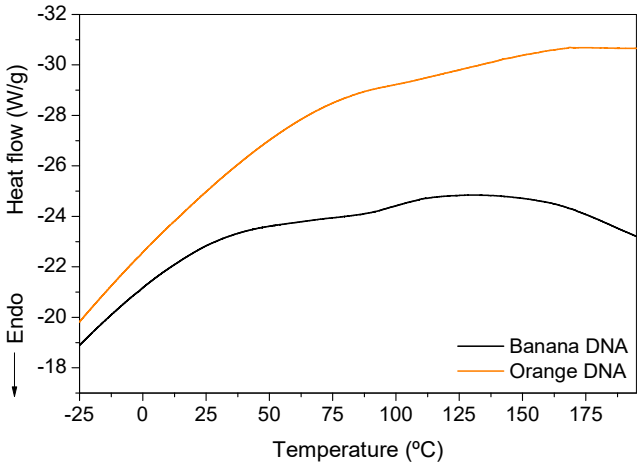

Figure 3. DSC curves of the DNA films.

3.4. Thermogravimetric analysis (TGA)

The TGA tracing of DNA is shown in **Figure 4**, showing the thermal stability and degradation profiles of the materials. The loss of mass (about 2 wt%) up to 100 ℃ can be attributed to water evaporation. The two samples showed a two-step loss of mass (about 60 wt%), which starts at 200 ℃, displaying the first peak at 220 ℃ and the second at 380 ℃. This two-step event can be attributed to the separation of the double-stranded DNA, followed by its degradation. The thermal decomposition process of both samples presented a single weight loss step profile.

The two-step degradation, most evident for the orange DNA, is strictly related to its structure, possibly because its composition is rich in C-G nitrogenous base pairs, which requires more energy to be broken when compared to the A-T-rich base pairs of the banana DNA, in agreement with the DSC results.

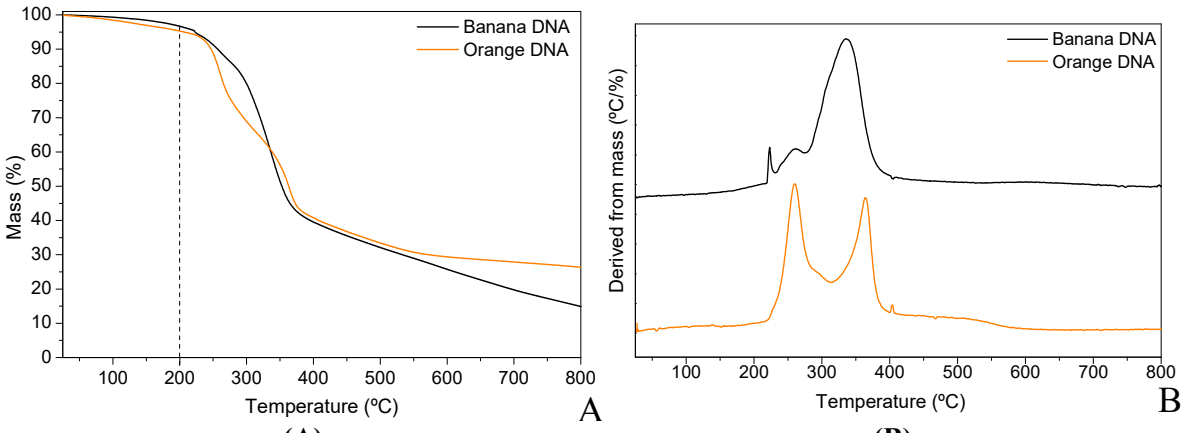

Figure 4. Thermogravimetric analysis (TGA); **(A)** orange and banana TGA curves; **(B)** orange and banana derivative.

The thermal features of the DNA indicated its good thermal stability, starting to degrade above 250 °C, similar to other natural polymers such as cellulose and nanofibrillated cellulose, which start to degrade at about 285 °C and 320 °C, respectively [23,24], pullulan, which starts to degrade at about 317 °C [25] and starch, which starts to degrade at about 325 °C [26]. The high thermal stability results reported here certainly reflect the excellent properties of DNA from waste fruits as a potential material to be used as reinforcing nanostructures/nanofibers for composite materials, for natural or synthetic matrices, replacing the conventional natural polymers.

3.5. Mechanical tests

The stress- strains of the DNA samples are shown in **Figure 5**. Both DNAs showed an almost linear behavior with fractures without large plastic deformations. The DNA samples display a stress-strain profile of a relatively brittle material. The most relevant differences between the DNA materials are the higher modulus and tensile strength of banana DNA with respect to orange DNA. Data for young modulus, tensile strength, and elongation at break are shown in **Table 1**. These mechanical properties of the films can determine their applications. The results showed the low elongation at break of both films and their relatively high Young´s modulus, suggesting applications as reinforcing materials for composite materials because of their high modulus and their use in new packaging or coatings because they self-assemble and are natural sources.

Table 1. Results of the mechanical tests of DNA films, mean and SD.

Results	Banana	Orange
Tensile strength (MPa)	2.16 ± 0.90	7.04 ± 1.59
Elongation at break (%)	2.45 ± 1.56	1.63 ± 0.31
Young's modulus (MPa)	157.50 ± 69.78	705.47 ± 90.75

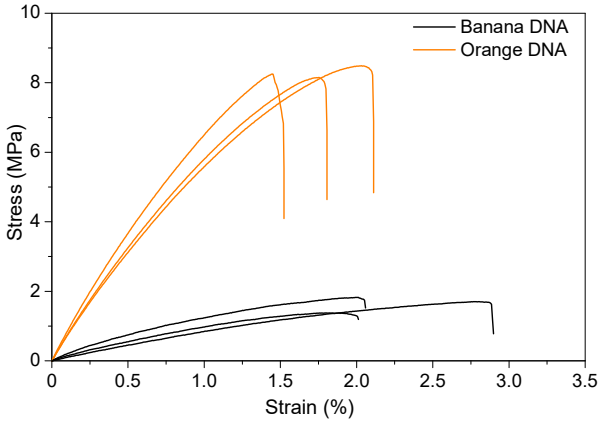

Figure 5. Stress-strain curves for DNA films.

In general, the results showed the use of biomass from the food and agroforestry industries to prepare self-assembled DNA films. The findings match the demand for the production of new sustainable materials using green methods. The methods for extraction and preparation are totally free of organic solvents and drastic procedures, contributing to the green tendency in material preparation. In addition, solvent-free

methods widen the range of applications of the material for use in medicine, food, and packaging. Its physicochemical properties also suggest its use in fields such as construction, transport, and manufactured goods. In addition, the sustainable utilization of biomass represents a strategy to valorize vegetable raw materials.

4. Conclusion

The extraction of DNA from bananas and oranges was efficient and cost-effective based on the yield, which was about 5 wt%. The characterization of DNA showed it can form films, and its thermal and mechanical properties are adequate for application in the materials science field. Its chemical structure and properties suggest it can be a potential raw material for innovative applications. In addition to the potential for the development of new materials, the reuse of vegetable residues can contribute to decreasing their disposal in nature, decreasing their associated environmental problems. In addition, the preparation of new materials from vegetable DNA can generate high-value products and contribute to the circular economy.

Author contributions: Conceptualization, ET and AJFC; methodology, TZdS; software, BFA; validation, TZdS, PNB and BFA; formal analysis, DdSC; investigation, BFA and PNB; resources, AJFC; data curation, DdSC; writing—original draft preparation, TZdS; writing—review and editing, ET; visualization, AJFC; supervision, ET; project administration, ET; funding acquisition, AJFC. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

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